

Negative Pressure Wound Therapy on Closed Surgical Wounds With Dead Space

Animal Study Using a Swine Model

Hyunsuk Suh, MD,* A-Young Lee, PhD,† Eun Jung Park, MS,† and Joon Pio Hong, MD, PhD, MMM†

Background: Closed incisional wound surgery frequently leaves dead space under the repaired skin, which results in delayed healing. The purpose of this study was to evaluate the effect of negative pressure wound therapy (NPWT) on incisional wounds with dead space after primary closure by evaluating the fluid volume through the suction drain, blood flow of the skin, tensile strength, and histology of the wounds.

Methods: Bilateral 25-cm-long incisional wounds with dead space were created on the back of 6 pigs by partially removing the back muscle and then suturing the skin with nylon sutures. NPWT (experimental group) or gauze dressing (control group) was applied over the closed incision for 7 days. Analysis of the wound included monitoring the amount of closed suction drain, blood perfusion unit, tensile strength of the repaired skin, and histology of the incision site.

Results: The drainage amount was significantly reduced in the experimental group (49.8 mL) compared to the control group (86.2 mL) ($P = 0.046$). Skin perfusion was increased in the experimental group with statistical significance compared to the control group ($P = 0.0175$). Collagen staining was increased in the experimental group. The tensile strength of the incision site was significantly higher in the experimental group (24.6 N at 7 days, 61.67 N at 21 days) compared to the control group (18.26 N at 7 days, 50.05 N at 21 days) ($P = 0.02$).

Conclusion: This study explains some of the mechanism for using NPWT in closed incision wounds with dead space. It demonstrates that NPWT significantly reduces drainage amount, increases skin perfusion, increases tensile strength, and has the tendency to promote collagen synthesis for closed wound with dead space indicating enhanced healing.

Key Words: negative pressure wound therapy, surgical wound dehiscence, primary closure, subcutaneous dead space

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Suture techniques have been used to close open wounds for more than 5,000 years.¹ Suturing can induce primary and spontaneous wound healing by approximating the wound edges generally with a needle and a thread. Despite significant advances in medical technologies including surgical techniques, suturing materials, and perioperative managements, unhealed wounds including dehiscence are

still critical issues. In recent decades, the incidence of abdominal wound dehiscence and mortality associated with it have not been improved.² Wound dehiscence can occur in any surgery, but its occurrence following open-heart operation or spinal implant surgery is particularly devastating. Also, abdominal, chest, and extremity wounds after bone surgery have high incidence rate of wound dehiscence and mortality related to it.^{2–5}

Negative pressure wound therapy (NPWT) is a dressing technique used globally for treating mainly open wounds by expediting the healing rate as a secondary intention. The mechanism is to promote angiogenesis, increase perfusion, and reduce edema.^{6–8} Using this mechanism to improve healing in closed incisional wounds, whether from clean surgery or trauma, has shown to be clinically effective by reducing infection and other related complications.^{9–12} However, detailed explanation of the possible mechanism or physiology behind this approach has been lacking. Thus, we designed an animal study aimed to test the use of NPWT over incisional wounds with dead space and to provide further evidence for this approach.

MATERIALS AND METHODS

Animal Model

Six Yorkshire pigs (XP Bio, Seoul, Korea) weighing 20 to 22 kg were used after approval of the animal protocols of Asan Medical Center Animal Care Committee (approval document 2011-11-129). All animals were acclimated for 1 week before surgery, kept in isolated cages at room temperature (28.5°C), and fed with commercial food. Before anesthesia, the pigs were fasted for at least 12 hours. Anesthesia began by intramuscular injection of atropine 0.1 mg/kg, Zoletil 50 (tiletamine/zolazepam; Virbac, France) 0.1 mL/kg, and Rompun (xylazine; Bayer Korea, Korea) 2.2 mg/kg followed by inhalation of enflurane 2 to 5 volume percent by an orotracheal tube.

The back and posterior neck of the animals were prepared for surgery. In prone position, 2 symmetrical areas (left and right) with 6-cm distance in between were designed over the back. One side was designated as control group where gauze dressing was used and the other as experimental group where NPWT was applied. A 25-cm incision was made on each area of the loin bilaterally and the loin and shoulder muscles beneath the incision were excised to create the dead space between the rib cage and vertebra (Fig. 1). The average weight of the excised muscle was 259 g [standard deviation (SD) = 47.8] for the experimental group and 266 g (SD = 33.1) for the control group without statistical significance between the two. After bleeding control with a bipolar coagulator, a closed suction drain (Sewoon Medical, Cheonan, Korea) was placed on each wound. The skin was closed with 3-0 nylon (Ailee, Busan, Korea) in a vertical mattress technique every centimeter resulting in 24 stitches for each site. The animal was free to move in the cage after the procedures. All animals were euthanized on day 21 after harvesting the tissue samples and observation of the wound.

Device Application and Wound Dressing

The experimental group was treated with CuraVAC (Daewoong Pharmaceutical, Co., Ltd., Seoul, Korea) according to the

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From the *Department of Plastic Surgery, Ewha Womans University Hospital, Seoul; and †Department of Plastic Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

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Reprints: Joon Pio Hong, MD, PhD, MMM, Department of Plastic Surgery, Asan Medical Center, Songpa-gu, Poongnap2-dong, 388-1, Seoul 138-736, Korea. E-mail: joonphong@amc.seoul.kr.

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FIGURE 1. Gross appearance of the swine's back after the operation. The wounds were closed with a nylon 3-0 vertical mattress suture. Note that there is an underlying dead space beneath the repaired skin.

manufacturer's instruction. A sponge foam (CuraVAC) of 25×5 cm and 3 cm thickness was applied over the closed incisional wound. Then a suction head was placed over the foam and covered together with an adhesive film drape (Daewoong) with a 3-cm border not crossing the midline. The suction head was connected to a tube from a portable negative pressure device (Curasys; Daewoong). A cyclic negative pressure oscillating between -50 and -125 mm Hg was applied.^{8,13} The NPWT was changed on the fourth day and reapplied for three more days. After the 7-day application, the wound was dressed with gauze for the remaining 2 weeks. In the control group, the wound was dressed with packed gauzes after applying ointment and petroleum jelly gauze. After dressing both sides, an elastic bandage (Peha-haft; Hartmann AG, Heidenheim, Germany) was applied with its original tension.

Visual Analysis

Condition of the dressing and the amount discharge through the closed suction drain and the canister from the NPWT device were monitored every 24 hours until removal. Wound characteristics including hematoma or fluid collection were observed on days 4, 7, 14, and 21.

Drainage Through Closed Suction Drain and NPWT Canister

Volume and quality of drainage through the suction drain and the canister from the NPWT device was checked daily. Monitoring was continued until the drainage amount reduced to less than 5 mL per day.

Blood Flow Evaluation

Skin perfusion was measured using a PeriScan PIM 3 System with 785 nm, 70 mW laser (Perimed AB, Stockholm, Sweden) at the following time points: before the operation; right after the operation; and on postoperative days 4, 7, and 21. The laser Doppler imaging system calculates blood perfusion as perfusion unit by counting the number, density, and speed of blood cells. The animals were sedated and anesthetized during evaluation. The animals were placed on standard prone position and room temperature (23.5 – 25°C), and the surface blood flow for a marked 3×3 cm on the middle of the incision wound was measured.

Histological Assessments

Histological examinations were performed at days 7 and 21. A full-thickness skin section with a size of 1×2 cm was symmetrically harvested from both incision sites. After initial processing, the fresh tissue specimens were embedded in paraffin and sectioned with $5\text{-}\mu\text{m}$

thickness. The sections were deparaffinized in xylene, rehydrated, and subjected to standard hematoxylin and eosin (H&E) staining, Masson's trichrome staining, or CD31 antibody staining. Pathologist in a single blinded evaluation reviewed the specimens.

Tensile Strength

On postoperative days 7 and 21, a full-thickness section including both fatty layer with a size of 5×1 cm was excised for the tensile strength test. The incision was located in the center of the specimen. The long axis of the harvested skin was perpendicular to the incision line. After removing the stitches, the specimen was wrapped in foil and stored at 4°C for 3 hours. The skin strips underwent a standard tensile strength test using a DTU-900 MH tensiometer (Daekyung Tech, Incheon, Korea) at room temperature. The technician performed a single blinded evaluation.

Statistical Analysis

The data regarding gross and histological observations were not statistically analyzed because of the descriptive nature of the data. All quantified values were represented as mean \pm SD. The average blood perfusion of the surgical wound, drainage amount, and tensile strength were compared between the 2 groups using the Wilcoxon signed-rank test. The tensile strength (N) was analyzed using the linear mixed model. Values of P less than 0.05 were considered statistically significant.

RESULTS

Gross Morphology

There were no apparent infections or instances of wound dehiscence in both groups. A minor blood clotting was observed on the margin of the sutured incisions in one wound of the control group (Fig. 2). There was no difference in color or skin texture between the 2 groups.

Drainage Through Closed Suction Drain and NPWT Canister

The closed suction drain was removed when the amount of drainage was less than 5 mL per day. Average time taken to remove the closed suction drainage was 3.0 days (SD = 1.095) for the experimental group and 3.3 days (SD = 1.033) for the control group. There was no significant difference between the groups ($P = 0.317$). However, the total drainage amount was 49.8 mL (SD = 35.7) in the experimental group and 86.2 mL (SD = 40.81) in the control group revealing statistical significance ($P = 0.046$) (Fig. 3). There was no fluid collected in NPWT canister and the tube. On day 7, while collecting tissue samples, one wound from the control group had collection of hematoma (10 mL) underneath the incision.



FIGURE 2. Gross appearance at day 4. A small amount of blood clot is seen at the margin of the sutured incision of the control group (right side).

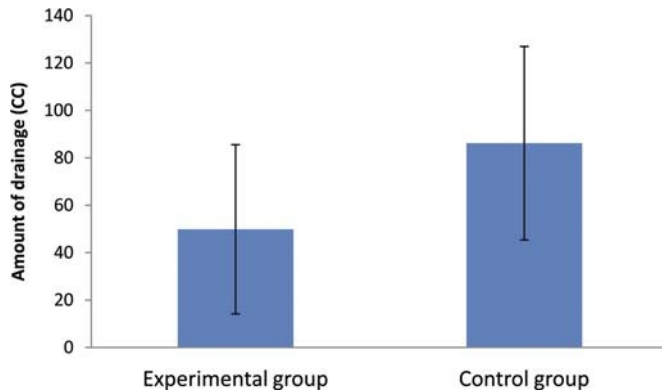


FIGURE 3. Averaged total drainage through the suction drain from each group. The values of the experimental group and the control group were 49.8 mL (SD = 35.7) and 86.2 mL (SD = 40.81), respectively. Significantly less amount of drainage was collected from the experimental group ($P = 0.046$).

Perfusion Analysis

There was no significant difference between the 2 groups in perfusion level measured before and immediately after the operation (data not shown). On postoperative days 4, 7, and 21, the experimental group showed significantly higher levels of perfusion unit compared to the control group ($P = 0.0175$) (Table 1 and Fig. 4).

Histological Assessments

H&E-stained specimens were observed by light microscopy for assessment of inflammatory and regenerative properties including tissue congestion, neutrophil infiltration, granulation tissue formation, and neovascularization. The collagen and collagen fibers were observed through Masson's trichrome staining. At day 7, inflammatory responses were evaluated by monitoring intravascular engorgement, perivascular leakage of red blood cells, and leakage of neutrophils and lymphocytes. The experimental group showed increased findings for inflammation and tissue regeneration compared to the control group (Fig. 5A). At day 21, the inflammatory cells disappeared and number of collagen fibers was increased (Fig. 6). The experimental group showed denser collagen fibers compared to the control group. The incision site of the experimental group was hard to distinguish from the surrounding area. On day 21, the experimental group showed increased CD31 antibody staining, suggesting higher level of newly formed vessels within the scar tissue compared to the control group (Fig. 7).

Tensile Strength

During the break-load test, the peak stress at the point of breakage of the incision site was interpreted as a tensile strength. The tensile strengths of the experimental and control groups are presented in Table 2. In both groups, the tensile strength steadily increased on day 21 compared to day 7. When comparing between the groups, the experimental group represented significantly greater tensile strength than the control group on both days 7 and 21 ($P = 0.02$). The specimen with the hematoma from the control group showed the lowest strength level (7.056 N). Thickness of excised tissue ranged from 6.3 to 7.8 mm by digital vernier caliper. There were no relevance between the use of negative pressure dressing and thickness of the skin and between tensile strength and thickness of excised skin. The animals were relatively thin and had small fatty component under the dermis compare to the thickness of the dermis.

DISCUSSION

Despite proper wound closure, certain surgeries or patient conditions are at risk for higher rate of wound breakdown. The orthopedic operations including tibial plateau reduction and pilon and calcaneus fractures are associated with the increased risk of wound dehiscence.^{10,11} The incidences of sternal wound infection after coronary artery bypass graft and abdominal wound dehiscence involving all layers of abdominal wall are 1% to 10% and 0.2% to 0.6%, respectively.^{2-5,14-20} Besides the surgical causes, there are several local and systemic factors favoring development of wound dehiscence.^{2-5,14,15} The risk factors include obesity, diabetes mellitus, smoking, hypertension, congestive heart failure, acute myocardial infarction, use of the internal mammary artery, and poor perfusion.^{15,17,18,20,21} Furthermore, wound dehiscence following major operations cause significant increase in postoperative cost and hospital stay, directly affecting the patient's life and the quality of life.^{2-4,14,15} The effort to minimize wound breakdown in complicated or in clean incision wounds continues and remains to be a challenge.

One option to decrease wound dehiscence after surgical incision in patients with systemic and surgical risk factors can be the clinical use of NPWT. Numerous reports on the clinical use to prevent wound complication and breakdown were shown to be beneficial.^{10,11,22,23} Although the NPWT was initially developed to accelerate healing of open wounds as a secondary intention by inducing increased blood flow, granulation tissue formation, and re-epithelialization, it was hypothesized that the same mechanism should reduce wound dehiscence when applied to high-risk patients after surgical closure.^{8,24-26} Currently, clinical reports have shown decreased incidence of wound dehiscence by reducing hematoma/seroma collection, increasing lymphatic drainage, and decreasing acute infection.^{9-11,27,28} To further understand the mechanism of applying NPWT on closed incision, we designed an animal model to mimic the clinical situation. By making a wide dead space and forming a flap-like lesion on the back, we considered that this wound would be similar to a complex wound such as avulsed wound or a post-surgical wound after retraction or elevation of the skin to maximize exposure. Using this model, we evaluated skin perfusion, hematoma/seroma drainage, wound tensile strength, and histology.

The application mode of the NPWT can be various. We chose the cyclic mode as it oscillates between -50 and -125 mm Hg to increase the mechanical stretch and minimize the pressure on the wound.^{13,29-31} Among many mechanisms behind the positive effect of NPWT over incision, we first looked at the change in perfusion of the skin after application. The wounds treated with NPWT showed greater perfusion. When measuring, we removed the NPWT foam dressing to focus on the actual change beneath the foam including the incision wound. Although factors regarding physical irritation from the dressing cannot be ruled out when evaluating perfusion, we fixed the dressing to minimize the irritation. There was a significantly increased level of perfusion on the NPWT applied wounds. This tendency is similar with the study by Timmers et al reviewing the result

TABLE 1. Average Value of Skin Perfusion

	Experimental Group	Control Group	<i>P</i>
	[Average Perfusion Unit (PU) ± SD]		
4 days after surgery	113.65 ± 36.90	109.51 ± 20.88	0.0175 ^a
7 days after surgery	101.55 ± 21.33	87.73 ± 22.16	
21 days after surgery	95.49 ± 22.20	78.80 ± 16.45	

Average value of skin perfusion unit (PU) was higher in experimental group compared to control group based on linear mixed model using mixed data of days 4, 7, and 21 ($P = 0.0175$).

^aLinear mixed model.

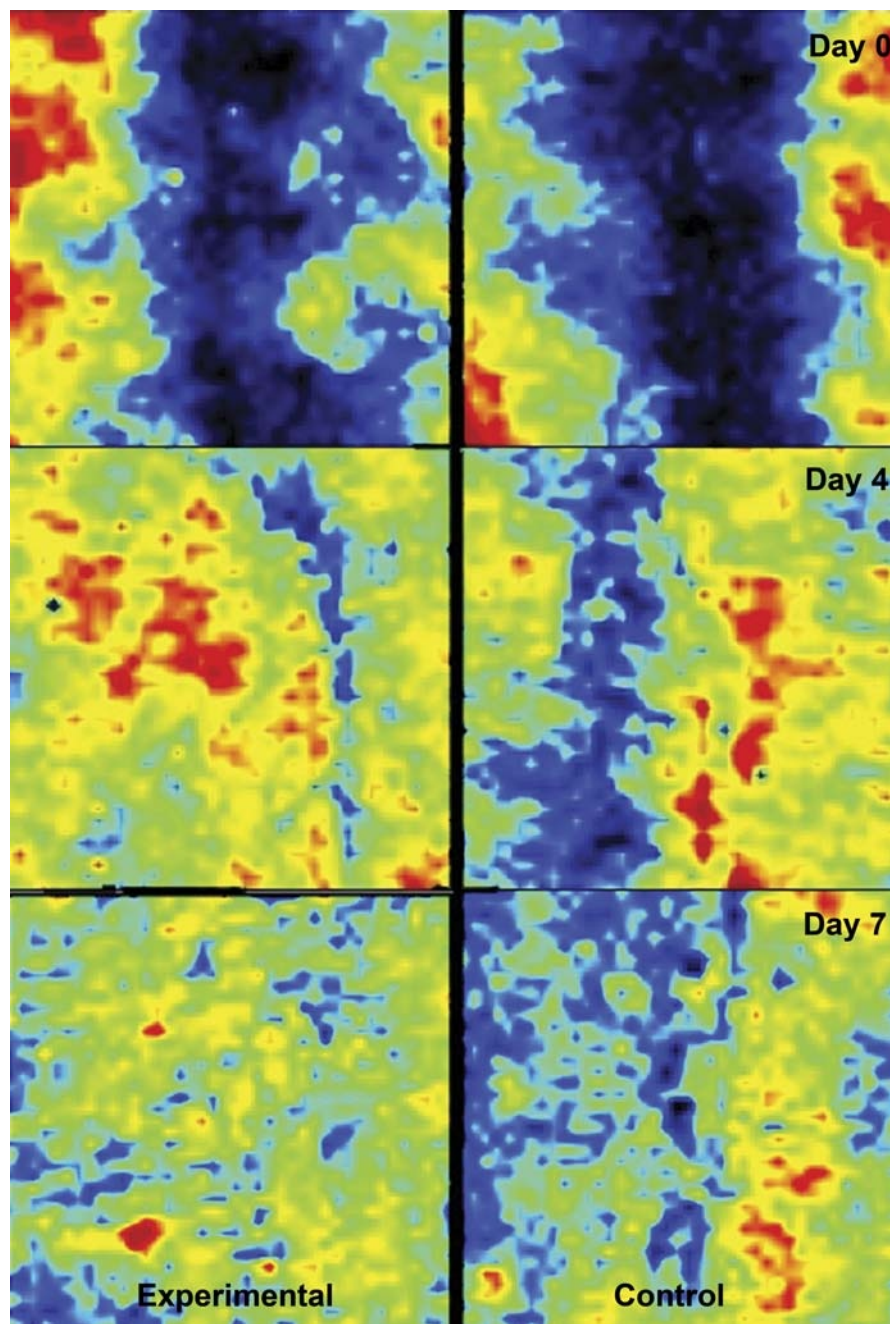


FIGURE 4. Images of surface perfusion unit of the experimental group (left) and the control group (right). Red color represents the areas with high perfusion unit and black color indicates the parts with less perfusion. The images of skin perfusion scan was taken immediately after the operation (above), postoperative day 4 (center), and postoperative day 7 (below).

from healthy forearm skin of 10 volunteers.³² These results indicate that the physical stimulation by negative pressure transmits through skin barrier and increases the cutaneous blood flow not only during the application but persists even after removal of the negative pressure. The prolonged increase in blood perfusion is thought to be a result of angiogenesis around the incisional margin. We were able to confirm this phenomenon by observing the increasing tendency of CD31 antibody staining compared to the control group. During the wound healing process, wounds are sensitive to hypoxia. In the early inflammatory phase, neutrophils provide nonspecific immunity to prevent infection and this nonspecific immunity of neutrophils

depends on a high content of tissue oxygen.³³ Increased angiogenesis may explain the effect of reduced infection in the previous clinical reports.^{10,11} High oxygen pressure also promotes superoxide availability, which favors increased bacterial killing capacity.^{34,35} In the proliferative phase, collagen production also requires oxygen.³⁶ Reversely, the increased amount of collagen observed in the experimental group supports the positive role of NPWT to increase the delivery of oxygen by increased angiogenesis. We also measured the thickness of the skin specimen between the groups after the application but did not find any statistical difference despite the increased tendency of collagen formation. Perhaps if we would have taken

specimens before the application and shown the difference in thickness after the application, it might have shown different results.

When wound breaks down after repair, it generally happens on 5 to 10 postoperative days, thus early application of NPWT can promote successful healing by increasing tissue perfusion and tissue oxygen pressure. Hence, to simulate the clinical setting, the NPWT was applied for 7 days.

Incisional closures can lead to various complications. Among them, formation of hematoma and seroma can lead to delayed healing and wound dehiscence.³⁷ As shown in one of the wounds from the control group that had hematoma collection, the tensile strength recorded the lowest among all specimens. With low force, this wound may easily lead to breakdown. Consistent with other clinical case series, the use of NPWT significantly reduced the amount of suction drainage and resulted in no subcutaneous hematoma or seroma formation.^{9,10,23,38} Based on this finding, we assumed that the increased pressure from the application of NPWT effectively compressed the wound to eliminate the dead space underneath the skin. Another assumption was that it acted as a splinting device to minimize sheering over the dead space allowing early vascularization from the wound bed to the skin flap as seen from histology.

NPWT also had the tendency to increase collagen fiber synthesis and significantly increase tensile strength on postoperative days 7 and 21. One interesting observation is early application of NPWT not only leads to statistically higher tensile strength immediately after application but this phenomenon is continued up to 3 weeks. This may allow early ambulation in the clinical setting with decreased risk for dehiscence and stable recovery. Currently, a long-term study is planned to evaluate the effect over closed incision wounds to have a better idea about the final wound tensile strength and the effect on scarring.

The application of NPWT on the closed incision wound with dead space was effectively supported by statistically increased perfusion, tensile strength, and reduced drainage. The observation of increased CD31 and increased collagen synthesis further supports

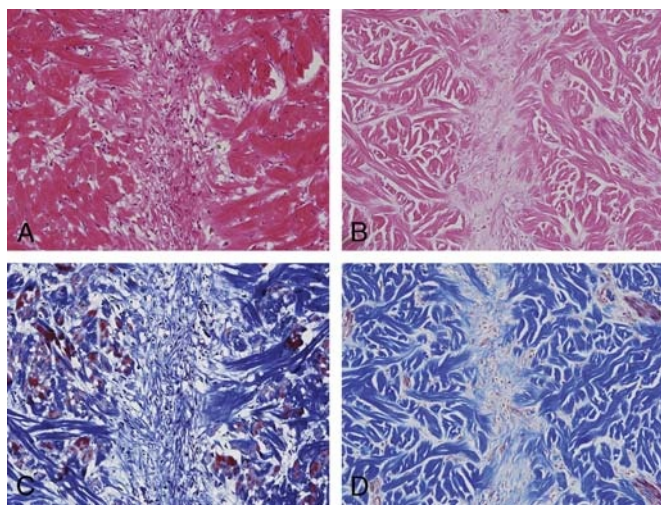


FIGURE 5. Histological staining results of the experimental group (A) and the control group (B) on postoperative day 7. H&E staining of the experimental group shows inflammatory response, such as intravascular engorgement and leakage of neutrophils and lymphocyte, between the fibroblast and collagen fibers with tissue edema (original magnification $\times 200$). Masson's trichrome staining of the experimental group (C) shows denser presentation of collagens compared to the control group (D) (original magnification $\times 200$).

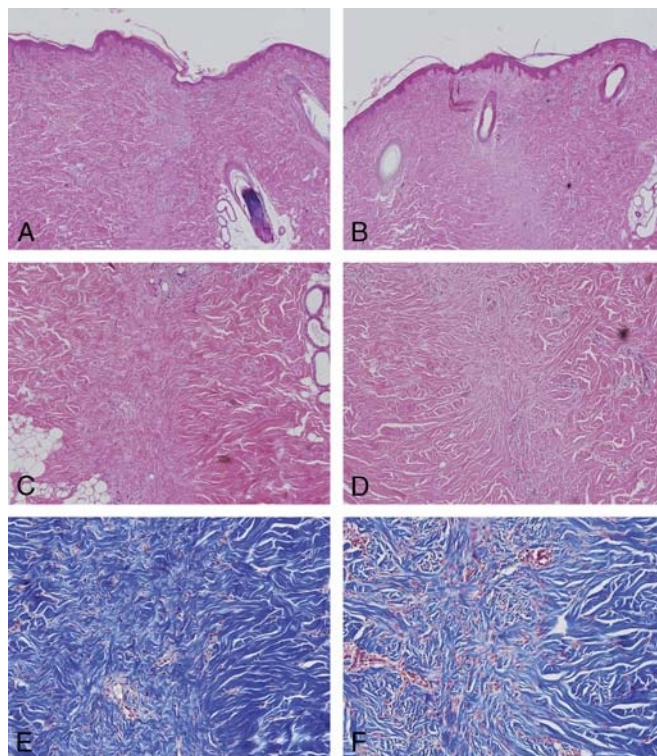


FIGURE 6. Histological staining results of the experimental group (left) and the control group (right) on Postoperative day 21. H&E and the Masson's trichrome staining the of the experimental group shows a dense and compact appearance compared to the control group. Also, note the pale and loose incision line of the control group. H&E staining—A and B: original magnification $\times 40$; C and D: original magnification $\times 100$. Masson's trichrome staining—E and F: original magnification $\times 200$.

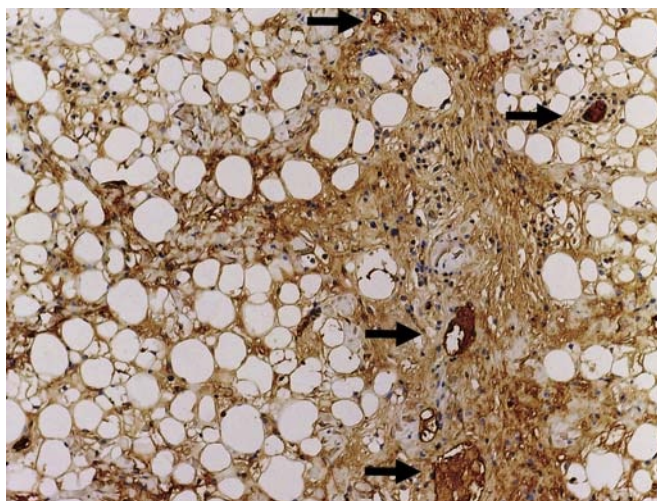


FIGURE 7. CD31 antibody staining of the experimental group on postoperative day 21 (original magnification $\times 100$). Several newly formed vessels (arrow) were shown at the incision margin within collagen fibers in the experimental group.

TABLE 2. Tensile Strength of the Wounds

	Experimental Group	Control Group	P
	[Average Tensile Strength (N) ± SD]		
After 7 days	24.60 ± 5.00	18.26 ± 8.24	0.02 ^a
After 21 days	61.67 ± 16.66	50.05 ± 10.83	

Tensile strength of experimental group was significantly higher than that of control group by linear mixed model with mixed data of 7 and 21 days.

^aLinear mixed model.

this effect. This animal study provides understanding on the application of NPWT on closed incision wounds and warrants clinical studies to treat high-risk patients prone to wound dehiscence after surgical repair.

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