Published in final edited form as:

Vet Surg. 2013 June ; 42(5): 511–522. doi:10.1111/j.1532-950X.2013.12005.x.

Effects of Negative Pressure Wound Therapy on Healing of Free Full-Thickness Skin Grafts in Dogs

BRYDEN J. STANLEY, BVMS, MVetSc, Diplomate ACVS, KATHRYN A. PITT, DVM, CHRISTIAN D. WEDER, BS, MICHELE C. FRITZ, BS, LVT, JOE G. HAUPTMAN, DVM, MS, Diplomate ACVS, and BARBARA A. STEFICEK, DVM, PhD, Diplomate ACVP

¹Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI.

²Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Abstract

Objective—To compare healing of free, full-thickness, meshed skin grafts under negative pressure wound therapy (NPWT) with bolster dressings in dogs.

Study design—Randomized, controlled experimental study, paired design.

Animals—Dogs (n = 5)

Methods—Full-thickness skin wounds (4 cm × 1.5 cm) were created bilaterally on the antebrachia of 5 dogs (n = 10). Excised skin was grafted to the contralateral limb. Grafts were randomized to NPWT or bolster dressings (control; CON). NPWT was applied continuously for 7 days. Grafts were evaluated on days 2, 4, 7, 10, 14 and 17, biopsied on days 0, 4, 7, and 14, and had microbial culture on day 7. Outcome variables were: time to first appearance of granulation tissue, percent graft necrosis, and percent open mesh. Significance was set at $P < .05$. Histologic findings, culture results, and graft appearance were reported.

Results—Granulation tissue appeared earlier in the NPWT grafts compared with CON grafts. Percent graft necrosis and remaining open mesh area were both greater in CON grafts compared with NPWT grafts at most time points. Histologic results showed no significant difference in all variables measured, and all cultures were negative.

Conclusions—Variables of graft acceptance were superior when NPWT was used in the first week post-grafting. Fibroplasia was enhanced, open meshes closed more rapidly and less graft necrosis occurred with NPWT application. More preclinical studies are required to evaluate histologic differences.

INTRODUCTION

A full-thickness skin graft (FTSG) is the preferred type of free cutaneous graft in small animal surgery because it includes the entire dermis and thus provide a hairs, glandular and robust wound coverage after healing.^{1,2} Full-thickness skin grafts are relatively easy to harvest and prepare compared to split-thickness grafts, requiring no specialized instrumentation (eg, dermatome) and the abundance of dog and cat truncal skin generally allows the donor site to be closed directly. However, the increased thickness of the FTSG

(compared to a split-thickness graft) tests the processes of graft survival. During the first 48 hours after transfer, free grafts survive by absorbing tissue fluid from the recipient bed via plasmatic imbibition and the joining of the graft and wound vessels by inosculation.² At ~ 72 hours, fragile capillary buds emerge from the recipient bed and start to vascularize the graft.² Graft survival depends upon successful vascular ingrowth, subsequent establishment of venous and lymphatic drainage, and development of a fibrous (collagenous) attachment.^{2,3} Causes of partial or total graft failure include fluid accumulation (seroma or hematoma) under the graft, movement of the graft, and infection.² Maintenance of a closely applied, immobile graft is vital to the final outcome.

Grafts are traditionally covered with a non-adherent primary layer, and then secured firmly by a bolster dressing to prevent shear movement. The large, firm secondary layer is comprised of an absorbent wrap, cast padding, and gauze wrap, acting to immobilize the graft and limb movement.^{1,2} Further immobilization can be achieved by incorporating some type of device with the tertiary bandage layer (e.g., splint, cast, external fixator). However, consistent and evenly distributed pressure can be difficult to maintain, especially in mobile and irregular areas. It is intuitive that if a dressing were capable of enhancing contact and minimizing motion between graft and recipient bed, it would result in a lower incidence of graft failures, and thus improved outcomes.

In the past decade, negative pressure wound therapy (NPWT) has become an increasingly popular adjunct in human medicine, used in a variety of wound healing and surgical applications.⁴⁻⁹ NPWT (also termed vacuum-assisted closure, topical negative pressure) involves the application of a regulated, sub-atmospheric pressure through a porous dressing placed over a wound bed that has been sealed from its atmospheric environment (Fig 1).¹⁰⁻¹² Experimentally, NPWT increases blood flow to the wound, stimulates formation of granulation tissue, and possibly reduces interstitial edema.¹³⁻¹⁷ Reduction of the bacterial load in wounds with NPWT has also been documented in animal models and human patients, although findings are inconsistent.^{13,18-21} Application of NPWT in human wound care is widespread, where it is used to improve healing in soft tissue trauma, compromised flaps, open fractures, surgical dehiscence and split-thickness skin grafts.^{7,22-35} Despite the plethora of publications documenting promising outcomes with NPWT, many surgeons remain unsure or skeptical of its effectiveness and specific indications because of the lack of randomized, controlled data in the literature.^{9,12,36-40} Furthermore, with respect to skin graft acceptance, skin grafting in people is typically split-thickness, with few reports documenting the effects of NPWT on the acceptance of FTSGs.⁴¹

There are several veterinary case reports demonstrating use of NPWT in open wounds, 1 case series and 1 controlled experimental study; all suggest positive effects of NPWT.⁴²⁻⁵⁰ In the case series reported by Ben-Amotz and Lanz et. al., the use of NPWT in 15 dogs with traumatic wounds, 10 cases subsequently underwent full-thickness skin grafting and NPWT was re-applied. All grafts were reported to have survived, although percentages were not documented. To our knowledge, no randomized, controlled study comparing NPWT to standard-of-care bolster dressings over FTSGs in dogs has been reported.

Given the positive results with NPWT in human grafting practices, we hypothesized that the NPWT would improve healing of FTSG in dogs. Our rationale was that by immobilizing and improving contact of the graft-bed interface, by preventing fluid accumulation beneath the graft, and increasing wound blood flow, NPWT would directly enhance the plasmatic imbibition and revascularization processes. To test our hypothesis we designed a prospective, randomized, controlled, experimental study in dogs. Our main objectives were to compare first appearance of granulation tissue, graft necrosis and the remaining area of open meshes of FTSGs, between NPWT and standard bolster dressings at 7 time points.

Secondary objectives were to report qualitative variables of graft acceptance at 7 time points, histologic findings at 4 time points, and bacterial cultures once.

MATERIALS AND METHODS

Purpose-bred adult female beagles ($n = 5$) weighing 9–10 kg, body condition score 4–5 out of 9, were acquired for study. All dogs had values within normal ranges on physical examination, complete blood count and serum biochemical profiles performed before study commencement. There was a 1 week acclimatization period, during which dogs were conditioned to the housing, feeding and social enrichment protocols.

On day 0, dogs were medicated with acepromazine maleate (0.07 mg/kg intramuscularly [IM]) and morphine sulfate (0.66 mg/kg IM). A saphenous intravenous (IV) catheter was placed and anesthesia was induced with thiopental (10–15 mg/kg IV to effect) and maintained with isoflurane (baseline concentration 2%) delivered in oxygen (30 mL/kg/hr). Lactated Ringer's solution (10 mL/kg/hr IV) was administered during anesthesia. After induction, cefazolin (22 mg/kg IV once) was administered. Thoracic limbs were clipped from mid-metacarpus to just above the radiohumeral joint, the dogs positioned in dorsal recumbency, and the skin prepared for aseptic surgery.

Using strict aseptic technique, bilateral 4.0×1.5 cm full-thickness skin wounds were surgically created on the dorsal aspect of each mid-antebrachium (ten wounds total), using sterile templates (Fig 2). Antebrachial fascia was excised to expose the underlying extensor carpi radialis muscle belly, which acted as the recipient bed. Hemostasis was achieved by applying pressure with gauze swabs. A 2 mm strip from a long edge of the excised skin was submitted as the day 0 histopathology sample.

Both excised skin sections were prepared for grafting by meticulous removal of all subdermal remnants and 8 consistent, longitudinal meshes were created using a #11 scalpel blade (Fig 3). After preparation, grafts were transposed (i.e., right antebrachium-derived skin into left antebrachial recipient bed and vice versa), and secured with 20 simple interrupted sutures of 4-0 nylon (Fig 4). Right and left antebrachial grafts were assigned to NPWT group or control (CON) group based on computer randomization (Microsoft Excel® Random Number Generation, Microsoft Corp., Seattle, WA). Randomization assignments were only revealed after grafting to eliminate any procedural bias of the surgeon (BJS). High-resolution digital photographs (3264×2448 pixels) were taken at this time (Sony DSC-T200, Sony USA, New York, NY), with a carefully positioned metric scale.

A single layer of petrolatum-impregnated knitted cellulose acetate dressing (Adaptic, Johnson & Johnson, Arlington, TX) was placed on top of the grafts in both groups. Negative pressure dressings were applied to the NPWT grafts in the following manner: skin surrounding the wound was gently wiped with a liquid medical adhesive (Mastisol, Ferndale Laboratories Inc, Ferndale, MI). An open weave gauze sponge (Venturi Dressings, Talley Medical, Hampshire, UK) was moistened with saline (0.9% NaCl) solution and contoured over the Adaptic-covered graft, including the edges. A flat, fenestrated drain was buried within this dressing and the tubing anchored to the skin with a hydrogel adhesive (Venturi Dressings, Talley Medical, Hampshire, UK). A transparent adhesive sheet was placed to cover the dressing, and an additional 3–5 cm border of periwound skin (Fig 5). The tubing was positioned such that it coursed proximally toward the trunk. The evacuation tubing was connected to a 3-way stopcock, incorporated into a thoracic bandage with coiled intravenous polyurethane tubing exiting upwards. The coiled tubing went to a swiveling fixture attached to a bar above the cage (Core Flex-coil, International WIN, Kennett Square, PA), then to the canister of the NPWT therapy unit (Venturi®, Talley Medical, Hampshire, UK) mounted on

the side of the cage (Fig 6). A continuous negative pressure of -65 mmHg was selected and the dressing observed for collapse and the development of raisin-like wrinkling (indicating subatmospheric pressure) (Fig 5). The machine setting was checked against a transducer (QA-PT Parameter Tester, Metron, Grand Rapids, MI) at the 3-way stopcock close to the level of the dressing, every 4 -6 hours throughout the duration of NPWT. CON grafts received only the petrolatum-impregnated dressing as a primary layer. Both groups were bandaged from digits to above the elbow with identical secondary and tertiary layer bandages (Specialist Cast Padding, BSN Medical Inc., Charlotte, NC; Kendall Conform Stretch Bandage, Covidien Inc., Mansfield, MA; PetFlex, Andover Healthcare Inc, Salisbury, MA).

Before recovery from anesthesia, a 2nd dose of morphine sulfate (0.33 mg/kg) was administered subcutaneously. Carprofen (4.4 mg/kg) was administered orally on the day before surgery and continued orally every 24 hours for 7 days. Elizabethan collars were placed on all dogs. On Days 2, 4, 7, 10, 14, and 17 all wounds underwent dressing changes, after administration of morphine (0.66 – 0.83 mg/kg IM) and acepromazine (0.07 mg/kg IM) to each dog. All dressing changes adhered to aseptic principles and antibiotics were not administered at any time. Care was taken not to disturb the grafts during these changes. On days 2 and 4, application of NPWT and bandaging was performed as previously described. NPWT was discontinued after day 7, based on the rationale that factors influencing graft acceptance are most critical during the initial week after grafting, and that most grafts will have either 'taken' or failed at that point.² From day 7 on, both groups were bandaged identically until the study termination on day 17. This bandaging consisted of a single layer of petrolatum-impregnated gauze dressing over the graft, followed by a 4×4 inch gauze, and identical secondary and tertiary layer bandages as previously described.

Dogs were housed individually in 4 × 6 ft cages, where they could interact vocally and visually with each other. They received environmental enrichment 2-3 times daily in the form of direct human contact not associated with dressing changes, usually at time of day time transducer checks. Enrichment consisted of playing, petting, and grooming. Dogs were provided with selected toys in each cage. Dogs were monitored for comfort, bandage integrity, and mechanical function of the NPWT machines every 4 hours during the day, and every 6 hours overnight. Consistent negative pressure at the level of the NPWT grafts was confirmed by checking that transducer readings concurred with machine settings at these time points. A negative pressure of -65 mmHg was maintained continuously until day 4, at which time the continuous negative pressure was changed to -45 mmHg until day 7. The pressure settings and continuous mode of NPWT were selected after consultation with the manufacturer.

High resolution digital photographs were taken at all dressing changes. The camera was angled to assume a straight, dorsoventral view of the graft, with a metric scale carefully positioned at the same level as the graft. The grafts were not disturbed; any wound fluid or crusting was gently removed with sterile saline-soaked gauze sponges. Subjective wound evaluations for recording qualitative variables were performed at all dressing changes (Table 1). Further to the day 0 sample, additional graft biopsies were obtained with a 4 mm disposable dermal biopsy punch from the corner of each graft on days 4, 7 and 14, in a systematic pattern (day 4, proximomedial; day 7 distolateral; day 14, proximolateral). Aerobic cultures were taken on day 7 by rolling a sterile culture swab over the surface of the graft and placing it into a commercial collection and transport system (BBL™ Culture Swab™; Becton, Dickinson and Company, Sparks, MA). Samples were stored at 4°C and plated within 4 hours of retrieval onto 5% enriched sheep-blood agar, Columbia CNA 5% blood agar with colistin and nalidixic acid, MacConkey agar, and thioglycollate broth.

Upon study completion, all images were computer randomized and coded. Wound planimetry was performed using downloadable software (Image J Software <http://rsbweb.nih.gov/ij/>), by an investigator blinded to the randomization and code (BJS).

Variables of Graft Healing

Graft acceptance was recorded both quantitatively and qualitatively. The first appearance of granulation tissue within the open meshes was recorded in days. The following quantitative variables were measured at each time point: 1) area of graft necrosis (cm^2); 2) total area of open mesh (i.e., not epithelialized) (cm^2); and 3) total graft area (cm^2). Necrosis was defined as black discoloration of the skin (epidermis and partial or full thickness dermis), eschar or slough of grafted epidermis or dermis. From these measurements, the percent necrosis ($\text{area necrosis}_{\text{Day } n} / \text{total graft area}_{\text{Day } n} \times 100$) and the percent open mesh area ($\text{area open mesh}_{\text{Day } n} / \text{area open mesh}_{\text{Day } 0} \times 100$) were calculated at each time point.

Qualitative assessments were made from review of the images and subjective wound evaluation forms (Table 1), for each dressing change, by an investigator blinded to the randomization (BJS). Ordinal scores of graft color were recorded at each time point, and an indication of graft mobility was obtained on Day 4. Mobility was assessed by applying gentle digital pressure on the graft, and pushing laterally, attempting to slide the graft over the bed. The presence of any seroma or hematoma beneath the graft, and bleeding from graft biopsy site was also noted.

Histologic Evaluation

Samples were fixed immediately upon collection in 10% neutral buffered formalin, and processed for light microscopy. Representative sections were stained with hematoxylin and eosin (H&E) and microscopically evaluated by a board certified veterinary pathologist (BAS) who was unaware of sample grouping. Histologic comparisons between groups were made at days 0, 4, 7 and 14. The following criteria of inflammation were evaluated, and scores (in parentheses) were attributed:

- a. The concentration of neutrophilic cellular infiltration into the dermis (0-3);
- b. The concentration of neutrophilic cellular infiltration into the hypodermis (0-3);
- c. Edema (0-3);
- d. Hemorrhage (0-3);
- e. Necrosis (0-3).

The criteria used to define the concentration of cellular infiltrates were as follows: 0 = within normal histologic limits; 1 = scattered; 2 = clustered or nodular; and, 3 = diffuse. Histologic evaluation of tissue edema was based primarily on distribution within the sections, with 0 = none; 1 = focal; 2 = localized (regional); and, 3 = diffuse. The degree or extent of hemorrhage within the tissue sections was subjectively and comparatively designated as: 0 = none; 1 = mild; 2 = moderate; and 3 = severe. The amount of necrosis was evaluated utilizing the following histopathologic criteria: 0 = none; 1 = focal; 2 = nodular/regional; and, 3 = diffuse (tracking along fascial planes). These 5 histologic features were weighted equally and plotted at each time point to check for any graphical interaction of each composite by group, before being summed to formulate an histologic acute inflammation score (HAIS; range 0-15).

Further histologic comparisons were made between groups by evaluating and scoring:

- a. epidermal devitalization, characterized as the degree of epidermal and follicular epithelial compromise, and scored: 0 = none/normal; 1 = superficial; 2 = full epidermis; and 3 = including follicular epithelium;
- b. epidermal hyperplasia, characterized as increase in number of keratinocyte layers when compared to day 0 specimen sections, and scored: 0 = none/normal; 1 = mild; 2 = moderate, and 3 = severe; and
- c. neovascularization within the wound bed and hypodermis, characterized as concentration of newly forming small caliber blood vessels compared to Day 0 specimen sections, and scored: 0 = none; 1 = mild, 2 = moderate; and 3 = marked.

Bacterial Evaluation

Samples were incubated and evaluated each day for quantity and species of bacteria. Bacterial isolates were to be enumerated and identified following this institution's Standard Operating Procedures for Wound Cultures. Cultures were incubated for 4 days on the Enriched Blood Agar and Thioglycollate broth, and for 2 days on Columbia CNA and MacConkey agar before considered negative.

Statistical Analysis

Each dog acted as its own control, receiving both NPWT and CON. The first appearance of granulation tissue was analyzed by means of the Wilcoxon signed rank test, and presented as median and range. The quantitative response variables of percent area necrosis, percent area open mesh, and total graft area (cm²) were measured 7 times (days 0, 2, 4, 7, 10, 14, and 17). The scores of the histologic variables of HAIS, epidermal devitalization, epidermal hyperplasia and neovascularization were measured at days 0, 4, 7 and 14. The factors that could influence each response variable were dog, group (NPWT/CON), and time. Data were analyzed by means of a 3 factor ANOVA with the fixed factors of group and time and the random factor of dog. The errors of the data were plotted for normality; normality was accepted if the plot was unimodal and approximately symmetrical. Post hoc comparisons were by means of t test (Group) or Bonferroni's t test for multiple comparisons (times vs. time₀). *P* values are stated for all analyses.

RESULTS

All NPWT dressings and apparatus were very well tolerated for the duration of the treatment, and pressure was maintained consistently over all wounds, confirmed by the transducer readings. The maximum disparity between transducer readings and the machine settings was 4 mmHg, with an average of 1.0 mmHg. Two minor line breaks resulted in short term loss of negative pressure. These were corrected at the next integrity check by simple line replacement. Because of the frequent monitoring of the animals, we are confident that this did not affect delivery of negative pressure to the grafts for significant periods of time. No major complications were encountered on dressing changes apart from small areas of skin erythema at the flexor aspects of the elbow from bandage rub.

Quantitative Variables

Granulation tissue was first noted in the meshes a median of 2 days in the NPWT grafts, and 7 days in the CON grafts (*P* = .04; Table 2; Fig 7). No graft experienced catastrophic failure, but greater necrosis was noted in the CON grafts on days 2, 4, 7 and 10, compared to the NPWT grafts (*P* < .01; Figs 8, 9). On days 2, 4, 7 and 10, the NPWT grafts had less open mesh area than CON grafts (*P* < .01; Figs 10, 11). The total graft area was not significantly different between NPWT and CON grafts at any time point, although all grafts steadily and significantly decreased in size during the study period. The mean total graft area for all

grafts was 6.47 cm² at day 0 and 4.46 cm² at day 17, which represents a 31% reduction in total graft area.

Qualitative Variables

Scores of graft color are presented in Table 3 (Fig 7). All (5/5) NPWT grafts and 2 (2/5) CON grafts were non-mobile by Day 4, indicating adherence to the underlying recipient bed. One (1/5) seroma was noted under the NPWT grafts (upon biopsy); 3 (3/5) seromas were recorded under the CON grafts. Seromas were noted at the day 4 or day 7 dressing change only, usually at time of biopsy. One CON graft seroma was present at both day 4 and day 7. Hemorrhage from the graft biopsy site was noted in four (4/5) of the NPWT grafts and one (1/5) of the CON grafts at day 4. On day 7, all (5/5) NPWT and 3/5 CON grafts hemorrhaged upon biopsy. Fluid amount, color or nature of the wound fluid on dressing changes were the same between groups. Typically the wound fluid was serosanguineous, pink or brown, and of minimal quantity. By days 14 and 17, all grafts were similar in appearance, and it was evident that the previously delineated areas of necrosis were epithelializing rapidly. Hair growth consistently appeared more robust on the NPWT grafts, compared to the CON grafts, which were less hirsute with more epithelial covering (Fig 12).

Histology

There was no significant difference in the HAIS between NPWT and CON grafts ($P=.26$). Histologic scores of epidermal devitalization, epidermal hyperplasia, and neovascularization were not different between groups ($P=.54$). On histologic sections, both NPWT and CON grafts showed marked epidermal devitalization at day 4, which progressively decreased on days 7 and 14. The epidermal necrosis presented histologically as full thickness devitalization of all keratinocyte layers, with patchy sparing of basal keratinocytes at the communication with the follicular ostia. In some areas, where epidermal architecture was still discernible, keratinocyte intracellular and intercellular edema was appreciated. Similar keratinocyte alterations extended to involve the follicular epithelium in more severely affected tissue specimen sections. Neovascularization slowly increased at each biopsy time point, with a mean of 0/3 on day 0, 1.5/3 on day 7, and 2.2/3 on day 14.

Bacterial Evaluation

All aerobic bacterial cultures were negative.

DISCUSSION

Results from this small experimental study show a clear difference in measured variables of graft healing when NPWT is applied to meshed, full-thickness, skin grafts, suggesting enhanced acceptance when this adjunct is used, compared with standard bolster dressings. Indicators of graft acceptance, including first appearance of granulation tissue within the mesh interstices, and closure of the open meshes were significantly superior when NPWT was applied to the grafts. Grafts treated with NPWT showed significantly decreased necrosis compared with CON grafts. These results show that the application of NPWT to FTSGs could be beneficial during the critical first week after grafting.

Most NPWT publications relate to the use of polyurethane open cell foam (V.A.C., Kinetic Concepts Inc., San Antonio, TX), rather than the gauze dressing we used. For this discussion, we have made the assumption that the mechanisms of action are similar for both types of contact dressing, based on recent studies showing equally effective delivery of negative pressure and deformation with either type.⁵¹⁻⁵³ The widely reported successes of NPWT are probably because of mechanisms of action that have been proven with *in vivo* and *in vitro* studies. These include increased wound blood flow, promotion of angiogenesis,

enhanced granulation tissue formation and stimulation of cellular proliferation and signaling pathways through the applied shear stress.^{13,14,54-58} Other putative mechanisms of action for NPWT, such as reduction of interstitial edema, enhanced bacterial clearance, and maintenance of a moist wound healing environment have not been proven in basic research studies.^{9,36,37} It is unknown which of these mechanisms of action, proven or otherwise, contribute most to benefit skin grafting, but one of the reasons for the improved graft acceptance we observed and suggested in the medical literature, may be in large part because of the simple immobilization of the graft when NPWT is used.²⁹⁻³⁴ In the early days after grafting, the graft-recipient bed interface is maintained only by a relatively weak fibrin network and whatever bolster or elastic conforming dressing is securing the site.² It is intuitive that the application of an evenly distributed negative pressure to press the graft firmly onto the recipient bed and increase contact area will bestow considerable benefit to the processes of graft 'take'. This contact may be further enhanced by the removal of excess fluid through the interstices of the meshes. These properties of NPWT alone would favor undisturbed growth of capillary buds from the recipient bed into the graft, and may be the reason why this modality has become so widely used in human medicine, before solid scientific proof of other mechanisms were validated.

Although increased split-thickness skin graft acceptance with NPWT has been widely claimed, this has not always been statistically significant, except in highly exudative grafts and when immobilization of the graft is difficult.^{31-33,36,59} This may be because of the high overall rates of success with split-thickness skin grafting in the medical field, and the lack of randomized, controlled, clinical trials in this area. Decreased rates of re-grafting and shorter hospitalization times have also been reported with NPWT and split-thickness grafting.^{30,31,33} Although full-thickness grafting is less commonly used in human reconstructive surgery, NPWT was used both before (to prepare the wound bed) and after full-thickness grafting in a non-controlled series of 24 pediatric patients, mostly with traumatic wounds or burn contracture excisions. Mean graft take was 95% and the authors concluded that NPWT enhanced full-thickness graft acceptance.⁴¹

The accelerated appearance of granulation tissue we observed was striking, and is consistent with what has been documented in open wounds treated with NPWT, both experimentally, and in most, but not all clinical studies in the medical literature.^{5,13,14,18,37,50,60} Rapid fibroplasia is also reported in the veterinary literature, with granulation tissue appearing several days earlier in open wounds treated with NPWT, compared to standard-of-care dressings.⁵⁰ Finite element (computer) modeling has shown that the strain levels induced by applied negative pressure (5% to 20% strain) are similar to the levels known to promote cellular proliferation.⁵⁶ Experimental studies have supported this theory, showing that the deforming forces applied to the extracellular matrix and cells during negative pressure will stimulate cellular proliferation, angiogenesis and increased capillary blood flow.^{16,54,55}

There was more necrosis (as evidenced by black discoloration, eschar, or slough) in the CON grafts than the NPWT grafts at most time points. Care was taken not to confuse the typical early discoloration of a compromised graft with necrosis. The difference observed was greatest at day 7, a time point at which it is generally evident whether grafted skin has 'taken' or failed. At that time, the mean percent necrosis of the NPWT grafts was < 1%, compared with 10% of the CON grafts. Although this difference was statistically significant, the difference between 1% and 10% graft necrosis may often not be considered clinically significant, especially for partial-thickness necrosis (which epithelializes well from follicular and glandular epithelium). However, the small grafts harvested and applied in our study were performed in young and healthy dogs under aseptic surgical conditions, and in an anatomic area where it is easy to apply and maintain uniform pressure. (This area was chosen to minimize the discomfort of the dogs and to enable accurate planimetry). In a large,

traumatic wound with an irregular wound bed, grafting can pose a significant challenge, especially so in an area difficult to immobilize. In these circumstances, the probability of partial or catastrophic graft failure is greater and the importance of optimizing graft contact even more crucial.

Mesh closure was also notably superior when NPWT was applied to the grafts, with the greatest difference again on day 7, at the time that NPWT was removed. At that time, the average percent open meshed area in the NPWT group (36.4%) was less than half of the CON group (98.1%). When measuring mesh closure in human split-thickness meshed grafts, the medical literature refers to 're-epithelialization' of the open mesh holes.⁶¹ In this study, which differs not only with respect to species, but also in the thickness of the grafts (full thickness) and type of meshing (by hand, compared to a mechanical mesher), it appeared to these authors that mesh closure was because of a combination of epithelialization and contraction. Mesh closure may be enhanced by the moist environment provided by the NPWT, and the lack of abrasive dressings to disrupt the fragile migrating epithelium. The earlier appearance of granulation tissue provides a smooth bed for epithelial migration, as well as enabling myofibroblast-mediated contraction.

Qualitative variables (graft color, mobility, bleeding on biopsy, seroma/hematoma) were not analyzed statistically because of their discrete nature and low number of dogs in our study. However, these subjective changes are typically what the clinician observes during early dressing changes, and we believe they are also interesting to note. Variables were different largely in the first week after grafting, and favored the NPWT grafts as evidenced by a pinker graft color, lack of mobility at day 4, early bleeding when biopsied (indicating revascularization), and decreased fluid accumulation beneath the graft. In a more challenging clinical situation, the presence of fluid underneath the graft, and/or increased movement in those first few days could make the difference between graft healing and catastrophic failure.

One of the advantages of using NPWT with skin grafting that has been noted in human studies is the improved quality of life immediately postoperatively. Patients receiving grafts in high-motion areas such as the neck, axilla, or perineum, require immobilization if a traditional bolster dressing is used. When NPWT is applied over the graft, these patients can have early, limited movement without compromising graft healing.^{30,34} We have also found this to be applicable to dogs and cats, allowing early ambulation and easier nursing care when NPWT is applied after grafting. When possible, the graft is also bolstered with a soft padded bandage, as was done in this study.

It was interesting to note that despite the quantitative and qualitative differences seen in the variables of graft healing, there was no statistically significant difference in any of the histologic scores between groups, at any time point. This lack of significance may be because of the infrequent number of biopsy time points, small numbers, or the fact that the ideal grafting conditions on the antebrachium did not challenge the treatment group enough to see a clear difference. Although not statistically significant, the mean HAIS was higher in the NPWT grafts on day 4 (4.8 v 3.6 out of 15), and the mean neovascularization score was higher in the NPWT grafts on day 4 (1.0 v 0.8 out of 3) and day 7 (1.8 v 1.2 out of 3). It is also of interest that although assessments of follicular and glandular epidermal devitalization were similar between groups, the NPWT grafts were noticeably more hirsute than the CON grafts (Fig 12). The more robust hair growth on the NPWT grafts reflects increased viable hair follicles associated with this modality. In contrast, the sparsely haired CON grafts are consistent with more compromise to and subsequent loss of hair follicles, possibly in the superficial dermis. Further investigation of the viability of adnexal structures in grafts under NPWT is indicated.

None of the grafts had clinical signs of infection, nor was there any bacterial growth on aerobic culture on day 7. This is likely because the wounds were created and grafted acutely under aseptic conditions. However, there was concern that if the NPWT dressings were too occlusive in nature it could increase the risk of graft maceration and subsequent infection. The role of NPWT on clearance of bacterial load from wounds remains unclear. Early investigations concluded that the application of NPWT decreased the bacterial burden of pig wounds that had been inoculated with human bacterial isolates.¹³ Subsequently, several clinical studies have failed to reach the same conclusion, although it has been shown that NPWT can be successfully used in infected wounds that have been appropriately debrided.^{18,20,21,28} Our study is not large enough to draw a conclusion concerning the effects of NPWT on contamination.

Although the number of dogs studied was small, it was appropriately controlled, randomized and blinded, and statistical significance of the quantitative variables was attained. The initial study design protocol was for 10 dogs (n=20), undertaken in 2 series of 5 (because of the intensity of monitoring). After analysis of results from these 5 dogs, however, the data were powerful enough to reach significance in the quantitative variables measured. This early analysis of data enabled the reduction of the total number of animals used. To identify any potential histologic or bioburden differences; however, larger numbers may be required. We did not attempt to undertake any cost comparison because there was no NPWT system marketed to the veterinary profession at the time of the study. The initial investment for the NPWT equipment is several thousand dollars, but a true analysis would need to weigh the cost of NPWT equipment and disposables against the cost of standard dressings, plus account for the cost of any revisional procedures in either group. Such an analysis would be useful and probably be best performed as part of a prospective clinical study.

Our results validate the use of NPWT over full-thickness skin grafts in clinical veterinary medicine, and this modality has become the standard-of-care when performing FTSGs in our teaching hospital. Further studies are now indicated in the form of randomized, controlled prospective clinical trials, to provide the most rigorous data. No such studies exist in veterinary medicine with respect to NPWT. Additional experimental veterinary investigations into this modality are also encouraged, refining its application, focusing on other potential indications, and evaluating overall cost-effectiveness.

Acknowledgments

We thank Eric Zellner, Humphrey Petersen-Jones, Duncan Petersen-Jones for. We thank the owners who adopted the dogs into private homes at the conclusion of the study.

Funded by the Michigan Animal Health Foundation, Michigan Veterinary Medical Association. Student funding was provided by NIH Grant 5T35RR017491-07 to Michigan State University.

REFERENCES

1. Pavletic, MM. Atlas of Small Animal Wound Management and Reconstructive Surgery. ed 3rd. Wiley-Blackwell; Ames: 2010. Free Grafts; p. 403-431. Vol.
2. Swaim, SF. Skin Grafts. In: Slatter, DH., editor. Textbook of Small Animal Surgery. ed 3rd. Vol. Vol 1. Saunders; Philadelphia: 2003. p. 321-338.
3. Waris T, Rechardt L, Kyosola K. Reinnervation of human skin grafts: a histochemical study. *Plast Reconstr Surg*. 1983; 72:439-447. [PubMed: 6351136]
4. DeCarbo WT, Hyer CF. Negative-pressure wound therapy applied to high-risk surgical incisions. *J Foot Ankle Surg*. 2010; 49:299-300. [PubMed: 20605565]
5. Dunn R, Hurd T, Chadwick P, et al. Factors associated with positive outcomes in 131 patients treated with gauze-based negative pressure wound therapy. *Int J Surg*. 2010

6. Petzina R, Hoffmann J, Navasardyan A, et al. Negative pressure wound therapy for post-sternotomy mediastinitis reduces mortality rate and sternal re-infection rate compared to conventional treatment. *Eur J Cardiothorac Surg.* 2010; 38:110–113. [PubMed: 20171898]
7. Pollak AN, Powell ET, Fang R, et al. Use of negative pressure wound therapy during aeromedical evacuation of patients with combat-related blast injuries. *J Surg Orthop Adv.* 2010; 19:44–48. [PubMed: 20371006]
8. Thompson JT, Marks MW. Negative pressure wound therapy. *Clin Plast Surg.* 2007; 34:673–684. [PubMed: 17967622]
9. Xie X, McGregor M, Dendukuri N. The clinical effectiveness of negative pressure wound therapy: a systematic review. *J Wound Care.* 2010; 19:490–495. [PubMed: 21135797]
10. Mendez-Eastman S. Guidelines for using negative pressure wound therapy. *Adv Skin Wound Care.* 2001; 14:314–322. quiz 324-315. [PubMed: 11794443]
11. Molnar JA. The science behind negative pressure wound therapy. *Ostomy Wound Manage.* 2004; 50:2–5. [PubMed: 15317235]
12. Venturi ML, Attinger CE, Mesbahi AN, et al. Mechanisms and clinical applications of the vacuum-assisted closure (VAC) Device: a review. *Am J Clin Dermatol.* 2005; 6:185–194. [PubMed: 15943495]
13. Morykwas MJ, Argenta LC, Shelton-Brown EI, et al. Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation. *Ann Plast Surg.* 1997; 38:553–562. [PubMed: 9188970]
14. Morykwas MJ, Faler BJ, Pearce DJ, et al. Effects of varying levels of subatmospheric pressure on the rate of granulation tissue formation in experimental wounds in swine. *Ann Plast Surg.* 2001; 47:547–551. [PubMed: 11716268]
15. Morykwas MJ, Simpson J, Pungert K, et al. Vacuum-assisted closure: state of basic research and physiologic foundation. *Plast Reconstr Surg.* 2006; 117:121S–126S. [PubMed: 16799379]
16. Scherer SS, Pietramaggiori G, Mathews JC, et al. The mechanism of action of the vacuum-assisted closure device. *Plast Reconstr Surg.* 2008; 122:786–797. [PubMed: 18766042]
17. Weston C. The science behind topical negative pressure therapy. *Acta Chir Belg.* 2010; 110:19–27. [PubMed: 20306904]
18. Braakenburg A, Obdeijn MC, Feitz R, et al. 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; 118:390–397. discussion 398-400. [PubMed: 16874208]
19. Malli S. Keep a close eye on vacuum-assisted wound closure. *Nursing.* 2005; 35:25. [PubMed: 15988191]
20. Moues CM, Vos MC, van den Bemd GJ, et al. Bacterial load in relation to vacuumassisted closure wound therapy: a prospective randomized trial. *Wound Repair Regen.* 2004; 12:11–17. [PubMed: 14974959]
21. Weed T, Ratliff C, Drake DB. Quantifying bacterial bioburden during negative pressure wound therapy: does the wound VAC enhance bacterial clearance? *Ann Plast Surg.* 2004; 52:276–279. discussion 279-280. [PubMed: 15156981]
22. Hinck D, Franke A, Gatzka F. Use of vacuum-assisted closure negative pressure wound therapy in combat-related injuries--literature review. *Mil Med.* 2010; 175:173–181. [PubMed: 20358706]
23. Goldstein JA, Iorio ML, Brown B, et al. The use of negative pressure wound therapy for random local flaps at the ankle region. *J Foot Ankle Surg.* 2010; 49:513–516. [PubMed: 20801691]
24. Uygur F, Duman H, Ulkur E, et al. The role of the vacuum-assisted closure therapy in the salvage of venous congestion of the free flap: case report. *Int Wound J.* 2008; 5:50–53. [PubMed: 18179554]
25. Parrett BM, Matros E, Pribaz JJ, et al. Lower extremity trauma: trends in the management of soft-tissue reconstruction of open tibia-fibula fractures. *Plast Reconstr Surg.* 2006; 117:1315–1322. discussion 1323-1314. [PubMed: 16582806]
26. Tang AT, Okri SK, Haw MP. Vacuum-assisted closure to treat deep sternal wound infection following cardiac surgery. *J Wound Care.* 2000; 9:229–230. [PubMed: 11933333]

27. Yuan-Innes MJ, Temple CL, Lacey MS. Vacuum-assisted wound closure: a new approach to spinal wounds with exposed hardware. *Spine*. 2001; 26:E30–33. [PubMed: 11224876]
28. Cowan KN, Teague L, Sue SC, et al. Vacuum-assisted wound closure of deep sternal infections in high-risk patients after cardiac surgery. *Ann Thorac Surg*. 2005; 80:2205–2212. [PubMed: 16305872]
29. Blackburn JH 2nd, Boemi L, Hall WW, et al. Negative-pressure dressings as a bolster for skin grafts. *Ann Plast Surg*. 1998; 40:453–457. [PubMed: 9600426]
30. Chang KP, Tsai CC, Lin TM, et al. An alternative dressing for skin graft immobilization: negative pressure dressing. *Burns*. 2001; 27:839–842. [PubMed: 11718987]
31. Llanos S, Danilla S, Barraza C, et al. Effectiveness of negative pressure closure in the integration of split thickness skin grafts: a randomized, double-masked, controlled trial. *Ann Surg*. 2006; 244:700–705. [PubMed: 17060762]
32. Moisidis E, Heath T, Boorer C, et al. A prospective, blinded, randomized, controlled clinical trial of topical negative pressure use in skin grafting. *Plast Reconstr Surg*. 2004; 114:917–922. [PubMed: 15468399]
33. Scherer LA, Shiver S, Chang M, et al. The vacuum assisted closure device: a method of securing skin grafts and improving graft survival. *Arch Surg*. 2002; 137:930–933. discussion 933–934. [PubMed: 12146992]
34. Schneider AM, Morykwas MJ, Argenta LC. A new and reliable method of securing skin grafts to the difficult recipient bed. *Plast Reconstr Surg*. 1998; 102:1195–1198. [PubMed: 9734445]
35. DeFranzo AJ, Marks MW, Argenta LC, et al. Vacuum-assisted closure for the treatment of degloving injuries. *Plast Reconstr Surg*. 1999; 104:2145–2148. [PubMed: 11149782]
36. Moues CM, Heule F, Hovius SE. A review of topical negative pressure therapy in wound healing: sufficient evidence? *Am J Surg*. 2011; 201:544–556. [PubMed: 21421104]
37. Gregor S, Maegele M, Sauerland S, et al. Negative pressure wound therapy: a vacuum of evidence? *Arch Surg*. 2008; 143:189–196. [PubMed: 18283145]
38. Mendonca DA, Papini R, Price PE. Negative-pressure wound therapy: a snapshot of the evidence. *Int Wound J*. 2006; 3:261–271. [PubMed: 17199762]
39. Hunter JE, Teot L, Horch R, et al. Evidence-based medicine: vacuum-assisted closure in wound care management. *Int Wound J*. 2007; 4:256–269. [PubMed: 17924882]
40. Vikatmaa P, Juutilainen V, Kuukasjarvi P, et al. Negative pressure wound therapy: a systematic review on effectiveness and safety. *Eur J Vasc Endovasc Surg*. 2008; 36:438–448. [PubMed: 18675559]
41. Landau AG, Hudson DA, Adams K, et al. Full-thickness skin grafts: maximizing graft take using negative pressure dressings to prepare the graft bed. *Ann Plast Surg*. 2008; 60:661–666. [PubMed: 18520204]
42. Adkesson MJ, Travis EK, Weber MA, et al. Vacuum-assisted closure for treatment of a deep shell abscess and osteomyelitis in a tortoise. *J Am Vet Med Assoc*. 2007; 231:1249–1254. [PubMed: 17937557]
43. Gemeinhardt KD, Molnar JA. Vacuum-assisted closure for management of a traumatic neck wound in a horse. *Equine Veterinary Education*. 2005; 17:27–33.
44. Guille AE, Tseng LW, Orsher RJ. Use of vacuum-assisted closure for management of a large skin wound in a cat. *J Am Vet Med Assoc*. 2007; 230:1669–1673. [PubMed: 17542735]
45. Harrison TM, Stanley BJ, Sikarskie JG, et al. Surgical amputation of a digit and vacuum-assisted closure (V.A.C.) management in a case of osteomyelitis and wound care in an Eastern Black Rhinoceros (*Diceros bicornis michaeli*). *Journal of Zoo and Wildlife Medicine*. 2011; 42:317–321. [PubMed: 22946413]
46. Lafortune M, Fleming GJ, Wheeler JL, et al. Wound management in a juvenile tiger (*Panthera tigris*) with vacuum-assisted closure (V.A.C. Therapy). *J Zoo Wildl Med*. 2007; 38:341–344. [PubMed: 17679522]
47. Mullally C, Carey K, Seshadri R. Use of a nanocrystalline silver dressing and vacuum-assisted closure in a severely burned dog. *J Vet Emerg Crit Care (San Antonio)*. 2010; 20:456–463. [PubMed: 20731813]

48. Owen L, Hotston-Moore A, Holt P. Vacuum-assisted wound closure following urine-induced skin and thigh muscle necrosis in a cat. *Vet Comp Orthop Traumatol*. 2009; 22:417–421. [PubMed: 19750293]
49. Ben-Amotz R, Lanz OI, Miller JM, et al. The use of vacuum-assisted closure therapy for the treatment of distal extremity wounds in 15 dogs. *Vet Surg*. 2007; 36:684–690. [PubMed: 17894595]
50. Demaria M, Stanley BJ, Hauptman JG, et al. Effects of negative pressure wound therapy on healing of open wounds in dogs. *Vet Surg*. 2011; 40:658–669. [PubMed: 21770975]
51. Malmström M, Ingemansson R, Martin R, et al. Wound edge microvascular blood flow: effects of negative pressure wound therapy using gauze or polyurethane foam. *Ann Plast Surg*. 2009; 63:676–681. [PubMed: 19887926]
52. Malmström M, Ingemansson R, Martin R, et al. Negative-pressure wound therapy using gauze or open-cell polyurethane foam: similar early effects on pressure transduction and tissue contraction in an experimental porcine wound model. *Wound Repair Regen*. 2009; 17:200–205. [PubMed: 19320888]
53. Malmström M, Lindstedt S, Ingemansson R. Influence on pressure transduction when using different drainage techniques and wound fillers (foam and gauze) for negative pressure wound therapy. *Int Wound J*. 2010; 7:406–412. [PubMed: 20649833]
54. Chen SZ, Li J, Li XY, et al. Effects of vacuum-assisted closure on wound microcirculation: an experimental study. *Asian J Surg*. 2005; 28:211–217. [PubMed: 16024319]
55. Labler L, Rancan M, Mica L, et al. Vacuum-assisted closure therapy increases local interleukin-8 and vascular endothelial growth factor levels in traumatic wounds. *J Trauma*. 2009; 66:749–757. [PubMed: 19276749]
56. Saxena V, Hwang CW, Huang S, et al. Vacuum-assisted closure: microdeformations of wounds and cell proliferation. *Plast Reconstr Surg*. 2004; 114:1086–1096. discussion 1097–1088. [PubMed: 15457017]
57. Timmers MS, Le Cessie S, Banwell P, et al. The effects of varying degrees of pressure delivered by negative-pressure wound therapy on skin perfusion. *Ann Plast Surg*. 2005; 55:665–671. [PubMed: 16327472]
58. Wackenfors A, Sjogren J, Gustafsson R, et al. Effects of vacuum-assisted closure therapy on inguinal wound edge microvascular blood flow. *Wound Repair Regen*. 2004; 12:600–606. [PubMed: 15555050]
59. Vidrine DM, Kaler S, Rosenthal EL. A comparison of negative-pressure dressings versus Bolster and splinting of the radial forearm donor site. *Otolaryngol Head Neck Surg*. 2005; 133:403–406. [PubMed: 16143190]
60. Fisher A, Brady B. Vacuum assisted wound closure therapy. *Issues Emerg Health Technol*. 2003; 1–6. [PubMed: 12653112]
61. Hauser J, Lehnhardt M, Daigeler A, et al. Photoplanimetric evaluation and impedance measurement of split-thickness skin grafts: a new model for objective wound-healing assessment in clinical trials. *Skin Res Technol*. 2009; 15:168–171. [PubMed: 19622126]

Figure 1. Application of negative pressure wound therapy, showing open cell foam (A) or gauze (B) as contact layer through which subatmospheric pressure is applied, once the area has been sealed with an impermeable, adhesive drape. As the negative pressure is applied, the dressing takes on a hard and wrinkled appearance.

Figure 2. Dorsal, mid-antebrachial wounds were created bilaterally on using 4.0×1.5 cm sterile templates.

Figure 3.
The excised skin sections were prepared for grafting by meticulous removal of hypodermal remnants with #11 scalpel blade (A), followed by meshing (B).

Figure 4.
Graft transposed and secured in the contralateral recipient defect.

Figure 5. NPWT dressing in place before the secondary and tertiary layer bandaging. Note the hard, wrinkled appearance of the dressing, indicating that a vacuum has been obtained.

Figure 6.

The negative suction tubing was secured around the thorax and exited dorsally through the coiled extension tubing to a connecting bar overhead. The NPWT machine can be seen secured to the wall on the left hand side of the cage. This dog is wearing an E-collar, and a baby “onesie” over the limb and thoracic bandage.

Figure 7.

Typical appearance of paired grafts at Day 2. Bright red granulation tissue is seen within the interstices of the meshes in the NPWT graft (A). In contrast, the muscle belly is still visible within the meshes of the CON graft (B). Note also the discoloration of the grafts: the NPWT graft (A) appears to be a healthy pink, compared to the more typical darker, mottled appearance of the CON graft (B).

Figure 8.
Typical appearance NPWT graft (A) and CON graft (B) in the same dog at Day 7, showing more compromise and partial necrosis in the CON graft.

Figure 9. Mean percent necrosis of meshed FTSGs under NPWT and standard-of-care bolster dressings (CON). Standard error bars are shown; asterisks indicate significance.

Figure 10.

Typical appearance of paired grafts in the same dog at day 7. Note the smaller size of the open meshes in the NPWT graft (A) compared to the CON graft (B). (Biopsy sites are marked with an asterisk).

Figure 11. Mean percent open mesh area of meshed FTSGs under NPWT and standard-of-care bolster dressings (CON) (n=10). Standard error bars are shown; asterisks indicate significance.

Figure 12. Typical appearance of both NPWT grafts (A) and CON grafts (B) at day 17. Note the superior hair growth on the NPWT graft. Biopsy sites are marked with an asterisk.

Table 1

Form used for subjective evaluation of grafts. After study completion the forms were randomized and analyzed without revealing the grouping (NPWT or CON).

Dog ID:				Right	Left		
Day:	0	2	4	7	10	14	17
Fluid amount	None	Minimal		Moderate		Excessive	
Fluid color	Clear		Pink/Red	Brown	Yellow	Green	
Nature of fluid	Serous	Serosanguineous	Sanguineous	Purulent +	Purulent ++	Purulent +++	
Appearance of secondary layer	Dry & clean		Dry & stained	Moist	Wet	Strike-through	
Graft adherence Day 4	Non-mobile			Mobile			
Graft color	1= Healthy pink	2= Mottled pink		3= Mottled, purple	4= Dark purple/black	5= Slimy white	
Notes on graft color:							
Peri wound	Normal	Edema	Erythema	Ulceration	Induration	Discoloration	
Graft hydration	Normal	Maceration +	Maceration ++	Maceration +++	Desiccation +	Dessication ++	

Additional notes:

Table 2

Day of first appearance of granulation tissue in the interstices of the meshed grafts in 5 dogs.

Dog ID	NPWT	CON
F	2	10
G	4	10
H	2	7
I	2	4
J	4	7

Table 3

Mean results of graft color of each group. 1= healthy pink; 2 = mottled pink; 3 = mottled, bruised; 4 = dark purple or black; 5 = slimy white.

	Day 0	Day 2	Day 4	Day 7	Day 10	Day 14	Day 17
NPWT	1	1.8	2.2	1	1	1	1
CON	1	2.6	3.0	2.2	1.2	1	1