Abstract: Negative pressure therapy (NPT), used on open wounds or postoperative infections, has not been evaluated on closed spinal incisions. This was analyzed after 3 days and 5 days of NPT application using biomechanics and histology in a porcine model. Methods. In 8 mature miniature pigs, 2 end-to-end midline spine incisions were closed in a standard fashion. Negative pressure (Prevena™ Incision Management System, KCI, San Antonio, TX) was applied to one incision (NPT group) while standard dry dressings were used on the other (control group). After 3 days or 5 days, all incisions underwent biomechanical (eg, failure load, failure energy, and stress), histological, and scar scale evaluation. Analysis. ANOVAs compared the groups (3-day vs 5-day, NPT vs control, \( P < 0.05 \)). Negative pressure therapy demonstrated a significantly improved scar scale height grade than the control (\( P = 0.026 \)). Failure load (4.9 ± 4.0 vs 16.5 ± 14.6 N), energy absorbed (8.0 ± 9.0 vs. 26.9 ± 23.0 mJ), and ultimate stress (62 ± 53 vs. 204 ± 118 N/mm²) were lower in the control group. Histological analysis revealed no differences in incision scar width. Conclusion. Negative pressure therapy application on closed incisions presented a trend toward improved early healing strength, and in significantly improved incision appearance. Clinically, NPT may improve incision integrity, minimizing the risk of dehiscence or subsequent infection. Patients at high risk of postoperative incision site complications may benefit from primary application of NPT.

P ostoperative wound drainage and infections are a major concern following spine surgery. In 2005, there were 575,000 spine surgery discharges in the United States, accounting for 1.5% of all acute discharges.¹ Postoperative spinal wound infection (SWI) can occur in 3% to 12% of cases.²³ Up to 11% of the patients with neuromuscular scoliosis may develop early SWI, with 82% being deep.⁴ Patients who smoke, are obese, or have diabetes, and individuals receiving revision surgeries, are at particular risk.⁵⁶

Prophylactic antibiotics are shown to significantly lower rates of wound infection,²⁷ but may promote antibiotic-resistant bacteria. Therefore, there is an increased effort to regulate the use and duration of antibiotics for pro-
phylactic purposes. Currently, NPTs have been effectively used to manage postoperative SWIs, allowing accelerated healing by secondary intention.8-11 Animal studies have demonstrated increased blood flow, granulation tissue formation, and reepithelialization following application of NPT on open wounds.12,13 In one series of 79 postoperative SWIs, Ploumis et al14 found that when NPT was used to treat wounds in conjunction with surgical debridement and antibiotic therapy, the wounds closed in an average of 7 days, with a range of 5 days to 14 days.

As current reports have focused primarily on the application of NPT to open wounds, no animal study has been performed to analyze the effects of NPT on sutured surgical incisions while studying the duration of NPT application.

Key Points

- Postoperative spinal wound infection (SWI) can occur in 3% to 12% of cases.2,3 Up to 11% of patients with neuromuscular scoliosis may develop early SWI, with 82% being deep.4
- Patients who smoke, are obese, or have diabetes, and individuals receiving revision surgeries, are at particular risk.5,6
- As current reports have focused primarily on the application of negative pressure therapy (NPT) to open wounds, no animal study has been performed to analyze the effects of NPT on sutured surgical incisions while studying the duration of NPT application.

Methods and Materials

The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC). Animals were housed for 1 week prior to surgery to allow for acclimation. After 1 week they were sedated, weighed, and anesthetized. Following sedation, approximately 30 minutes prior to surgical incision, preoperative antibiotics consisting of IM Baytril (5 mg/kg) were given for prophylaxis. Following this, the animals were positioned in prone position and the back prepped with Chlorhexidine scrub and ChloraPrep (Enturia, Leawood, KS). Using standard sterile surgical techniques, 2 spinal incisions, each 20 cm long, were made posteriorly end-to-end, leaving 15 cm of intact skin in between. Exposure of the spine at each site was along the spinous processes to the sub-fascial layer exposing the vertebral bone. Closure was performed using 1 Vicryl suture (Ethicon, Inc, Somerville, NJ) for the subcutaneous layers followed by interrupted 2-0 Prolene (Ethicon, Inc, Somerville, NJ), 7 mm to 8 mm apart, to close the skin. The more cephalad incision in half of the animals (n = 4), and the more caudal incision in the other half of the animals (n = 4), had NPT incision management dressings applied (Prevena Incision Management System, KCI Inc, San Antonio, TX) with continuous, -125 mm Hg pressure exerted via portable NPT units (Prevena, KCI Inc, San Antonio, TX) (NPT group), that remained in place until the conclusion of the study (Figure 1). The remaining incisions (control group) had a standard sterile dry dressing (ABD dressing) applied.

Specially designed neoprene vests that secured the NPT units and tubing were placed around each pig postoperatively. Postoperative antibiotics consisted of Baytril (5 mg/kg), IM, (or 5 mg/kg, po, once the animal began eating well) once a day until the end of the study (3 days or 5 days postoperatively). Animals were euthanized after 3 days (n = 4) or 5 days (n = 4). High resolution digital pho-
Photographs were taken of each incision, cropped (so that only the incision was visible), de-identified, and graded by a single blinded observer using a modified Vancouver Scar Scale\textsuperscript{19} which assessed pigmentation, vascularity, pliability, and height of each healing incision.

**Tissue preparation.** Skin through the superficial fascia and fat layers was excised, including at least 5 cm proximal and distal to the incision, taking care to never stretch the skin tissue. Skin samples were cut into equal halves (Figure 2); half of each skin sample was wrapped in a saline-soaked towel and frozen (-20°C) prior to biomechanical testing. The other half was placed directly into 10% neutral buffered formalin for histological processing.

**Biomechanical testing.** Frozen specimens were cut into uniformly thick slabs (8 mm - 9 mm thick), and then cut to shape. A dumbbell shape was chosen to promote a homogeneous force distribution across the test area (incision), and to minimize edge-effects due to clamping. Samples were thawed overnight in a refrigerator. Markings approximately 10 cm above and 10 cm below the incision were made on each specimen. Before performing biomechanical testing, the following was recorded for each sample: the number of sutures, sample dimensions (measured with electronic digital calipers [Chicago Brand, Fremont, CA]) including distance between markings, width and thickness at incision, and the sample temperature.

Tensile testing was performed on all 16 skin samples using a table mounted biaxial servohydraulic test frame (MTS858, MTS, Co, Eden Prairie, MN), using a high accuracy load cell (3397, Columbus, OH) and specially designed soft tissue clamps. The clamps allowed for even load distribution while preventing skin slippage and damage at the points of fixation. The samples were mounted onto the testing frame under zero load and then the sutures across the incision were removed. Samples were frequently sprayed with normal saline throughout the testing regimen to maintain tissue hydration. Distraction until failure was applied at a rate of 0.5 mm/s along the long axis of the skin sample until failure (Figure 3). Displacement and force data were collected at 100Hz, and force ($F_f$), and displacement ($d_f$) at sample failure were recorded.

**KEYPOINTS**

- Using standard sterile surgical techniques, 2 spinal incisions, each 20 cm long, were made posteriorly end-to-end, leaving 15 cm of intact skin in between. Exposure of the spine at each site was along the spinous processes to the sub-fascial layer exposing the vertebral bone.
- The more cephalad incision in half of the animals ($n = 4$), and the more caudal incision in the other half of the animals ($n = 4$), had NPT incision management dressings applied with continuous, -125 mm Hg pressure\textsuperscript{13} exerted via portable units that remained in place until the conclusion of the study (Figure 1).
- The remaining incisions (control group) had a standard sterile dry dressing (ABD dressing) applied.
The raw data were analyzed without filtering or other post-processing. The following parameters were calculated using a custom written analysis routine in MatLab (MathWorks, Natick, MA): the total mechanical energy absorbed until tissue failure $E_f$, also called the modulus of toughness (mJ); yield point ($F_y$ and $\sigma_y$); stress $\sigma_f$ (kPa) and strain $\varepsilon_f$ (%) at failure; Young’s modulus $E$ (MPa); and the material stiffness $k$ (N/mm). These parameters summarize the most important biomechanical skin properties and allow for conclusions regarding failure resistance to stretching and incision site toughness. The load-displacement curve (Figure 4) was used to derive the different parameters. Energy to failure is the area under the F-$\delta$ curve, calculated using trapezoid integration. The yield point (mm) is the point at which the tissue material properties transition from elastic to plastic. The elastic modulus as a measure of the linear stiffness was determined from the slope of the nearly linear portion of the F-$\delta$ curve. The failure load (F) is the maximal load that can be borne by the sample (ultimate load), and failure displacement ($\delta$) is the corresponding displacement; failure stiffness is calculated as $F/\delta$. The stress is calculated by dividing force by cross-sectional area, where the area is defined by the width and thickness of the sample. However, the authors note that as the sample is stretched, the area changes, which was not taken into consideration. Strain is defined as the change in length divided by its initial length. Intact skin was also tested on “normal” specimens to identify parameters of the uninjured skin.

**Key points**

- Tensile testing was performed on all 16 skin samples using a table mounted biaxial servohydraulic test frame, using a high accuracy load cell, and specially designed soft tissue clamps.
- The samples were mounted onto the testing frame under zero load and then the sutures across the incision were removed.
- Samples were frequently sprayed with normal saline throughout the testing regimen to maintain tissue hydration.

**Histological evaluation**. The formalin fixed tissue samples were submitted for routine paraffin infiltration, embedment and sectioning (4µm thick). Hematoxylin-eosin (HE) and Mason’s trichrome stains were applied to assess the incision repair and collagen deposition and orientation. Immunohistochemical stains were employed for assessing angiogenesis and lymphangiogenesis during incision repair. Specifically, the following markers for vascular growth were employed: CD-31 as a pan endothelial marker for capillaries and vascular endothelial growth factor (VEGF) for capillary and lymphatic microvessel formation.

Hematoxylin-eosin and Mason’s trichrome slides were evaluated by a blinded clinical anatomist. All slides were analyzed with light microscopy for incision repair staging, including hemostasis, inflammation, proliferative, and remodeling phases. Negative pressure therapy and control slides from the same animal were compared and histologically graded with regards to presence of reepithelialization, thickness of remaining scar within the dermal layer, hemostasis, and general progression of incision healing. The following morphological measurements were made at 10x or 20x magnification using a Leica micrometer reticle: thickness of epidermis at 5 mm on either side of the healing incision; thickness of epidermal re-epithelialization at the incision; thickness of epidermal reepithelialization at the thinnest point; dermal thickness at 5 mm on either side of the healing incision; superficial fascia thickness at the incision; and thickness of superficial fascia that is intact. Also, the width of the incisional repair site, as determined by identification of the margins of either fibrin deposition with hemorrhage, or the presence of granulation tissue within the incisional defect, was measured at 5 random locations initiating from the stratum germinativum, and extending 2 mm into the underlying dermis. Immunohistochemical slides were examined qualitatively for presence and extent of staining for...
angiogenesis and lymphangiogenesis (Grade 0 = no label present; 1 = weak label present; 2 = moderate label present; 3 = label abundantly present).

**Statistical methods**

Statistical Package for Social Sciences (SPSS, Chicago, IL) was used for all analyses. Continuous data were checked for normality and equal variances. ANOVAs compared perioperative data between groups (3 days vs 5 days, or NPT vs control incisions). Multivariate ANOVA (MANOVA) was used to compare the biomechanical parameters between the 4 groups (3 days vs 5 days, or NPT vs control incisions). MANOVA was also used to compare the biomechanical parameters between the control and NPT treatment groups. Results are reported as means with standard deviation (SD). Chi-squared analysis was used to evaluate the distribution of Vancouver Scar Scale between the control and treatment group. The alpha level for all analyses to declare significance was set at 0.05.

**Results**

Perioperative data were similar in the 3-day and 5-day groups with respect to age, weight, and body length (Table 1). Negative pressure therapy and control-treated incisions had similar surgical times from first incision to final suture placement, blood loss, number of skin sutures, and increase in incision length, following 3-day or 5-day treatment (Table 2).

Using a modified Vancouver Scar Scale, all 16 skin samples (8 NPT, 8 control) received scores of 0 (“normal”) for pigmentation, vascularity, and pliability. Scar height grades (0 = normal, 1 = less than 2 mm high at the highest point) for control samples were 3/8 with a score of 0, and 5/8 received a score of 1 (Figure 5). All 8 NPT samples had a scar height grade of 0, which was statistically better than the controls ($P = 0.026$).

**Biomechanical Results.** There was no statistically significant difference between the 4 groups (control at 3 days or 5 days, and NPT at 3 days or 5 days) regarding the dimensions of the samples used for biomechanics. Thickness at the incision, average width, and area were likewise similar for the control and NPT groups.

Data for intact skin mechanical behavior were used to determine all described parameters. Intact skin failed at a load of 285 N with a maximum stretch displacement of 13.78 mm (Figure 4). As to be expected, intact skin was able to resist much higher loads before failure and was more extensible than the healing incisions. The healing skin ruptured before reaching the yield point along the intact skin curve. The most appropriate factors to describe the force-displacement curves and their differences were determined to be: Force at Failure (N), Energy to Failure (mJ), and Stress at Failure (kPa).

In all considered parameters, the incisions treated with NPT demonstrated greater biomechanical parameter values on average when compared to the control group (ie,
NPT-treated incisions trended toward higher failure load, toughness, and extensibility than the control group [Figure 6]; however, this pilot study was underpowered for statistical significance. The average differences in biomechanical parameters between the NPT and control (ratio of NPT Failure Load/Control Failure Load, NPT Energy to Failure/Control Energy to Failure, and NPT Stress/Control Stress) was greater at 3 days than at 5 days (Figure 7), although no statistical differences were detected. Trends towards significance were reached when 3-day and 5-day samples were combined for material stiffness ($P = 0.065$), ultimate/failure load ($P = 0.059$), $F/\delta$ ($P = 0.069$), energy to failure ($P = 0.089$), and stress ($P = 0.057$).

**Histological Results.** After 3 days, all control incisions had a better histological grading than the NPT, whereas after 5 days, 3 of the 4 NPT incisions were graded better than the control. Reepithelialization had occurred at 3 days in 50% of the control group, compared to 13% of the NPT group. After 5 days, all control slides demonstrated full reepithelialization (100%, Fig-
Discussion

This study was prepared to help evaluate the complex events that take place in and around healing incisions when comparing NPT to standard dressings. Biomechanically, the current study suggests tensile strength in the NPT group was similar, and perhaps improved, compared to the control group. As incisions heal, their strength increases; once an incision heals adequately, the sutures that provide early tensile strength are not needed. The difference between the 2 groups was more noticeable after 3 treatment days compared to 5 days. This suggests that NPT may accelerate the healing responsible for maintaining incision integrity. It is not clear, however, if the incision strength will be different between the 2 groups at the completion of the healing process.

Although this study is preliminary in terms of statistical power of biomechanical and histological measurements, there was a clearly visible difference between NPT-treated incisions and incisions treated in a standard manner. This was shown by the NPT group having a higher rating using the Modified Vancouver Scar scale, demonstrating a “flatter” surface. The authors acknowledge that the difference of one grade is indeed a crude metric, and that this scar scale was designed to be used for healing wounds and not incisions; however, in the absence of incisional healing scales in the literature, it allows a prescribed analysis of the visual effects of the treatment groups.

At 5 days, the histological data suggest that NPT may possibly work to bring the 2 sides of an incision closer together at a deeper dermal level. The lack of reepithelialization of the epidermis at 3 days in the NPT-treated samples may have allowed for this reaproximation in the dermis; however, this certainly warrants further investigation. Once reepithelialization at the incision occurs, it is not clear that the

Keypoints

• In all considered parameters, the incisions treated with negative pressure therapy (NPT) demonstrated greater biomechanical parameter values on average when compared to the control group (ie, NPT-treated incisions trended toward higher failure load, toughness, and extensibility than the control group [Figure 6]); however, this pilot study was underpowered for statistical significance.

• After 3 days, all control incisions had a better histological grading than the NPT, whereas after 5 days, 3 of the 4 NPT incisions were graded better than the control.

• Reepithelialization had occurred at 3 days in 50% of the control group, compared to 13% of the NPT group. After 5 days, all control slides demonstrated full reepithelialization (100%, Figure 8) and only 1 of the 4 NPT incisions did not (75%).

Figure 8) and only 1 of the 4 NPT incisions did not (75%). Reepithelialization was always accompanied by granulation tissue within the dermis (ranging from early to advanced stages), demonstrating the proliferative stage of healing. Samples without reepithelialization showed various stages of hemostasis and inflammation, and some showed granulation. No samples demonstrated late stages of healing characterized by remodeling of the collagen fibers. After 5 days, the thinnest epithelial incision bridge was 44 μm ± 10 μm vs 71 μm ± 32 μm for the NPT vs control; not significantly different (P = 0.2, Figure 9). There was no significant difference in “incision width” (NPT: 236 μm ± 172 μm vs Control: 93 μm ± 50 μm, P = 0.2).

Normal skin from each animal demonstrated weak CD-31 labeling of scattered vessels, homogeneously distributed. All of the control and NPT-treated incisions had at least some CD-31 labeling of the granulation tissue scar. At 3 days, the NPT group had CD-31 moderate labeling (Grade 2) in all samples; whereas the control group were Grade 2 in 50% of the samples, with weak labeling (Grade 1) in the other 50%. At 5 days, all samples had moderately present (Grade 2) CD-31. Vascular endothelial growth factor was either absent (Grade 0) or weakly present (Grade 1) at 3 days in both groups; and either absent or moderately present (Grade 2) at 5 days in both groups, showing a slightly stronger presence at 5 days.

Keypoints

• Biomechanically, the current study suggests tensile strength in the NPT group was similar, and perhaps improved, compared to the control group.

• The difference between the 2 groups was more noticeable after 3 treatment days compared to 5 days. This suggests that NPT may accelerate the healing responsible for maintaining incision integrity. It is not clear, however, if the incision strength will be different between the 2 groups at the completion of the healing process.

• Although this study is preliminary in terms of statistical power of biomechanical and histological measurements, there was a clearly visible difference between NPT-treated incisions and incisions treated in a standard manner.
dermis would experience any further effects from NPT. Negative pressure therapy certainly does not prevent re-epithelialization, as only one sample did not complete this process at 5 days. In addition, studies of NPT management of open wounds show that wound healing (including reepithelialization) is accelerated.

It is the principal investigator's experience that significant reepithelialization of open wounds occurs following discontinuation of NPT. This process could occur in closed incisions as well. Ultimately, it is not the epithelial layer that is responsible for the visible scar but the thickness of the scar, possibly derived from the granulation tissue below. Immunohistochemical labeling did not show any statistical or remarkable differences between the NPT and control groups; however, some subtle differences did emerge. CD-31 labeling, indicating general epithelial formation, showed there might have been a greater angiogenesis with NPT early on (3-day group) with no difference after 5 days. Certainly, further study with larger numbers of samples would be needed to confirm these observations.

Among the study limitations was the use of an animal model instead of human tissue. Involving human subjects early in the process of treatment and product evaluation is complicated from the practical, ethical, and moral standpoint. Animal models and in vitro biomechanical testing are important to obtain valid preclinical information for improved surgical care. Translational research helps obtain data that can lead to sound clinical trials and is an indispensable tool for researchers. Porcine skin is similar to human skin and has been shown to be an excellent tool to evaluate wound healing therapies.17,18 Similarities between human and porcine skin have been found in epidermal thickness and dermal-epidermal thickness ratios,16 dermal collagen and a dermal elastic content,15 and physical and molecular responses to various growth factors.23

Another major limitation was the sample size. Considering this was the first study to evaluate NPT on closed incisions in a porcine model, 4 animals were chosen at 2 different time points to gain experience with the model and determine the effect. While there was a strong trend between the 2 groups, the sample size prevented the results from reaching statistical significance. Power analysis showed that 15-18 samples per group would be sufficient to reach statistical significant difference for further studies.

Time to harvest was another limitation to the study. There were many benefits in analyzing the tissue at 3 days and 5 days; the NPT did not have a negative effect on incision healing. While the 3 day histological analysis did suggest some early decrease in reepithelialization of NPT compared to control, the 5 day analysis was not discerning. Considering the biomechanical properties of the 2 groups at 3 days, the epithelial layer is not responsible for the strength of the healing incisions. Upon visual inspection of the incisions, the NPT group had an improved appearance. While this is suggestive of an improved final scar, further studies with longer follow-up will be needed to support this finding. Histological evaluation following the remodeling phase of the incision will also be needed to determine if the decreased width of the granulation tissue seen in the dermal layer will result in decreased width of the scar.

Conclusion

Patients requiring spine surgery, especially those with significant comorbidities (eg, diabetes, obesity, and neuromuscular diagnoses), are at increased risk of postoperative incision site complications, such as infection and delayed healing.24 These problems can lead to prolonged hospitalization, revision surgeries, and ultimately, poor outcomes. Currently, practices including extended (> 24 hours postoperative) perioperative antibiotics, and surgical drains to minimize these complications, even though these practices are not supported by evidence-based medicine.25,26 Methods to improve or accelerate incision healing may minimize the incision site complications, which may ultimately decrease surgeons' need for extended use of perioperative antibiotics or surgical drains. This study presents preliminary data suggesting NPT may be one method of improving incision healing.

Disclosure

This study was supported by a research grant from KCI Medical, San Antonio, TX.

Acknowledgements

The authors would like to recognize and thank Tracey Bastrom, MA, for statistical analysis; JD Bomar for figure development; and KCI Medical, Inc for research support.

References

3. Sasso RC, Garrido BJ. Postoperative spinal wound infec-


