A histological analysis of chronic wounds treated with negative pressure wound therapy to aid healing: a case series

Objective: Our aim was to examine histopathological changes in three chronic wounds, and to assess whether the application of negative pressure wound therapy (NPWT) changes the wound bed environment.
Method: -
Results: We recruited three patients were recruited who had a non-healing wounds in excess of eight weeks. Histopathological changes in the wound bed supported the evidence that NPWT changes the wound bed environment by reducing inflammatory damage and facilitating wound bed perfusion with angiogenesis.
Conclusion: We suggest that NPWT is a valuable adjunct to aid chronic wound healing. In this case series, we observed histopathological changes and improvement in the wounds, following one week's treatment with NPWT. Further analysis should be done to demonstrate any interaction between the cells involved in the wound healing process is enhanced, growth factor performance is optimised and cell migration is achieved following NPWT, in order to facilitate the healing process.
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angiogenesis • chronic wounds • histopathological change • negative pressure wound therapy

Negative pressure wound therapy (NPWT) is recognised as an important adjunct in supporting and promoting wound healing.[1,2] Although NPWT comes in different sizes and interface mediums (foam or gauze), the mechanism is the same—a vacuum is applied to the wound bed which creates a negative pressure environment. However, the way in which it stimulates healing are not completely understood.[1,2] The frequently cited explanation of the mechanical effects of NPWT is based on reviews by Ingber,[3] which describes how NPWT's physical forces impact on biochemical responses at a cellular level. According to Ingber's theory, NPWT stimulates chemical messengers essential for wound healing by a means of interstitial pressure on the wound memberane.[3,4] These messengers lead to the immediate activation of immediate early (IE) genes, followed by matrix molecule synthesis and cell proliferation.[5,6]

A numbr of reports, in different woudn types, have shown NPWT reduceds exudate levels and oedema. In patients with bilateral hand burns, the removal of exudate by NPWT resulted in increased perfusion, and reduced oedema.[7] In an experimental study on septic open porcine abdomen, it was shown that the NPWT-treated wounds had less tissue oedema than those treated by passive drainage.[8] In the periwound tissue in a small group of pressure ulcer (PU) patients, treated with NPWT, high-frequency ultrasound has been used to quantify the level of oedema reduction.[9] The literature suggests that oedema and exudate are reduced, both directly through mechanical removal of excess fluid, and indirectly through altered microcirculation.[4,10] The cellular impact of NPWT on the wound has yet to be documented.[4,7,11,12]

NPWT is a treatment used on complex and chronic wounds. It enhances healing through several mechanisms of action including:[3,7,8,13–16]

- Decreases tissue oedema in extravascular space
- Increases vascular flow and promotion of angiogenesis
- Reduction of the bacterial load and inflammatory response
- Provides a moist wound environment that increases fibroblast proliferation
- Decreases metalloproteinase (MMP) activity and enhances macrophage activity.

Aim

The purpose of this research was to assess the cellular impact of NPWT in chronic wounds, by examining the histopathological changes and assessing the evidence as to whether the application of NPWT changes the wound bed environment.

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Fig 1. Case 1, a 70-year-old female with diabetic foot ulcer (DFU) of two months' duration. Before negative pressure wound therapy (NPWT) (**a**). The DFU one week after treatment with NPWT (**b**)

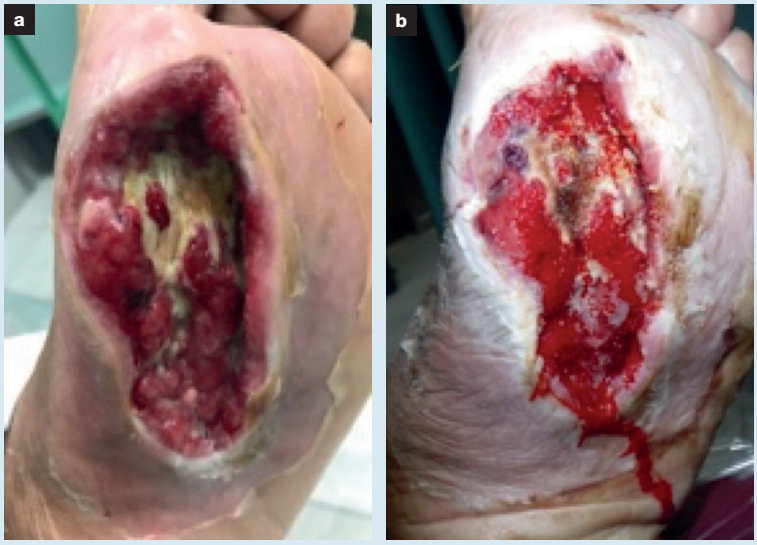
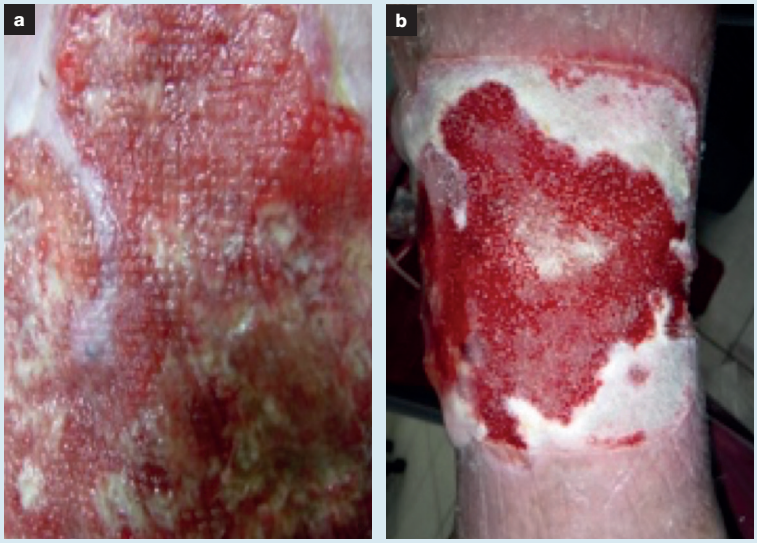


Fig 2. Case 2, a 58-year-old male with venous leg ulcer (VLU) of four years' duration. Before negative pressure wound therapy (NPWT) (**a**). The VLU, one week after treatment with NPWT (**b**)



Method

A histological analysis was conducted between December 2016 and March 2017 in the outpatient wound clinic at Hospital Mexico, San Jose, Costa Rica. Wound assessments and photographs were taken at the initial patient assessment, and at the end of the evaluation period, using local Trust policies and guidelines from the hospital's Wound Clinic, which is part of the hospital's dermatology service.

Inclusion and exclusion criteria

All patients were identified as suitable for this evaluation following a comprehensive medical assessment by the medical team. Patients were attending the hospital's wound clinic and were included in the study if they had a chronic wound in excess of eight weeks, were able to understand and comply with the evaluation protocol, and were willing to give written informed consent to punch biopsy and application of NPWT. For this study, we defined a chronic wound as a wound that fails to heal in an orderly set of stages and in a predictable amount of time.[17] All participating patients gave full consent for photographs and entry into the evaluation.

Patients were excluded if they were under 18 years of age, were on anticoagulant therapy, were immune compromised, if they had a previous history of cancer, had any known allergies to dressing components, and/or were unable to give informed consent. All patients were free to withdraw at any time from the study or when clinically indicated. All patients' medications and standard treatments were reviewed by the medical team, with advice given on diet and supplements suitable for glycaemic control.

Treatment protocol

Patients were treated in the outpatient wound clinic, three times a week for one week, during the study period, were followed-up as outpatients and were given standard treatments, such as foams and alginate dressings with compression bandages. Case 3 had a successful skin graft and was followed up until complete healing had occurred.

Four punch biopsies were taken from the border and wound bed of each patient before treatment with NPWT (Genadyne XLR8, Genadyne Biotechnologies, Hicksville, NY, US). Similar biopsies were taken at each NPWT dressing change at two days, four days, and six days. Wounds were measured and photographed at each outpatient visit, and at the time of punch biopsy.

Appropriate education and training was given to staff, patients and family members, on the use of the NPWT device before and during treatment. Family members were key in supporting home care as nurses were not available for house visits. Patients' comorbidities also continued to be managed during their treatment with NPWT.

Histopathological analysis

Histopathological analyses for granulation tissue, inflammatory cells, angiogenesis and extracellular matrix (ECM) deposition were performed. For each patient, an intial biospy (basal) was taken before treatment with NPWT. Subsequent biopsies were taken on days two, four and six of NPWT treatment. Each biopsy was an incisional biopsy using a skin punch of 4mm diameter. The formalin-fixed tissue was hemi-section and processed in paraffin-embedded blocks. For each specimen, six step-sections were taken and stained using the haematoxylin-eosin technique. All slides

were evaluated by a certified pathologist using a light micrscope (Olympus CX-30).

Histological parameters evaluated in the different biopsy times for each case presented included:

- Inflammatory cells
- New blood vessels
- ECM deposit
- Oedema.

Each parameter was graded as follows:

- 0—no evidence
- 1—occasional
- 2—moderate
- 3—abundant.

Results

A total of three adult patients were assessed for eligibility and recruited to the study. Wound types included:

- Case 1: diabetic foot ulcer (DFU)
- Case 2: chronic venous leg ulcer (VLU)
- Case 3: cutaneous *Leishmaniasis* ulceration of the lower limb.

All of the patients in this study had wounds that had failed to heal from two months to four years. All patients completed the study.

Table 1 depicts the results for the histopathological parameters of the healing process evaluated at the different biopsy timepoints for each case presented. Evaluation covered four basic parameters (all contained in the granulation tissue). The inflammatory cells (leucocytes), angiogenesis (new blood vessels), deposit of new extracellular matrix (by fibroblasts) and oedema. In all cases, there was a boost in all parameters (more pronounced in Cases 1 and 2).

Case 1

A 70-year-old female with a past medical history of hypertension, type 1 diabetes with nephropathy, and osteomyelitis of the metatarsal on a Wagner 2 neuropathic DFU (Fig 1). The patient had been treated with alginates at home for one month. Following a further month's treatment at the wound clinic, with no granulation visible, and persistent slough at the wound bed, the patient was admitted to hospital for management with IV antibiotics. After two weeks treatment with anitbiotics, NPWT was started. Wound size before treatment with NPWT was 7.5x4cm. After one week's treatment with NPWT, a good granulation bed was obtained. Although the wound size had only reduced by a small fraction (to 7x4cm), granulation tissue had risen about three-quarters of the depth of the wound (Fig 1b). After completion of antibiotic treatment (four weeks in total) the patient was able to continue treatment as an outpatient, controlling their diabetes through the standard treatment method. Skin closure was obtained with alginates.

Case 2

A 58-year-old male with type 2 diabetes, on oral hypoglycaemic medication since 2007, and with a deep vein thrombosis (DVT) of the left leg in 2012 (Fig 2). The patient had a chronic leg ulcer of four years' duration which was not responding to traditional wound care including primary dressing, alginates and compression therapy. Wound size at the start of treatment with NPWT was 9x12cm. After one week's treatment with NPWT, exudate had decreased, no pain present and the patient was able to return to his normal activities. The wound had reduced in size to 8x10cm, with granulation tissue almost to the level of the wound bed borders, and re-epithelialisation from the wound borders to the centre. The patient then continued on compression therapy. However, since this study, the patient has, unfortunately, regressed as they did not feel able to continue with the compression therapy, leading

Fig 3. Case 3: 25-year-old male with three-year-old chronic leg ulcer with *Leishmaniasis* (**a**). Day two, following applicaton of negative pressure wound therapy (NPWT) (**b**). Day four, post NPWT (**c**). At six days post NPWT (**d**)

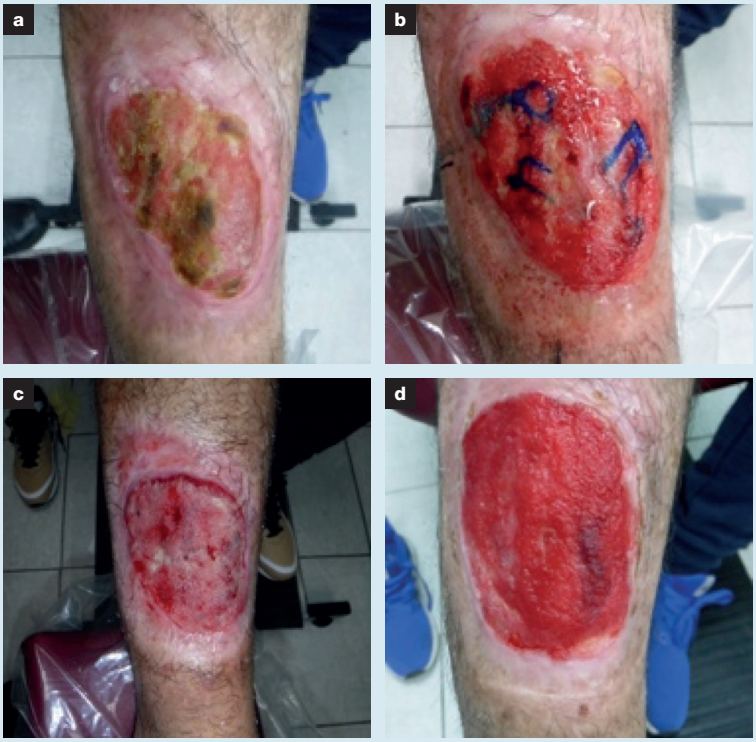


Table 1. Histopathology results

	Case 1 Diabetic foot ulcer				Case 2 Venous leg ulcer				Case 3 Leishmania ulceration			
Day	**0**	**2**	**4**	**6**	**0**	**2**	**4**	**6**	**0**	**2**	**4**	**6**
Inflammatory cells	1	3	3	2	1	3	3	2	1	2	2	2
New blood vessel	0	3	3	1	1	3	3	1	0	2	3	1
ECM deposit	0	2	3	2	0	2	3	1	1	2	2	2
Oedema	0	2	2	2	0	3	3	2	0	2	2	1

ECM—extracellular matrix; 0—no evidence; 1—occasional; 2—moderate; 3—abundant

Fig 4. Histological presentation of the basal and two-day biopsies in two cases. The pre-treatment biopsy of case 1 showed a diffusely hyalinized stroma with almost no-inflammatory infiltrate (**a**). The two-day post-treatment biopsy exhibits a diffuse mixed inflammatory response, with new blood vessels and oedema (granulation tissue) (**b**). These same changes were observed in case 2, in both the pre-treatment biopsy (**c**), and two-days post-treatment biopsy (**d**)

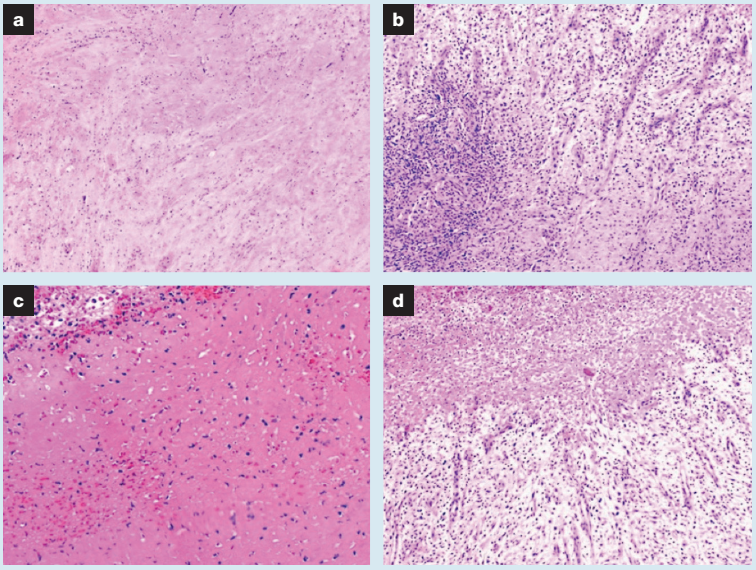
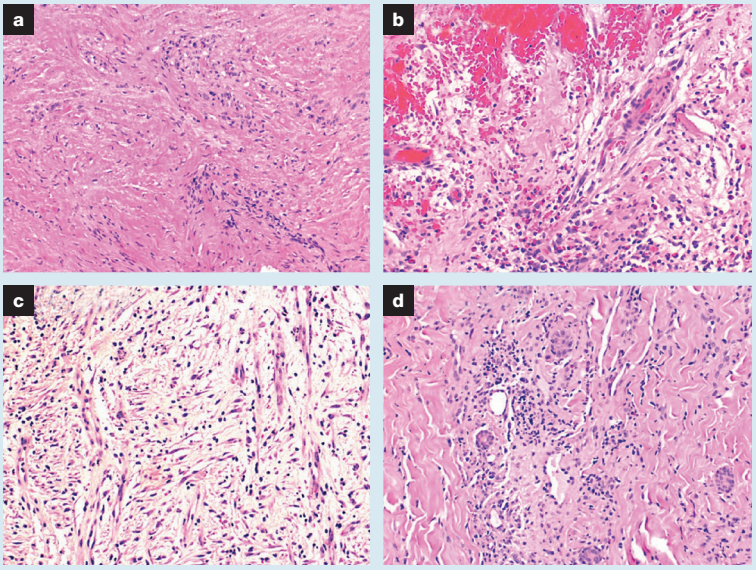


Fig 5. Chronological evolution of the different histological parameters evaluated (inflammatory infiltrate, neovascularisation and oedema) in case 3. The most prominent changes were documented at two and four days, with a brisk inflammatory response and formation of new capillaries. Before NPWT (**a**), and at two days post-NPWT (**b**), day four, post-NPWT (**c**) and six days post-NPWT (**d**)



to a deterioration of the wound. The patient continues to be followed up in the chronic wound clinic.

Case 3

A 25-year-old-male with no known comorbidities. A diagnosis of *Leishmaniasis* on a three-year-old chronic leg ulcer was made and glucantime treatment was initiated (Fig 3). Due to a poor response after 80 ampules of glucantime, a polymerase chain reaction (PCR) diagnosis of *Leishmania (Viannia) panamensis,* a resistant strain of *Leishmaniasis*, was made and treatment with mefloquine was initiated. At the start of treatment the wound size was 5.5x7cm. Although the wound size did not decrease following treatment with NPWT, wound bed preparation with increased granulation, and re-epithelialisation and systemic therapy was achieved six days post-NPWT treatment. The patient went on to have a successful skin graft, one month post-treatment, which healed successfully, and has been discharged from the clinic.

Histology biopsy results

In all cases, the biopsies taken before treatment with NPWT showed a paucicellular background composed mainly of hyalinized stroma with sparse lymphocytes and few (small calibre) blood vessels. The two-day biopsies displayed a marked change in cellularity, with a diffuse inflammatory infiltrate (mixed type), neovascularisation and oedema (compatible with granulation tissue). These changes were sustained in the four-day biopsies, and declined in the six-day biopsies (with less inflammatory response and oedema) (Fig 4).

Following one week's treatment with NPWT, a reduction in the inflammatory or senescent cells, improvement in angiogenesis and granulation, and the presence of fibroblasts were observed, consistent with the early healing process, as seen in Figs 4 and 5.

Discussion

Histopathological findings on tissue biopsies, taken on all wound types on entry to this study, before the application of NPWT, showed a basal chronic wound bed composed mainly of senescent cells with sparse lymphocytes and few (small calibre) blood vessels. After the use of NPWT, there was a visual improvement in both angiogenesis and proliferation in granulation, correlating with the clinical finding of the first stages of the healing process.

The two-day biopsies displayed a marked change in cellularity, with visible granulation tissue, and wound contraction. These cellular changes were sustained in the four-day biopsies and declined on the six-day biopsies with less inflammatory response and oedema.

The authors examined the literature in relation to histopathology in patients with diabetes treated with NPWT that may support the findings in this paper.[18,19] Walgenbach et al.[20] suggest that there was a proliferation of endothelial cell activity in the newly formed granulation tissue after the application of NPWT. Walgenbach's finding also showed evidence of endothelial cells proliferation in the wound exudate of patients with neuropathic DFUs.[20] This was also the case for the punch biopsies taken from the patient with a DFU during this case study. In a randomised controlled

trial (RCT) of 30 patients with DFUs, Kopp et al.[21] demonstrated an increase of growth factors within the wound fluid of those treated with NPWT, compared with the control group treated with a hydrocolloid dressing (it should be noted that the group treated with NPWT was also treated with a hydrocolloid dressing). In a RCT of 162 patients, where 77 patients had NPWT compared with 85 patients receiving standard care treatment, Armstrong et al.[22] concluded that 'NPWT seems to be a safe and effective treatment for complex diabetic foot wounds. Treatment with NPWT resulted in a higher proportion of wounds that healed, faster healing rates, and potentially fewer re-amputations than with standard treatment'.

The European Wound Management Association (EWMA)[11] suggests caution in that the use of NPWT in non-healing neuropathic or neuro-ischaemic ulcers, in both cases, possible ischaemia and infection must be addressed before applying NPWT. With regards to the DFU in this study, the underlying infection and neuro-ischaemia was addressed before the application of NPWT, in line with national guidelines.[23]

The decision to use NPWT for the patient with the chronic VLU (Case 2) was in order to relieve the symptoms of excessive wound exudate which was having a significant adverse effect on the patient's quality of life. The findings in the literature supported the challenges the authors of this paper experienced with regards to Case 2, including periwound maceration, leaking and soiled dressings associated with malodour.[11,23] While the initial histopathology was encouraging, the findings suggest that chronic VLUs are complex, and cellular changes alone cannot indicate healing. It was beyond the remit of this study to explore if the extended use of NPWT, compared with compression alone, would have reduced symptoms and improved healing rates over a longer period of time.

With regards to the selection of the patient with *Leishmaniasis*, this patient was chosen as they met the criteria of a chronic wound with a duration of three years. In the authors' experience, *Leishmaniasis* cases usually heal with a combination of glucantime and standard dressings. *Leishmaniasis* is a tropical disease transmitted to human skin by the bite of a sandfly. The disease then invades human macrophages and replicates intracellularly.[24,25] A raised, red lesion develops at the site of the bite (often weeks or sometimes years afterwards). The lesion then ulcerates and may become secondarily infected with bacteria. In many species, the infected lesion often spontaneously heals with atrophic scarring,[26] which in some species (for example, *Leishmania braziliensis*) may reappear elsewhere.[26] Infection with different *Leishmania* spp. protozoa can lead to an inflammatory response in the skin as neutrophils are the first inflammatory cells to migrate to the site of infection.[25,26] The gold standard for diagnosis is PCR.[25] Treatments that work for one species of *Leishmaniasis* may not work for another and requires specialist tropical medical advice.[26]

Limitations

A limitation of this evaluation was that patients were evaluated for only a short period of time, and it was focused on a small sample size. Although all patients had encouraging responses pathologically, it was beyond the remit of this evaluation to continue the NPWT. Calculating the cost of treatment before entry to the evaluation was not possible, and further robust data to assess the possible cost-effectiveness of using NPWT in the treatment of chronic wounds would be required.

Conclusions

The role of NPWT as a way to quickly stimulate healing in chronic wounds has not been established. However, this study demonstrates that one week of NPWT exerts a beneficial effect on chronic wound healing by decreasing inflammatory cells, and increasing angiogenesis and ECM deposition, creating a wound environment suitable for the use of another standard dressing.

NPWT is a technology that has the potential to reduce the healing time in hard-to-heal wounds. We suggest that although some studies state that NPWT improved cell communication, cytokine and growth factor expressions profiles to promote wound healing, the exact mechanism and timing in the wound healing process is still not clear.

This one week study of the use of NPWT in wound healing would suggest that it probably helps to 'turn on' the signalling processes required to booster the healing process.[27] These ECM interactions not only guide and regulate cullular morphology, but also cellular differentiation, migration, proliferation, and survival during tissue development[28] probably due to cellular micro-deformation induced during NPWT[29] that contributes to the angiogenesis, ECM remodelling and deposition of granulation tissue obtained. Once a good granulation bed is obtained, other wound care modalities can be applied to continue the healing process.

Further analysis should be carried out to better understand how the interaction between cells involved in the wound healing process is enhanced, growth factor performance is optimised, and how cell migration is achieved in order to facilitate the healing process. JWC

Reflective questions

- How does negative pressure wound therapy (NPWT) enhance healing in chronic wounds?
- What were the histopathological findings of NPWT in this study?
- In your own practice, could NPWT be used to in the way presented in this paper?

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