

REVIEW ARTICLE



Revisiting negative pressure wound therapy from a mechanobiological perspective supported by clinical and pathological data

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Abstract

Negative pressure wound therapy is used often in the management of surgical incisions, chronic wounds and subacute lesions, and there are numerous publications discussing its clinical application and outcomes. However, whilst clinical use and associated literature have expanded since these systems became commercially available in the 90s, important research and discussion around the mode of action have waned, leading to a deficit in the understanding of how this important therapy influences healing. Further, much research and many publications are predominantly reflective, discussing early theorem, some of which have been proven incorrect, or at least not fully resolved leading to misunderstandings as to how the therapy works, thus potentially denying the clinician the opportunity to optimise use towards improved clinical and economic outcomes. In this narrative review, we discuss established beliefs and challenges to same where appropriate and introduce important new research that addresses the manner in which mechanical strain energy (i.e., deformations) is transferred to tissue and how this influences biological response and healing. In addition, we assess and discuss the effect of different negative pressure dressing formats, how they influence the mode of action and how this understanding can lead to more efficient and effective use and clinical economic outcomes.

Abbreviations: CB, canister-based; CDU, Colour Doppler ultrasound; CL, canisterless; MOA, mechanism of action (or mode of action); MLD, multilayer dressing; NPWT, negative pressure wound therapy; suNPWT, single-use negative pressure wound therapy; tNPWT, traditional negative pressure wound therapy; VEGF, vascular endothelial growth factor.

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KEYWORDS

chronic wound care, influence zone theory, mechanisms of action, neo-angiogenesis, ultrasound Doppler

Key Messages

- Microdeformations at the wound environment stimulate granulation tissue growth.
- Shifting from traditional to single-use negative pressure wound therapy (NPWT) during treatment may improve healing.
- The influence zone concept explains a broader mechanical stimulation in single-use NPWT.
- New insights are reported on NPWT-induced angiogenesis following the hypoxic phase.
- Integrating mechanobiology concepts in single-use NPWT will refine treatment protocols.

1 | INTRODUCTION

1.1 | Overview of the known mechanisms of action of negative pressure

Since the first description of the utility of negative pressure wound therapy (NPWT) and its ability to accelerate wound healing, published by Morykwas et al.¹ and Argenta and Morykwas² in 1997, wound care has been revolutionised through the application of this technology to the management of acute and chronic wounds. However, although many agree that there is no unifying theory as to the mechanisms/modes of action (MOAs) of NPWT,³ and to a large extent the complete set of MOA is still unknown, particularly concerning cell scale and molecular scale effects,^{4,5} the well-known and generally perceived MOAs at the secondary effect level, which at the forefront include a tendency towards increased blood flow and tissue perfusion, have been questioned in some quarters.^{6–10} On this point, much of the historical evidence regarding NPWT has been derived from a fundamental misunderstanding of the biological and physiological processes taking place, particularly with respect to the influence of NPWT systems on soft tissue pressure levels and more generally speaking, internal tissue loading changes^{6,8,9,11} and the consequent influence of the system on wound perfusion.⁷ Hence, there remains considerable debate regarding the MOAs of this now commonly used intervention.

Kairinos⁶ and Macionis¹¹ recognise a lack of consensus with respect to the biophysics and biomechanics underlying the MOAs of NPWT, particularly with reference to whether it reduces or increases soft tissue pressures under suction; Kairinos et al.⁷ note that a correct understanding of processes in play with respect to tissue pressures will lead to a better understanding of perfusion

changes under NPWT. The importance of clarifying the controversy surrounding the impact of NPWT, whether negatively or positively, on tissue pressures, and consequently perfusion, lies in the fact that it will influence other aspects of NPWT, including the direction that future research should take to elucidate its MOA as well as enabling the better formulation of indications and contraindications for its use.

A widely accepted and cited aspect of NPWT hinges on reports of up to fourfold increases in tissue perfusion.¹ In this narrative review work, dissecting the MOAs of NPWT, we will weigh the above publications against other, more recent evidence which potentially reverses the widely held belief that this increased perfusion is a fundamental MOA type supposedly occurring due to blood vessel dilation under NPWT.⁷ Accordingly, our guiding research question was ‘*What are the primary and secondary MOAs in NPWT, and how does the evolving understanding of these mechanisms impact clinical effectiveness and optimization of NPWT systems and treatments?*’ To address this question, we conducted a thorough literature review using the following databases: PubMed/Medline, Cochrane Library, Scopus, Google Scholar and the Web of Science. We focused on peer-reviewed original research articles, literature reviews, evidence synthesis, systematic reviews, meta-analyses, scoping reviews, narrative reviews, critical reviews, chapters in books and formal publications of professional medical societies. We excluded non-peer-reviewed sources such as conference proceedings, meeting abstracts and posters. Our review was limited to studies involving adult populations requiring advanced wound care, and we excluded non-English publications and studies published more than 30 years ago. An iterative process was used to identify key search terms, starting with the keywords: angiogenesis; cellular mechanotransduction; exudate management; foam dressing; granulation tissue formation; histopathology;

macro-deformation; MOA; mechanobiology; micro-deformation; multilayer dressing (MLD); NPWT; single-use NPWT (suNPWT); tissue perfusion; traditional NPWT (tNPWT); wound contraction; wound environment optimization; wound healing and wound microenvironment. Rather than a limited macroscale analysis, we place particular emphasis on the micro-scale mechanobiological impact of NPWT and the cellular changes resulting (Figure 1).⁷ We support our hypotheses by Doppler ultrasound measurements of open wounds and histopathology studies conducted by the clinical authors, however, as will be discussed further, additional controlled clinical studies are still needed to validate these findings.

The objective of this narrative review supported by wound case and pathological studies is therefore to investigate and elucidate the MOAs underlying NPWT through a comprehensive analysis of previous literature, to better distinguish between primary and secondary effects of NPWT, aiming to provide a deeper

understanding of how NPWT promotes wound healing and its potential implications for clinical practice. It is our hope that the current observations, in terms of what is actually occurring within the wound under NPWT, in light of a new mechanobiological understanding of at least some aspects of its MOA, can re-ignite interest in this wound care research and development area.

1.2 | The classic mechanisms of action suggested for NPWT

The concept of NPWT was first described by Morykwas et al.¹ and Argenta and Morykwas² in 1997 who conducted a series of animal and human studies, respectively, using a novel subatmospheric pressure system developed by KCI under the trade name Vacuum Assisted Closure System. Although the basic principles of applying subatmospheric pressure or suction (often wrongly referred to as 'negative pressure', as physical pressure can only be a positive value^{12,13}) to various types of the wound are the same, the technique is referred to by a variety of different terms including micro-deformational wound therapy, negative pressure therapy and NPWT.¹² However, for the purpose of the current work we will use the term NPWT generically throughout, even though the majority of early studies were conducted with the KCI Vacuum Assisted Closure technology.

In general terms,^{1,2,12,14,15} the application of tNPWT systems involves conveying subatmospheric pressure, either continuously or intermittently,¹ to the wound bed by way of foam^{1,2} or gauze dressings (Figure 1A). The wound dressing behaves as a pressure manifold, to which negative pressure is applied, and commonly takes the form of reticulated, open-cell polyurethane foam, in which the open pore structure of the dressing provides the pressure transduction. Once in place the foam dressing is covered and sealed with a semi-occlusive polyurethane film, or drape, through which a hole is cut, and a suction pad attached from which an outlet tube leads to a vacuum pump and fluid collection canister. Optionally, a wound contact layer is applied at the skin-foam interface to avoid tissue ingrowth into the open pores of the foam which causes disruption to the tissue, and often incomplete re-epithelialisation and pain to the patient on removal of the dressing.^{16,17} Under the suction created by the vacuum pump the foam dressing collapses into the wound, effectively forming a closed wound, at which point as the foam is pushed into the wound the skin is compressed into the intra-strut spaces of the sponge, and in doing so, the sponge stretches and stimulates the skin.¹⁵ As these mechanical forces are applied it results in tension being applied to the tissue substrate (Figure 1).

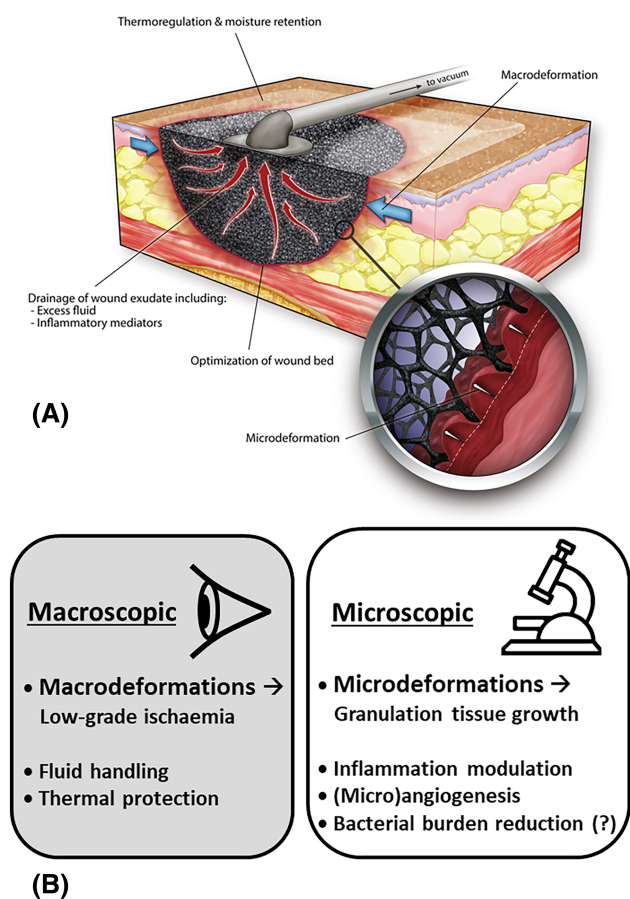


FIGURE 1 The modes/mechanisms of action (MOAs) of negative pressure wound therapy: (A) a conceptual description reproduced from Huang et al.¹² Under a Creative Commons licence and (B) the MOAs classified according to their presence in the macroscopic versus the microscopic scales as per the current analysis of literature.

Using the dressing construct described above, and with pressure levels of 125 mmHg below ambient pressure, Morykwas et al.¹ conducted four studies utilising a pig model to assess the effect of NPWT on (1) laser Doppler velocimetry-measured blood flow in wounds and adjacent tissues; (2) rate of granulation tissue formation; (3) bacterial clearance from infected wounds and (4) quantification of nutrient flow through random-pattern flap survival. In the work of Morykwas et al.,¹ the blood flow (or perfusion) levels increased fourfold (400% above baseline) when 125 mmHg subatmospheric pressure was delivered to the foam above the wound site. Rates of granulation tissue formation increased significantly when negative pressure was applied either continuously or intermittently. Bacterial counts in tissue decreased significantly after 4 days, and the random-pattern flap survival was significantly increased. It is worth noting that the findings of tNPWT effects on bacterial load are inconclusive, with studies other than those of Morykwas et al.¹ recording no influence on the bioburden levels in the studied wounds, or a tendency towards an increased bioburden.¹⁸ A more recent innovation, one form of NPWT that has been demonstrated to be highly effective in reducing bioburden and supporting to resolution of infections is the so-called NPWT instillation.¹⁹ This innovation supports the intermittent instillation and evacuation of non-medicated solutions such as normal saline and fluids containing antimicrobial agents, and in many institutions, this is now the front-line therapy in addressing complex infected wounds.

From the landmark study of Morykwas et al.,¹ it was concluded that ‘application of controlled subatmospheric pressure creates an environment that promotes wound healing’. Of 300 wounds treated in a clinical study conducted by Argenta and Morykwas,² 296 wounds responded well to NPWT as indicated by increased rates of granulation. Thus, the now widely held consensus on primary mechanisms of action of NPWT encompasses: (1) promotion of increased perfusion¹; (2) creation of mechanical forces that stimulate a mechanobiological response in tissues and cells²; (3) reduction of oedema and removal of exudate^{1,2}; (4) alteration of wound fluid composition and (5) reductions in bacterial load.¹

1.3 | Histopathology of a NPWT environment during healing

Huang et al.¹² evolved, with others¹³ compartmentalising MOA into primary MOA and secondary effects. Primary MOA encompasses (1) macrodeformation or induced wound shrinkages; (2) microdeformation; (3) fluid removal and (4) optimisation/alterations of the wound

environment. Secondary effects are cited to include (1) modulation of inflammation; (2) cellular responses including division, migration and differentiation; (3) angiogenesis; (4) formation of granulation tissue; (5) peripheral nerve response and (6) changes in bioburden.¹² Through histological assessment, in their study on pressure ulcers/injuries, Salvati et al.²⁰ confirmed and quantified these MOA, additionally noting there appears to be a point at which NPWT no longer elicits a cellular response, possibly indicating that there is a normalisation to the mechanical stressors leading with time to a quiescence state. Specifically, the Salvati et al. study²⁰ investigates the anatomo-pathological evaluation of scarring in 250 elderly patients with chronic cutaneous wounds following NPWT treatments. The authors observed a reduction in wound areas, particularly in the first few weeks of treatment. The biopsies acquired at different time points to assess the wound evolution revealed distinct histological changes over the treatment period, culminating in the observation of an ‘immature scar’ at approximately 21 days post-treatment (Figure 2). Salvati et al.²⁰ concluded that NPWT is most effective within the first 3 weeks but requires further research to elucidate underlying cell signalling and molecular mechanisms affecting regeneration beyond this period. Histological findings of our expert micropathologist author SR (Figure 2) further revealed consistent repair responses in biopsies in which necrobiotic tissue and ulceration were initially observed and wounds subjected to NPWT: first, an inflammatory stage occurs with increased inflammatory cells and granulation tissue formation; then the inflammation resolves and follows by the proliferation of fibroblasts and myofibroblasts, leading to scar formation; finally, the wound healing progresses into scar formation with decreased cellularity and collagen deposition (Figure 2). The biopsy findings in Figure 2 were overall robust and reproducible, albeit dependent on the specific area and cause of wounds. Our current histological evaluation highlights the effectiveness of NPWT in accelerating the healing process (with different behaviours observed in epidermal and dermo-epidermal layers).

2 | PRIMARY MECHANISMS OF ACTION

2.1 | Macro-deformations and their effect on perfusion levels in the wound environment

Macro-deformation describes the biomechanical effects of NPWT leading to wound shrinkage with a decreased wound surface area and depth,^{5,12} or contraction,^{5,18}

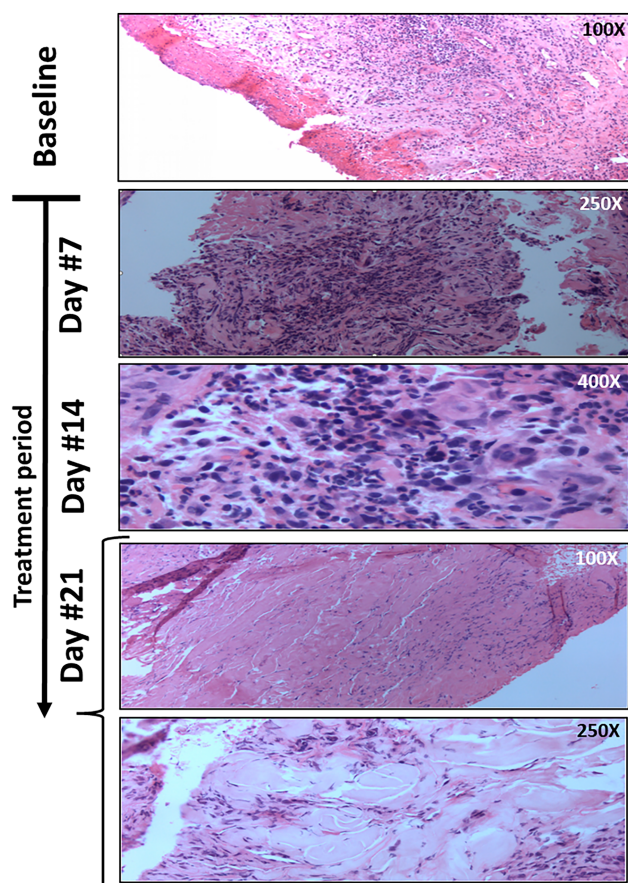


FIGURE 2 Histological weekly progression of wound healing under negative pressure wound therapy over 21 treatment days, demonstrated using Haematoxylin and Eosin staining: at baseline, ulceration with dermal necrosis and fibrinous tissue (fibrin, neutrophils and cellular debris) is shown (magnification level, ML = 100 $\times$). After 1 week, fibromyxoid stroma with increased vascularity, that is, granulation tissue, and inflammatory cells promoting the myofibroblast development-inflammatory stage are demonstrated (ML = 250 $\times$). After 2 weeks, myofibroblasts, the central cells driving fibrosis, appear as morphologically enlarged and irregular (star or web-shaped) spindle cells; these are surrounded by oedema towards the primary extracellular matrix-proliferative stage (ML = 400 $\times$). After 3 weeks, as the wound healing approaches completion, the formerly cellular tissue is transformed into a poorly cellular scar. In the top frame corresponding to day #21, an increased collagen fibre deposition in the dermis is exhibited. The myofibroblasts disappear as the healing progresses. The fibroblasts display a slender fusiform, spindle shape and smooth-contoured nucleus at this stage and the collagen fibres maintain an approximately parallel array, with fascicles that are demarcated by small, thin-walled blood vessels (ML = 100 $\times$). In the bottom frame, which was also acquired on day #21, there is a focus on the transition between immature and mature scar tissues, also showing scattered residual myofibroblasts surrounding a few collagen bundles (ML = 250 $\times$). These histological slides were provided and interpreted by author SR who is an expert pathologist.

induced by the collapse of foam dressing pores and centripetal forces acting on the wound surface whilst under NPWT.^{12,21} This results in the wound edges being drawn together and the peri-wound skin being remodelled to extend accordingly.¹⁷ Mechanical deformations of tissues at the wound edge, if not excessively low or high, are also known to stimulate the formation of granulation tissue.^{1,2,5,12,21,22,23} Observations from *in vitro*⁹ and *in vivo*⁸ studies with NPWT, conducted by Kairinos et al., indicate an increased extracellular pressure in tissues below the wound bed, which is thought to be due to macro-deformation inducing the tissue compression.¹² This latter point is of considerable importance because before Kairinos et al. published their findings on tissue pressure changes under NPWT, the general assumption was that pressure decreases. Orlov and Gefen further demonstrated, by means of computer (finite element) modelling, that the soft tissue volume under the wound bed is subjected to a complex, compound loading state which involves simultaneous compression, tension and shear and is therefore highly nonuniform.²¹ In other words, the uniform negative pressure delivered to the surface of the wound by the NPWT is transformed into inhomogeneous macro-deformations within the tissue depth.²¹ In this context, and with respect to the clarification of how NPWT influences tissue stress states, traditional theories of the effects of NPWT on blood flow need to be revisited and revised, since the tissue loading and blood flow/perfusion quality are inextricably linked.^{8,9,21}

2.2 | Fluid handling of excessive wound exudate and thermal protection of the wound

Fluid handling represents a key treatment element leading to a wound environment that favours healing.¹² Foam dressings and semi-occlusive drapes used in NPWT, which exhibit properties of minimal permeability to water vapour and other gases, provide thermal insulation, so maintaining warmth to the wound,^{12,24} and further maintain a moist and stable wound environment conducive to healing as proposed in the principle of moist wound healing developed by Winter et al.^{12,13,24,25} The evacuation of excess fluids with any accompanying proteins and electrolytes theoretically stabilises oncotic and osmotic gradients at the surface of the wound.^{12,13} Regarding the influence of fluid handling by NPWT, which promotes effective and continuous evaporation of exudate from the external dressing surface utilising the entire surface of the dressing (compared to conservative dressings),^{14,26} it is often the case that chronic wounds

and oedema occur concomitantly, such as in venous leg ulcers.^{12,26} Argenta and Morykwas² noted in a clinical study of NPWT application to chronic wounds, including pressure ulcers/injuries, that chronic oedema was abolished and led to increased localised blood flow. As the application of NPWT to the wound interface facilitates the removal of excess tissue fluids, this leads to a reduction in interstitial fluid contents and as a result, to lower interstitial pressure levels, which in turn reopens obstructed or occluded capillaries, thus restoring blood flow to the peri-wound tissues.²² This is a separate mechanism perceived to increase blood flow than that supposedly occurring by the dubiously proposed reductions in pressure below the NPWT dressing, which purportedly allows dilation of blood vessels with a consequent increase in blood flow/perfusion. We are now questioning the latter mechanism as the findings of Kairinos et al.¹⁰ point to blood flow being in fact reduced by application of NPWT due to the now proven increases in tissue pressures^{8,9} and the compound loading state reported by the Gefen group,²¹ with consequent compression, tension and shearing of blood vessels occurring under the NPWT dressing.

2.3 | Micro-deformation at the wound bed and their impact on granulation tissue growth

Micro-deformation at the foam-wound interface is caused by the microtopography of the porous¹² foam dressings used in the NPWT treatment process,¹² which mechanically stimulates the surface of the wound bed.³ Such 'micromechanical' distortions applied to the wound bed surface are thought to lead to an array of mechanobiological effects useful in the healing process.¹² Predominantly, the micro-deformational signals are thought to influence the development of granulation tissue through promotion of cell proliferation and migration.¹⁴ Ingber²⁷ conclude from their studies that integrins linking to the plasma membrane and actin fibres in the cell cytoskeleton play a key role in cellular mechanotransduction, which is defined as the process by which cells convert a mechanical deformation signal, into a biochemical²⁷ or biological¹² response, such as modulation of proliferation,¹³ differentiation and/or alterations in gene expression or the intracellular environment¹²; mechanotransduction is thought to be integral to the MOAs of NPWT (Figure 3).¹² In this context, based on a computational finite modelling study, Saxena et al.²⁸ proposed that application of micro-deformational forces to the wound site represents the most significant MOA of NPWT and allude to in vitro studies^{29,30} demonstrating

that when cells are subjected to certain substrate deformations there is a tendency for them to divide where soluble mitogens are present. Alternatively, the Gefen group more recently reported that for mechanoresponsive and motile cells such as dermal fibroblasts, micro-deformations promote cell migration to close gaps caused by mechanical damage in confluent cultures (Figure 3).²³

Kinases are a group of enzymes that enable the phosphorylation of molecules to form biochemically active molecules. With respect to this, cell deformation studies further imply that stretching of dermal fibroblasts increases the mitogen-activated protein kinase activity, with one study showing that 1 h after stretching, expression of two types of kinases, namely 'extracellular regulated kinase' and 'Jun N-terminal protein kinase' were increased.²² Both of these kinases, which are upregulated by either tensional stretch of a soft solid substrate or fluid flow-induced shear stresses, are associated with cell division (as well as with having an involvement in the vascular endothelial growth factor (VEGF) pathway that ultimately leads to new vessel growth,²² as discussed further when addressing the secondary result of NPWT treatments in promoting angiogenesis). In elemental terms, soft tissues respond to mechanical forces by adapting their structure, composition and function with fibroblasts transducing the applied forces into biomechanical signals ultimately resulting in their migration and collagen synthesis at the destination for tissue repair; NPWT is the generator of the forces in tissues which drive this soft tissue remodelling via fibroblast activation.^{23,31,32}

3 | SECONDARY MECHANISMS OF ACTION

3.1 | Modulation of inflammation and potential influence on the bacterial burden

Consistent with its effect of inducing micro-deformational signals and reducing oedema, NPWT has been demonstrated to modulate the local inflammation, that is, dampen the pro-inflammatory response.^{14,33} Specifically, in full-thickness porcine wounds, NPWT was shown to reduce the numbers of peripheral neutrophils and blood monocytes during the inflammatory phase.³³ Additionally, serum proinflammatory cytokine levels, including interferon-gamma and interleukin-6, were reduced, as were the concentrations of interleukin-8, transforming growth Factor- β 1 and TNF- α in the wound fluid.³⁴

Surgical site infections alone kill between 6 and 30 out of each 10 000 surgical patients^{35,36} and so the potential of NPWT in this arena is vast. The influence of

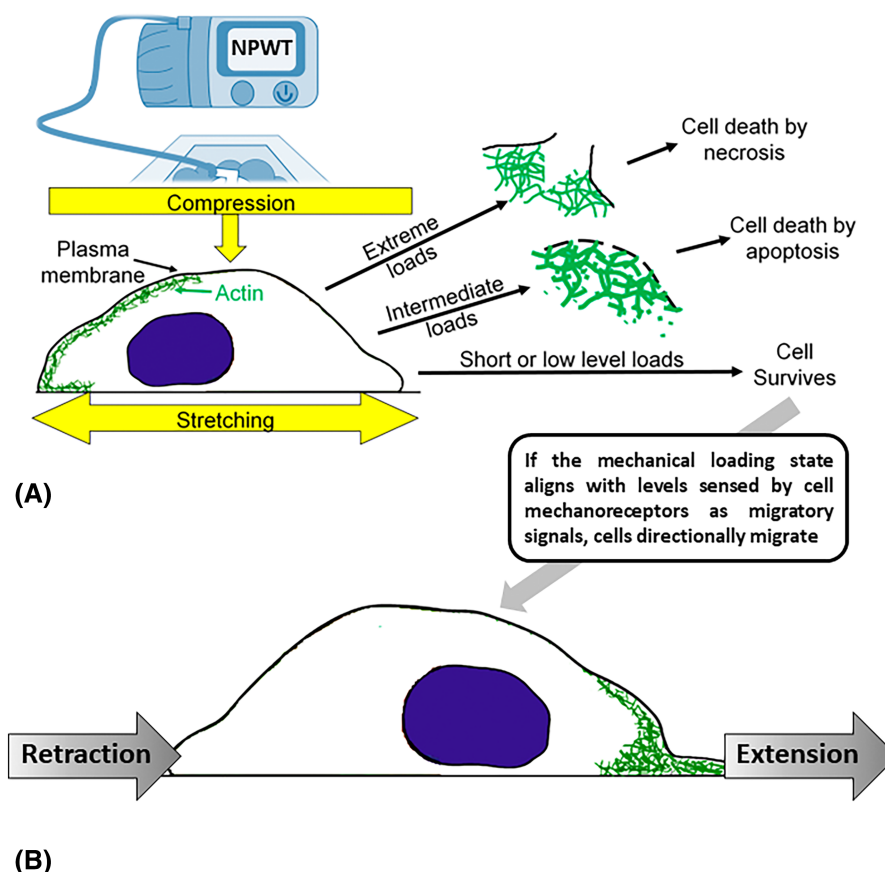


FIGURE 3 Schematic depicting potential cellular responses to mechanical forces within the framework of negative pressure wound therapy (NPWT). (A) Cells adapt their cytoskeleton in response to the mechanical microenvironment generated by the NPWT system, potentially resulting in successful or unsuccessful adaptation (material reproduced from figure 1 in Gefen et al.⁴⁹ with permission from the publisher). If the mechanical loading from the NPWT system remains within tolerable limits and corresponds to stimuli levels sensed by cell mechanoreceptors as migratory-promoting signals (i.e., within their sensitivity range for activating migration), cells may exhibit migratory responses (B). These responses are marked by the assembly of contractile cytoskeletal elements, predominantly actin filaments, at the leading edge of the cell, whilst detachment occurs posteriorly. This mechanism facilitates cell body advancement along the direction of the force (or stress) gradient and is an important mechanism of action of NPWT at the cell scale.

NPWT on bacterial burden is a controversial issue.¹² With some studies indicating decreased bacterial levels¹ whilst others demonstrate no change, increased bioburden or comparable levels between NPWT and controls.¹² Evidence-based recommendations for NPWT published in 2011 state that ‘evidence suggests that reduction of bacteria in wounds is not a major mode of action of NPWT’.¹⁸ Consistently, Birke-Sorensen et al.¹⁸ suggested that the action of NPWT on bacterial burden might be secondary to other benefits of NPWT, including the formation of granulation tissue, wound contraction, increase in wound environment perfusion and control of oedema. With that said, it is possible that NPWT, particularly NPWT systems delivering intermittent pressures, are inducing an environment that is not convenient for bacteria to form biofilms in the wound as the tissue substrata for these are mechanically unstable (i.e., are moving and not stationary). Further, if reduction in bacterial

load is a primary clinical objective, then a system designed specifically for this action, NPWT-instil has been demonstrated to be highly effective, particularly as the instillation of fluids containing antimicrobial agents is enabled.¹⁹ Lastly, if bacteria- or fungi-binding dressing materials are combined with the NPWT,³⁷ that creates an opportunity for double protection: trapping pathogens in the dressing or alternatively, carrying them away from the wound into a wound fluid collection canister.

3.2 | Angiogenesis

Sufficient blood flow into the wound site is of utmost importance to wound healing as it allows the provision of oxygen, circulating inflammatory cells, glucose and other essential nutrients to the tissue, as well as removing waste products detrimental to wound healing. As such,

evidence of angiogenesis, and consequent increased blood flow being stimulated by NPWT, implies that this is one of the most beneficial MOAs of NPWT.³ Angiogenesis at the site of wounds is promoted by micro-deformations, specifically, the application of tension and shear²⁸ leading to mild hypoxia, or low-grade ischaemia. These do not typically form at a level that causes massive and immediate cell death.^{9,10} Rather, the low-grade hypoxia and ischaemia are likely causing a biological mitigation response of the body, which puts into play VEGF release and formation of a gradient of this growth factor^{12,38} that may, in turn, encourages directionalised growth of blood vessels to create additional redundancy in the vascular network, and eventually, bypass the obstructed pathways for renewing normal tissue perfusion.¹² This is a well-established mechanism in cardiology in the context of partially blocked coronary vessels and of course, applies also to the microcirculation as related to NPWT. Specifically, Kairinos et al.^{9,10} have demonstrated temporarily decreased blood flow at wound edges under NPWT and it has subsequently been hypothesised^{12,38} that the resultant mild hypoxia induces upregulation of hypoxia-inducible factor-1 α and subsequent stimulation of VEGF expression and as a result angiogenesis is stimulated via this hypoxia-inducible factor-1 α -VEGF (HIF/VEGF) pathway.

4 | SCIENTIFIC, TECHNOLOGICAL AND CLINICAL PROGRESS IN VIEW OF THE MOAs

Over the course of several days, NPWT typically results in a decrease in the size of the treated wound by way of granulation tissue formation, which, as explained above, is a major and primary MOA of NPWT; this may benefit surgical closure, particularly by secondary intention.^{18,39} In a study of NPWT amongst 30 patients with infected chronic wounds including pressure ulcers/injuries, Isago et al.⁴ reported reduced wound area and stimulation of granulation tissue formation. Reductions in the wound area were lowest at -25 mmHg and progressively higher at -50 , -75 and -125 mmHg; however, the differences between the latter three pressure settings were statistically indistinguishable. These data point to the existence of a non-zero (absolute) negative pressure threshold below which NPWT is likely to be clinically ineffective and further suggest that this threshold is below -50 mmHg.

Granulation tissue growth stimulated by NPWT may fill in large tissue defects partially or completely¹⁸; however, full wound closure can only be achieved through cell proliferation from the peri-wound in addition to the formation of granulation tissue. A combination of a

treatment by a tNPWT system designed for inpatient use (which includes a canister for fluid collection from the wound) followed by treatment in the community using a suNPWT system, is promising in improving clinical outcomes and reducing wound care costs.^{40–42} The suNPWT systems are typically utilising MLDs which extend over greater margins to the peri-wound, and thereby, distribute the mechanical strain energy (i.e., the tissue deformations) beyond the wound margins.^{43,44} This enables more effective recruitment of dermal fibroblasts from the peri-wound dermis, specifically from within what is known in the work published by Gefen as the ‘influence zone’.^{43,44} Describing the peri-wound as the biological reservoir for fibroblasts and myofibroblasts essential for tissue repair and healing, Gefen showed using a computational modelling framework that there is an optimum level of peri-wound tissue deformations for gaining the benefits of NPWT, and that the size of this influence zone – in which optimal tissue deformation levels apply – is affected by the specific size, shape and materials of the MLD serving as the vessel for pressure delivery and the absolute pressure magnitude. The delivered pressure magnitude needs to be neither too much nor too little, and be consistent over the entire period of therapy.^{43,44} Gefen specifically reported that negative pressures below 40 mmHg are understimulating the tissues and therefore, ineffective.^{43,44}

4.1 | The transition from traditional to suNPWT

Since the development and validation of conventional, tNPWT systems^{1,2} which are reusable and incorporate a canister and foam wound filler with film dressing to cover, suNPWT systems have been developed, which are either canister-based (CB) or lack a fluid collection canister and are therefore named canisterless (CL). Both system types utilise MLDs through which to convey therapy, and in the case of CL systems, to exclusively manage wound drainage. Using an ex vivo pre-clinical skin model, Wilkinson et al.⁴⁵ compared between tNPWT and a CL suNPWT system with respect to the induction of tissue, cellular and molecular changes. They reported that the suNPWT system caused less damage to wound edge tissue than traditional application, demonstrated by improved skin barrier, reduced dermal–epidermal junction disruption and a dampened damage response.⁴⁵ These authors further conducted transcriptional profiling which confirmed significantly less activation of multiple pro-inflammatory markers in wound edge skin treated with suNPWT versus tNPWT.⁴⁵ Matrix metalloproteinase levels were also studied and found to be higher with the tNPWT system, which could lead to delays in healing by

way of subjecting matrix components to excessive proteolysis.⁴⁵ Overall, compared with tNPWT, the studied suNPWT induced substantially less inflammation, epidermal damage and apoptosis.⁴⁵

A primary difference between tNPWT and suNPWT systems stems from how the negative pressure is delivered to the wound environment, and the resulting tissue response. In tNPWT, a film-foam dressing is used whereas in suNPWT systems the wound is covered by an MLD. The bottom layer of MLDs, that is, the wound pad, does not collapse or contract under the negative pressure as does foam, but predominantly compresses. Hence, contraction at the wound site may not occur to the same extent under MLDs as in tNPWT systems.^{9,10} The MLD

construction further leads to microdeformational signals over a larger area of the wound and neighbouring skin, so that the impact of the pressure therapy typically reaches 2–3 cm beyond the wound edges (Figure 4).^{43,44} Importantly, the overall degree of soft tissue deformations and mechanical tractions under MLDs is likely to be less than that for the film-foam format used in tNPWT. In other words, based on the literature reviewed above, we propose that in suNPWT systems, the dominant primary MOAs are fluid handling and microdeformation, not macrodeformation. We also hypothesise that because blood vessels are distorted, obstructed and occluded around the wound edges under film-foam-based tNPWT, the consequent hypoperfusion zone at the

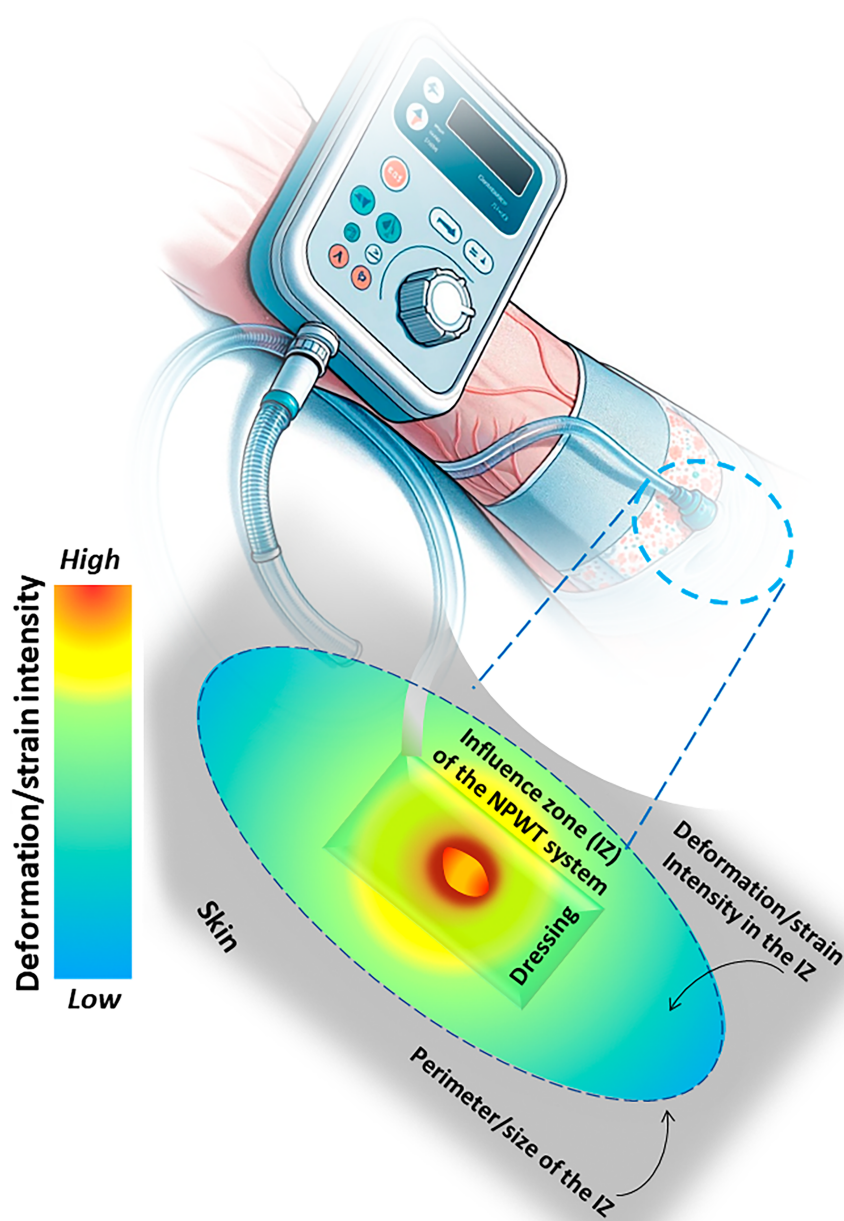


FIGURE 4 Concept of the influence zone (IZ) induced by a negative pressure wound therapy (NPWT) system. According to the IZ theory developed and published by author Gefen,⁴³ cells sense and respond to mechanical cues within their microenvironment, which may extend beyond the immediate area of direct negative pressure application by the NPWT system (adapted from Gefen et al.⁴³ with appropriate modifications made to emphasise the current clinical context).

wound margins abates cellular activity, so negating the processes of cell migration and epithelialisation, thus further diminishing the benefits of the microdeformation NPWT MOA. The clinical hope is therefore that by judiciously combining tNPWT with MLD-based suNPWT in smaller and shallower cavity wounds, complete wound closure can be achieved without the need for grafting.^{40–42} We suggest here that suNPWT not only promotes the microdeformational MOA but also, in particular, alleviates the distortions leading to obstruction or occlusion of blood vessels feeding the wound and the consequent hypoperfusion, by redistributing tractional forces (and related tissue stress concentrations) directly over the wound margins.^{43,44} Clinical studies would be required to prove the above hypotheses.

With regards to CB versus CL design concepts for suNPWT, both CB and CL systems have their advantages and limitations. In principle, the choice between the two types depends on multiple clinical and cost factors including the mobility of the individual, their physical and psychological comfort with the selected system type, the ease of use, financial considerations in the specific setting and of course, the clinical experience and effectiveness for managing the particular wound aetiology and severity. From a technology perspective, CL systems rely solely on the evaporation of excess exudate from the MLD to the environment, whereas CB systems constantly transfer the wound fluids directly into a collection canister, thereby ensuring that the performance of the system is not affected by, for example, clothing, compression bandaging, or any other objects potentially covering the dressing, and are also independent of the ambient conditions, particularly the relative humidity.⁴⁶ A CB suNPWT system therefore delivers intrinsically stable provision of the intended wound bed negative pressure,⁴⁶ however, this system type may not fit all patients, for example, due to psychological (body image) impact as the canister is a potential source of visual and odour awareness of fluids drained from the wound.^{47,48} With that said, for infected wounds in particular, CB systems have the capacity to transport infectious materials away from the dressing, into the canister.⁴⁶

4.2 | The ‘influence zone’ concept linked with single-use systems and MLDs

Unlike traditional film–foam dressings used in tNPWT, which focus negative pressure only on the wound bed, absorptive MLDs used in the suNPWT systems have the potential to distribute the negative pressure over the peri-wound skin area as well as the wound bed.^{43,44} This means that absorptive MLD dressings, distributing the pressure to both the peri-wound skin and the wound bed,

have a wider ‘influence zone’, which has been defined by the Gefen group as the peri-wound skin ‘strain state’ (of the dermis), than traditional dressings.^{43,44} Accordingly, their combined computational modelling work and animal studies of suNPWT systems with MLDs indicate that these systems likely enable stimulation of more responsive and available factors in the peri-wound tissues which include a substantial reservoir of fibroblasts potentially available for recruitment from the dermis during the wound-healing process.^{43,44} According to Orlov and Gefen,⁴⁴ for fibroblast stimulation and their en-mass migration to the wound bed, the optimum mechanical strain, that is, the optimal microdeformation level, lies between 0.5% and 3%.⁴⁴ Since a discrete strain threshold was required for a computational finite element analysis in their work, the mid value (1.75%) was taken. If the 1.75% strain threshold was exceeded it was taken to be associated with the likely induction of a fibroblast migratory response; however, with strains below this value such a migratory response was thought to be less likely.⁴⁴ These finite element analysis simulations, of the ‘influence zone’ as a function of the negative pressure applied, successfully demonstrated that consistent delivery of negative pressure and the resulting mechanostimulation of the peri-wound skin throughout treatment is critical to clinically successful outcomes.⁴³ With loss of the intended pressure for any reason, there would be a reduction in dermal stimulation levels at the peri-wound, and with severe pressure losses, below a $|-40|$ mmHg pressure delivery, the microdeformation stimulation level at the peri-wound may approach zero.^{43,44}

4.3 | Clinical cases demonstrating the proposed angiogenesis mechanism in practice

Colour Doppler ultrasound (CDU)^a is widely used in wound care⁷ as an effective method for quantifying the likely hypoxias and induced neogenesis in the wound environment as hypothesised by Kairinos et al.^{8,9} We have thus conducted a number of cases (Figures 5 and 6; work led by clinical co-author MC) demonstrating successful outcomes in progressing wounds to closure in a timely way through the use of a combination of film–foam tNPWT applied in early phases of healing which was later replaced by a CB suNPWT system with MLDs. As explained above, the change from tNPWT to suNPWT has likely altered the manner in which pressure is transduced to both the wound and peri-wound, specifically expanding the influence zone to recruit more cells necessary for wound closure, as per the Gefen theory.^{43,44} Whilst the maturity and size of each treated wound

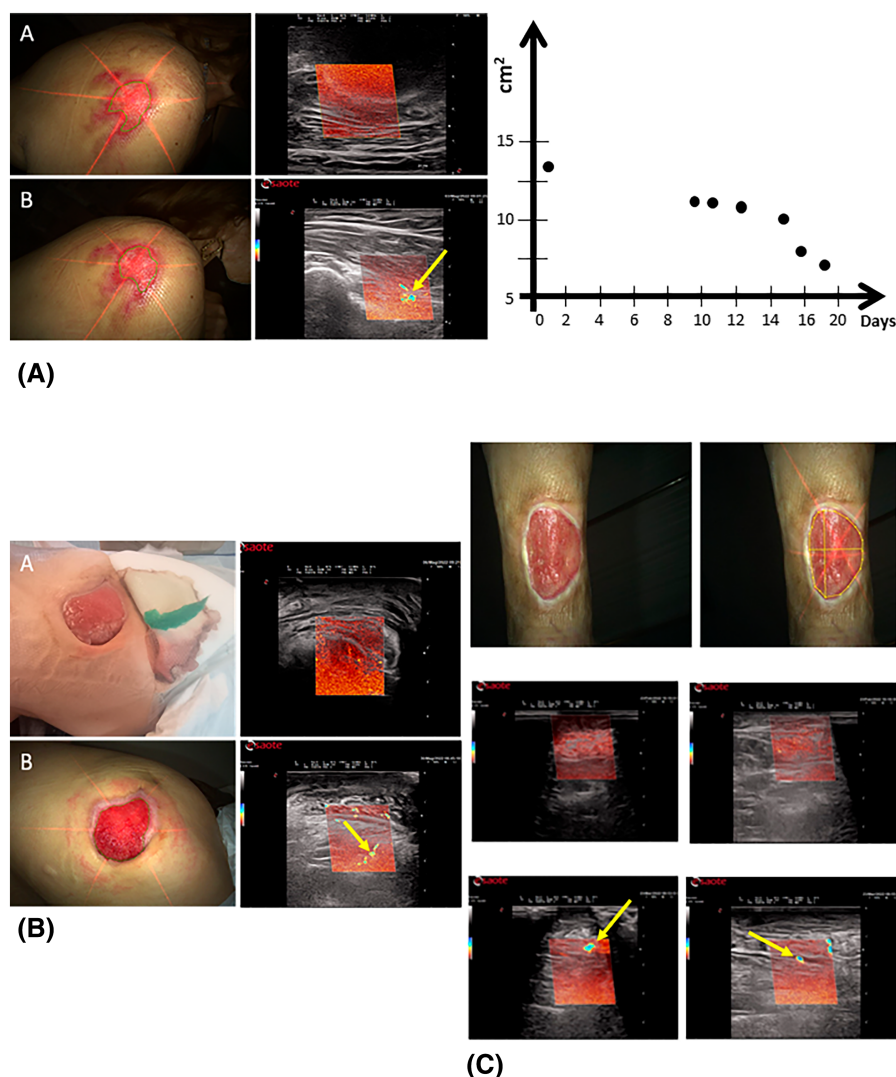


FIGURE 5 Cases demonstrating, by means of colour Doppler ultrasound (CDU), that under negative pressure wound therapy (NPWT), hypoxia can induce neo-angiogenesis: (A) progression of a burn injury healing on the right shoulder (left frames) over 19 days of NPWT. Indication for new vessels appears (as coloured spots) in the CDU images (centre frames) at the end of this period (bottom-centre image, marked with yellow arrow) compared to very poor microcirculation at baseline (top-centre image); concurrently, the wound size and depth reduced from baseline values of 11.9 cm² and 1 mm to 7.9 cm² and ~0 mm, respectively (area reduction of 44%; wound size-time plot shown in the right frame). (B) Evolution of necrotising fasciitis (left frames) wound healing over 17 days of NPWT delivery, from a baseline wound size of 25.7–18.3 cm² by day 17 (area reduction of 28.8%), which was accompanied by a reduction in wound depth from 3.0 to ~0 mm. Again, new vessels are evident in the CDU examination (yellow arrow in bottom-left image) after poor microcirculation at baseline (top-left image). (C) A 4-year-old vascular ulcer (top frames) treated by means of NPWT reduced in size from 17.7 to 15.6 cm² after 27 days (area reduction of 11.8%), during which the wound depth reduced from 15 to ~0 mm. As in the previous two cases, neo-angiogenesis is demonstrated in the bottom CDU images post the NPWT treatment (bottom frames), compared to poor vascularisation when the treatment began (centre frames).

ultimately dictated the specific course of treatment according to clinical experience, the cases reported below are compelling examples of implementation of the Gefen influence zone theorem, and in particular, how a change in the pressure transduction vehicle (i.e., the dressing) can have a profound positive effect on the cellular activity stimulated. The cases further exemplify the fact that not all NPWT systems/formats/treatment protocols are

the same and/or will illicit the same healing response. This is not to say however that one specific embodiment of NPWT is superior to the others, but that they are different and there is a clear clinical benefit to recognising differences in MOAs and considering these alternative presentations as constructive compliments or for future improvements of NPWT technologies. Future controlled clinical studies can provide further support to this theory.

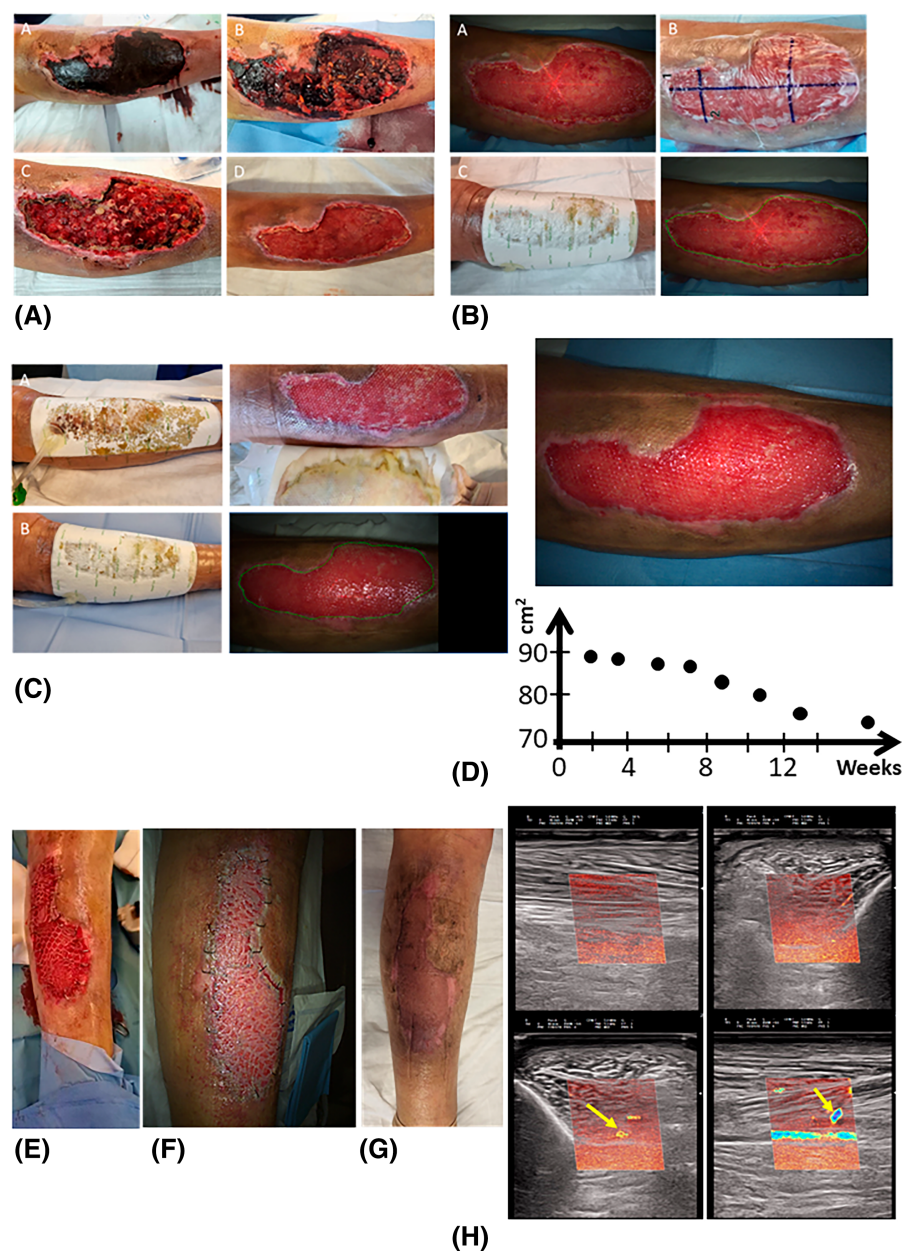


FIGURE 6 Wound progression and response to negative pressure wound therapy (NPWT) over a 43-day period in a 63-year-old woman with a trauma wound following a motorcycle accident.

(A) Initial presentation of a 3-month-old wound. (B) After debridement on the first day. (C) Following 14 days of treatment by means of traditional NPWT. (D) After a further 10 days of traditional NPWT therapy using a film-foam dressing (top frame) the wound size continued to reduce (bottom frame). (E) Clean, healthy granulation tissue after 14 days of NPWT therapy with traditional film-foam dressing, with wound area and depth mapped prior to switching to single-use NPWT (suNPWT) with a multilayer dressing. (F) Progression after seven additional days of treatment using the suNPWT system, with ongoing regrowth and mapping of wound area and depth; the reduction in wound area (19%) and depth (86%) is evident. (G) Final assessment after 43 days of suNPWT therapy, demonstrated considerable improvement. (H) The Doppler ultrasound images taken after 4 weeks (bottom frames) demonstrate increased perfusion of the wound bed with neo-angiogenesis (yellow arrows), resulting in an evident uniform perfusion profile (i.e., no hypoperfusion zones) compared to poor perfusion at baseline (top frames). Aspects of these cases have been reproduced from Henriksson et al.⁵⁰ with permission from the publisher and new Doppler data in the context of the current article were added to them.

The three cases in Figure 5 use CDU to support the hypothesis that under NPWT, hypoxia can induce neo-angiogenesis. The case in Figure 5A shows the progression of a burn injury healing on the right shoulder (Figure 5A; left frames) over 19 days of NPWT. Post-treatment, coloured spots appeared in the CDU images which could represent new vessel formation (Figure 5A; centre frames) at the end of this period (Figure 5A; bottom-centre image, marked with yellow arrow), compared to very poor microcirculation at baseline (top-centre image). Concurrently, the wound size and depth reduced from baseline values of 11.9 cm² and 1 mm to 7.9 cm² and nearly zero, respectively (area reduction of 44%; wound size-time plot shown in Figure 5A, right frame). The second case, in Figure 5B,

shows the evolution of necrotising fasciitis (Figure 5B; left frames) wound healing over 17 days of NPWT delivery, from a baseline wound size of 25.7–18.3 cm² by day 17 (area reduction of 28.8%), which was accompanied by a reduction in wound depth from 3 mm to again nearly zero. The new colour signal evident in the CDU examination again suggests angiogenesis (Figure 5B; yellow arrow in bottom-left image) after poor microcirculation at baseline (Figure 5B; top-left image). The third case is of a 4-year-old vascular ulcer (Figure 5C; top frames) treated by means of NPWT. The wound reduced in size from 17.7 to 15.6 cm² after 27 days (area reduction of 11.8%), during which the wound depth reduced from 15 mm to nearly zero. As in the previous two cases, neo-angiogenesis is demonstrated in the bottom CDU images

post the NPWT treatment (Figure 5C; bottom frames), compared to poor vascularisation when the treatment began (Figure 5C; centre frames).

A fourth, and more detailed case description, of wound progression and response to NPWT over a 43-day period in a 63-year-old woman with a trauma wound following a motorcycle accident is depicted in Figure 6, from the initial presentation of a 3-month-old wound (Figure 6A), to post-debridement on the first day (Figure 6B), then following 14 days of treatment using film-foam tNPWT (Figure 6C), and after further 10 days of tNPWT during which the wound size continued to reduce (Figure 6D) and eventually considered sufficiently mature for switching to CB suNPWT with MLDs (Figure 6E). The wound continued to progress to healing and after seven additional days of treatment using the suNPWT system, exhibited ongoing regranulation and smaller wound area and depth (Figure 6F); the reductions in wound area (19%) and depth (86%) were evident. Upon final assessment after 43 days of suNPWT therapy, a considerable improvement was documented (Figure 6G). Importantly, CDU images taken after 4 weeks (Figure 6H; bottom frames) demonstrated increased perfusion of the wound bed with neo-angiogenesis (Figure 6H; yellow arrows), resulting in an evident uniform perfusion profile, that is, no hypoperfusion zones, compared to poor perfusion at baseline (Figure 6H; top frames).

The fifth and last case presented here nicely depicts the absence of hypoperfusion whilst treating a chronic wound with suNPWT (Figure 7). Specifically, a 70-year-old patient with a history of heart disease and diabetes was presented with a 4-month-old vascular malleolar ulcer (Figure 7A). Microcirculation was evaluated using Laser Doppler in both tNPWT and suNPWT. Initial assessment with tNPWT revealed poor microcirculation in the peri-wound area with a considerable gradient between the central wound and surrounding areas (Figure 7B), however, after switching to suNPWT, there was a notable improvement in microcirculation in both the wound and peri-wound areas, with relatively small areas of localised ischaemia confined only to the wound edges (Figure 7C).

These cases altogether serve as practical, graphic examples of the clinical benefits of a deeper understanding of the MOAs of NPWT, specifically angiogenesis and microdeformations, and motivate an appreciation for how the manner in which the intended therapy is delivered can yield beneficial clinical (and then, likely, better cost-effectiveness) outcomes. Lastly, it is important to recognise that clinical case studies are inherently uncontrolled, meaning that other interventions performed before or during the NPWT treatment, as well as throughout the evaluation period, may also have

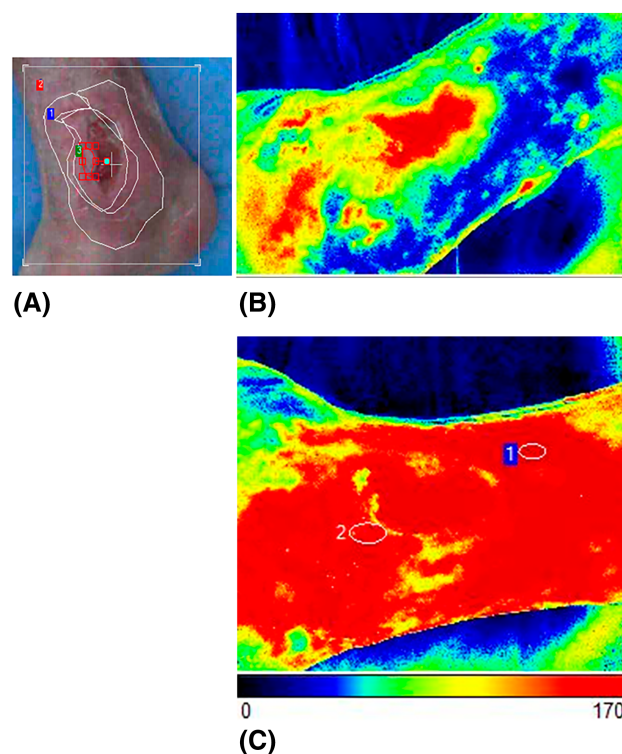


FIGURE 7 A 70-year-old patient with a history of heart disease and diabetes was presented with a 4-month-old vascular malleolar ulcer (A). Microcirculation was evaluated using Laser Doppler in both traditional negative pressure wound therapy (tNPWT) and single-use NPWT (suNPWT). Initial assessment with tNPWT revealed poor microcirculation in the peri-wound area with a considerable gradient between the central wound and surrounding areas (B), however, after switching to suNPWT, there was a notable improvement in microcirculation in both the wound and peri-wound areas, with relatively small areas of localised ischaemia confined only to the wound edges (C).

influenced new blood vessel formation. Therefore, our current findings underscore the need for further controlled clinical trials to validate the hypotheses proposed in this study.

5 | SUMMARY AND CONCLUSIONS

Since its introduction nearly three decades ago, the technology of NPWT has undoubtedly revolutionised all the subfields of wound care, in both chronic and acute contexts. Whilst the effectiveness of NPWT technologies in promoting wound healing is now widely acknowledged, the exact MOAs remain an ongoing subject of debate (Figure 1). This narrative review examined and analysed the existing literature to elucidate the primary and secondary MOAs of NPWT. We further used original

histopathology (Figure 2) and clinical data (Figures 5 and 6) collected by our co-authors with the respective expertise to support new arguments.

All NPWT systems essentially apply subatmospheric pressures to the wound bed using dressings for fluid handling and modulation of the wound environment. However, growing evidence challenges the notion that increased perfusion is a primary MOA in the modulation of the wound environment, suggesting in fact that microdeformations play a more significant role than thought before (Figure 1). Our analysis of the literature combined with clinical experience indicates that microdeformations at the wound bed, which are known to stimulate cell proliferation and migration and are therefore crucial for granulation tissue growth, are best achieved using suNPWT with MLDs. In fact, changing from tNPWT to suNPWT during the course of treatment, which eliminates the macrodeformations associated with foam material shrinkage in the tNPWT route, promotes angiogenesis after the mild hypoxia-induced VEGF expression under the tNPWT, which then enhances tissue perfusion. Additionally, suNPWT systems facilitate better mechanostimulation of the peri-wound skin, promoting fibroblast migration and tissue repair according to the Gefen influence zone theorem (Figures 3 and 4).^{43,44} This newer suNPWT technology therefore currently offers advantages over tNPWT alone, or at least, it complements it when used in combined therapy. The newer technology of suNPWT shows promise in optimising tissue response and further promoting wound healing, likely based on a better understanding of how the sizes, shapes and materials of MLDs interact with different wound aetiologies and types. Further research is much needed in this area, to fully understand MOAs and refine applications for improved clinical outcomes.

Most importantly, although many of the proposed MOAs of NPWT are generally accepted to be with good foundation, some aspects of this knowledge are now believed to be flawed, specifically with regard to blood perfusion. Although there is no doubt that perfusion at distances greater than 1 cm from the wound edges is eventually increased after NPWT, the mechanism by which this occurs is now thought more likely to be due to increases in tissue pressures under tNPWT leading to low-grade ischaemia, which stimulates angiogenesis and new vessel growth. Further research should be focused on how to best utilise the low-grade ischaemia to stimulate (micro)angiogenesis, either by creating new suNPWT systems that will deliberately cause that at the first phase of treatment, then utilise the microdeformation MOA for further granulation tissue growth, or more short-term, by combining existing tNPWT with suNPWT systems for optimal treatment protocols.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest other than those stated in the Acknowledgements.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ENDNOTE

^a Using sound waves, CDU provides real-time imaging of blood vessels and can detect areas of reduced or increased blood flow which helps monitoring effectiveness of chronic wound treatments, and can guide clinical decisions regarding interventions to improve circulation such as debridement or vascular surgery. It is particularly valuable in detecting suboptimal blood perfusion that may impair wound healing.

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