Negative pressure wound therapy using gauze and foam: histological, immunohistochemical, and ultrasonography morphological analysis of granulation and scar tissues

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ORIGINAL PAPER

Negative pressure wound therapy using gauze and foam: histological, immunohistochemical, and ultrasonography morphological analysis of granulation and scar tissues

Second phase of a clinical study

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Abstract
Background Negative pressure wound therapy (NPWT) is becoming routine for the preparation of wounds prior to grafting for wound closure. With this purpose, we have been using both foam and gauze-based NPWT obtaining similar proportions of closed wounds and observing less pliable scar tissue on the foam-treated patients. The aim of this study was to compare this two different fillers and to identify if there are different indications for their use according to anatomical areas in relation to the type of granulation and scar tissue obtained.
Methods Both foam and gauze patients were compared in terms of depth and wound location, patients' age, and comorbidities. All foam patients were treated at 125 mmHg for an average of 25 days before skin grafting, while gauze patients were treated at 80 mmHg for an average of 21 days before skin grafting. Biopsies of granulation and scar tissues were taken and stained with hematoxylin-eosin and Masson's trichrome stainings, investigating vascular endothelial growth factor (VEGF) and metalloproteinase (MMP). An ultrasound analysis of the closed wounds was also conducted.
Results Histological, immunohistochemical, and ultrasonographical results after gauze-based NPWT showed a minor tissue thickness and disorganization and less sclerotic components.
Conclusions These results support the hypothesis that different fillers generate different scar tissues. The choice of the filler to apply negative pressure should be dictated by the anatomical areas affected by the lesion.
Level of Evidence: Level IV, therapeutic study.

Keywords Foam · Gauze · Negative pressure wound therapy · Wound healing

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Introduction

Negative pressure wound therapy (NPWT) is widely used in managing and accelerating wound healing. Despite the quick introduction of this device into clinical practice, the mechanism by which this method stimulates wound healing has not been fully defined.

To apply negative pressure on the surface of the wound, polyurethane (PU) or polyvinylalcohol foam and polyhexamethylene biguanide (PHMB) pre-impregnated gauze are available. These fillers are introduced in the wound and fixed with the use of an adhesive dressing. A negative pressure is used to achieve suction and drainage. The optimal pressure range to obtain a good clinical result for the foam is

between 80 and −125 mmHg and for the gauze it is between −40 and −80 mmHg.

NPWT is becoming routine for the preparation of wounds prior to grafting for wound closure. We have been using both foam and gauze-based NPWT to prepare wounds for closure prior to skin grafting and have obtained similar proportions of closed wounds [1]. In our follow-up consultations, we observed that scar tissue on the foam-treated patients were less pliable than those on the gauze-treated patients.

We noticed some macroscopic differences on the wound bed. Therefore, we led a histological and immunohistochemical evaluation after taking biopsies of the wound beds.

From 6 to 15 months after healing, an ultrasonographic (US) study was performed with the aim of appraising the pattern of the newly reconstructed tissue followed by an echocontrastography to assess the revascularization. A histological and immunohistochemical evaluation was performed later on the biopsies taken from the scar tissue.

In this article, we will discuss our results regarding the differences in granulation and scar tissue obtained after NPWT with gauze or foam using histological, immunohistochemical, and ultrasonographical findings.

Materials and methods

The study was conducted in the Department of Reconstructive and Aesthetic Plastic Surgery at the University Hospital Citta della Salute e della Scienza Turin, Italy during the period from May 2008 to September 2013.

The treatment with RENASYS™ (Blue Sky Medical/SMITH&NEPHEW, London, UK) uses a gauze impregnated with 0.2 % polyhexamethylene biguanide with a spiral pattern, placed on the bottom of the wound. This method needs a negative pressure of 80 mmHg transferred by a drain according to the Chariker–Jeter method. The treatment with vacuum assisted closure (V.A.C) therapy uses a PU polyether foam with a pore size of 400–600 mm (V.A.C.® (KCI, San Antonio, TX, USA) pack dressing) to transfer a constant negative pressure of 125 mmHg. In both methods, a transparent adhesive was used to fix the dressing around the drain to complete the seal in accordance with the manufacturer's guidelines. Dressing was changed once every 3 days and a wound measuring system with laser and a digital camera was used weekly to evaluate the macroscopic changes on the wound bed.

One hundred twenty Caucasian patients admitted to our department and treated them with gauze-based NPWT were evaluated. After passing the inclusion/exclusion criteria, 13 of them were selected and compared to 16 patients selected from our 184 long-term case histories of foam-treated patients. Inclusion criteria were acute posttraumatic lower limb (car/motorcycle accident or surgical complication) wounds up to the muscular band, age range 18–80 and wound size from 30 cm2. Exclusion criteria were chronic wounds, diabetes, and pregnancy.

After 20 to 25 days of NPWT application, one biopsy of granulation tissue was taken from the wound bed of each patient at the moment of reconstruction. Out of the 29 granulation tissue biopsies, 13 were taken on 13 patients treated with gauze (two female and 11 male; average age, 49; average time of treatment, 21 days); 16 were taken on 16 patients treated with foam (seven male and nine female; average age, 58; average time of treatment, 25 days) (Table 1).

A histological and immunohistochemical evaluation was performed by a pathologist blinded to the treatment [1]. Formalin fixed, paraffin-embedded tissue sections, 3-μ thick, were pretreated for antigen retrieval with Dako Target Retrieval Solution pH 9 (S2368/S2367) for 15 min at boiling point. After thermal treatment, the slides were stained for immunohistochemical procedure with Polyclonal Rabbit Anti-Human Matrix Metalloprotease 9 (Dako a0150) at a dilution of 1:50 for 15 min at room temperature. Monoclonal Mouse Anti-Human Vascular Endothelial Growth Factor (Dako M7273) at a dilution of 1:25 for 30 min at room temperature. Visualization of both reactions was performed according the avidin–biotin method, (Kit Dako LSAB2 System HRP: code K0672–K0673–K0675). A semi-quantitative evaluation (0 none–3 high) of VEGF and Metalloproteases was also performed. The values were analyzed using STATA statistical software.

From 6 to 15 months after healing, on selected and homogeneous patients, a US examination was performed to evaluate the thickness and the ultrasonographical pattern of the newly reconstructed skin, comparing it with the contralateral physiological one. We analyzed a total of 23 areas in 23 patients, one area in each patient. Twelve areas in 12 patients treated with gauze (six male and six female; average age, 53; average time of treatment, 20 days; average months after healing, 9) and 11 areas in 11 patients treated with foam (nine male and two female; average age, 47; average time of treatment, 19 days; average months after healing, 10). The US examination was performed using ESAOTE TECHNOS device (ESAOTE S.p.A, Geneva, Italy) supported by a high-frequency probe (10–13 MHz) to study the superficial structures and color Doppler module to evaluate tissue vascularization. We evaluated thickness, echoicity, and skin vascularization after reconstructive treatment. The evaluation was performed by scanning different planes and comparing neighboring and contralateral symmetric cutaneous normal areas with the new constructed tissue. They were analyzed by the same sonographer blinded to the treatment.

To view and analyze the newly formed vessels, from 12 to 22 months after healing, we continued our study using the echocontrastography to assess the neovascularization of the newly regenerated tissue. The echocontrastography examination was performed in single blind using the MyLab 70 XVG

Table 1 Patients' baseline characteristics and examinations performed on each patient

Pat.	Age	Sex	Wound locat.	Filler used	Duration (days) NPWT	Healed	Type of healing	Biopsies on granul.ti.	Biopsies on scar ti.	US exam	CEUS
1	40	M	Leg	Gauze	49	Yes	SG	yes	no	Yes	Yes
2	79	F	Leg	Gauze	6	Yes	SG	Yes	No	Yes	yes
3	80	F	Foot	Gauze	27	Yes	SG	Yes	No	No	No
4	80	F	Leg	Gauze	30	Yes	SG	Yes	No	Yes	Yes
5	29	M	Foot	Gauze	21	Yes	SG	Yes	Yes	Yes	Yes
6	79	F	Leg	Gauze	30	Yes	SG	Yes	No	Yes	Yes
7	30	F	Leg	Gauze	10	Yes	SG	Yes	No	Yes	Yes
8	43	M	Leg	Foam	27	Yes	SG	Yes	No	Yes	Yes
9	34	M	Leg	Foam	16	Yes	SG	Yes	No	Yes	Yes
10	60	M	Foot	Foam	5	Yes	2nd int.	Yes	No	No	No
11	80	M	Foot	Foam	29	Yes	SG	Yes	No	No	No
12	33	M	Leg	Foam	36	Yes	SG	Yes	No	Yes	Yes
13	69	M	Leg	Foam	39	Yes	SG	Yes	No	Yes	Yes
14	44	M	Leg	Foam	18	Yes	SG	Yes	No	Yes	Yes
15	63	M	Foot	Foam	50	Yes	SG	Yes	No	No	No
16	40	M	Foot	Foam	5	Yes	SG	Yes	No	Yes	Yes
17	20	M	Leg	Foam	45	Yes	SG	Yes	No	No	No
18	67	M	Foot	Foam	15	Yes	SG	Yes	Yes	Yes	Yes
19	33	M	Leg	Gauze	30	Yes	SG	Yes	No	Yes	Yes
20	56	M	Foot	Foam	19	Yes	SG	Yes	No	Yes	Yes
21	49	F	Leg	Gauze	10	Yes	SG	Yes	No	Yes	Yes
22	79	F	Foot	Foam	30	Yes	SG	Yes	No	No	No
23	80	F	Leg	Foam	20	Yes	SG	Yes	No	Yes	Yes
24	54	F	Leg	Foam	21	Yes	SG	Yes	No	Yes	Yes
25	34	M	Leg	Foam	19	Yes	SG	Yes	No	Yes	Yes
26	47	F	Leg	Gauze	15	Yes	SG	Yes	No	Yes	Yes
27	45	M	Leg	Gauze	14	Yes	SG	Yes	No	Yes	Yes
28	53	M	Leg	Gauze	25	Yes	SG	Yes	No	Yes	Yes
29	41	M	Leg	Gauze	18	Yes	SG	Yes	No	Yes	Yes

SG skin graft, *granul. ti* granulation tissue, *scar ti* scar tissue, *pat* patient

ESAOTE (Esaote SpA) equipment supported by two probes with 6–18 MHz and 4–13 MHz frequencies. Before the examination, we injected the contrast agent SonoVue (Bracco International, Amsterdam, The Netherlands), consisting of microbubbles of sulpfur hexafluoride, into the veins of selected patients. SonoVue is a contrast agent for ultrasound used in the exploration of the great vessels or organs. It is a loss of millions of microbubbles, each one smaller than a red blood cell. The bubbles reflect the ultrasound signal and increase the echogenicity of blood with respect to other body tissues. The agent consists of powder and solvent for injection.

To support these data, 12 to 15 months after healing, we also took five biopsies on scar tissue of three patients (in all three cases skin grafts were taken from the anterolateral side of the thigh), three biopsies were taken from two patients treated with gauze (one male and one female; mean age, 54; mean time of treatment, 13.5 days) and two from a patient treated with foam (male; age, 63; duration of treatment, 50 days). Then, we led histological and immunohistochemical analyses on these biopsies. The difficulty in obtaining biopsy is to perform a surgical procedure on a healthy person; hence, the number of the biopsies from scar tissue is low. These biopsies were taken from patients who were already having other surgical procedures done close to the lesion, so it was possible, after informed consents, to obtain the biopsies without any further anesthesia.

Results

Biopsies of granulation tissue prior to skin grafting revealed more rounded shaped blood vessels in the gauze-treated patients. The histological and immunohistochemical analyses of biopsies evidence a similar pattern in patients treated with

gauze compared with those treated with foam in regard to inflammatory cells and myofibroblast [1].

After analyzing the data achieved from the semi-quantitative evaluation of VEGF, the average score for the gauze-treated patients was 2 (0 none–3 high); SD, 0.88. For the foam-treated patients, the average score was 0.81; SD, 0.98. The p value was calculated in 0.0165. Analyzed data achieved from the semi-quantitative evaluation of matrix metalloproteases revealed an average score of 2.5 (0 none–3 high); SD, 0.66 in the gauze-treated patients and 1.18; SD, 0.655 in the foam-treated patients. This data show a more concentration of VEGF and matrix metalloproteases in the gauze-treated patients' tissue. (Figs. 1, 2, 3, and 4).

The US examination of selected and homogeneous patients reveals that the newly reconstructed tissue of patients treated with gauze is more similar to the physiological one. Ultrasound analysis of the skin-grafted wounds showed an average depth of scar tissue (mean) of 18 mm, median 20, and SD 5 in the beds of the foam-treated wounds and an average depth of 7 mm, median 7, and SD 0.8 in the gauze-treated ones. Ultrasonography showed that the scar tissue thickness after treatment with foam is approximately twice the scar tissue obtained after treatment with gauze. Hypoechoicity is much more evident in foam-treated patients: it means that the fibrotic tissue is more represented (Table 2).

From the data gathered from echocontrastography, it appears that neovascularization after treatment with gauze is higher. The presence of less scar tissue after NPWT with gauze is accompanied by an increased formation of new mini-vessels. The presence of this blood supply leads to the restoration of the physiological condition.

In the three biopsies taken on the scar tissue after treatment with gauze, we confirmed a minor tissue thickness and disorganization and less sclerotic components compared with the two biopsies taken on the new reconstructed tissue after treatment with foam.

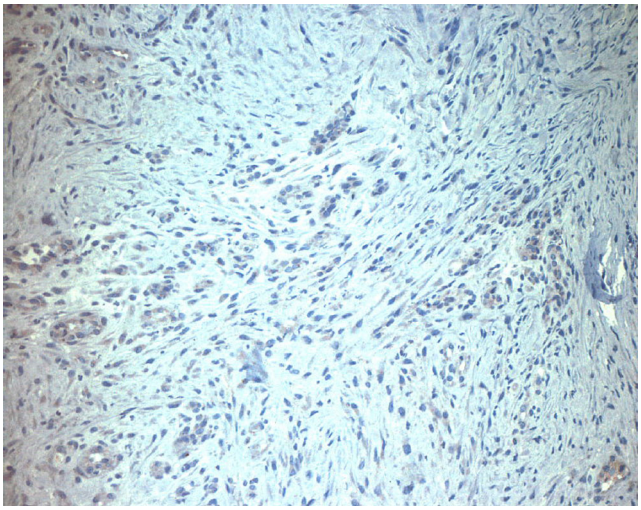

Fig. 2 Vascular endothelial growth factor visualization after foam using avidin–biotin method. (patient 15, Table 1)

Discussion

The aim of this study was to compare the two different fillers presently available, used to apply negative pressure on the wound bed, and to identify if there are different indications for their use according to anatomical areas in relation to the type of granulation and scar tissue obtained.

In our case studies, we noticed a different granulation tissue on the wound bed; using foam, we observed an irregular patchy wound bed which needed a second surgical procedure to flatten the receiving area before skin grafting; using gauze, we observed a uniform

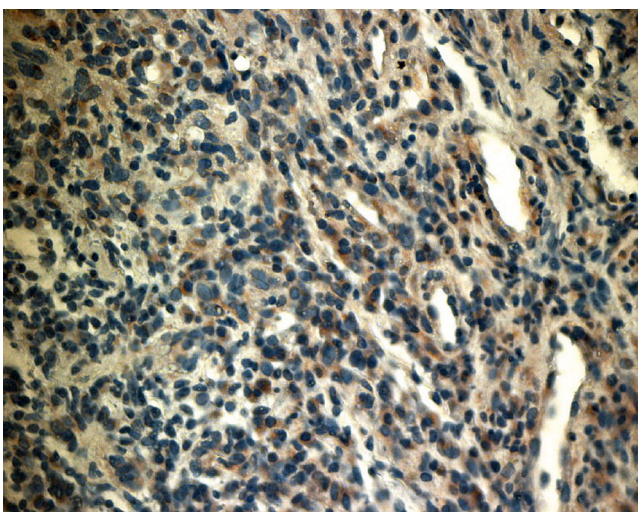

Fig. 1 Vascular endothelial growth factor visualization after gauze using avidin–biotin method. (patient 19, Table 1)

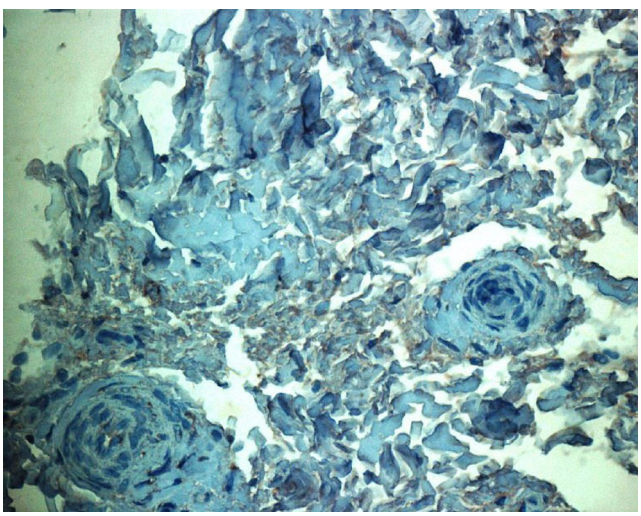

Fig. 3 Metalloproteases visualization after gauze using avidin–biotin method. (patient 4, Table 1)

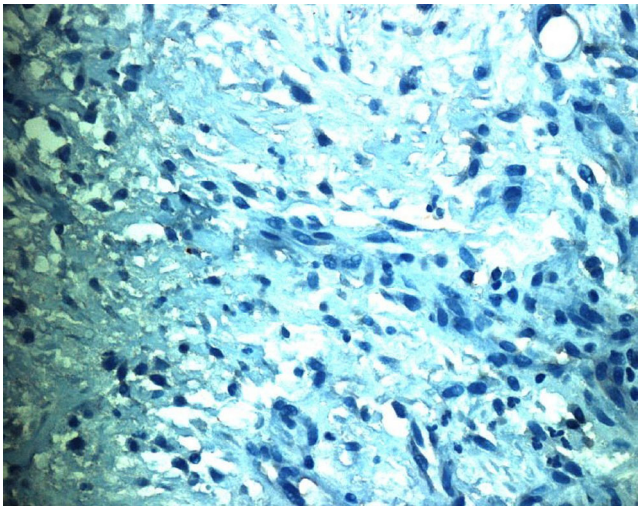

Fig. 4 Metalloproteases visualization after foam using avidin–biotin method. (patient 23, Table 1)

wound bed which was then more adaptable for skin graft. Considering this clinical evidence, we studied if these data coincided with histological specimens [1]. It was the clinical observation that urged us to perform this study. This observation was also supported by the experimental studies obtained from literature which state that there are anatomical differences between the two fillers. Due to the presence of micropores, the foam allows the ingrowth of the granulation tissue (this probably is the reason for the irregular patchy wound bed observed when using the foam) while the gauze because of its dense lines does not permit this ingrowth of the granulation tissue [2].

Table 2 US semi-quantitative evaluation

Patient	Months after healing	Thickness	Hypoechoicity
1	15	+	No
2	9	+	No
4	12	+	No
5	15	+	No
6	15	+	No
7	6	+	No
8	6	+++	Yes
9	15	+++	Yes
12	14	+++	Yes
13	9	+++	Yes
14	6	+++	Yes
16	15	++	Yes
18	15	+	Yes
19	6	+	No
20	6	++	Yes
21	6	+	No
23	7	+++	Yes
24	6	+++	Yes
25	6	++	Yes
26	7	+	No
27	6	+	No
28	7	+	No
29	6	+	No

+5–10 mm, ++15–20 mm, +++20–25 mm. Patients numbers were taken from Table 1

The vascular endothelial growth factor is a signal protein produced by the cells, which stimulates vasculogenesis and angiogenesis. The correlation between NPWT and VEGF is already reported in the literature by two articles. The first one [3] compares a pool of posttrauma patient treated with VAC therapy to a control group. The result of this study revealed a greater level of VEGF in the VAC therapy-treated group. The second one [4] carried on a population of experimental mice after treatment with the foam leads to the same conclusions as the first. The increase in VEGF during the negative pressure is mainly due to two factors:

- The hypoxic area created between the filler and the tissue determines an increase of HIF (hypoxia inducible factor) which in turn determines a greater production of VEGF [4].
- Cellular distortion associated to the fluid removal by the negative pressure, causes an increased production of VEGF by the cells [5].

The matrix metalloproteinases (MMP) are part of a large family of metalloproteinase enzymes that play an important role in wound healing. They are produced by activated inflammatory cells (macrophages and neutrophil) and by cells of the skin (mobile epithelial cells, fibroblasts, and vascular endothelial cells) [6]. There is only one article in the literature that describes the correlation between MMP and NPWT [7] which gains data from patients treated with polyurethane foam at −125 mmHg. This paper reveals a decrease in MMP after negative pressure. This result, in contrast with the data from our work, is explained by the fact that the samples for immunohistochemical examination are taken from the wound exudate and within the first ten days of wound healing; in the early stages of healing process. Obviously, the MMP are low because of the active phase of wound healing. Our samples for immunohistochemical examination are taken from the wound bed after an average of more than 20 days of negative pressure, in a more advanced stage of the wound healing. The MMP play an important role in all stages of wound healing [6], but particularly in the proliferative and especially in scar remodeling phase, through the degradation of the extracellular matrix. The correlation between high level of MMP and

VEGF is explained by the fact that the expression of MMPs is given by inflammatory cells in response to cytokines/growth factors; VEGF is a growth factor [8]. A few articles in the literature, two in particular, both carried out on experimental rats, report that elevated levels of MMPs are able to reduce the formation of scars [9]; up to talk about healing without scar formation [10].

From these results, the elements of a puzzle are slowly reassembling to form a completed picture. In the tissues treated with NPWT with gauze, we observed high levels of VEGF which is correlated to the presence of more rounded and elongated blood vessels, showed with anti cd34 examination. In addition, the echocontrastography study of the tissues treated with gauze demonstrated a greater neovascularization and biopsies of the same scar tissues revealed more rounded and elongated blood vessels [1]. High levels of VEGF (after gauze) explain high levels of MMP in the same treated tissues. This result justifies the lower thickness and the hypoechoicity showed by the ultrasound examination of the tissues treated with gauze. Even biopsies on newly formed tissues treated with gauze revealed a lower thickness [1].

One of the absolute limits of NPWT use is the excessive scar tissue formation. After these results, we can hypothesize that different filler generates different scar tissue. The choice of the filler in order to apply the negative pressure should be dictated by the anatomical areas affected by the lesion. Obviously, less pliability of the wounds means less scar tissue: certain anatomical areas like flexor, extensor regions, the main function of which is movement, are more important than the permanent mechanical strength of the wound. In the areas where the mechanical strength of the wound is more important, we used foam as it gives a better clinical result.

Further studies, which we have already started but still in embryonic stage, will help us understand where is the filler or the level of pressure to determine certain results and if the physical characteristics of fillers are able to give different biological responses [11–15].

Ethical Standards The study has been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Patients gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study were omitted.

Conflict of interest None

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