Effects of topical negative pressure therapy on tissue oxygenation and wound healing in vascular foot wounds

Nathaniel Chiang, MBChB, FRACS,[a] Odette A. Rodda, MBBS,[b] Jamie Sleigh, MBChB, MD, FANZCA,[a] *and* Thodur Vasudevan, MD, FRACS,[a] *Hamilton, New Zealand; and Melbourne, Victoria, Australia*

ABSTRACT

Objective: Topical negative pressure (TNP) therapy is widely used in the treatment of acute wounds in vascular patients on the basis of proposed multifactorial benefits. However, numerous recent systematic reviews have concluded that there is inadequate evidence to support its benefits at a scientific level. This study evaluated the changes in wound volume, surface area, depth, collagen deposition, and tissue oxygenation when using TNP therapy compared with traditional dressings in patients with acute high-risk foot wounds.

Methods: This study was performed with hospitalized vascular patients. Forty-eight patients were selected with an acute lower extremity wound after surgical débridement or minor amputation that had an adequate blood supply without requiring further surgical revascularization and were deemed suitable for TNP therapy. The 22 patients who completed the study were randomly allocated to a treatment group receiving TNP or to a control group receiving regular topical dressings. Wound volume and wound oxygenation were analyzed using a modern stereophotographic wound measurement system and a hyperspectral transcutaneous oxygenation measurement system, respectively. Laboratory analysis was conducted on wound biopsy samples to determine hydroxyproline levels, a surrogate marker to collagen.

Results: Differences in clinical or demographic characteristics or in the location of the foot wounds were not significant between the two groups. All patients, with the exception of two, had diabetes. The two patients who did not have diabetes had end-stage renal failure. There was no significance in the primary outcome of wound volume reduction between TNP and control patients on day 14 (44.2% and 20.9%, respectively; $P = .15$). Analyses of secondary outcomes showed a significant result of better healing rates in the TNP group by demonstrating a reduction in maximum wound depth at day 14 (36.0% TNP vs 17.6% control; $P = .03$). No significant findings were found for the other outcomes of changes in hydroxyproline levels (58.0% TNP vs 94.5% control; $P = .32$) or tissue perfusion by tissue oxyhemoglobin saturation (19.4% TNP vs 12.0% control; $P = .07$) at day 14. At 1 year of follow-up, there were no significant outcomes in the analysis of wound failure, major amputation, and overall survival rates between the two groups.

Conclusions: In this pilot study, applying TNP to acute high-risk foot wounds in patients with diabetes or end-stage renal failure improved the wound healing rate in reference to wound depth. This suggests that TNP may play a role in enhancing wound healing. This study sets the foundation for larger studies to evaluate the superiority of TNP over traditional dressings in high-risk foot wounds. (J Vasc Surg 2017;66:564-71.)

From the Department of Vascular Surgery, Waikato Hospital, Hamilton[a]; and the Department of Vascular Surgery, St. Vincent's Hospital, Melbourne.[b]

This work was supported by the Waikato Medical Research Foundation, Faculty Research Development Fund, Braemar Charitable Trust Research Grant, Bayers Health Care Fund, Department Grant-in-aid, Performance-Based Research Fund Allocation, Postgraduate Student Fund, and the PReSS Fund. There were no significant relationships with HyperMed Inc (developer of OxyVu), Intermed (manufacturer of VAC), and ARANZ (manufacturer of the FastScan and Silhouette Mobile). The devices were bought and leased using independent research funds. There were no conflicts of interest that were directly relevant to the content of this manuscript.

Author conflict of interest: none.

Presented at the 2015 Annual Scientific Meeting of the Australian and New Zealand Society for Vascular Surgery (ANZSVS), Wailea, Hawaii, September 21-24, 2015.

Correspondence: Odette A. Rodda, MBBS, Department of Vascular Surgery, St. Vincent's Hospital, 41 Victoria Parade, Fitzroy VIC 3065, Australia (e-mail: odette.rodda@svha.org.au).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214

http://dx.doi.org/10.1016/j.jvs.2017.02.050

The management of foot wounds is a major concern in vascular surgery owing to the unpredictable time required for healing. Chronic illnesses, such as diabetes, along with our aging population, exacerbate the effect and incidence of lower limb ulcers.[1-3]

Interventions that enhance wound healing after surgical débridement or minor amputations of a foot (eg, toe or forefoot amputations) may salvage limbs. Providing there is an adequate blood supply, the main objective would be to apply appropriate dressings dictated by the condition of the wound. Commonly used dressings can be subdivided into two groups: traditional dressings comprising a range of products, such as hydrogels and hydrofibers, and topical negative pressure (TNP) or vacuum-assisted closure (VAC; Intermed, Albany, Auckland, New Zealand). The use of TNP has seen a paradigm shift in the management of many wound types worldwide. However, systematic reviews concluded that evidence for its benefits in wound healing is still lacking, especially at a scientific level, for wounds in vascular patients where the healing potential is often compromised.[4-6]

Most study end points have been time to heal, time to secondary closure, wound complications, and economic costs, which are not objective and reliable. Furthermore, these do not provide direct evidence that TNP would enhance wound healing to a greater extent than traditional dressings. A small number of studies focused on change in wound surface area or depth. However, skin wounds are three-dimensional and irregular in shape, and the deposition of granulation tissue would start at the wound base, resulting in changes in depth before contraction of the edges. Volume measurement would therefore be more reliable in the evaluation of wound healing.

Only four randomized clinical studies have investigated wound dimensions in TNP therapy, and the results were mixed. Two studies, involving 41 patients, used early stereophotographic systems to show a reduction in depth and volume with TNP therapy.[7-9] The others used fluid installation for assessment of volume and did not show a significant difference in wound volume.[10,11]

The aims of this study were to demonstrate the effects of TNP on healing of acute wounds of the foot by measuring the change in wound volume and collagen deposition. In addition, this study sought to determine the effects of TNP on skin perfusion around the wound. The hypotheses were that TNP would enhance the healing rate of acute wounds as assessed by wound volume and collagen deposition compared with traditional dressings. Volumetric reduction was the primary outcome. Furthermore, we hypothesized that TNP would enhance blood flow dynamics around the wound, thereby promoting granulation tissue formation.

METHODS

Study design. The protocol and informed consent for this pilot study were approved by the local Northern Y Ethics Committee (NTY/08/11/104), and all participants gave informed consent.

Vascular patients presenting to Waikato Hospital, Hamilton, New Zealand, from March 2010 to June 2011 with a wound on a lower extremity were assessed by a vascular surgeon. The inclusion criterion was an acute wound after surgical débridement or minor amputation that had an adequate blood supply without requiring further revascularization procedures and was deemed suitable for TNP therapy. This included patients who had undergone recent revascularization to assist wound healing.

Exclusion criteria were:

- Previous treatment with corticosteroids, immunosuppressive drugs, chemotherapy, VAC therapy, hyperbaric oxygen therapy, growth factors, or other bioengineered tissue products in the previous 30 days.
- An acute wound with signs of infection or osteomyelitis or necrotic tissue that would not be suitable for TNP therapy.

ARTICLE HIGHLIGHTS

- **Type of Research:** Single-center pilot randomized controlled trial
- **Take Home Message:** In patients with acute high-risk foot wounds, topical negative pressure improved wound healing rate in reference to wound depth.
- **Recommendation:** The data of this pilot study suggests that topical negative pressure therapy may help wound healing.

- Known ankle pressure <50 mm Hg or toe pressure <30 mm Hg.
- Wounds from chronic venous insufficiency.
- Being unsuitable for the trial in the opinion of the operating surgeon, based on clinical equipoise which, for example, excluded patients with a wound size too small for a TNP dressing and wounds with inadequate perfusion or active infection.

Main outcomes. The primary end point was volumetric assessment of the wound at 2 weeks using a modern stereophotographic wound measurement system, FastScan (Polhemus Inc, Colchester, Vt). Secondary end points included biochemical analyses of hydroxyproline (OHP) levels at baseline and on day 14. Tissue oxygenation surrounding the wound measured using OxyVu, a hyperspectral transcutaneous (HT) oxygenation measurement system (HyperMed Inc, Burlington, Mass) was compared between baseline and day 14. Wound failure, limb loss, and overall survival rates at 12 months were calculated. OxyVu has received 510(K) clearance from the Food and Drug Administration (FDA) for use, while FastScan is not yet approved for medical use in the United States.

Study protocol. Consented patients were randomly allocated to a treatment group to receive TNP according to routine practice or to a control group to receive regular topical dressings guided by the condition of the wound. Randomization codes were formulated by SPSS software (IBM Corp, Armonk, NY) on a 1:1 basis. Neither the investigators nor the patients were blinded; however, the outcomes were objectively measured.

On day 0, relevant demographic information was collected, including age, comorbidities, cardiovascular risk factors, ankle-brachial pressure index (ABI), body mass index, and drug history, in particular, use of steroids.

The wound was cleaned appropriately to prepare for dressing application. Light débridement over the wound surface was permitted to remove superficial debris or slough, if necessary. Baseline dimensions of the ulcer were measured using the innovative volumetric wound measurement device FastScan. The FastScan laser scanner was swept across the wound repeatedly until a clear three-dimensional image of the wound was produced. Images were opened in Delta software (JEOL USA, Inc,

Peabody, Mass) to be fashioned appropriately for volumetric measurements attained using the FastScan Volumator program. The imaging for each wound was repeated three times, and the mean values were used for analyses. Outcomes recorded were wound surface area (area of the wound surface), wound cap surface area (area of the wound defect at skin surface), maximum depth, mean depth, and volume.

After wound depth was calculated, a 3-mm punch needle was used to extract three biopsy specimens from the center of the wound under aseptic technique. Urogel with lidocaine was applied topically if required. Tissue samples were placed in a sterile test tube and held at −80°C in a freezer for analysis by OHP assay based on principles described in previous OHP studies.[12,13] OHP is an amino acid confined exclusively to collagen, connective tissue selenoproteins, and elastin. More than 60 published studies on wound healing have used OHP for histologic assessment of granulation tissues and study of collagen synthesis.[12] OHP is considered a reliable surrogate marker of wound healing.[14,15] Spectrophotometry was used to quantify OHP content.[16]

HT oxygenation values were calculated by the OxyVu over a fixed area of 204 mm^2 1 cm around a target in a doughnut contour at two sides of the wound. OxyVu measures the HT concentration of oxyhemoglobin (HT-Oxy) and deoxyhemoglobin (HT-Deoxy) in arbitrary units (AU). From this, the oxyhemoglobin saturation (HT-Sat, %) and the total amount of hemoglobin (HT-Sum, AU) in the skin area could be determined.

In the treatment group, TNP was applied by ward nurses with the settings on continuous suction at −125 mm Hg for the first 24 hours and intermittent thereafter. In the control group, modern traditional dressings, typically topical hydrofiber or hydrogel dressings, were applied. Dressings were changed every 48 hours in each group unless advised by the surgeon or the wound care nurse specialists.

On day 14, FastScan was used to calculate wound dimension. Three punch needle biopsy specimens were taken at the center of the wound bed for assessment of biochemical properties. Tissue oxygenation around the wound was recorded with OxyVu.

TNP was switched to traditional dressings before day 14 if the surgeon could no longer justify its use clinically, such as when the wound improved significantly in line with current clinical practice. Patients were allowed to be discharged <day 14 with wound dressings changed by district nurses or the ward nurses if an arrangement was made. Patients were excluded if the study protocol was violated or if the wound required significant débridement or failed to heal during the study. Participants had to have attended the wound assessment clinic on day 14 to complete the study.

At 12 months, patients were reviewed in the outpatient clinic or by telephone to record the progress of the wound. The review for six patients was by telephone because they had relocated to a different catchment area, and the rest were reviewed in the clinic.

Data analysis. Data were collected in Excel software (Microsoft, Redmond, Wash). Statistical analyses were performed using SPSS 22 software. A type I error of 5% ($P \leq .05$, two-tailed) was considered to be statistically significant.

Descriptive statistics are described as the mean with the range and standard deviation (SD). When the means of two groups of continuous variables were compared, parametric analysis (the Student *t*-test) was used assuming the data followed a normal distribution. When means were compared for discrete variables, χ^2 and Fisher exact tests were applied, depending on the size of the sample. Wound failure, major amputation, and overall survival rates at 12 months were calculated using Kaplan-Meier survival analysis. Wound failure was defined as a requirement for further surgical débridement. Mixed-effects regression model tested the significance of the rate of change in wound dimension between the groups.

RESULTS

Recruitment commenced in March 2010 and ended in June 2011. Twenty-two patients (14 men [63.6%]) completed the study, with 12 patients in the treatment group and 10 in the control arm (Fig 1). TNP was used for an average of 10.6 (SD, 3.7) days in the treatment group. Seven patients (31.8%) were Maori. Patients were a mean age of 61.5 years (SD, 13; range, 41-83 years). Comorbidities included diabetes in 20 patients (86.4%), of whom 14 were on insulin, and renal impairment in 12 patients (54.5%), nine of whom were dialysis dependent. The two patients who did not have diabetes had end-stage renal failure. Table I details the basic demographic and clinical characteristics of the two arms. There were no differences between the two groups, including for wound location, history of major and minor amputations, and ABI. The erroneously normal values for ABI probably reflected the stiff calcified vessels in the diabetic and renal patients.

Changes in wound dimension. At day 0, there were no differences in wound surface area, depth, and volume between the TNP group and the control group. Wound surface area was irregular, with areas of concavity and convexity. Therefore, this was larger than the cap surface area (surface area of the wound at the skin surface level) that was assumed to be a flat, smooth surface by the computer software.

Table II reports the degree of reduction of wound dimensions as a result of the specific regimens for wound dressing and describes absolute and relative reductions. Although the primary outcome in wound volume reduction at day 14 was not significant to suggest

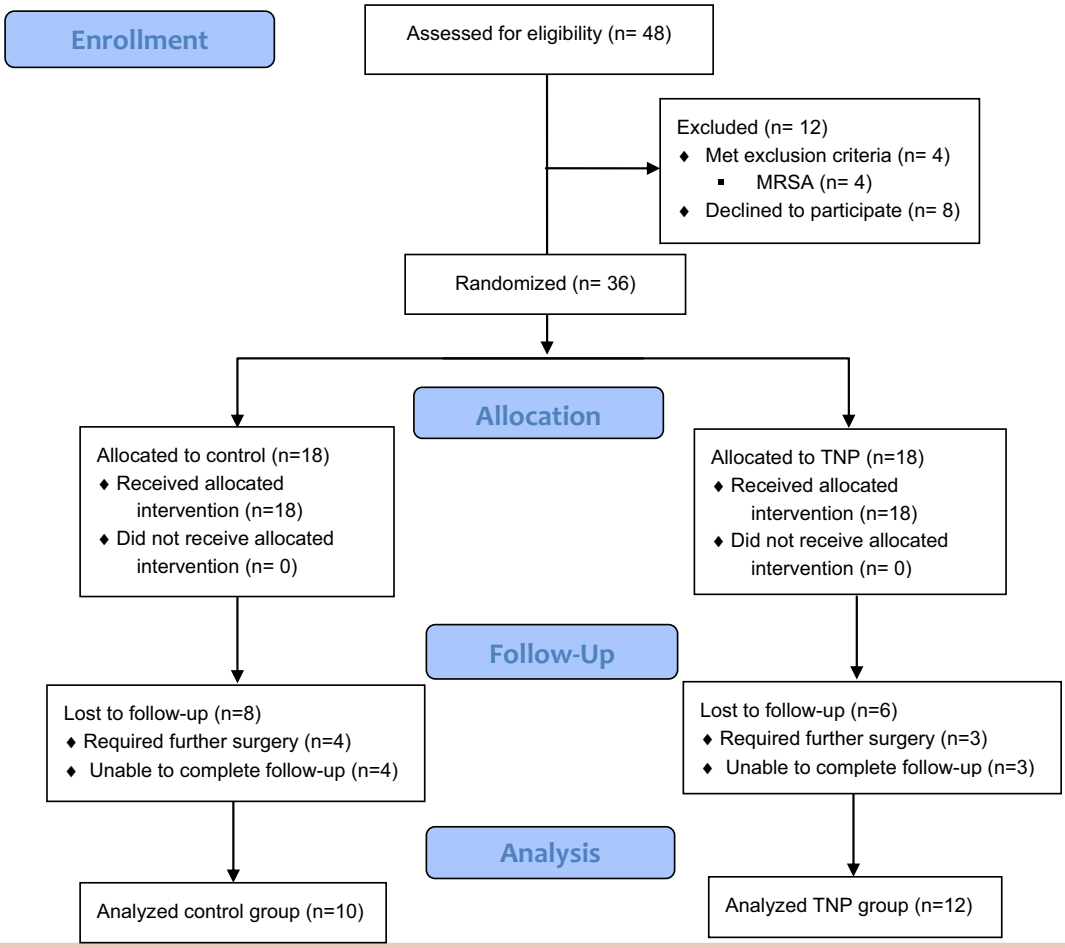

Fig 1. Flow diagram of the recruitment process. *MRSA,* Methicillin-resistant *Staphylococcus aureus*; *TNP,* topical negative pressure.

TNP therapy expedites wound healing (44.2% TNP vs 20.9% control; $P = .15$), there were trends indicating TNP enhanced wound dimension reduction in wound depth compared with traditional dressings. The relative reduction in maximum depth in the TNP group was faster than in the control group (39.0% and 17.4%, respectively; $P = .03$; Fig 2). A mixed-effects regression model showed TNP therapy was 18% quicker in wound depth reduction each day ($P = .003$). No significant interactions were found with wound surface areas and volume ($P = .10$ for volume reduction). TNP did not significantly change the surface area of the wound.

Although wound location between the groups was not significant, there appeared to be more wounds in the TNP group at the forefoot and the heel (n = 7) than in the control group (n = 3). One might argue that the findings in Table II could represent false positives because the healing potential at the two sites might be different; therefore, wound healing rates at the toes and above the toes (forefoot and heel) were compared.

As expected, surface areas of the toe wounds at day 0 were smaller than at the forefoot and heel; however, depth and volume measurements were similar between the two groups (Table III). Wound-healing rates at the toes were not significantly different from those at the forefoot and heel. This would suggest that the findings observed in Table II comparing healing potential were not influenced by the location of the wounds.

Changes in collagen content of granulation tissue. The OHP content in tissue samples was expressed in micrograms of collagen per milligram of granulation tissue. There was no significance in OHP levels between the groups at baseline (Table IV). Irrespective of the type of dressing used, the mean increase in OHP over 14 days was 0.62 (SD, 1.02) μg/mg ($P = .01$). In contrast with the study hypothesis, the degree of collagen deposition was not significant between the two arms (58% TNP vs 94.5% control; $P = .32$).

Changes in skin perfusion. The variation of skin perfusion around the wound between the two groups was not significant (Table V). When patients in both groups were considered together, skin perfusion decreased at the end of the study with reductions in HT-Oxy

Table I. Patient demographics and clinical characteristics of the 22 patients who completed the study

Variable	Control group (n = 10)	TNP group (n = 12)	*P* value
Male, No. (%)	6 (60)	8 (66.7)	.75
Maori, No. (%)	3 (30)	4 (33.3)	.87
Age, mean (SD), years	62.0 (13.9)	61.0 (12.9)	.86
BMI, mean (SD), kg/m^2	27.1 (6.8)	27.4 (6.8)	.93
Smoking history			.35
Active smoker, No. (%)	4 (40)	2 (16.7)	
Former smoker, No. (%)	3 (30)	7 (58.3)	
Nonsmoker, No. (%)	3 (30)	3 (25)	
Diabetes mellitus, No. (%)	10 (100)	10 (83.3)	.24
Insulin-dependent, No. (%)	7 (70)	7 (58.3)	.61
Renal impairment, No. (%)	4 (40)	8 (66.7)	.24
Dialysis-dependent, No. (%)	3 (30)	6 (50)	.41
Ischemic heart disease, No. (%)	8 (80)	7 (58.3)	.28
COPD, No. (%)	3 (30)	3 (25)	.79
Hypertension, No. (%)	10 (100)	12 (100)	1.00
Dyslipidemia, No. (%)	7 (70)	10 (83.3)	.46
Previous amputations, No. (%)	6 (60)	7 (58.3)	.94
Location of wound			.26
Toe, No. (%)	7 (70)	5 (41.7)	
Forefoot, No. (%)	3 (30)	5 (41.7)	
Heel, No. (%)	0 (0)	2 (16.7)	
ABI, mean (SD)	1.21 (0.70)	1.18 (0.41)	.93
Current antibiotics, No. (%)	8 (80)	6 (50)	.25

ABI, Ankle-brachial index; *BMI,* body mass index; *COPD,* chronic obstructive pulmonary disease; *SD,* standard deviation; *TNP,* topical negative pressure.

Table II. Summary of baseline (day 0) wound dimensions and absolute and relative reduction in wound dimensions after 14 days between the topical negative pressure (*TNP*) and control groups

Variable	Control, mean (SD)	TNP, mean (SD)	*P* value
Summary of wound dimensions at day 0			
Wound surface area, cm^2	32.9 (16.2)	38.8 (16.6)	.41
Cap surface area, cm^2	27.0 (14.9)	29.5 (13.2)	.69
Maximum depth, mm	14.0 (5.1)	13.6 (6.4)	.89
Mean depth, mm	2.9 (1.6)	2.7 (2.0)	.85
Volume, cm^3	7.1 (4.6)	6.3 (4.3)	.70
Absolute reduction from day 0 to day 14			
Wound surface area, cm^2	2.7 (7.1)	7.0 (9.7)	.24
Cap surface area, cm^2	1.6 (5.7)	2.2 (3.9)	.77
Maximum depth, mm	2.3 (2.6)	4.9 (4.0)	.09
Mean depth, mm	0.5 (0.8)	1.2 (1.2)	.11
Volume, cm^3	1.5 (2.8)	3.2 (3.3)	.21
Relative reduction from day 0 to day 14			
Wound surface area, %	7.9 (17.7)	18.7 (20.8)	.20
Cap surface area, %	5.7 (15.2)	10.7 (15.9)	.46
Maximum depth, %	17.6 (17.7)	36.0 (18.5)	.03[a]
Mean depth, %	17.4 (35.7)	39.0 (32.8)	.16
Volume, %	20.9 (36.1)	44.2 (36.6)	.15

SD, Standard deviation.
[a]Statistically significant ($P < .05$).

(22.1 [SD 29.4] AU; $P = .02$), HT-Sum (29.3 [SD 38.2] AU; $P = .02$), and HT-Sat (8.2% [SD 13.7%]; $P = .04$). This indicates that oxygen saturation and blood flow around the wound decreased.

Clinical outcome. Wound failure rate, major amputation rate, and overall survival rates between the two groups did not detect significance (Table VI).

DISCUSSION

Recruitment lasted 12 months and was slower than anticipated. As a result of the relatively strict inclusion/exclusion criteria, there were significant numbers of dropouts and refusals to participate. Nevertheless, this study is significant given the number of patients with high-risk vascular foot wounds.

In this study cohort, there was no significant improvement in wound volume reduction with TNP therapy compared with traditional dressings. However, TNP achieved a greater relative reduction in maximum depth wound of 36% by day 14 compared with an 18% reduction using traditional dressings. Wound depth recovered at a greater rate than surface area, and the volumetric findings in our study were similar to those in other studies published,[17] suggesting that wounds heal from the floor to the roof.

McCallon et al[7] focused on reduction in surface area and the time taken for the ulcer to heal and did not detect significance between the treatment and control groups. In contrast, Etöz et al[18] studied 24 patients with diabetic foot ulcers, presumably chronic wounds. The reduction in wound surface area and time taken for the wound bed to be covered with granulation tissue were more rapid in the TNP group than in the group treated with saline gauze dressings.[18]

Eginton et al[11] compared TNP with hydrocolloid wound gel and gauze dressings in diabetic foot wounds. Only six of their 10 patients completed the study. Although TNP decreased wound volume and depth to a greater degree than moist gauze dressings, all wounds received 2 weeks of TNP and traditional dressings.

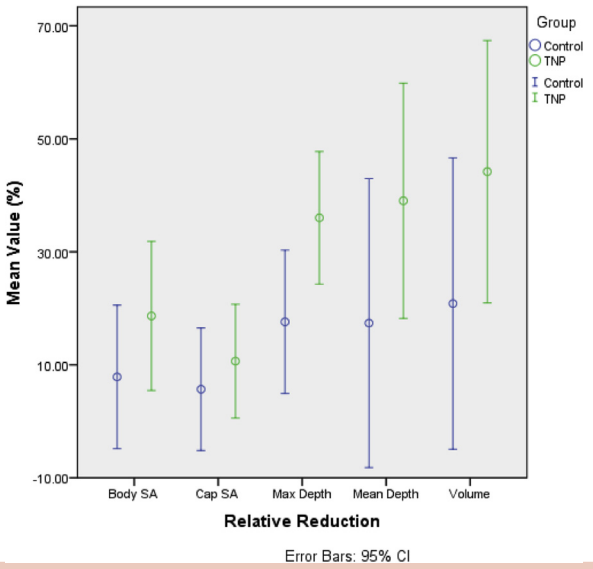

Fig 2. Error bar graph shows the mean (*circles*) and 95% confidence interval (*bars*) of the relative reduction of various wound dimensions between the topical negative pressure (*TNP*) therapy group (*blue*) and the control (*green*) groups. *SA*, Surface area.

Table III. Wound dimensions at baseline (day 0) comparing toe wounds and forefoot/heel wounds, along with the absolute and relative reductions in wound dimension between wounds at the toes and those at the forefoot/heel

Variable	Toe, mean (SD)	Forefoot/heel, mean (SD)	*P* value
Wound dimensions at day 0			
Wound surface area, cm^2	26.8 (14.7)	47.3 (10.1)	.001
Cap surface area, cm^2	20.9 (13.5)	37.3 (7.2)	.002
Maximum depth, mm	13.6 (4.6)	13.9 (7.1)	.91
Mean depth, mm	3.3 (1.7)	2.2 (1.9)	.18
Volume, cm^3	6.1 (3.4)	7.3 (5.2)	.55
Absolute reduction from day 0 to 14			
Wound surface area, cm^2	5.1 (6.3)	5.0 (11.3)	.98
Cap surface area, cm^2	2.7 (4.6)	1.0 (4.9)	.41
Maximum depth, mm	4.4 (3.9)	2.9 (3.3)	.32
Mean depth, mm	1.0 (1.1)	0.7 (1.0)	.58
Volume, cm^3	2.4 (2.6)	2.5 (3.8)	.97
Relative reduction from day 0 to 14			
Wound surface area, %	17.7 (19.3)	9.0 (20.2)	.32
Cap surface area, %	11.7 (15.7)	4.4 (14.8)	.28
Maximum depth, %	31.4 (19.4)	23.1 (20.8)	.35
Mean depth, %	31.5 (30.8)	26.5 (41.2)	.76
Volume, %	37.7 (32.7)	28.7 (43.7)	.60

SD, Standard deviation.

Joseph et al[9] studied 36 wounds that were not arterial in nature, such as pressure wounds, venous ulcers, and traumatic wounds. Eighteen wounds were treated with saline-soaked gauze dressings three times a day, and the rest were randomized to TNP. Wound volumes were assessed by volume displacement of alginate impression molds, and punch biopsies were used to obtain tissue samples for histologic analysis. Volume reduction was greater in the TNP group at 3 and 6 weeks. The chief characteristic of the TNP group was formation of granulation tissue, whereas inflammation and fibrosis were seen in wounds treated by gauze dressings.

A randomized study conducted by Ford et al[10] included 35 pressure wounds, with eight wounds located below the ankle. Traditional dressings were Iodosorb (Smith & Nephew Wound Management, Hull, United Kingdom), Iodoflex (Smith & Nephew Wound Management), or Panafil (Healthpoint, Ltd, Ft. Worth, Tex). Tissue biopsy and wound assessments were conducted at 3 and 6 weeks. Wound volumes measured by displacement of plaster impressions were not significant between the groups.

Paola et al[19] assessed diabetic foot wounds after débridement and compared TNP with modern (non-gauze) dressing in 130 patients. End points were time needed for complete coverage of exposed bone with granulation tissue, healing time, and number of surgical procedures. TNP was superior, but the end points were arguably weak and prone to bias.[20]

Although previous studies suggest that would healing is better with TNP than with traditional dressings, the studies were weak, small, and at risk of performance bias. This study addressed the heterogeneity that was evident in previous studies and used products that are relevant to modern clinical practices. This study was novel in its approach in quantifying changes in wound volume after TNP therapy in high-risk vascular wounds using accurate and objective wound-measurement devices. FastScan has been shown to be reliable and to correlate strongly with other methods used to measure wound dimensions like three-dimensional computed tomography reconstruction.[21,22]

Several patients had negative values for wound dimension reduction, indicating an increase in the depth of the wound. For example, the wound in one control patient was in fact dying back as a result of ongoing tissue ischemia from small-vessel disease despite adequate proximal revascularization, and the wound was surgically débrided on day 17. Another patient in the TNP group had a forefoot wound that over granulated above the level of the skin surface to a greater height than the original depth of the wound. Thus, the device recorded a negative volume when comparing this to the original wound dimension.

Malmsjo et al[23-27] published several reports relating to microvascular blood flow at the wound edge during TNP as measured with laser Doppler flowmetry. In summary, cutaneous blood flow nearly doubled at 2.5 cm from the skin edge but halved at 0.5 cm from the skin

Table IV. Summary of hydroxyproline (*OHP*) levels at baseline and percentage increase at 14 days for the topical negative pressure (*TNP*) group and the control group

Variable	Control group, mean (SD)	TNP group, mean (SD)	*P* value
OHP at day 0, μg/mg	1.29 (0.51)	1.97 (1.61)	.21
OHP at day 14, μg/mg	2.25 (1.10)	2.32 (0.97)	.89
Relative increase in OHP, %	94.5 (86.7)	58 (68)	.32

SD, Standard deviation.

Table V. OxyVu[a] findings of the 22 patients at baseline and percentage decrease at 14 days

Variable	Control group, mean (SD)	TNP group, mean (SD)	*P* value
At baseline: Day 0			
HT-Oxy, AU	83.5 (23.0)	82.3 (22.4)	.92
HT-Deoxy, AU	62.3 (15.8)	60.7 (19.1)	.86
HT-Sat, %	56.1 (2.9)	56.9 (6.5)	.71
HT-Sum, AU	146 (38.3)	143 (35.4)	.89
Relative change (%): Day 14			
HT-Oxy	−28.4 (31.0)	−22.4 (33.4)	.79
HT-Deoxy	−25.0 (20.4)	−4.2 (19.4)	.21
HT-Sat	−19.4 (28.3)	−12.0 (20.0)	.70
HT-Sum	−27.3 (25.0)	−15.3 (24.9)	.52

AU, Arbitrary units; *HT,* hyperspectral transcutaneous oxygenation measurement system; *HT-Deoxy,* deoxyhemoglobin; *HT-Oxy,* concentration of oxyhemoglobin; *HT-Sat,* oxyhemoglobin saturation; *HT-Sum,* total amount of hemoglobin; *SD,* standard deviation.
[a]HyperMed Inc, Burlington, Mass.

edge as a direct result of negative pressure. The degree of reduction was proportional to the increase in pressure. In contrast with laser Doppler flowmetry, OxyVu quantified oxyhemoglobin and deoxyhemoglobin for each wound image captured. Skin perfusion surrounding the wound decreased by the end of study, but there was no significance between the groups. This possibly reflects a resolution of inflammation around the wound with less vasodilation.

The clinical application of the OxyVu to wound healing has been explored. Greenman et al[28] compared patients with diabetes and ulcers and monitored them for 6 months. OxyVu was able to predict ulcer healing at 6 months based on readings from the first visit with a sensitivity of 93% and specificity of 86%. Nouvong et al[29] conducted another study that included 73 diabetic foot ulcers in 54 patients. Again, OxyVu could predict ulcer healing with a sensitivity of 86%, specificity of 88%, and positive predictive value of 96%.[29]

This was a pilot study, and the efficacy of the intervention could not be formally tested; therefore, interpretation of the *P* values should be taken with caution. The wound-volume reduction in the TNP group was twice that of the control group, but yet statistically

Table VI. Survival analysis outcomes between the topical negative pressure (*TNP*) and control groups

Variable	Control group		TNP group		*P* value
	At 1 month	At 12 months	At 1 month	At 12 months	
Wound failure, %	20	60	8.3	49.1	.70
Amputation, %	0	30	8.3	49.1	.29
Survival, %	100	70	91.7	75.0	.95

insignificant, which presents a high likelihood of a type II error. Future study should attest the validity of this study.

Assuming a minimum of 20% greater reduction in wound volume at 2 weeks in the TNP group to be clinically relevant, a randomized controlled study with 82 patients in each arm would be required to test at 80% power at a 5% significance level. Post hoc analysis with 1000 simulated data sets suggested strong likelihood in demonstrating enhanced wound-depth reduction in the TNP group of similar effect size in a study of larger scale ($P = .03$). Power analyses with other wound dimension variables did not yield significant findings ($P = .17$ for "relative volume reduction"). Thus, this study sets the foundation for larger studies to evaluate the superiority of TNP over traditional dressings in high-risk foot wounds. Further observations, such as stratifying wounds of different healing rates, could be examined in future studies.

CONCLUSIONS

This study was based on modern clinical practices in the management of high-risk acute vascular foot wounds. The changes in OHP levels or tissue oxygenation were not significant, but there was a significant reduction in maximum wound depth in patients who received TNP compared with those who received traditional dressings at day 14. This suggests that TNP may play a role in enhancing wound healing.

We gratefully acknowledge Dr Christina Buchannan (Department of Molecular Medicine and Pathology, University of Auckland) for her support and guidance on OHP analysis.

AUTHOR CONTRIBUTIONS

Conception and design: NC, TV
Analysis and interpretation: NC, OR, JS
Data collection: NC, OR
Writing the article: NC, OR
Critical revision of the article: NC, OR, JS, TV
Final approval of the article: NC, OR, JS, TV
Statistical analysis: NC
Obtained funding: NC
Overall responsibility: NC

REFERENCES

1. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, et al. Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. Ann Intern Med 2004;141:421-31.
2. Apelqvist J, Castenfors J, Larsson J, Stenstrom A, Agardh CD. Prognostic value of systolic ankle and toe blood pressure levels in outcome of diabetic foot ulcer. Diabetes Care 1989;12:373-8.
3. Boulton AJ. The diabetic foot: from art to science. The 18th Camillo Golgi lecture. Diabetologia 2004;47:1343-53.
4. Ubbink DT, Westerbos SJ, Nelson EA, Vermeulen H. A systematic review of topical negative pressure therapy for acute and chronic wounds. Br J Surg 2008;95:685-92.
5. Vig S. A systematic review of topical negative pressure therapy for acute and chronic wounds. Br J Surg 2008;95: 1185-6.
6. Noble-Bell G, Forbes A. A systematic review of the effectiveness of negative pressure wound therapy in the management of diabetes foot ulcers. Int Wound J 2008;5: 233-42.
7. McCallon SK, Knight CA, Valiulus JP, Cunningham MW, McCulloch JM, Farinas LP. Vacuum-assisted closure versus saline-moistened gauze in the healing of postoperative diabetic foot wounds. Ostomy Wound Manage 2000;46: 28-32, 34.
8. Ellison TL, Elliott R, Moyes SA. HbA1c screening for undiagnosed diabetes in New Zealand. Diabetes Metab Res Rev 2005;21:65-70.
9. Joseph E, Hamori CA, Bergman S, Roaf E, Swann NF, Anastasi GW. A prospective randomized trial of vacuum-assisted closure versus standard therapy of chronic nonhealing wounds. Wounds 2000;12:60-7.
10. Ford CN, Reinhard ER, Yeh D, Syrek D, De Las Morenas A, Bergman SB, et al. Interim analysis of a prospective, randomized trial of vacuum-assisted closure versus the healthpoint system in the management of pressure ulcers. Ann Plast Surg 2002;49:55-61.
11. Eginton MT, Brown KR, Seabrook GR, Towne JB, Cambria RA. A prospective randomized evaluation of negative-pressure wound dressings for diabetic foot wounds. Ann Vasc Surg 2003;17:645-9.
12. Jorgensen LN. Collagen deposition in the subcutaneous tissue during wound healing in humans: a model evaluation. APMIS Suppl 2003:1-56.
13. Chiariello M, Ambrosio G, Cappelli-Bigazzi M, Perrone-Filardi P, Brigante F, Sifola C. A biochemical method for the quantitation of myocardial scarring after experimental coronary artery occlusion. J Mol Cell Cardiol 1986;18:283-90.
14. Gross JP, Piez KA. The nature of collagen. I. Invertebrate collagens. In: Sognaes RF, editor. Calcification in biological systems. Washington DC: Washington, D. C., American Association for the Advancement of Science; 1960. p. 395-409.
15. Viljanto J. Biochemical basis of tensile strength in wound healing: an experimental study with viscose cellulose sponges on rats. Acta Chir Scand (Suppl) 1964:333.
16. Woessner JF. Determination of hydroxyproline in connective tissues. The methodology of connective tissues research. Oxford, UK: Johnson-Bruvvers Ltd; 1976. p. 235-45.
17. Venturi ML, Attinger CE, Mesbahi AN, Hess CL, Graw KS. Mechanisms and clinical applications of the vacuum-assisted closure (VAC) device: a review. Am J Clin Dermatol 2005;6:185-94.
18. Etöz A, Özgenel Y, Özcan M. The use of negative pressure wound therapy on diabetic foot ulcers: a preliminary controlled trial. Wounds 2004;16:264-9.
19. Paola LD, Carone A, Ricci S, Russo A, Ceccacci T, Ninkovic S. Use of vacuum assisted closure therapy in the treatment of diabetic foot wounds. J Diabet Foot Complications 2010;2: 33-44.
20. Dumville JC, Hinchliffe RJ, Cullum N, Game F, Stubbs N, Sweeting M, et al. Negative pressure wound therapy for treating foot wounds in people with diabetes mellitus. Cochrane Database Syst Rev 2013;10:CD010318.
21. Chiang N. Validation of Silhouette and FastScan. Presented at 2010 Australian and New Zealand Society for Vascular Surgery (ANZSVS) conference. Gold Coast, Queensland, Australia: August 2-5, 2010.
22. Edgar D, Day R, Briffa NK, Cole J, Wood F. Volume measurement using the Polhemus FastSCAN 3D laser scanning: a novel application for burns clinical research. J Burn Care Res 2008;29:994-1000.
23. Anesater E, Borgquist O, Hedstrom E, Waga J, Ingemansson R, Malmsjo M. The influence of different sizes and types of wound fillers on wound contraction and tissue pressure during negative pressure wound therapy. Int Wound J 2011;8:336-42.
24. Borgquist O, Anesater E, Hedstrom E, Lee CK, Ingemansson R, Malmsjo M. Measurements of wound edge microvascular blood flow during negative pressure wound therapy using thermodiffusion and transcutaneous and invasive laser Doppler velocimetry. Wound Repair Regen 2011;19:727-33.
25. Borgquist O, Ingemansson R, Malmsjo M. The effect of intermittent and variable negative pressure wound therapy on wound edge microvascular blood flow. Ostomy Wound Manage 2010;56:60-7.
26. Borgquist O, Ingemansson R, Malmsjo M. Wound edge microvascular blood flow during negative-pressure wound therapy: examining the effects of pressures from -10 to -175 mmHg. Plast Reconstr Surg 2010;125:502-9.
27. Malmsjo M, Ingemansson R, Martin R, Huddleston E. Wound edge microvascular blood flow: effects of negative pressure wound therapy using gauze or polyurethane foam. Ann Plast Surg 2009;63:676-81.
28. Greenman RL, Panasyuk S, Wang X, Lyons TE, Dinh T, Longoria L, et al. Early changes in the skin microcirculation and muscle metabolism of the diabetic foot. Lancet 2005;366:1711-7.
29. Nouvong A, Hoogwerf B, Mohler E, Davis B, Tajaddini A, Medenilla E. Evaluation of diabetic foot ulcer healing with hyperspectral imaging of oxyhemoglobin and deoxyhemoglobin. Diabetes Care 2009;32:2056-61.

Submitted Dec 1, 2016; accepted Feb 27, 2017.