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Does treatment of split-thickness skin grafts with negative-pressure wound therapy improve tissue markers of wound healing in a porcine experimental model?

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Abstract

Background—Negative-pressure wound therapy (NPWT) has been used for to treat wounds for more than 15 years and, more recently, has been used to secure split-thickness skin grafts. There are some data to support this use of NPWT, but the actual mechanism by which NPWT speeds healing or improves skin graft take is not entirely known. The purpose of this project was to assess whether NPWT improved angiogenesis, wound healing, or graft survival when compared with traditional bolster dressings securing split-thickness skin grafts in a porcine model.

Methods—We performed two split-thickness skin grafts on each of eight 30 kg Yorkshire pigs. We took graft biopsies on postoperative days 2, 4, 6, 8, and 10 and submitted the samples for immunohistochemical staining, as well as standard hematoxylin and eosin staining. We measured the degree of vascular ingrowth via immunohistochemical staining for von Willenbrand's factor to better identify blood vessel epithelium. We determined the mean cross-sectional area of blood vessels present for each representative specimen, and then compared the bolster and NPWT samples. We also assessed each graft for incorporation and survival at postoperative day 10.

Results—Our analysis of the data revealed that there was no statistically significant difference in the degree of vascular ingrowth as measured by mean cross-sectional capillary area ($p = 0.23$). We did not note any difference in graft survival or apparent incorporation on a macroscopic level, although standard hematoxylin and eosin staining indicated that microscopically, there seemed to

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be better subjective graft incorporation in the NPWT samples and a nonsignificant trend toward improved graft survival in the NPWT group.

Conclusion—We were unable to demonstrate a significant difference in vessel ingrowth when comparing NPWT and traditional bolster methods for split-thickness skin graft fixation. More studies are needed to elucidate the manner by which NPWT exerts its effects and the true clinical magnitude of these effects.

Level of Evidence—Not applicable.

Keywords

Negative-pressure wound therapy; split thickness skin graft

Negative-pressure wound therapy (NPWT) has been in use for nearly 20 years. Early animal studies demonstrated that, compared with traditional dressings, the application of NPWT to surgically created wounds resulted in increased blood flow and granulation tissue within the wound.¹ In humans, early forms of NPWT were first used in the treatment of open fractures and other severe soft tissue injuries.² During the years, the indications and applications of this therapy have expanded. This has led to the application of NPWT as a postoperative dressing to stabilize split-thickness skin grafts (STSG).³

Traditionally, STSGs have been fixed with a bolster technique, where sutures are used to fix pressure dressings over the top of recently placed grafts. Proponents of NPWT in the use of STSG fixation note that there are some data to suggest that the rates of engraftment are higher and the need for reoperation is lower with the use of NPWT when compared with traditional bolster-type fixation.⁴

Despite the widespread use of NPWT, there is a paucity of data supporting its use in many of the clinical scenarios in which it is used. There is a great deal of anecdotal data suggesting the effectiveness of NPWT. However, there are far fewer studies that have prospectively examined the true clinical effects of NPWT or attempted to explain the mechanisms by which it exerts these effects. This is especially true when examining the role of NPWT as a postoperative dressing for STSG. Some studies have suggested that when used in this manner, NPWT improves engraftment by reducing the rate of seroma or hematoma formation, removing excess interstitial fluids, and reducing shear forces between the graft and wound bed.⁵ Other investigators have suggested that NPWT induces angiogenesis or improves blood flow via existing channels through biomechanical effects or induction of moderate hypoxia in the wound bed.⁶

In this context, we sought to assess whether better wound healing or angiogenesis, as measured by immunohistochemistry (IHC) and clinical assessment, could be observed in STSGs treated with NPWT. Using pigs as our experimental model, we compared STSGs fixed with traditional bolster dressings with those fixed with NPWT. We hypothesized that we would see improved wound healing and vessel ingrowth in the grafts treated with NPWT.

Materials and Methods

Before initiating the study, approval to use animal subjects was obtained by the institutional animal care and use committee. Before the study was begun, a power analysis was performed under the assumption that there would be a large treatment effect on vessel ingrowth and angiogenesis, as demonstrated by another study examining the effect of NPWT on traumatic wounds.⁷ We set our threshold for statistical significance at $p < 0.05$,

and using data from the previously mentioned study as a rough guide, we calculated that eight subjects would be required to arrive at a power of 0.8. Eight 30-kg Yorkshire pigs were used each receiving two 6 × 8-cm skin grafts to each side of the dorsolateral back, with skin from one side serving as the graft for the other. The skin grafts were 0.01-inch thick, harvested using an air-driven dermatome and meshed at a 1:1.5 ratio. On each pig, one graft was secured using a gauze-based NPWT system (Renasys, Smith and Nephew, London, United Kingdom) set to 80 mm Hg continuous suction, with the remaining side fixed with a bolster dressing. The bolster dressing consisted of several layers of dry gauze overlaying a piece of petrolatum-impregnated gauze. This dressing was then fixed in place with occlusive plastic sheeting stretched across the top, which was secured to the skin around the graft with the aid of topical adhesive. Regardless of subsequent fixation method each graft was covered by petrolatum-impregnated gauze. Random 2-mL punch biopsy specimens were obtained from each graft on postoperative days 2, 4, 6, 8, and 10. Care was taken to disturb the grafts as little as possible. Biopsy specimens were obtained by making small “windows” in the overlying dressings by incising the overlying plastic dressings, sweeping aside the next layers of gauze dressing, and making a small incision in the underlying petrolatum gauze. Once a small area of the graft was exposed we obtained a punch biopsy specimen and patched the hole in the dressing after ensuring hemostasis with light pressure alone. All biopsy specimens were preserved in formalin and processed according to the IHC protocol mentioned later, and underwent standard hematoxylin and eosin staining. Examination and analysis of both hematoxylin and eosin-stained and IHC-stained sections was supervised by a pathologist who was blinded to the method of STSG fixation.

For immunohistochemical staining, a three-step avidin-biotin-peroxidase procedure was used. Sections were first deparaffinized rinsed and dried and antigen retrieval was accomplished using a steamer for 30 minutes. The sections were next rinsed in 0.5% Tween 20 (Merck, Whitehouse Station, NJ) and PBS (Sigma-Aldrich, St. Louis, MO) and nonspecific binding sites blocked with 2% goat serum/1% PBS at room temperature for 30 minutes. The sections were incubated overnight with rabbit polyclonal anti-von Willenbrand's factor (1:1000) (Dako, Glostrup, Denmark) in 2% goat serum/1% PBS at 4°C. The sections were next washed thoroughly with 0.5% Tween 20 and then incubated with biotinylated goat anti-rabbit immunoglobulin as the secondary antibody for 30 minutes. After further washing and blocking of endogenous peroxidase with 3% H₂O₂ in PBS for 15 minutes, the sections were treated with a Vector Avidin/Biotin Complex (Vector Laboratories, Burlingame, CA) amplification reagent for 30 minutes. The sections were then incubated in 3,3'-Diaminobenzidine. The sections were then washed and counterstained with Gill Hematoxylin for 90 seconds and were dehydrated and the slides were mounted.

The biopsy specimens were analyzed after IHC staining as detailed previously. Blood vessel endothelium was identified and the area encompassed by the lumens of identified blood vessels was then measured. This technique is described elsewhere.⁷ In brief, representative sections of areas near the graft-wound bed interface were identified and then at 200 × magnification, the proportion of total cross-sectional area of blood vessel out of total tissue on each section was then calculated. The means of these proportions were then calculated and compared for both the control and experimental arms of the study.

The pigs were killed on postoperative day 10, at which point the percent graft “take” was determined by one or more investigators with surgical clinical experience, final biopsy specimens were obtained and photographs of the skin grafts were taken for later reference and review. Graft take was assessed by calculating the ratio of failed graft to incorporated graft, then expressed as a percentage total graft take, rounded to the nearest 5%. During the assessment of graft take, the investigators were not blinded to the method of treatment because the investigators were blinded to the primary end point as measured by quantitative

analysis of vessel ingrowth, and the graft take was considered a secondary end point. We compared the average percentage take and rates of graft survival for each arm of the study.

Data were statistically analyzed. Univariate analysis of continuous variables was done using a two-tailed *t* test. For dichotomous variables, a Fisher's exact test was used. A $p < 0.05$ was designated as statistically significant.

Results

We found that there was no statistically significant difference in pooled mean cross-sectional area of blood vessel lumens when the two groups were compared ($p = 0.23$). In fact, there was a trend toward increased mean blood vessel cross-sectional area in the control group (Fig. 1), but at no point was the difference between the means statistically significant. There were two graft failures in the control group, and none in the NPWT group, although this difference was not statistically significant ($p = 0.47$). There was no significant difference in percentage graft take, although there was a trend favoring the NPWT group over the control group (mean [SEM], 0.99 [0.6] vs. 0.74 [16.1]; $p = 0.16$).

Standard histologic analysis of representative sections of the graft-wound interface did reveal some modest, subjective differences (Fig. 2 and 3). There appeared to be less edema, inflammatory infiltrate, and better apposition of the graft in the NPWT group when compared with control.

Discussion

There is mounting evidence and clinical experience suggesting that NPWT is beneficial when used to fix STSG when compared with traditional methods. A handful of prospective studies have demonstrated favorable outcomes with this technique.^{8–10} Numerous retrospective studies have demonstrated a significant beneficial effect of NPWT when used to fix STSG in a variety of scenarios.^{11–13} Within the last year, a panel of experts in wound management arrived at a broad, global consensus for the use of NPWT in the fixation of STSG, among other uses.¹⁴ However, a recent systematic review of randomized controlled trials found no clear evidence of benefit for any use of NPWT.¹⁵ In the current climate of health care, there are lingering doubts about the cost-effectiveness of NPWT and the precise clinical scenarios in which it should be used.

There are a few scenarios in which NPWT is used so frequently that it has become the standard of care. Aside from its use in the fixation of STSG, several studies suggest that NPWT confers considerable benefit in the treatment of diabetes-associated leg wounds, severe soft tissue injuries, and open fractures.^{7,11,16} A recent animal study demonstrated increased blood flow and angiogenesis in open wounds treated with NPWT when compared with other methods.¹⁷ Studies conducted on human subjects indicate that NPWT applied to traumatic wounds increases levels of interleukin 8, vascular endothelial growth factor, von Willenbrand's factor, and CD34, all of which are vital in the ordered healing of wounds.⁷ NPWT also probably induces biomechanical pathways that lead to improved cell migration and differentiation.¹⁸

It is not clear, however, that the same mechanisms at work in open wounds are applicable to the effect of NPWT on STSG. Most studies conducted to date on the use of NPWT for STSG fixation have focused on clinical outcomes, usually in a retrospective manner, in human subjects. Many investigators have theorized that NPWT reduces seroma formation, stabilizes grafts, and leads to improved neovascularization and graft take. These theories make intuitive sense. However, there are few data to back up these claims because few

studies have examined the cytologic or molecular basis for the changes that NPWT presumably induces.

It is in this context that we present our results. We found that, in our experimental model, there was no significant difference in the degree of angiogenesis or wound healing in skin grafts treated with NPWT or bolster-type dressings.

It is worth noting that although there appeared to be no difference in neovascularization between the two groups, the control group had two of eight grafts fail, whereas none failed in the NPWT group. There was a trend toward improved graft survival and take in the experimental group, which given a larger sample size, might have become significant. As far as graft survival and take are concerned, our results are consistent with most previously published literature. There probably is a difference in graft survival when NPWT is applied. Our study suggests that this difference is not accounted for by neovascularization alone.

Instead, our study suggests that other pathways are at work, which account for these differences in outcome. The environment created by NPWT is different from that created by a traditional bolster dressing. This different environment undoubtedly induces changes in skin grafts, which we did not measure. Other studies have demonstrated improved migration and proliferation of epithelial cells across skin substitutes when NPWT is applied.¹⁹ Other studies have also noted increased levels of proinflammatory cytokines in wound beds treated with NPWT.⁷ A recent study by Chen et al.¹⁷ demonstrated impressive changes in wound beds treated with NPWT. That study examined the effects of treatment on open wounds without STSG but did demonstrate differences in wound healing, fibroblast migration and differentiation, and neovascularization. Although wound beds treated with STSG differ from open wounds, there are likely common processes at work in each scenario. Had we designed our study to examine the presence of specific cells known to be important in STSG engraftment and found to be effected by NPWT in other studies, such as macrophages or fibroblasts, we may have discovered a significant difference.

Our study had other limitations. We used an animal model, and although porcine models have been used extensively in the study of NPWT, there are intrinsic differences in the physiology between species, and the effect of those differences is difficult to quantify. The behavior of live, large animals also introduces a degree of uncertainty and variability, especially in this study examining dressings overlying fragile skin grafts, which are easily dislodged. Our study was also limited by its small sample size. A power analysis performed after the study was completed indicated that the study was underpowered to detect the observed difference. A sample size of about 26 additional animals ($N = 34$) would be required to arrive at a power = 0.8 and to detect a statistically significant difference ($p = 0.05$) between the two groups for graft survival or take. Compounding this is the problem of sampling error, in that biopsy specimens were taken at random locations and the degree of vessel ingrowth was likely not uniform throughout each sample. Using punch biopsies, the depth of each sample could not be exactly calibrated, although subsequent analysis was directed to the wound-graft interface evident in each sample.

Although our study does not demonstrate a significant advantage to NPWT as a postoperative dressing for STSG, there is some clinical experience and evidence to the contrary. However, the mechanisms by which NPWT improves STSG take continue to be incompletely understood. Further investigation into the effects of NPWT as well as the specific patient populations and problems that are best suited to the therapy are needed.

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Figure 1.
Comparison of mean cross-sectional blood vessel area over time. There was no statistical difference between the means at any time.

Figure 2. Representative photomicrograph (original magnification, $\times 100$) of a biopsy sample from a STSG treated with a control dressing. *Arrows* indicate the graft-wound interface.

Figure 3.
Representative photomicrograph (original magnification, $\times 100$) of a biopsy sample from a STSG treated with a NPWT dressing. *Arrows* indicates the graft-wound interface.