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**Title:** Pre-Clinical Assessment of Single-Use Negative Pressure Wound Therapy During *In Vivo* Porcine Wound Healing.

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**Running Title**: Single-Use NPWT in Porcine Wound Healing.

**Key Words**: NPWT, Porcine, Wound Healing, Pressure.

**ABSTRACT**

**Objective:** Traditional negative pressure wound therapy systems can be large and cumbersome, limiting patient mobility and adversely affecting quality of life. PICO™, a no canister single-use system offers a lightweight, portable alternative to traditional negative pressure wound therapy, with improved clinical performance. The aim of this study was to determine the potential mechanism(s) of action of single-use negative pressure wound therapy versus traditional negative pressure wound therapy.

**Approach:** Single-use negative pressure wound therapy and traditional negative pressure wound therapy were applied to an *in vivo* porcine excisional wound model, following product use guidelines. Macroscopic, histological and biochemical analyses were performed at defined healing time-points to assess multiple aspects of the healing response.

**Results:** Wounds treated with single-use negative pressure displayed greater wound closure and increased re-epithelialisation versus those treated with traditional negative pressure. The resulting granulation tissue was more advanced with fewer neutrophils, reduced inflammatory markers, more mature collagen and no wound filler-associated foreign body reactions. Of note, single-use negative pressure therapy failed to induce wound edge epithelial hyperproliferation, while traditional negative pressure therapy compromised

peri-wound skin, which remained inflamed with high transepidermal water loss; features not observed following single-use treatment.

**Innovation:** Single-use negative pressure was identified to improve multiple aspects of healing versus traditional negative pressure treatment.

**Conclusion:** This study provides important new insight into the differing mode of action of single-use versus traditional negative pressure and may go some way to explaining the improved clinical outcomes observed with single use negative pressure therapy.

## INTRODUCTION

Our skin has evolved an innate ability to rapidly and efficiently repair injury and damage. This wound healing response is both complex and dynamic, requiring initial inflammation followed by granulation tissue formation, angiogenesis, re-epithelialisation and dermal remodelling (1). In the elderly and diabetic these normal reparative processes are substantially impaired, increasing the risk of developing non-healing, "chronic" skin wounds (2). Chronic wounds are a significant socio-economic and clinical burden, estimated to cost the UK's National Health Service more than £5 billion per year (3). The development and clinical implementation of therapies designed to address this ever-increasing and largely underappreciated area of clinical need remains a challenge (4, 5).

Negative pressure wound therapy (NPWT) is one of the most effective and widely-used interventions for problematic wounds (6). In traditional application, foam or gauze is used to fill the wound to allow negative pressure transmission through to the wound bed. A drape is then applied to form a sealed system. NPWT devices generate negative pressure between -50 and -175 mmHg (7), removing excess wound exudate via a drainage tube and preventing bacterial contamination (8,9). Early studies demonstrated that traditional NPWT (tNPWT)

promotes granulation tissue formation in pigs (10) and in the clinical setting (11,12). Additional, healing promoting effects of NPWT also include pulling wound margins together to accelerate contraction, stimulating cellular proliferation via microdeformation (13,14) and increasing tissue perfusion (15). Thus, NPWT is indicated for a plethora of wound types, including acute surgical, chronic, trauma, burns and skin grafts (7).

Despite its versatility, traditional NPWT (tNPWT) is not without limitations (16). For example, tNPWT devices can be seen as cumbersome, requiring large canisters, power supplies and drainage tubes. The utilisation of wound fillers (e.g. foam) add complexity of use, create longer application times and can cause discomfort and pain upon dressing changes. Indeed, it has been noted that there is potential for filler fragments to remain in the wound bed (17). Recently, lightweight negative pressure modalities have been developed to overcome some of these challenges inherent to larger tNPWT devices. One such example is a single-use NPWT system (sNPWT, PICO™, Smith & Nephew Wound Management, Hull UK), which is canister-free, portable and disposable.

Here, we present a direct comparison of sNPWT to tNPWT in an *in vivo* porcine injury model, with a focus on elucidating the effects of sNPWT on specific aspects of the wound repair response.

## CLINICAL PROBLEM ADDRESSED

tNPWT has been shown to influence multiple aspects of the wound repair process, but comes with clinical limitations. sNPWT has been developed to overcome these limitations, but the mode of action remains poorly understood. Here a standardised and reproducible *in vivo* porcine wound model is used to explore the effects of sNPWT on specific aspects of the healing response, with direct comparison to tNPWT. This porcine study, in a close model of human wound repair, provides significant new insight into the effects of sNPWT on healing and should inform future treatment innovations.

## MATERIALS AND METHODS

**Animal Experimentation:** Young (12-14 weeks) female Landrace x Large White x Duroc farm pigs (n=12, ~40 kg) were prepared for surgery via intramuscular injection of Azaperone (2 mg/kg) and Midazolam (0.3 mg/kg) and anaesthetised with isoflurane and oxygen. Prophylactic amoxicillin (15 mg/kg) was administered subcutaneously on the day of wounding, and buprenorphine (0.01 mg/kg) was given intramuscularly post-operatively and subsequently according to clinical need. Back and flank skin was clipped, wet-shaved and disinfected with 5% chlorhexidine, and the skin wound site swabbed with 70% ethanol immediately prior to the creation of full-thickness, 3 cm diameter excisional wounds (two wounds per flank on each pig). Digital photographs were then taken for macroscopic wound analysis.

Contralateral wounds were treated with sNPWT (PICO™ system with no filler, Smith & Nephew Wound Management, Hull, UK; -80 mmHg) or tNPWT (V.A.C Via™ system with Granufoam™ wound filler, KCI Medical Ltd., West Sussex, UK; -125 mmHg continuous mode). The PICO™ sNPWT system consists of a silicone wound interface dressing to transmit even pressure across the wound bed, while negative pressure in the traditional device was transmitted from the wound filler. sNPWT was changed every 6 days, while tNPWT was changed every 3 days, as per the 'Instructions for Use' provided with each device (**Supplemental Figure 1A**). A purpose made swine jacket with pockets (Lomir Biomedical Inc., Quebec, Canada) was used to support the NPWT device pumps on the animals.

**Wound Planimetry Analysis**: Wounds were digitally photographed at day 0 (n=12 pigs/24 wounds per treatment group), day 6 (n=12 pigs/24 wounds per treatment group) and day 12 (n=8 pigs/16 wounds per treatment group). Macroscopic wound closure analysis was

performed using Image Pro Plus v.4.1.0 (Media Cybernetics, Maryland, USA). The wound area remaining open, and the contribution of re-epithelialisation and contraction to overall wound closure were measured as described below (see also **Figure 1A**):

$$\% \text{ wound area remaining open} = \frac{\text{open wound area at day x}}{\text{original wound area at day 0}} \times 100$$

$$\% \text{ contraction} = \frac{\text{contracted wound area at day x}}{\text{original wound area at day 0}} \times 100$$

$$\% \text{ re-epithelialisation} = \frac{\text{contracted wound area at day x - open wound area at day x}}{\text{original wound area at day 0}} \times 100$$

**Skin and Wound Assessment**: Surface wound damage was determined at each dressing change, where 0 = no bleeding, 0.5 = removal of surface tissue without bleeding, 1 = minimal bleeding, 2 = moderate bleeding and 3 = substantial bleeding. Skin colour measurements were taken using a spectrophotometer (X-Rite Sp68 Sphere, Manchester, UK) and expressed using the CIELAB colour notation system. A Tewameter® TM 300 was used to measure transepidermal water loss (TEWL) and skin hydration, and a Mexameter® MX 18 probe (both Courage and Khazaka, Germany) was used to measure erythema (using a redness index). These measurements were made, after sNPWT dressing or tNPWT drape removal, in two regions around the wound: a) the peri-wound (approximately 0.5-1 cm away from wound edge) and; b) the extended zone (2.5-3 cm away from wound edge). For TEWL, an average of the first 30 readings was taken following skin acclimatisation post-dressing removal. For skin hydration (skin surface moisture), a reading was taken immediately following dressing removal with no acclimatisation (at the extended zone site only). Wound depth was assessed in three defined wound regions using a depth gauge. These measurements were taken on n=12 pigs (24 wounds) at day 6 and n=8 pigs (16 wounds) at day 12.

**Tissue Collection**: Histological samples were collected from n=4 pigs harvested at day 6 (n=8 wounds per treatment group) and n=4 pigs harvested at day 12 (n=8 wounds per

treatment group), pre-selected during study planning. Strips (1 cm wide) of wound tissue and marginal skin (cranio-caudal orientation) were harvested and placed in 10% buffered formal saline for histological analysis. Wound tissue (n=4, one wound per pig per time-point) was placed in RNA*later*™ (Thermo Fisher Scientific, Paisley, UK) and frozen at -80ºC for PCR Array profiling. Normal skin, peri-wound skin (immediately adjacent to the wound) and extended zone skin (under the sNPWT dressing island, or tNPWT drape) was also collected for comparison.

**Histology**: Paraffin embedded sections (6 μm thick) were dewaxed and rehydrated prior to staining. Haematoxylin and eosin (H&E) was used to visualise trapped filler material and to quantify granulation tissue depth and re-epithelialisation via Aperio ImageScope image analysis software (Leica Biosystems, Milton Keynes, UK). Picrosirius Red (PSR) colour analysis allowed histological assessment of matrix maturity, where immature (green birefringence) and mature (red birefringence) fibres were visualised via polarising light and quantified (as in 18). For BrdU analysis, proliferating cells were labelled 1 hour prior to culling via i.p. injection of 1 mg 5-Bromo-2'-deoxyuridine (BrdU, B5002, Sigma-Aldrich) in 100 mL physiological saline. BrdU was traced using an anti-BrdU (Bromodeoxyuridine) antibody (GE Life Sciences, Buckinghamshire, UK). Neutrophils were stained using an anti-NGAL (neutrophil gelatinase-associated lipocalin) antibody (Enzo Life Sciences Inc, New York, USA). Bound antibodies were detected via ABComplex and 3,3′-Diaminobenzidine (DAB; Vector Laboratories Ltd., Peterborough, UK). The number of BrdU+ve and NGAL+ve cells were determined using Image Pro Plus.

**Transcriptional Profiling**: Porcine skin and wound tissue was homogenised (T10 basic, IKA, Oxford, UK) in TRIzol® reagent (Thermo Fisher Scientific). Chloroform was added for

phase separation and RNA removed and purified using a PureLink RNA Mini Kit (Thermo Fisher Scientific) following manufacturer's instructions. RNA concentration was determined using a SimpliNano nanodrop (Biochrom, Cambridge, UK) and adjusted to 1 µg/µL. Reverse transcription was performed with Random Primers (Promega, Southampton, UK) and Bioscript reverse transcriptase (Bioline, London, UK). cDNA was diluted in nuclease free water and each sample plated in $RT^2$ Profiler™ PCR array plates (Pig Wound Healing; Qiagen, Manchester, UK) with 2X Takyon SYBR mastermix (Eurogentec, Hampshire, UK). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed and data was analysed in CFX Manager software on a CFX connect thermocycler (Biorad Laboratories Ltd., Hertfordshire, UK).

**Statistical Analyses**: All data are presented as mean +/- standard error of the mean (SEM). Pair-wise *t* tests were performed on data sets comparing sNPWT and tNPWT at one time-point. One-way ANOVA was performed on qRT-PCR data comparing normal skin and day 12 treatments. Two-way ANOVA was performed on all other data sets with appropriate *post-hoc* analysis (Tukey or Sidak). Statistical analysis was performed in GraphPad Prism v.7.0 (GraphPad Software, CA, USA). Data were considered significant at the $P < 0.05$ level.

## RESULTS

**sNPWT leads to greater wound closure than tNPWT.** Planimetric analysis was performed on scaled macroscopic wound images taken at day 0, day 6 and day 12 (**Figure 1A**). The area of the wound remaining open, determined as a percentage of day 0 wound area, was significantly smaller following sNPWT than tNPWT at both day 6 (70.06% vs 78.55%; $P < 0.001$) and day 12 (18.56% vs 33.36%; $P < 0.001$) post-injury (**Figure 1B**). Similarly, macroscopic quantification demonstrated significantly greater re-epithelialisation with sNPWT compared to tNPWT at days 6 (4.46% vs 0.55%; $P < 0.01$) and 12 (22.73% vs 8.4%; $P < 0.001$; **Figure 1C**). Wound contraction was greater at day 6 for sNPWT treated wounds ($P < 0.05$; **Figure 1D**) versus tNPWT, and was found to be similar between treatments at day 12. Accelerated re-epithelialisation following sNPWT treatment was confirmed by histological analysis of H&E stained tissue sections at day 12 ($P < 0.001$; **Figure 1E-F)**. Interestingly, the neo-epidermis of wounds under tNPWT was extremely hyperproliferative at day 12, as demonstrated by increased numbers of proliferative (BrdU+ve) cells ($P < 0.001$; **Figure 1G, I**) and increased peak epidermal thickness (**Figure 1H-I)**. Collectively, these data demonstrate that sNPWT accelerates wound closure compared to tNPWT, with increased epithelial migration and reduced wound edge hyperproliferation.

**Reduced wound bed inflammation following sNPWT treatment.** Immunohistochemistry for neutrophils was performed to assess the level of early inflammatory cells in porcine NPWT-treated wounds. Quantification showed significantly higher neutrophil numbers in tNPWT treated wounds compared to sNPWT at day 12 post-wounding (**Figure 2A**; $P < 0.001$). Transcriptional profiling revealed statistically significant upregulation of a number of pro-inflammatory cytokines including *CXCL11* (Day 6), *CSF2*, *IL-1α* and *IL-1β* (day 12) in

tNPWT treated wounds (**Figure 2B-H**). Collectively, these findings support higher wound bed inflammation in tNPWT versus sNPWT treated wounds.

**sNPWT promotes granulation tissue maturation and causes less damage to the wound bed than tNPWT.** Similar wound filling was observed between sNPWT and tNPWT at day 6. However, by day 12 tNPWT led to significantly reduced macroscopic wound depth ($P < 0.001$; **Figure 3A**) and increased wound granulation tissue deposition (measured by histology; $P < 0.001$; **Figure 3B)** compared to sNPWT. While wounds filled faster under tNPWT, the quality and maturity of granulation tissue formed in these wounds was inferior to that following sNPWT application. Picrosirius red (PSR) staining determined granulation tissue extracellular matrix maturity in discrete upper wound regions (**Figure 3C-F**). Here, sNPWT led to significantly increased total collagen deposition (brightfield; $P < 0.05$; **Figure 3E**). Polarising light microscopy revealed that sNPWT treatment increased both immature (green birefringence) and mature (red birefringence) collagen fibre deposition compared to tNPWT ($P < 0.001$; **Figure 3D, F**).

Wound maturation was evaluated by measuring the level of granulation tissue cellular proliferation. Here, sNPWT treated upper wound tissue contained fewer BrdU+ve cells versus tNPWT ($P < 0.001$; **Figure 3G-H**). qRT-PCR array analysis further substantiated increased maturity of sNPWT wounds, with higher expression of wound matrix components, *COL1A2* ($P < 0.01$; **Figure 3I**) and *COL3A1* ($P < 0.001$; **Figure 3J**), granulation-promoting factors, *CTGF* ($P < 0.001$; **Figure 3K**), and proteoglycans, *DCN* (**Figure 3L**), in sNPWT wounds. By contrast, tNPWT treated wounds displayed substantially elevated levels of the tissue remodelling matrix metalloproteinases, *MMP3* ($P < 0.01$; **Figure 3M**) and *MMP9* ($P < 0.05$; **Figure 3N**), but not *MMP2* (**Supplementary Figure 2A**).

Evaluation of H&E stained sections revealed trapped filler material/foreign body reactions in 50% of tNPWT treated wounds (representative images in **Figure 4A**). By contrast, no trapped filler material or foreign body reactions were detected in any sNPWT treated wounds. Additionally, tNPWT dressing removal resulted in significantly more wound surface damage with noticeable bleeding compared to removal of sNPWT dressing at day 6 ($P < 0.001$) and day 12 ($P < 0.001$; **Figure 4B**). Overall, these data reveal that sNPWT increased granulation tissue maturation, without the trapped filler and damage observed following tNPWT.

**Reduced surrounding skin disruption with sNPWT versus tNPWT.** Skin barrier function and erythema were assessed in the peri-wound skin and the extended zone (schematic, **Supplementary Figure 1B**) to determine whether NPWT application affected the function of skin surrounding the wound. Extended zone skin hydration, measured immediately after dressing or drape removal, revealed significantly higher moisture content in skin under tNPWT compared to skin under sNPWT ($P < 0.001$ at days 6 and 12; **Figure 5A**). In addition, TEWL, a direct measure of skin barrier, was significantly elevated in peri-wound skin under the tNPWT drape compared to that under the sNPWT dressings ($P < 0.001$; **Figure 5B**). The redness (erythema) of tNPWT treated peri-wound skin was significantly greater than sNPWT treated skin ($P < 0.001$; **Figure 5C**), with a non-significant trend towards an increased erythema index (**Figure 5D**). Collectively, these *in vivo* data suggest that sNPWT causes far less disruption to the skin surrounding a wound.

**tNPWT, but not sNPWT, causes heightened proliferation and inflammation in peri-wound skin.** Next, the cellular correlates to the observed reduced redness and TEWL in sNPWT treated peri-wound skin were assessed histologically. Reduced epidermal BrdU+ve (proliferating) cells were observed in the sNPWT treated peri-wound tissue at day 6 ($P <$

0.05) and day 12 ($P < 0.05$; **Figure 6A-B**), commensurate with reduced tissue damage. Transcriptional analysis revealed elevated inflammation in tNPWT treated peri-wound skin with upregulation of the inflammatory markers *CSF2* ($P < 0.05$; **Figure 6C**), *IL-1α* (**Figure 6D**), *IL-1β* ($P < 0.05$; **Figure 6E**). In addition, *MMP2* was specifically upregulated at day 6 ($P < 0.05$; **Figure 6F**; **Supplementary Figure 2**). Taken together, these data suggest that tNPWT treatment adversely influences the peri-wound skin region, while sNPWT supports a pro-healing wound edge environment.

## DISCUSSION

Traditional NPWT devices were successfully implemented in wound treatment over 20 years ago (11,19). In these applications a wound filler (foam or gauze) is required to deliver negative pressure to the wound bed and to serve as a fluid conduit. As previously mentioned, the PICO™ sNPWT system uses a very different technology, a silicone wound interface dressing with an incompressible airlock layer that transmits pressure evenly across the wound bed, peri-wound and wider skin region (20). Interestingly, a recent randomised control trial demonstrated that sNPWT achieved greater wound closure (45%) compared to tNPWT (22%) with fewer adverse events, such as wound maceration (21). Concurrent cost-based analysis revealed that sNPWT was more cost-effective than tNPWT (22), suggesting benefits that extend beyond the patient.

The current study was specifically designed to explore the mode of action of sNPWT compared to tNPWT using an *in vivo* porcine wound repair model. Note, previous studies (e.g. 23) have performed direct porcine side-by-side comparison of tNPWT devices demonstrating relative equivalence. Here we report, for the first time, detailed macroscopic and histological comparison of sNPWT and tNPWT *in vivo*. We show that sNPWT

accelerated wound closure, promoted wound re-epithelialisation and increased granulation tissue maturity when compared with tNPWT. In fact, tNPWT actively prevented re-epithelialisation, inducing substantial wound edge epidermal hyperproliferation. tNPWT treated wounds also displayed extensive filler material trapped in the granulation tissue (observed in earlier studies; in pigs (24,25) and in patients (26-28)), which may contribute to heightened local tissue inflammation (29).

NPWT-associated healing has previously been linked to dampened pro-inflammatory cytokines, *TNFα* and *IL-1β*, compared to non-NPWT treatment (30,31). In our study, tNPWT treated wound granulation tissue displayed increased inflammation and upregulation of the pro-inflammatory the cytokines, *CSF2*, *CXCL11*, *IL-1α* and *IL-1β*, compared to sNPWT treatment. The heightened damage response following tNPWT extended to the peri-wound skin region, which also contained increased pro-inflammatory marker expression and increased redness, indicative of erythema (32). This local damage likely results from both the differential forces experienced by tNPWT and sNPWT treated wounds, and the differences in frequency of dressing change. Interestingly, Karaback et al. (33) report that compressional injury from tNPWT drape drainage tubes can cause spontaneous wound formation in compromised skin surrounding a wound.

Higher TEWL and excessive hydration were observed in the skin surrounding tNPWT treated wounds. TEWL is a direct measure of skin barrier integrity, where higher TEWL is associated with a compromised barrier (34,35). High TEWL is a potential indicator of skin maceration risk, an observation noted by Kirsner et al. (21) in their randomised control trial following tNPWT use. Loss of barrier integrity also increases the risk of infection (36), a common problem when negative pressure application fails and wound exudate is not

effectively managed (26). Indeed, in the present study, sNPWT delivered active therapy 97% of the time requiring only 14 device-related interventions, versus 24 interventions to correct leaks and blockages under tNPWT.

In addition to neo-epidermal hyperproliferation, tNPWT caused excessive cellular proliferation in the peri-wound region and in the wound granulation tissue. High levels of epidermal proliferation are a hallmark of wound pathology, and have previously been observed following tape stripping (37), suggesting that tNPWT dressing removal may cause similar damage. By contrast, mature wound granulation tissue is typically characterised as relatively acellular (38). In the present study, granulation tissue of tNPWT treated wounds was highly proliferative, with fewer mature collagen fibres and increased wound matrix metalloproteinases, indicating reduced maturation compared to sNPWT. Overall, these data suggest that sNPWT independently promotes granulation tissue maturation and re-epithelialisation. Indeed, these two aspects could be closely linked, with a mature wound bed important to permit active re-epithelialisation.

Data now show a direct link between compromised skin barrier and subsequent wound recurrence (39), a significant consideration for chronic wound management. Although several clinical studies have demonstrated enhanced wound closure and faster granulation with tNPWT (40,41), few studies have performed follow-up assessments to determine rates of wound recurrence (42). A future pre-clinical *in vivo* investigation would provide a unique opportunity to explore recurrence, an important and often overlooked aspect of wound healing studies.

## INNOVATION

The results of this pre-clinical *in vivo* study clearly demonstrate that sNPWT, which delivers negative pressure using a unique multi-layered interface technology, promotes faster healing than tNPWT. While tNPWT treatment led to wound damage and inflammation, sNPWT stimulated faster re-epithelialisation and promoted granulation tissue maturation. This study therefore provides new mechanistic insight that informs the enhanced wound healing outcomes of sNPWT observed in the clinical setting.

## KEY FINDINGS

- Single-use NPWT (sNPWT) promotes a faster rate of wound closure than traditional tNPWT (tNPWT) in an *in vivo* porcine model.
- sNPWT improves multiple aspects of healing, promoting re-epithelialisation, dampening inflammation, and increasing granulation tissue maturity.
- sNPWT avoids the detrimental effects of tNPWT on the peri-wound epithelium.
- sNPWT appears to circumvent the foam-trapping and foreign body reactions that are frequently observed with tNPWT.

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## AUTHOR DISCLOSURE AND GHOSTWRITING

The content of this study was exclusively contributed and written by the authors listed. Varuni R. Brownhill, Elizabeth Huddleston and Iain Webster are employees of TJ Smith & Nephew Ltd. The authors declare no other conflicts of interest. No ghost writers were involved in the completion of this article.

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## ABBREVIATIONS AND ACRONYMS

BrdU – Bromodeoxyuridine

H&E – Haematoxylin and eosin

NPWT – Negative pressure wound therapy

PSR – Picrosirius red

qRT-PCR – quantitative real-time polymerase chain reaction

sNPWT – Single-use negative pressure wound therapy

tNPWT – Traditional negative pressure wound therapy

TEWL – Transepidermal water loss

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## FIGURE LEGENDS

**Figure 1. Single-use negative pressure wound therapy accelerates porcine wound closure.** Single-use negative pressure wound therapy (sNPWT) or traditional NPWT (tNPWT) was applied to 3 cm diameter full-thickness excisional wounds. Representative macroscopic images showing the impact of treatment over time (**A;** Bar = 1 cm). Macroscopic analysis was performed to determine original wound area (day 0; outer dashed line), wound area remaining open (inner dotted line) and wound contraction (central solid line). Quantification of wound area (**B**), percentage re-epithelialisation (**C**) and wound contraction (**D**) over time. Representative day 12 H&E images (**E;** Bar = 1 mm, Arrows = length of neo-epithelium), and quantification of histological re-epithelialisation (**F**). Neo-epidermal proliferation (**G**) and peak neo-epidermal thickness (**H**) with representative BrdU staining at day 12 (**I**; Bar = 1 mm, Arrows = peak thickness). Dotted line separating epidermal and dermis (**I**). Mean +/- SEM. **B-D**, n=8-12 pigs (16-24 wounds per treatment group), **F-H**, n=4 pigs (8 wounds per treatment group). * = $P < 0.05$, ** = $P < 0.01$, *** = $P$

< 0.001. Two-way ANOVA was performed on **B, C, D, F**. Independent two-tailed student's *t* test performed on **G** and **H**.

**Figure 2. Single-use negative pressure dampens inflammation in porcine wounds**. Wounds treated with traditional NPWT (tNPWT) showed increased neutrophil infiltration at day 12 (**A**), and higher cytokine marker expression (qRT-PCR) at day 6 and day 12 post-wounding (**B-H**). Mean + SEM. n=4 pigs (**A**, 8 wounds per treatment group, **B-H**, 4 wounds per treatment group). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Independent two-tailed student's *t* test was performed on **A**. Two-way ANOVA was performed on all other data sets.

**Figure 3. Porcine wound maturation is accelerated with single-use negative pressure wound therapy compared to traditional application**. Increased wound depth (**A**) and reduced granulation tissue (GT) depth (**B**) were shown following single-use negative pressure wound therapy (sNPWT) compared to traditional NPWT (tNPWT). Schematic depicting outer (O), inner (I), and central (C) histological assessment regions of wounds (**C**). Wound maturation was assessed at day 12. Picrosirius red staining under brightfield (BF) and polarised (Pol) light (**D**). Bar = 50 μm. sNPWT treatment increased BF matrix deposition (**E**) and increased immature (green birefringence) and mature fibres (red birefringence; **F**). Cell proliferation (**G**, quantified in **H**) within granulation tissue was higher in wounds treated with tNPWT. Bar = 200 μm. Arrows = proliferative cells. PCR array analysis demonstrated elevated matrix gene expression in sNPWT day 12 wounds (**I-L**) and reduced matrix metalloproteinases (**M-N**). Mean + SEM. **A**, n=8-12 pigs (16-24 wounds per treatment group), **B, E-F**, n=4 pigs (8 wounds per group), **I-N**, n=4 pigs (4 wounds per group). * = $P <$

0.05, ** = $P < 0.01$, *** = $P < 0.001$. Two-way ANOVA used on data sets **A, B, E, F, H**, one-way ANOVA used on data sets **I-N**.

**Figure 4. Filler foam in traditional negative pressure wound therapy causes wound damage**. Traditional negative pressure wound therapy (tNPWT) left foam in wounds (**A**, H&E staining) and caused more wound surface damage on removal than single-use NPWT (**B**). 0 = no damage, 3 = substantial bleeding. Black stars = filler material. Yellow stars = foreign body reactions. Bars = 100 μm. Mean + SEM. n=8-12 pigs (16-24 wounds per treatment group). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Two-way ANOVA with Tukey's *post-hoc* test was performed.

**Figure 5. Traditional negative pressure wound therapy increases erythema and transepidermal water loss in peri-wound skin.** Traditional negative pressure wound therapy (tNPWT) increased hydration in the extended zone region (**A**) and increased TEWL in the peri-wound skin (**B**). tNPWT also caused more erythema (**C-D**) in the peri-wound skin region. NS line = normal skin value. Mean + SEM. n=8-12 pigs (16-24 wounds per treatment group). *** = $P < 0.001$. Two-way ANOVA was performed with Sidak *post-hoc* analysis.

**Figure 6. Traditional negative pressure wound therapy causes inflammatory damage to peri-wound skin.** Traditional negative pressure wound therapy (tNPWT) increased peri-wound epidermal proliferation at day 6 and day 12 (**A,** quantified in **B**). Bar = 200 μm. Arrows = BrdU+ve cells. PCR array demonstrated upregulation of inflammatory genes in the peri-wound skin at day 6 post-wounding (**C-F**). Mean + SEM. **B**, n=4 pigs (8 wounds per treatment group), **C-F**, n=4 pigs (4 wounds per group). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Two-way ANOVA was performed on **B**. Paired *t* tests were performed on **C-F**.

**Supplementary Figure 1. Experimental setup and live phase assessments.** Single-use negative pressure wound therapy (sNPWT) or traditional NPWT (tNPWT) was applied to 3 cm diameter full-thickness excisional wounds. Numbers indicate day post-injury. sNPWT was changed every 6 days (blue arrows) and tNPWT was changed every 3 days (red arrows) as per instructions for use. At day 6 and 12, live phase assessments and macroscopic measurements were performed (all animals), while tissue was collected for histology and qRT-PCR (**A**). For live phase assessment, transepidermal water loss (TEWL) and hydration measurements (T), and colour and erythema measurements (C), were taken from the peri-wound (green *), extended zone (purple *) and normal skin (red *) regions (**B**).

**Supplementary Figure 2. Negative pressure therapy differentially alters MMP expression.** PCR arrays were used to assess the expression of *MMP2*, *MMP3* and *MMP9* in wounds at day 12 (**A**) and in the peri-wound region at day 6 (**B**). NS = normal skin. tNPWT = traditional negative pressure wound therapy. sNPWT = single-use NPWT. Mean + SEM. n=4 pigs (4 wounds per group). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. One-way ANOVA was performed on **A**. Paired *t* tests were performed on **B**.