

## Tissue Ingrowth Into Foam but Not Into Gauze During Negative Pressure Wound Therapy

Ola Borgquist, MD;<sup>1</sup> Lotta Gustafsson, MSc, PhD;<sup>1</sup> Richard Ingemansson, MD, PhD;<sup>2</sup> Malin Malmsjö, MD, PhD<sup>1</sup>

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From the <sup>1</sup>Department of Ophthalmology and <sup>2</sup>Department of Cardiothoracic Surgery, Lund University Hospital, Sweden

Address correspondence to:

**Malin Malmsjö, MD, PhD**

Associate Professor, Department of Ophthalmology  
Lund University  
Lund, Sweden  
Phone: 004 673 356 5650  
Email: malin.malmsjo@med.lu.se

**Abstract:** *Background.* Foam and gauze are two types of wound fillers used for negative pressure wound therapy (NPWT). Differences in the wound healing effects of foam and gauze have been observed clinically. The aim of the present study was to examine the effects of NPWT on the wound bed using foam and gauze. *Methods.* A porcine peripheral wound model was treated with NPWT at 0, -75 mmHg, or -125 mmHg for 72 hours. The effects of foam and gauze on the wound bed were compared, and the force required to remove the dressings was measured. Sections of biopsies from the wound bed with an overlying dressing were stained with hematoxylin-eosin and Giemsa and were examined histologically. *Results.* The force ratio needed to remove the wound filler from the wound bed after treatment with negative pressure was greater for foam than for gauze. NPWT caused the wound bed tissue to grow into the foam, while there was no such ingrowth into gauze. Furthermore, beneath the foam there was more leukocyte infiltration, tissue disorganization, disruption of contact among cells, and differences in size among cells. The results were similar regardless of the level of negative pressure. *Conclusion.* More force was required to remove foam compared to gauze following NPWT, which may have been due to greater ingrowth into foam. These findings may explain the patient discomfort and wound bed disruption upon removal of foam. The observed differences in wound bed tissue morphology under foam and gauze are in accordance with the clinically observed differences in quality of granulation tissue formation.

The use of negative pressure wound therapy (NPWT) has evolved over the last decade due to its remarkable effects on the healing of chronic and difficult wounds.<sup>1,2</sup> Although the precise mechanism by which NPWT brings about wound healing is not known, it has been shown that it promotes a moist wound healing environment,<sup>3</sup> reduces bacterial colony counts,<sup>4</sup> increases granulation tissue formation,<sup>4</sup> removes edema,<sup>5</sup> stimulates cell-mediated immune response,<sup>6</sup> induces mechanical deformation of the wound edge tissue,<sup>1,4,7</sup> decreases the permeability of blood vessels,<sup>8</sup> and stimulates angiogenesis and blood flow to the wound margins.<sup>9,10</sup>

NPWT consists of a wound filler material covered with an adherent air-

tight drape that is connected to a negative pressure pump. The wound filler material is either foam or gauze. Paglinawan et al<sup>11</sup> showed that both gauze and foam result in increased granulation tissue formation, yet it is believed that there are differences in the granulation tissue formed by foam and gauze. In clinical practice it has been observed that the granulation tissue formed after NPWT using foam is thick but fragile, whereas the granulation tissue is thinner and denser following NPWT using gauze. Furthermore, foam tends to adhere to the wound. Upon removal, the patient may experience pain, the tissue in the wound bed may be disrupted, and pieces of the foam may remain in the wound. Morykwas<sup>12</sup> showed that granulation tissue grows into the wound filler during NPWT using foam and that this effect may be responsible for the clinically observed problems. No study has yet been performed to examine the effects of gauze on tissue ingrowth. Furthermore, the difference in structure of the wound bed after NPWT using foam and gauze has not been elucidated.

The aim of the present study was to evaluate features of the wound bed after treatment with NPWT using either foam or gauze. Continuous negative pressure therapy was applied for 72 hours in a porcine peripheral wound model. First, the force needed to remove the wound dressing after NPWT was determined. Second, the amount of ingrowth of tissue into the dressing was evaluated in hematoxylin-eosin and Giemsa stained sections of biopsies from the wound bed with the overlying dressing. Third, the wound bed was examined histologically regarding morphology, leukocyte infiltration, and tissue disorganization.

## Materials and Methods

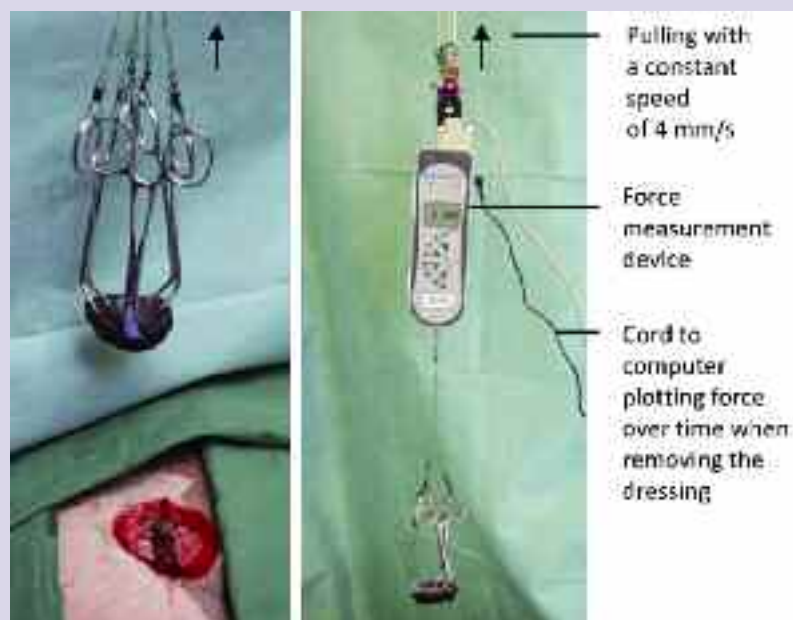
**Animals.** Healthy domestic pigs of both sexes with a mean body weight of 70 kg were fasted overnight with free access to water. The Ethics Committee for Animal Research (Lund University, Sweden) approved the experimental protocol for this study. All animals received humane care in compliance with the European Convention on Animal Care.

**Anesthesia and surgical procedure.** Premedication was performed with an intramuscular injection of xylazine (2 mg/kg) mixed with ketamine (20 mg/kg). Two peripheral veins in the pig's ear were cannulated for induction and maintenance of anesthesia and for fluid administration. Anesthesia was maintained with a continuous infusion of ketamine (0.4–0.6 mg/kg/h). Complete neuromuscular blockade was achieved with a continu-

ous infusion of pancuronium bromide (0.3–0.5 mg/kg/h). The animals were orally intubated with cuffed endotracheal tubes. Mechanical ventilation was established with a Siemens-Elema ventilator (Siemens-Elema AB, Solna, Sweden) in the volume-controlled mode (65% nitrous oxide, 35% oxygen). Ventilatory settings were identical for all animals (respiratory rate, 15 breaths/min; minute ventilation, 12 L/min). A positive end-expiratory pressure of 5 cmH<sub>2</sub>O was applied. A Foley catheter was inserted into the bladder through a suprapubic cystostomy. Fluid loss was compensated by a continuous infusion of Ringer's acetate with a rate of 200 mL/h for the first 24 hours, followed by 110 mL/h for the remainder of the experiment. The animals received total parenteral nutrition. Antibiotics were given once daily as intravenous bolus injections (Streptocillin Vet 250 mg/mL + 200 mg/mL; Boehringer Ingelheim Vetmedica, Malmö, Sweden; 10 mL). Once the experiments were complete, the animals were euthanized with a lethal dose (60 mmol) of intravenous potassium chloride.

**Force measurements.** Circular wounds, 5-cm in diameter going into subcutaneous tissue, were created on each pig's back. Kendall AMD<sup>®</sup> gauze (Covidien, Mansfield, MA) or foam (V.A.C.<sup>®</sup> GranuFoam<sup>®</sup>, KCI, San Antonio, TX) was used as wound filler. The gauze was soaked with saline. AMD gauze contains polyhexamethylene biguanide, which is an antimicrobial component proven to resist bacterial colonization within the dressing and reduce bacterial penetration through the dressing.<sup>13</sup> The foam has an open cell structure that allows pressure transduction to the wound bed. The volume of wound filler material was approximately one and a half times larger than the wound volume to permit volume reduction during negative pressure application. A drainage tube was inserted into the gauze/foam and connected to a vacuum source (Prospera<sup>®</sup> PRO-III, Prospera, Fort Worth, TX). The wound was then sealed with transparent adhesive drape, which overlapped the wound margins by 10 cm. After 72 hours of continuous NPWT at 0, -75, or -125 mmHg, the adhesive drape that covered the wound was cut open at the borderline between the tissue and the wound filler and the drain was cut off. The wound filler was attached to a force measurement device and pulled away from the wound at a constant speed of 4 mm/s. The force needed to remove the wound filler was plotted over time using a computer. The experimental setup is shown in Figure 1.

**Ingrowth and histological processing.** A 1 cm x 1 cm x 2 cm strip of the wound filling material was sewn



**Figure 1.** Experimental setup for measurements of the force needed to remove foam or gauze after 3 days of NPWT of a porcine peripheral wound. The left photo shows how the wound filler material (in this case foam) is attached to three forceps for an even distribution of the pulling forces. The wound filler is pulled out of the wound at a constant speed (4 mm/s). The right photo shows the wound filler below the force measurement device.

onto the bottom of each wound. After discontinuation of NPWT, the strip and the underlying wound bed tissue was cut away with a scalpel. The tissue was then treated in 4% paraformaldehyde, dehydrated, and finally embedded in paraffin overnight. Sectioning was performed with a rotary microtome HM 355 (ThermoFisher Scientific, Waltham, MA).

**Giemsa staining.** Glass slides with 4- $\mu$ m thick tissue sections were deparaffinized (5 minutes in xylene, 4 minutes in 99.5% ethanol, and 4 minutes in distilled H<sub>2</sub>O). The tissue sections were stained for 1 hour in a staining solution (10% Giemsa and 90% distilled H<sub>2</sub>O). The slides were differentiated by dipping in HAc (16 drops of 100% HAc in 100-mL distilled H<sub>2</sub>O) followed by dipping in 86% ethanol. The slides were dipped twice in isopropanol followed by xylene treatment (4 minutes) for mounting.

**Hematoxylin and eosin staining.** Slides with 4- $\mu$ m thick tissue sections were deparaffinized for 2 x 4 minutes in xylene, 2 x 3 minutes in 99.5% ethanol, and 3 minutes in tap water. The slides were stained for 12 minutes in Mayer's hematoxylin and then treated with tap water for 8 minutes, erythrosin (1.5 g in 500-mL H<sub>2</sub>O) for 6 minutes, tap water for 3 minutes, 99.5% ethanol for 2 x 3

minutes, and xylene for 4 minutes.

Biopsy sections were evaluated for ingrowth into the wound filler and morphology of the underlying tissue. The following measurements were performed:

1. Ingrowth into the wound filler (micrometers into the wound filler).
2. Disorganization of the cells in the wound bed—ie, disruption of contact between the cells and differences in cell size (micrometers into the tissue). Tissue disorganization was examined in the wound bed tissue underlying the wound filler.
3. Leukocyte count (leukocytes per micrometer squared).

### Statistical Analysis

Eight wounds were created for each pressure level and wound filler. Calculations and statistics were performed using GraphPad 5.0 software (GraphPad Software Inc., La Jolla, CA). Statistical analysis was performed using the Mann-Whitney test when comparing

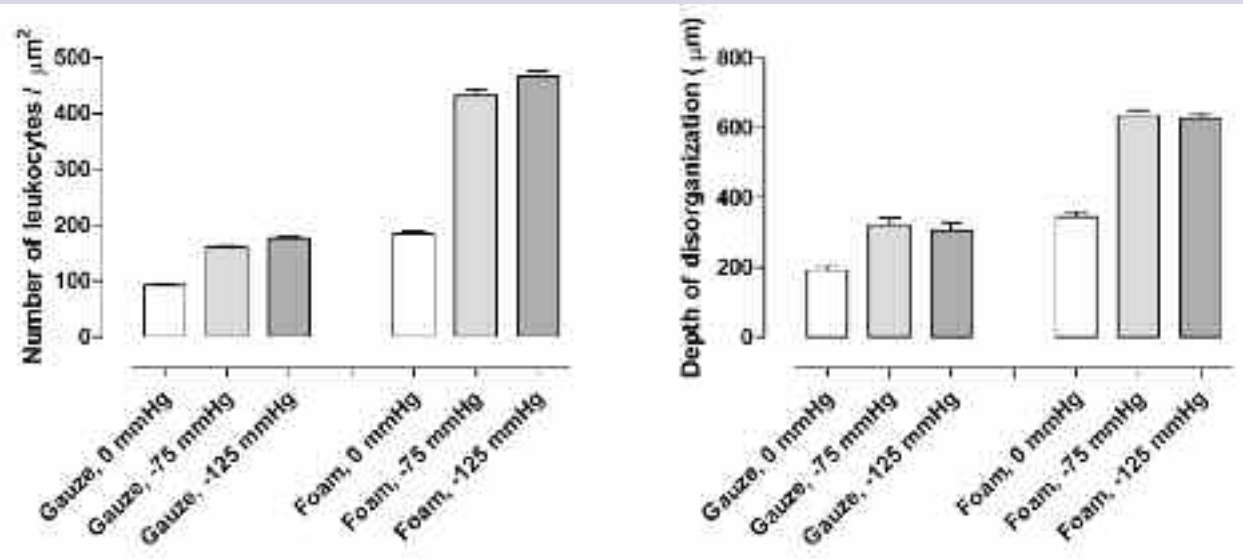
two groups, and the Kruskal-Wallis test with Dunn's post-test for multiple comparisons when comparing three groups or more. Significance was defined as  $P < 0.05$ . Results are presented as mean  $\pm$  the standard error of the mean (SEM).

### Results

#### Force needed to pull away the wound filler.

Greater force was needed to release foam than to release gauze from the wound bed tissue after treatment with negative pressure (force ratio  $3.1 \pm 0.4$  for foam and  $1.8 \pm 0.2$  for gauze after -75 mmHg;  $P < 0.001$  [Figure 2]). Treatment at -75 mmHg and -125 mmHg resulted in similar attachment of the wound filler to the wound bed (force ratio  $1.8 \pm 0.2$  after treatment at -75 mmHg and  $1.5 \pm 0.1$  after treatment at -125 mmHg for gauze;  $P > 0.30$  [Figure 2]).

**Ingrowth into the wound filler.** NPWT caused the wound bed tissue to grow into the foam ( $342 \mu\text{m} \pm 16 \mu\text{m}$  at -75 mmHg). There was no such ingrowth into gauze ( $92 \mu\text{m} \pm 12 \mu\text{m}$ ;  $P < 0.001$  compared to foam at -75 mmHg). The extent of ingrowth into foam was similar when comparing -75 mmHg and -125 mmHg ( $342 \mu\text{m} \pm$



**Figure 2.** Force required to remove foam and gauze after 3 days of NPWT at 0, -75, or -125 mmHg in a porcine peripheral wound. The force was plotted over time and the area under the curve was calculated. The force needed to pull out each wound filler after treatment at subatmospheric pressure was calculated as a quotient of the force needed after treatment at atmospheric pressure. The results are shown as means  $\pm$  SEM.

16  $\mu\text{m}$  for -75 mmHg and  $332 \mu\text{m} \pm 13 \mu\text{m}$  for -125 mmHg for foam;  $P > 0.30$  [Figures 3, 4]).

**Wound bed tissue morphology.** The tissue morphology and the cellular infiltrate in the wound bed underlying the wound filler was examined using histology. The wound bed was mainly composed of subcutaneous tissue. A morphological difference could be observed between the wounds treated with foam and gauze, both after NPWT and after treatment at atmospheric pressure. After treatment at atmospheric pressure, there were more leukocytes in the tissue (leukocyte count =  $185 \pm 4$  per  $\mu\text{m}^2$  for foam and  $92 \pm 2$  per  $\mu\text{m}^2$  for gauze;  $P < 0.001$ ). The tissue was also disorganized to a greater extent with disruption of the contacts between cells and differences in cell size (tissue disorganization =  $344 \mu\text{m} \pm 12 \mu\text{m}$  into the tissue for foam and  $191 \mu\text{m} \pm 11 \mu\text{m}$  for gauze;  $P = 0.0094$  at 0 mmHg). After NPWT, the number of leukocytes and tissue disorganization increased slightly in the gauze-treated wounds and to a greater extent in the foam-treated wounds (leukocyte count =  $432 \pm 10$  per  $\mu\text{m}^2$  for foam and  $161 \pm 2$  per  $\mu\text{m}^2$  for gauze [ $P < 0.001$ ], and tissue disorganization =  $636 \mu\text{m} \pm 14 \mu\text{m}$  for foam and  $320 \mu\text{m} \pm 22 \mu\text{m}$  for gauze [ $P < 0.001$ ] at -75 mmHg [Figures 5, 6]).

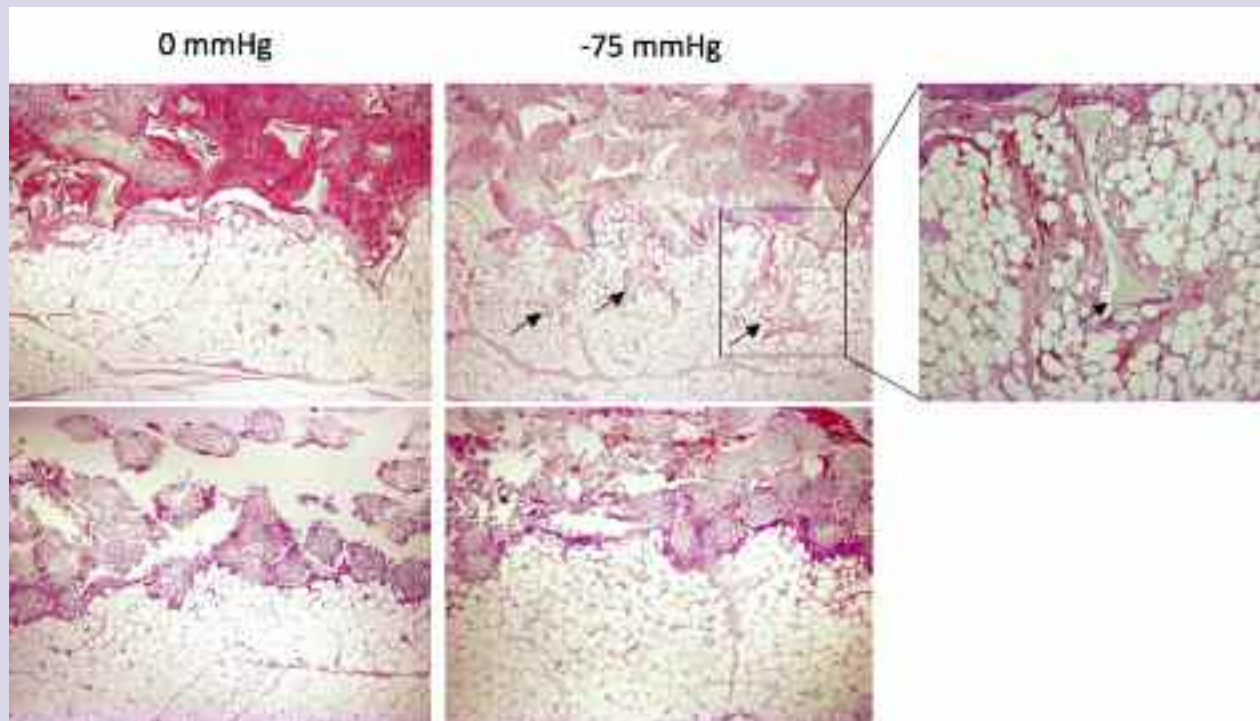
## Discussion

In clinical practice, NPWT is performed using either

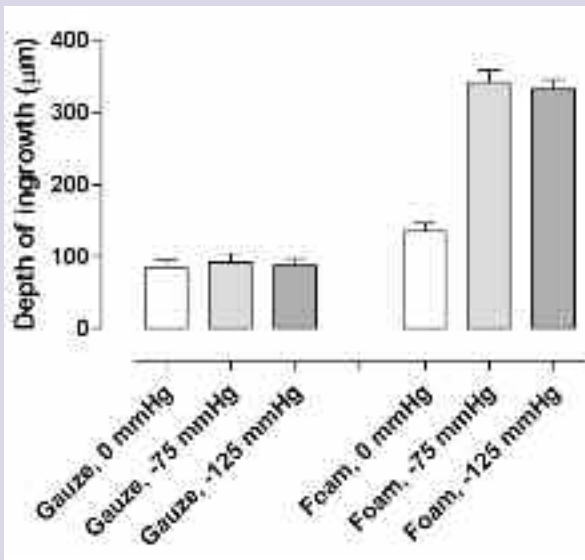
foam or gauze. Paglinawan et al<sup>11</sup> have shown that both gauze and foam increase granulation tissue formation. However, many clinicians using NPWT observe differences in the quality of granulation tissue formed by gauze or foam, although this has not yet been examined in detail in a controlled study. The present study shows that wound bed tissue grows into foam but not into gauze and that a greater force ratio is needed to remove foam than to remove gauze from the wound. This is in agreement with clinical observations that foam forms a strong mechanical bond with the wound bed tissue, and upon removal, the wound bed may be disrupted, pieces of the foam may remain in the wound, and that patients regularly experience pain.<sup>14</sup>

Campbell et al<sup>15</sup> have described ingrowth of granulation tissue into foam. The present study showed that the wound bed tissue extended approximately  $400 \mu\text{m}$  into the foam after 3 days of therapy. Morykwas et al<sup>12</sup> found that granulation tissue grew approximately  $1000 \mu\text{m}$  into foam after 4 days of continuous NPWT at -125 mmHg. The present study found no such ingrowth into gauze, which corresponds with clinical observations that gauze is easier to remove and does not disrupt the wound bed or cause patient pain during dressing changes.<sup>14</sup>

The mechanism that governs ingrowth into foam may be related to the mechanical deformation of tissue upon NPWT application. NPWT results in both macromechan-



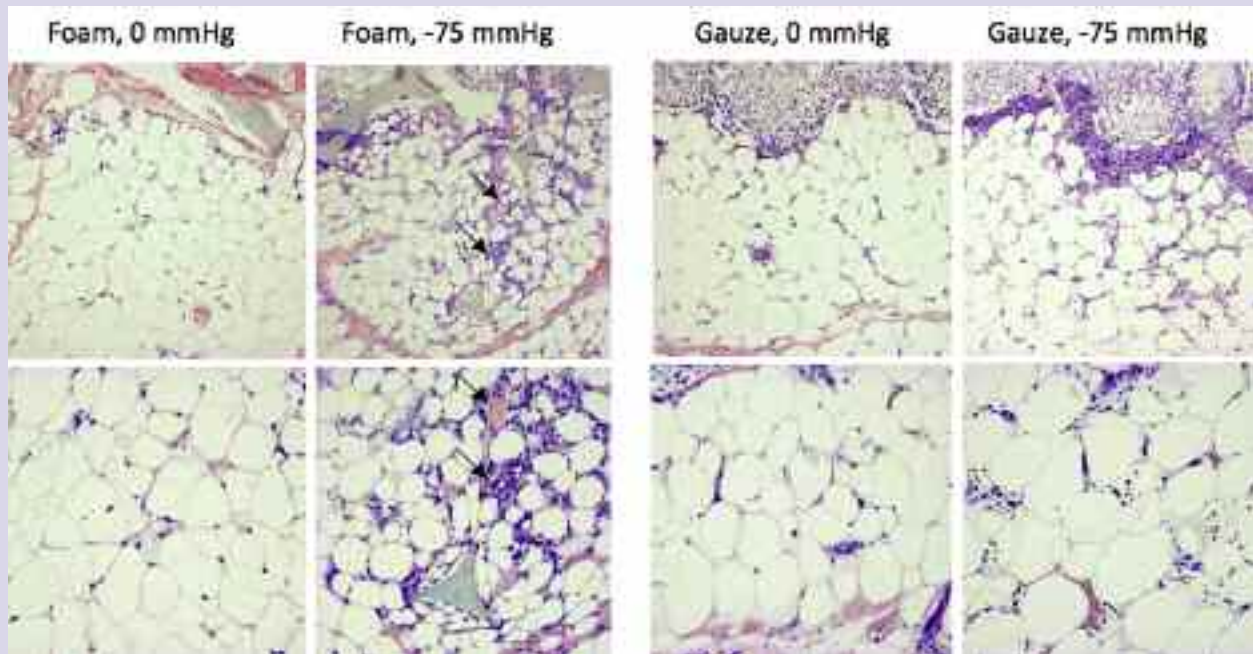
**Figure 3.** Hematoxylin and eosin stained sections of biopsies from the wound bed with the overlying wound filler, foam (top panels) and gauze (bottom panels), after 3 days of treatment with NPWT at 0 or -75 mmHg in a porcine peripheral wound. Note the tissue ingrowth into foam (arrows) but not into gauze. The right panel is a magnification of a wound bed treated with NPWT and foam at -75 mmHg and shows an ingrown piece of foam (arrow).



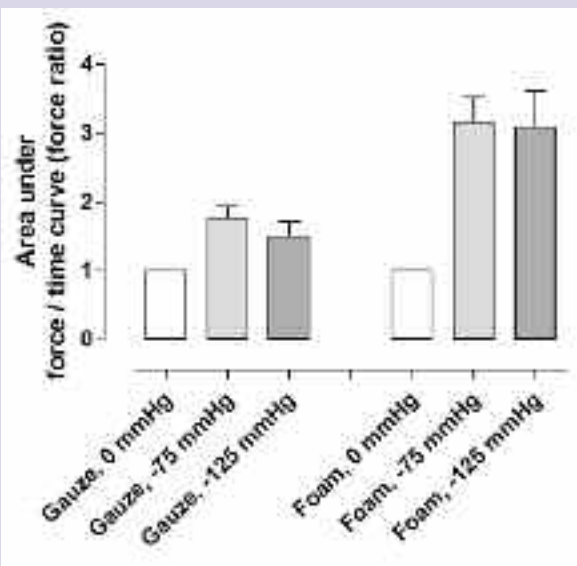
**Figure 4.** The depth of tissue ingrowth into gauze and foam after 3 days of NPWT at atmospheric (0 mmHg) or subatmospheric (-75 or -125 mmHg) pressure in a porcine peripheral wound. The results are presented as means  $\pm$  SEM.

ical effects, such as wound contraction, and micromechanical effects as a result of the interaction of the tissue and dressing at a microscopic level.<sup>1</sup> It is believed that these mechanical effects affect the cytoskeleton,<sup>16</sup> which initiates a signaling cascade that ultimately leads to granulation tissue formation. Differences in the mechanical properties of foam and gauze may result in ingrowth differences. Other factors that may be of importance are the chemical nature of the material, the surface properties of the wound fillers, and the geometry of the pores of the wound filler.

In the present study, there was a morphological difference in the wound bed underlying foam and gauze. Foam-treated wounds had more leukocyte infiltration into the tissue and the tissue was also more disorganized. The reason for increased leukocyte infiltration is not known. The mechanical effects on the wound bed are similar for gauze and foam, and thus it seems more probable that the chemical or the geometrical properties of the wound filler plays a role. One possible mechanism could be that the wound filler causes a foreign body reaction. Leukocytes first infiltrate the wound bed.



**Figure 5.** Giemsa stained sections of biopsies from the wound bed with the overlying wound filler, foam (left panels) and gauze (right panels), after 3 days of treatment with NPWT at 0 or -75 mmHg, in a porcine peripheral wound. The bottom panels are magnifications of the top panels. Widespread leukocyte infiltration (arrows), tissue disorganization with disruption of the contacts between the cells, and differences in cell sizes in the foam-treated wound bed are seen.



**Figure 6.** Diagrams showing the number of leukocytes per micrometer squared and the depth of tissue disorganization after 3 days of NPWT at 0, -75, or -125 mmHg in a porcine peripheral wound. A greater degree of leukocyte infiltration and tissue disorganization is seen under foam compared to gauze. Results are shown as means  $\pm$  SEM.

Leukocytes then release cytokines in order to promote granulation tissue formation. The first step in the process of granulation tissue formation is disorganization of the tissue as the cells turn into fibroblasts.<sup>17</sup> Once formed, fibroblasts create granulation tissue that organizes. Tissue disorganization is one of the initial events in generating granulation tissue formation. Disorganization was seen in the sliced sections of the wound bed in the present study, with disruption of the contacts between the cells and differences in cell sizes.

While granulation tissue formation is an important element of wound healing, it also results in fibrosis. Fibrosis leads to scarring and contraction of the wound as the healing tissue organizes. The fact that the tissue is more disorganized after NPWT with foam than after NPWT with gauze may be related to the fact that many clinicians observe more fragile granulation tissue in wounds treated with foam than in wounds treated with gauze.

The differences in the wound bed after NPWT treatment with gauze or foam can be seen in the clinical setting. NPWT with foam results in thick granulation tissue, but with more tissue ingrowth compared to NPWT with gauze. When foam is removed, the wound bed tissue is

disrupted resulting in nonselective mechanical debridement, which may be painful for patients. Extensive granulation tissue formation results in scarring that will contract the wound during the healing process. Foam may be advantageous when treating wounds that benefit from thick granulation tissue and where scarring does not pose a problem, such as in sternotomy wounds or wounds that benefit from contraction (ie, upper or lower limb compartment syndrome). Gauze offers less thick granulation tissue without ingrowth into the wound filler, which may make dressing changes less painful compared to foam with less scarring and thereby less contraction upon healing. Less scarring may be preferable when preparing the wound bed for skin grafting, especially over joints where mobility is a concern.

In clinical practice, measures are undertaken to prevent tissue ingrowth into the foam. First, the wound bed may be covered with an interface dressing. It is plausible that an interface dressing would have partly prevented ingrowth. Second, the recommended time between dressing changes is 2 days for foam in order to hinder ingrowth. The present experiment lasted 72 hours, because in clinical practice, the time between dressing changes is usually longer than 2 days and commonly lasts for at least 3 to 4 days.

The two different levels of negative pressure examined in the present study, -75 mmHg and -125 mmHg, showed similar results regarding both the degree of ingrowth and also the pressure needed to remove the wound dressing. Negative pressure levels ranging from -10 mmHg to -175 mmHg have been studied with regard to biological effects in the wound edge, including blood flow and macrodeformation.<sup>18</sup> The results show that -80 mmHg has maximal effects in the wound edge and increasing negative pressure to more than -80 mmHg does not enhance the effects of NPWT. The results from the present study support this conclusion. Negative pressure of -80 mmHg may be sufficient to achieve many of the biological effects that are thought to be important for wound healing.

## Conclusion

Gauze and foam are currently being used as wound filler materials for NPWT. This study shows that these materials have different properties. More force is needed to remove foam than gauze and there is greater ingrowth into foam. This may explain the clinical observations that the wound bed is disrupted and the patient experiences pain during dressing changes after treatment with foam.

The morphology of the wound bed tissue differs when comparing foam and gauze; there is a greater degree of leukocyte infiltrate and tissue disorganization under foam. Leukocytes promote the formation of granulation tissue. This newly formed tissue may promote faster healing, while extensive granulation tissue formation results in scarring that will contract the wound during the healing process. The greater degree of leukocyte infiltration and tissue disorganization underneath foam may explain the difference in wound bed quality upon healing.

## Acknowledgements

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