Green foam, black foam or gauze for NWPT: effects on granulation tissue formation

- **Objective:** To compare the effects of green foam with black foam and gauze during negative pressure wound therapy (NPWT), with regard to wound bed appearance and granulation tissue formation, and monitoring of wound exudate.
- **Method:** Wounds on the backs of eight pigs underwent 72 hours of NPWT plus either green polyurethane foam with an open pore structure, black polyurethane foam with an open pore structure or saline-moistened AMD gauze. Sections of biopsies from the wound bed, including the overlying dressing, were examined histologically with regard to microdeformation of the wound bed and granulation tissue formation. The force required to remove the wound fillers was measured.
- **Results:** Wound exudate and bleeding could be easily seen when using gauze and green foam, but were not visible under the black foam. Such visibility facilitates monitoring of the wound status. No difference was found in the quantity or characteristics of the granulation tissue formed under the green foam or black foam. Both green foam and black foam resulted in more pronounced granulation tissue formation than gauze under negative pressure. There was also more leucocyte infiltration and tissue disorganisation under green foam and black foam than under gauze. All three wound fillers created microdeformation within the wound bed surface. Similar forces were required to remove green foam and black foam (5.0 ± 0.6 N for green foam and 4.0 ± 0.4 N for black foam), while less force was needed for gauze (2.1 ± 0.2 N). This may be a result of tissue ingrowth into the foam (357 ± 12μm for green foam and 362 ± 14μm for black foam), but not into gauze (0μm), as shown by examination of biopsy sections from the wound bed.
- **Conclusion:** Green foam and black foam have similar biological effects on the wound bed. Bleeding and exudate can be more easily monitored when using green foam or gauze. Differences in the wound bed tissue morphology when using foam or gauze plus NPWT support clinical observations that granulation tissue under foam is thick but fragile, whereas that under gauze is thinner but denser.
- **Conflict of interest:** The study was supported by Mölnlycke Health Care AB.
research

Anaesthesia
The pigs were fasted overnight, with free access to water. Premedication was performed with an intramuscular injection of xylazine (Rompun vet. 20mg/ml; Bayer AG, Leverkusen, Germany; 2mg/kg), mixed with ketamine (Ketaminol vet. 100mg/ml; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20mg/kg). Two peripheral veins in the pigs’ ears were cannulated for induction and maintenance of anaesthesia, and for fluid administration.

Anaesthesia was maintained with a continuous infusion of ketamine (Ketaminol vet. 50mg/ml; Farmaceutici Gellini S.p.A.; 0.4–0.6mg/kg/h). Complete neuromuscular blockade was achieved with a continuous infusion of pancuronium bromide (Pavulon; N.V. Organon, Oss, the Netherlands; 0.3–0.5mg/kg/h).

Fluid loss was compensated for by continuous infusion of Ringer’s solution at a rate of 200ml/kg/h for the first 24 hours, followed by 110ml/h for the remainder of the experiment. The animals received total parenteral nutrition (Kabiven; Fresenius Kabi AB, Uppsala, Sweden). Antibiotics were given once daily as intravenous bolus injections (streptocillin vet. 250mg/ml and 200mg/ml; Boehringer Ingelheim Vetmedica, Malmö, Sweden; 10ml).

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 litres/min). A positive end-expiratory pressure of 5cm H₂O was applied. A Foley catheter was inserted into the urinary bladder through a suprapubic cystostomy.

After the experiments were completed, the animals were euthanised with a lethal dose (60mmol) of intravenous potassium chloride.

Negative pressure wound therapy
Circular wounds, 6cm in diameter, extending into the subcutaneous tissue, were created on each pig’s back using a scalpel. The wounds were immediately sealed for NPWT, as described below. The wounds were filled with saline-moistened AMD gauze (Kendall Kerlix AMD, Tyco Healthcare Group, Mansfield, MA, USA), green polyurethane foam with an open pore structure (Avance foam, Mölnlycke Health Care AB, Gothenburg, Sweden), or black polyurethane foam with an open pore structure (VAC Granufoam, KCI, San Antonio, TX, USA). The green and black foam are both an open pore structure polyurethane foam. Besides colour, the only difference between them is that the green foam has higher tensile strength than the black foam (165kPa versus 108kPa), which may lead to fewer problems with foam residues in the wound bed. For gauze and green foam, drainage tubes were placed over the top of the wound filler and connected to the vacuum source (Avance NPWT system, Mölnlycke Health Care AB, Gothenburg, Sweden). For the wounds filled with black foam, a drain (T.R.A.C. pad, KCI, San Antonio, TX, USA) was attached to the top of the dressing and connected to the vacuum source (VAC ATS pump, KCI, San Antonio, TX, USA). For all three fillers, this was in accordance with the manufacturers’ instructions. The wounds were treated for 72 hours at either 0mmHg or -120mmHg.

Quantity of granulation tissue formed
The quantity of granulation tissue formed during NPWT was graded on a scale from 0–5 by two different surgeons. Grading was performed blinded, and separately, by each surgeon, as described in a previous study. The scale ranged from zero (a pale wound bed without granulation) to five (fully granulated tissue and a vascularised wound bed).

Force measurements
After 72 hours of NPWT (as described above), the adhesive film dressing that covered the wound was cut along the borderline between the tissue and the wound filler, and the drain was cut off. The wound filler was attached to an advanced force gauge (AFG) (Mecmesin, UK) and withdrawn at a constant speed of 4mm/s. The force required to remove the wound filler was plotted as a function of time, using a computer.

Histological examination
The tissue morphology and the cellular infiltrate in the wound bed underlying the wound filler were examined histologically. A strip of the wound filler material (1 x 1 x 2cm) was sutured onto the bottom of each wound before the NPWT dressings were applied. Post-NPWT, the strip and the underlying wound bed tissue were excised with a scalpel. The tissue was then treated in 4% para-formaldehyde, dehydrated and finally embedded in paraffin and left overnight. Biopsies were sectioned (4µm thick) using a rotary microtome (HM 355, ThermoFisher Scientific, MA, USA), mounted on glass slides and stained with routine haematoxylin and eosin stainng. All wound samples were subject to histological examination.

Characteristics of the granulation tissue formed
Biopsy sections were evaluated with regard to microdeformation (wound bed surface undulations), ingrowth into the wound filler, and morphology of the underlying tissue, including disorganisation of the cells in the wound bed (disruption of the contacts between the cells and differences in cell size) and leucocyte count (number per µm²).

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Statistical analysis
Results are presented as means ± the standard error of the mean (SEM).
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. All differences referred to in the text have been statistically verified.

Results
Similar results were found for green foam and black foam with regard to all of the parameters measured. However, wound exudate and bleeding could easily be seen using green foam, whereas this was not possible with black foam (Fig 1).

Quantity and characteristics of granulation tissue
No differences were observed in the quantity or characteristics of the granulation tissue formed under green and black foam during NPWT. Both kinds of foam resulted in more granulation tissue than gauze during NPWT (Figs 2 and 3). Leucocyte infiltration and tissue disorganisation were more prominent under green and black foam than under gauze (Fig 4).
Microdeformation

Both foams and the gauze compressed the wound bed so that small tissue blebs were drawn into the empty spaces of the gauze and the pores of the green and black foam, as indicated by the arrows in Fig 5.

Ingrowth and force required to remove the filler

The wound bed tissue grew into both kinds of foam (357 ± 12µm for green foam and 362 ± 14µm for black foam), but not into the gauze (Fig 6). Similar forces were required to remove green and black foam (5.0 ± 0.6 N for green foam and 4.0 ± 0.4 N for black foam), while less force was needed to remove gauze after NPWT (2.1 ± 0.2 N for gauze, Fig 7).

Discussion

An advantage of green foam and gauze is that wound exudate and bleeding can be monitored more easily than with black foam. The biological effects studied, including granulation tissue formation, microdeformation, ingrowth and the force required to remove the wound filler, were similar for green and black foam, but differed for gauze.

Granulation tissue formation

The quantity and characteristics of the granulation tissue formed under green and black foam during NPWT were similar. Slightly more granulation tissue was formed under green and black foam than under gauze. Furthermore, there was more leucocyte infiltration and tissue disorganisation under green and black foam than under gauze.

It has been shown previously, both experimentally and in patients, that the amount and character of granulation tissue formed under black foam and gauze may differ. As stated above, granulation tissue formed under foam is thicker than that produced under gauze. Thick granulation tissue is beneficial for fast wound healing, but may lead to problems such as fibrosis, scarring and contractures as the wound heals.

Gauze is often used in NPWT because of its conformability and ease of application. It has become especially popular among plastic surgeons for wound bed preparation before grafting, and is a good choice when the cosmetic result is more important than the speed of wound healing, or in cases where scar tissue may restrict movement, for example over joints.

Foam is suitable for wounds that benefit from thick granulation tissue and where scarring does not pose a problem, such as in sternotomy wounds, fasciotomy wounds in upper or lower limb compartment syndrome, where contraction is beneficial, or to provide a bridging therapy in acute wounds with large tissue loss.
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Microdeformation

The properties of the wound–filler interface determine many of the effects of NPWT on the wound bed, as the tissue surface is stimulated by the structure of the wound dressing. The interaction between the filler and the wound bed has been described in detail for black foam and gauze. Histological examination of cross sections of the wound bed in the present study showed that the use of green foam, black foam and gauze all resulted in an undulating wound bed surface and that small tissue blebs or ‘tissue mushrooms’ were drawn into the pores of the green and black foam dressing and between the threads of the gauze. The mechanical effects stimulated by the pulling of tissue blebs into the pores of the foam dressing and between the threads of the gauze are believed to be one of the fundamental mechanisms by which NPWT promotes healing.

These deformational forces at the wound-foam interface are thought to initiate a series of inter-related biological effects that affect the cytoskeleton and stimulate angiogenesis and granulation tissue formation. This microdeformation is thought to result in shearing forces at the wound–dressing interface, which affect the cytoskeleton and initiate a series of biological effects, including the stimulation of angiogenesis and the promotion of granulation tissue formation, leading to wound healing.
Ingrowth and force needed to remove the filler
The results show that the wound bed tissue grew into both black and green foam, but not into gauze. Similar forces were required to remove green and black foam, while less force was needed to remove gauze. This is probably due to the tissue ingrowth into the green and black foam, but not into the gauze, as seen in biopsy specimens from the wound bed.

A number of complications are associated with ingrowth into foam. First, the patient may experience pain during dressing changes as the ingrown tissue is torn away from the wound, requiring the administration of strong analgesics.17,18 Second, wound-bed disruption and mechanical tissue damage may arise as the foam is removed from the wound bed during dressing changes. Finally, pieces of foam may become stuck in the wound bed and, if not removed, will act as foreign bodies that may impede wound healing.

A non-adherent wound contact layer, therefore, is often placed over the wound bed, underneath the wound filler, when the clinician anticipates such complications.19 A wound contact layer may be placed over vulnerable structures, such as blood vessels or nerves,19 and over the wound bed itself to prevent the ingrowth of granulation tissue into the wound filler.

The mechanism governing ingrowth into foam is likely related to the interaction between the tissue and dressing at a microscopic level.20 The differences in ingrowth observed in this study are probably due to differences in the physical properties of the foam and gauze dressings. The chemical nature of the material may also be of importance.

Conclusion
Green foam and black foam have similar biological effects on the wound bed. However, the wound status (bleeding and exudate) can easily be monitored with green foam, but not with black foam.

Differences were observed in the morphology of the wound bed tissue when using foam and gauze plus NPWT, which is in accordance with clinical observations that granulation tissue under foam is thick but fragile, whereas that under gauze is thinner but denser.

Treatment of the patient can be optimised by choosing the appropriate wound filler material for individual wounds and patients, depending on the desired effects in the wound bed.

References
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