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Histological change of cbronic ulcers following negative-pressure wound therapy

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Abstract

We treated various ulcerative lesions associated with different causes of ulceration using negative-pressure wound therapy, and investigated the characteristics of the histological changes during the treatment period. The wound surface was then entirely sealed and maintained with a closed dressing material and managed by applying approximately 125 mmHg of continuous negative pressure to the wound surface. Tissue was collected from the central region of the ulcer floor and the collected specimen was macroscopically and histologically evaluated. Using this device, the remission of edema, neutrophil infiltration, capillary blood vessel formation, and collagen fibers advancing in the vertical direction from the wound floor were observed regardless of the cause of ulceration. The results of histological examinations also confirmed that favorable granulation is achievable in chronic ulcers. Although this method necessitates the monitoring of the negative pressure load and infection level in the wound during device application, it is simple, less invasive, and effective.

Key words：chronic ulcer, negative-pressure wound therapy, biopsy, granulation

Introduction

Negative-pressure wound therapy has recently been indicated for acute wounds including sternal osteomyelitis and mediastinitis after open heart surgery, which are associated with high fatality rates. The application of negative pressure to open wounds induces granulation in the early phase in a minimally invasive manner. It is very effective in cases exhibiting difficulties with fistula and wound closure[1,2] for favorable wound granulation appropriate for skin grafting[3]. The efficacy of negative-pressure wound therapy has been clinically verified; however, the mechanisms promoting wound healing and the effects on chronic ulcers have not yet been elucidated in detail[4-6].

Although chronic ulcers are being increasingly treated using this device, the therapeutic effects are suspected to vary due to the different causes of ulceration. Unfortunately, the influence of negative pressure on granulation in chronic ulcers has not been investigated and the histological changes during negative-pressure wound therapy remain to be elucidated.

In the present study, we treated various ulcerative lesions associated with different causes of ulceration using negative-pressure wound therapy, and investigated the characteristics of the histological changes during the treatment period.

Methods

The study-subjected patients were provided with written informed consent prior to this study. The first step in negative-pressure wound therapy involved the removal of unfavorable granulation tissue. The wound surface was then entirely sealed and maintained with a closed dressing material and managed by applying approximately 125 mmHg of continuous negative pressure to the wound surface. The dressing material was

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changed every week, and tissue was collected from the central region of the ulcer floor using Derma Punch with a diameter of 3 mm. The collected specimen was macroscopically and histologically evaluated. HE staining was performed in the histological examination. In order to assess the granulation tissue that was formed, immunostainings for Factor VIII and type Ⅲ collagen were performed.

The collected specimen were fixed by immersion in 10% neutral formalin for 24 hours and dehydrated with an ethanol series. The specimens were paraffin-embedded and 5-um thin sections were prepared. The sections were extended (42 ℃, 16 hours) and deparaffinized. After blocking endogenous peroxidase with 3% hydrogen peroxide (5 min), the sections were reacted with anti-type III and anti-vWF antibodies as the primary antibodies at room temperature for 60 min (type III:1:20, vWF:1:1000). For the secondary antibody, Envision reagent (Dako) was used, and the color was developed using DAB (Nichirei) followed by nuclear staining with hematoxylin.

Case 1: A 49-year-old male with a sacral pressure ulcer

History of the present illness: With malignant rheumatoid arthritis as the cause of ulceration, chronic ulcer had developed in the sacral region. The patient was being treated with an oral adrenocortical steroid at a dose of 20 mg/day to control arthiritis. Although conservative treatment had been performed for approximately 6 months, the ulcer became aggravated and had expanded. Negative-pressure wound therapy was continued for 3 weeks which was followed by split- thickness skin grafting. The grafted skin did not take; however, the treatment of the arthritis with an immunosuppressant was effective, leading to the tapering of the adrenocortical steroid dose, and the wound spontaneously healed after one year (Fig. 1C).

Histological findings: Necrosis and fibrin deposits were noted in the outermost layer of the ulcer floor prior to the introduction of negative-pressure wound therapy. Infiltrations of lymphocytes and edema were detected underneath them. Edema subsided and the outgrowth of capillary blood vessels was noted from one week after the introduction of negative-pressure wound therapy, while cell infiltration mainly with lymphocytes and neutrophils and the outgrowth of abundant capillary blood vessels were observed to form favorable granulation in weeks 2 and 3. Collagen fibers were arranged vertical to the wound (Fig. 2).

Case 2: A 43-year-old male with a diabetic foot ulcer

History of the present illness: Chopart amputation was performed due to the diabetic foot ulcer. Ulcer with severe infection occurred in the weight-bearing region after wearing a lower- extremity prosthesis for one year. Conservative treatment was performed for 3 months, but did not result in epithelization (Fig. 3A, B). Subsequently, negative-pressure wound therapy was introduced.

Histological findings: Collagen fibers accompanied by cell infiltration mainly with neutrophils, lymphocytes, and plasma cells, were observed in the wound prior to negative-pressure wound therapy (Fig. 3B). One week after the introduction of the negative-pressure wound therapy, the outgrowth of capillary blood vessels and interstitial edema were noted on the wound surface. Over the course of negative-pressure wound therapy, outgrowing capillary blood vessels and collagen fibers extended in a direction that intersected the ulcer to form favorable granulation. Immunostaining revealed that type Ⅲ collagen was strongly expressed around the capillary blood vessels, suggesting the proliferation of fibroblasts. The positive expression continued to increase over the treatment course (Fig. 4).

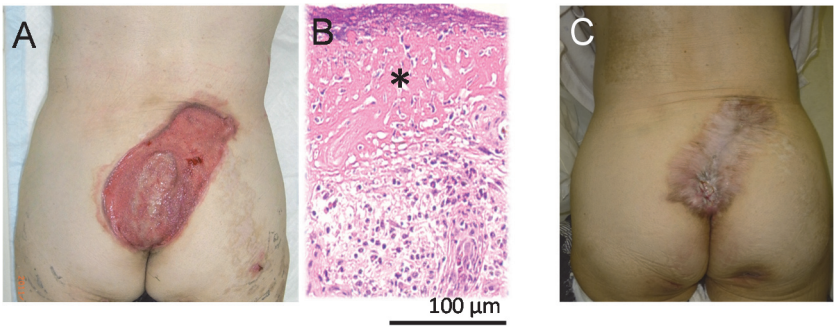

Fig. 1 Case 1
A Macroscopic findings
B Histological findings before negative pressure wound therapy was introduced
C Macroscopic findings after the treatment
(*) Represents necrosis and fibrin deposits

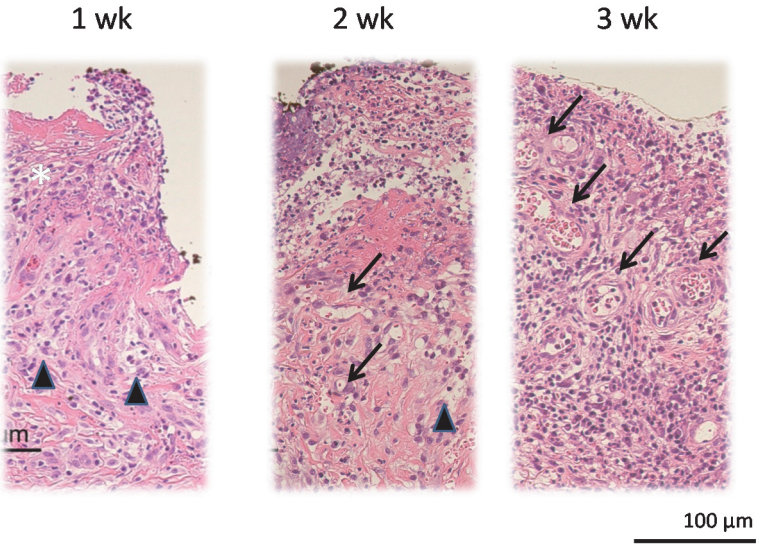

Fig. 2 Histological findings after the introduction of negative-pressure wound therapy
(▲) Represents interstitial edema
(↑) Represents capillary blood vessels

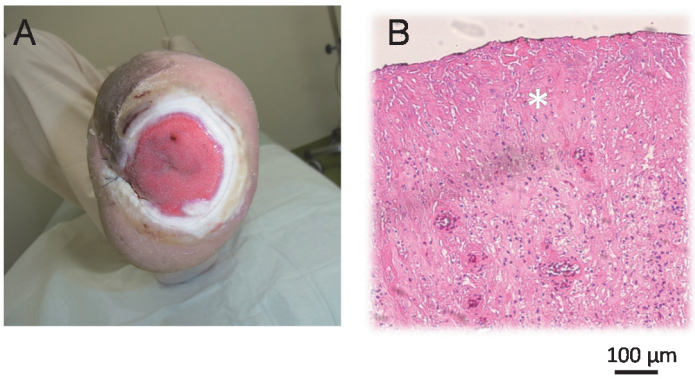

Fig. 3 Case 2
A Macroscopic findings
B Histological findings
(*) Represents collagen fibers accompanied by inflammatory cells

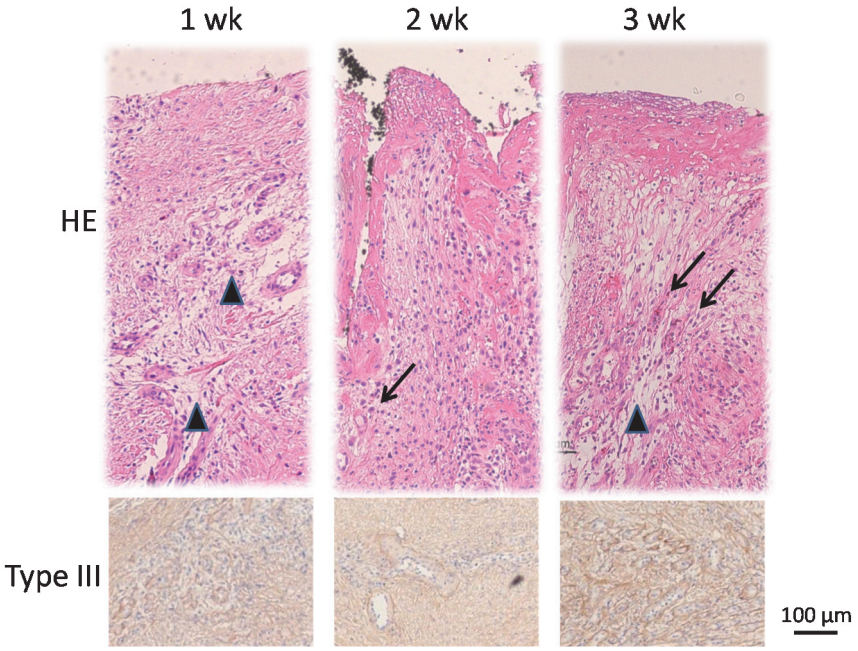

Fig. 4 Histological findings after the introduction of negative-pressure wound therapy
(▲) Represents interstitial edema
(↑) Represents capillary blood vessels

Case 3: A 71-year-old female with a 3rd-degree burn on the right forearm
History of the present illness: The patient was found in the kitchen due to brain hemorrhage accompanied with a 3rd-degree burn on the right forearm. Conservative treatment of the forearm burn was continued for one month until general improvement in brain hemorrhage was achieved, leaving the wound unhealed. Negative-pressure wound therapy was introduced after debridement (Fig. 5).

Histological findings: Interstitial edema and the infiltration of lymphocytes and neutrophils were observed in the wound prior to the introduction of negative-pressure wound therapy (Fig. 5). Interstitial edema subsided over the course of negative-pressure wound therapy, collagen fibers accompanied by lymphocyte and plasma cell infiltration were observed, and favorable granulation was noted. Immunostaining for Factor Ⅷ revealed the outgrowth of capillary blood vessels toward the superficial layer of the ulcer (Fig. 6).

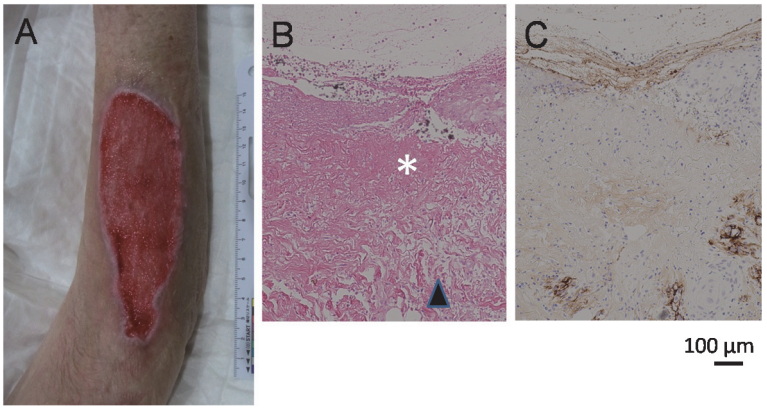

Fig. 5 Case 3
A Macroscopic findings
B Histological findings before negative pressure wound therapy was introduced (HE staining)
C Immunostaining for Factor VIII
(▲) Represents interstitial edema
(*) Represents neutrophil and lymphocyte infiltration

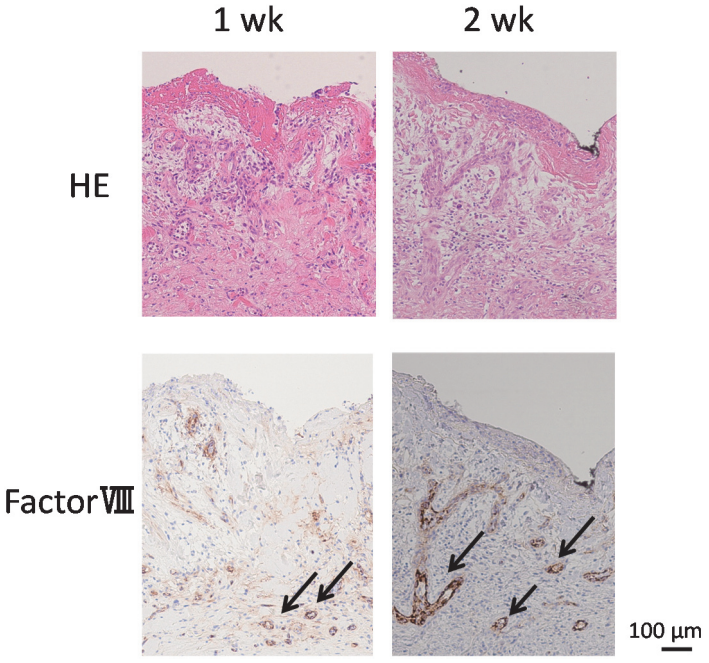

Fig. 6 Histological findings after the introduction of negative-pressure wound therapy
(↑) Represents capillary blood vessels

Discussion

Negative-pressure wound therapy was reported by Argenta and Morykwas et al. in 1997 as a new minimally-invasive wound-healing device that loads a negative pressure to promote granulation [3]. Freischemann et al. subsequently demonstrated that the removal of a harmful exudate by loading a negative pressure on a wound is possible and exerts beneficial effects, such as the removal of interstitial fluid and improvements in blood flow by mechanical stimulation[7]. The efficacy of negative-pressure wound therapy for acute open wounds has already been demonstrated; however, the mechanisms promoting wound healing and the effects on chronic ulcers remain unclear [4-6].

Recent studies on wound healing in a closed negative-pressure environment revealed that a negative-pressure load increases tissue microcirculation in the wound margin and the floor. The highest local blood flow was obtained at 125 mmHg. Although Argenta et al. proposed 125 mmHg as the optimum negative pressure, a similar effect was achieved at 50 mmHg in another study [9]. Clear criteria have yet to be established for the negative-pressure load level, and further investigation is needed.

In chronic ulcers, local microcirculation is insufficient in the wound floor, and favorable granulation is generally not expected. However, using this device, the remission of edema, neutrophil infiltration, capillary blood vessel formation, and collagen fibers advancing in the vertical direction from the wound floor were observed regardless of the cause of ulceration. Furthermore, the results of histological examinations confirmed that favorable granulation is achievable in chronic ulcers. Basic studies on the mechanisms responsible for the remission of interstitial edema induced by negative-pressure loading have recently progressed, and they suggest that interstitial edema is not due to the movement of interstitial fluid, but is affected by changes in vascular endothelial cells due to negative-pressure-induced shear stress [10,11].

In Case 1, in which a steroid was administered at a high dose for malignant rheumatoid arthritis, wound infection was induced after the application of the device. Although granulation was macroscopically and histologically observed, the grafted skin did not take, indicating that the effect of negative-pressure wound therapy is limited by infection. Prior to applying the device, careful consideration is needed when infection control has been insufficient.

Although this method necessitates monitoring of the negative pressure load and infection level in the wound during device application, it is simple, less invasive, and effective. Favorable granulation is achievable in chronic ulcers with various causes, suggesting that this is a useful method capable of shortening the overall healing period necessary for chronic ulcers.

Disclosure Statement

The authors declare no competing interests and there was no financial support.

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