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Effective negative pressure wound therapy for open wounds: The importance of consistent pressure delivery

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Abstract

Two distinct design concepts exist for single-use negative pressure wound therapy systems: Canister-based versus canisterless. The canister-based technology provides intrinsic stable delivery of the intended negative pressure, because exudate is constantly transferred from the wound into a canister, thereby preventing dressing saturation. In contrast, with a canisterless system, where delivery of the negative pressure depends on continuous evaporation of wound fluids from its dressing, loss of the intended wound-bed pressure may occur due to dressing saturation. To investigate whether these two designs differ in their mechanobiological effect with respect to magnitudes and distributions of tissue strain fields under the absorptive dressing, termed the *influence zone,* we integrated computational modelling with an animal study. This influence zone must be of biologically influential strain levels and extend sufficiently into the peri-wound for stimulating fibroblasts to migrate and progress the healing. We found that an effective influence zone requires continuous delivery of the intended pressure to the wound-bed. Loss of negative pressure at the wound-bed below 40 mmHg adversely lowered the peri-wound stimulation around a 120 × 70 mm sized wound to less than one-third of the baseline stimulation, and further pressure decreases to 20 mmHg or lower resulted in complete lack of peri-wound mechano-stimulation.

KEYWORDS

animal study, bioengineering laboratory research of chronic wounds, diabetic foot ulcer, finite element modelling, pressure ulcer/injury

Key Messages

- single-use negative pressure wound therapy systems are applied on open wounds
- two distinct system design concepts exist: canister-based versus canisterless

LIST OF ABBREVIATIONS: CB, Canister-based; CI, Cell ingrowth; CL, Canisterless; CRI, Collagen remodelling index; DFU, Diabetic foot ulcer; ECM, Extracellular matrix; FE, Finite element; FRS, Feulgin & Rossenbeck stain; FSD, Full-size dressing; IZ, Influence zone; NPWT, Negative pressure wound therapy; PRS, Picrosirius red stain; PU/PI, Pressure ulcer/injury; RSD, Reduced-size dressing; suNPWT, Single-use negative pressure wound therapy.

- the influence zone is the peri-wound skin strain state induced by each system
- computer and animal models compared influence zone results between the systems
- consistent delivery of the intended pressure sets an effective influence zone

1 | INTRODUCTION

Open wounds, particularly deep chronic wounds such as pressure ulcers/injuries (PUs/PIs) classified as category-3/4 and diabetic foot ulcers (DFUs) continue to be a major and snowballing threat to global public health and the economy. For example, the prevalence of PUs/PIs in the general population is 11 of 100 000 people, but rises to approximately 5% and 10% for community and adult/paediatric acute healthcare settings, respectively, rendering this condition as the third most costly disease to treat after cancers and cardiovascular diseases.[1-6] Likewise, the global prevalence of DFUs is approximately 6% (630 per 100 000 diabetic individuals) and the total cost for management of diabetic foot disease ranges from 9 to 13 billion dollars only in the United States, in addition to the cost of managing the diabetes alone.[7-9] It is well established that negative pressure wound therapy (NPWT) is an effective wound care approach for treating and managing these tissue damage conditions. In NPWT, the wound is covered with an airtight dressing, and negative pressure is applied to remove the exudate and to mechanically stimulate the wound and peri-wound tissues, to initiate and progress the tissue repair. The latest embodiment of this commonly used wound care technology, which exists in the commercial medical device arena since the 1990's,[10,11] is single-use NPWT (suNPWT) systems, which are meant to be used by an individual patient, are simple to operate and are disposed post-treatment.

There is a consensus in the contemporary medical literature that with respect to traditional wound care, suNPWT systems are overall highly effective in: (i) Decreasing the wound size and depth; (ii) Increasing the amount of perfusion into the wound-bed and of newly-generated granulation tissue; (iii) Providing a high-level of patient satisfaction with low complication frequencies and their associated costs; and (iv) Leading to higher rates of success in limb salvage.[12-14] Battery-powered suNPWT systems can be classified into two fundamentally different design concepts. One suNPWT system type utilises a canister for clearing excess wound fluids from the dressing and is hence termed a canister-based (CB) suNPWT system. A second suNPWT system type is dependent on the evaporation of exudate from the dressing to the environment - to clear the dressing from excess wound fluids, and this type is therefore known as a canisterless (CL), or canister-free design class.[15] Performance differences between CB versus CL suNPWT systems with absorptive multilayer dressings were investigated and analysed in our published work, in the context of treatment of surgical incisions.[15] Specifically, comprehensive, rigorous and clinically-relevant laboratory testing of a commercial, market-popular CL suNPWT system demonstrated losses of the intended negative pressure as fluid saturates its dressing.[15] These pressure losses were observed to increase in the CL system as its dressing exceeded a critical absorbency level of approximately 50%, reaching negative pressure values that were as low as 15% of the intended nominal pressure towards the end of the test time (72 h) of simulated use.[15] The pressure drops from the pump of the CL suNPWT system to its dressing are likely caused by gel blocking in the absorbent dressing, a phenomenon that occurs when regions of the dressing swell, and thereby, impede flow of further liquid into the inner dressing space.[16-18] Clearly, when loss of the negative pressure at the wound-bed occurs, the inherent benefits of NPWT are compromised, and particularly, the ability of the suNPWT system to positively stimulate wound healing-related biological activities in the peri-wound declines, as explained further below.

1.1 | The peri-wound as a biological reservoir for wound healing

During the proliferative phase of wound healing, dermal fibroblasts proliferate and migrate from the peri-wound into the wound-bed to synthesise growth factors and produce new extracellular matrix (ECM) there, in the form of thick collagen bundles that ultimately form scar tissue.[19-21] As such, fibroblast migration from the peri-wound skin into the wound-bed is vital for healing.[22] Accordingly, the peri-wound is considered to be the biological and cellular reservoir for healing and tissue repair. In clinical terms, the peri-wound is the skin region that is within approximately 4 cm of the wound edges.[23] The peri-wound region further contains the vascular supply to the wound, and indeed, peri-wound hypoxia delays

wound healing,[24] whereas stimulating the vasculature supports healing and promotes angiogenesis.[25]

In the peri-wound tissues, micro- and macro-deformations induced by NPWT mechanically stimulate fibroblasts to proliferate and migrate into the wound-bed, and promote their biological activities of cytokine signalling, production of basic fibroblast growth factor, expression of collagen type-I and matrix protein secretion, and ultimately, collagen fibrillogenesis towards scarring and wound closure.[26-30] In particular, stimulating fibroblasts to proliferate, migrate and produce collagen, by inducing micro-deformations at the cell environment and within cell bodies, is essential for promoting healing in general, and predominantly in wounds with impaired healing.[30-32] However, the mechanical stimulation needs to reach a sufficient level to be detected by mechanoreceptors on the plasma membrane of these cells, for example, by primary cilia[33,34]; once detected, that stimulation triggers and maintains collective fibroblast migration targeted and directed specifically towards the wound-bed, where organised collagen deposition can commence.[31] Another important aspect is that sufficient and consistent mechano-stimulation is required for both angiogenesis and maturation of the microvasculature in and around the wound, which in turn, facilitates macroscopic tissue growth on the basis of the neovascular supply.[25,29,35] In the particular context of the mechanobiology of fibroblast behaviour under NPWT conditions, we previously reported that exposing fibroblast cultures to a static 3% strain level increased their migration speed and reduced gap closure times relative to unstretched control cultures,[32,36] however, exposure to 9% strain or higher strain levels led to plasma membrane poration followed by apoptotic cell death.[37] Accordingly, as per the well-known Goldilocks principle, there exists an optimal range of positively-stimulating strains that, when induced in the peri-wound, will activate and accelerate fibroblast proliferation and migration, whereas subcritical strains will not be detected by the fibroblasts, whilst excess strains may cause cell and tissue damage. That specific optimal range of peri-wound skin strains can be approximated from contemporary, published literature which is reviewed below.

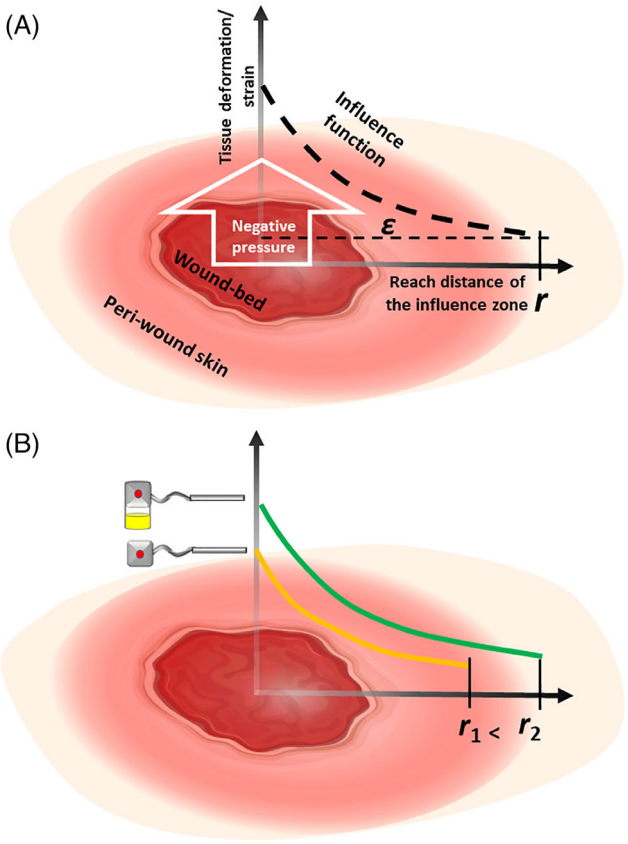

FIGURE 1 The influence zone (IZ) theory: A, The IZ magnitude and area depend on the tissue strain state induced by the single-use negative pressure wound therapy (suNPWT) system with its absorptive dressing; the larger the (absolute value of the) nominal pressure, the greater and the farther is the impact of the IZ in the peri-wound tissues. B, An illustrative example of a schematic comparison of the sizes of the IZs induced in peri-wound skin by canister-based versus canisterless suNPWT systems. The canister-based suNPWT system with its consistent delivery of −125 mmHg induces a larger IZ in the peri-wound skin, that is, with influence radius $r_2 > r_1$ where r_1 is the IZ radius of the canisterless suNPWT system operating at −80 mmHg. The critical strain level ε which affects the reach distance of the IZ, r, is the strain sensitivity threshold for triggering the mechanotaxis of fibroblasts and myofibroblasts, which is set in this study as 1.75% for the purpose of the current computational simulations (please see the body of the text for the rationale for choosing this value)

1.2 | The optimal peri-wound strain state for promoting healing: *The influence zone*

Our published research to determine the strain sensitivity threshold which triggers or promotes the en-mass migration of fibroblasts revealed that 0.5% strains are a sub-optimal stimulus, and so, while not damaging the cells, this strain level does not have a positive effect either.[32] Subjecting fibroblast cultures to a 3% strain level increased the rate at which they migrate to close a gap formed by an in vitro injury, but the above effect was reduced under 6% strain.[36] Other evidence reviewed in our aforementioned works consistently indicated that strains above 5% slow the cell migration. A conservative assumption, based on the above published research, is that the optimal range of applied mechanical strains for fibroblast stimulation is between 0.5% and 3%. For practical purposes, and particularly since the finite element (FE) computations of the current study required a

discrete strain-stimulation threshold value, the midrange of the above optimal value was taken, that is, $\varepsilon = 1.75\%$ strain. In the current work, peri-wound skin strains equal to or exceeding the 1.75% strain threshold were associated with a high-likelihood to induce a fibroblast migratory response, whereas strains below 1.75% were considered unlikely to achieve such a response. However, as the strain field decays with the distance from the site of the applied negative pressure, there must exist an *influence zone* (IZ) that is specific for the NPWT system type used to treat a given wound, and it is essential for this IZ to be of adequate magnitude and area, that is, to reach sufficiently far into the peri-wound region, in order to promote the healing. This IZ* is therefore defined as the effective peri-wound region size from which it is possible to recruit dermal fibroblasts by promoting their migration into the wound-bed through mechanical stimulation (Figure 1A).

Supporting evidence concerning the importance of the magnitude of the applied negative pressures for positive biological influence on the wound healing process exist in multiple clinical and laboratory studies reported in the literature, such as for treating diabetic foot ulcers,[38] infected wounds in a pig model[39] and non-infected wounds in a rabbit model.[40] All these published studies consistently indicated that a potential decrease (loss) of the absolute negative pressure delivered to the wound de facto, will likely slow or halt the healing process. With absorptive dressings, there is a potential for the negative pressure to also be distributed over the peri-wound area (in contrast to traditional foam and film dressings that concentrate the application of negative pressure at the wound-bed only). In other words, a larger area and magnitude of the IZ, provided by an absorptive dressing which covers both the wound-bed and the peri-wound, implies stimulation of more available and responsive biological resources (in the peri-wound tissues), particularly including a larger reservoir of fibroblasts that can potentially be recruited and made available for the wound healing process.

1.3 | Hypothesis and objective

In this study, we investigated and compared the IZs induced by suNPWT systems that are CB or CL, and with advanced absorbent dressings that cover the wound and its margins (ie, the peri-wound), as per clinical practice, using computational modelling reinforced by an animal study of open wound healing. We hypothesised that the reach distance of the IZ determining its size (Figure 1A) is a function of: (*i*) the level of the negative pressure delivered to the wound and peri-wound; (*ii*) the consistency in delivery of the intended negative pressure; and (*iii*) the size of the applied dressing. Importantly, we surmise that if a CL suNPWT system loses wound-bed pressure due to dressing wetness as explained above, its IZ will consequently decrease, which will thereby reduce the healing potential of the delivered treatment (Figures 1B and 2). Our objective in developing the current integrated experimental-computational research approach for testing the above hypothesis here, was therefore, to determine whether pressure losses in a CL suNPWT system can indeed decrease the IZ such that it may have a negligible effective size, or even no presence in the peri-wound.

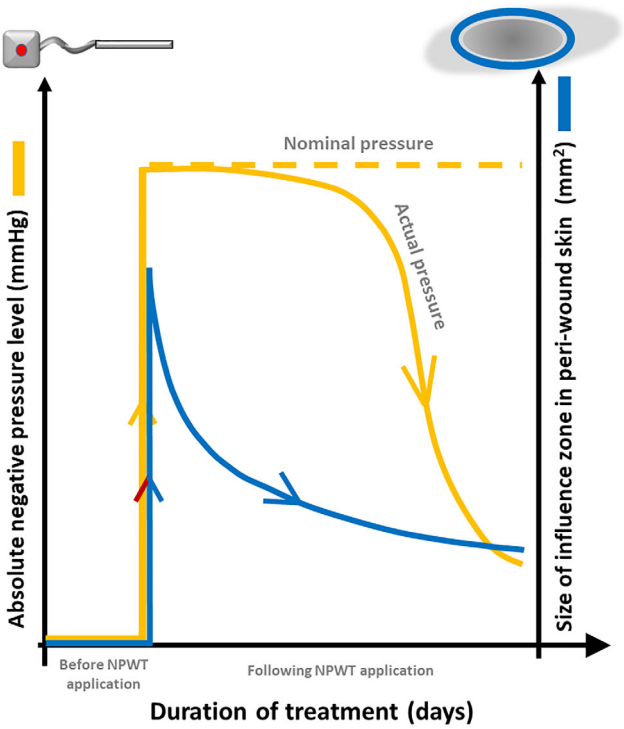

FIGURE 2 Conceptualization of the effect of the magnitude of the absolute negative pressure level produced by the canisterless single-use negative pressure wound therapy (suNPWT) system (left vertical axis) on the size of the influence zone in the peri-wound skin (right vertical axis) over a time course of its application, considering a scenario where the canisterless suNPWT system, at times of impaired fluid management, delivers pressures that are lower than the nominal (expected) absolute pressure level

2 | METHODS

2.1 | Geometry of the wound treatment model

An elliptically-shaped soft tissue defect with dimensions of 120 × 70 mm (length×width) that extends into

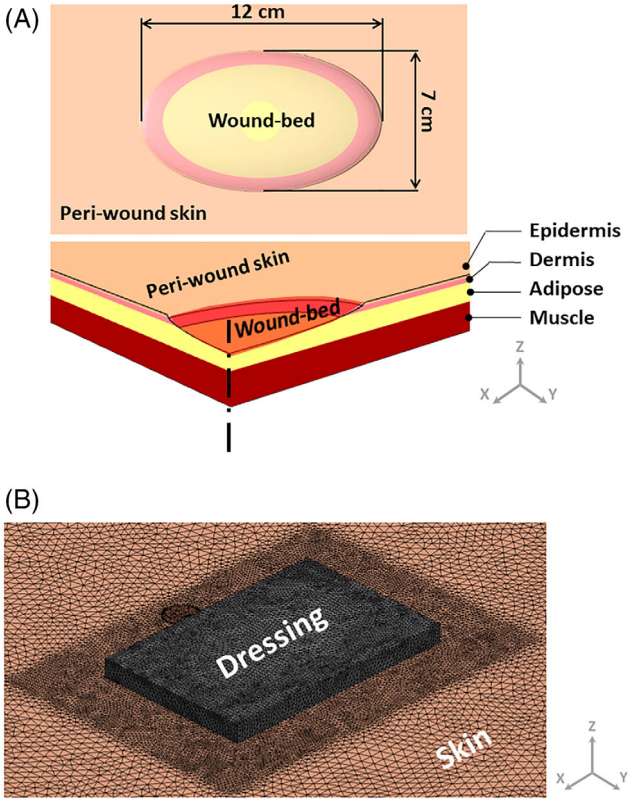

FIGURE 3 Geometry of the computational, finite element (FE) open wound model from top and cross-sectional views A, and the FE mesh around the wound-bed with the applied dressing, showing an increased mesh density around the applied dressing for accuracy of the calculations

subepidermal adipose tissue, and which represents an open cavity wound such as a category-3 PU/PI or a plantar DFU, was modelled using the FE method (Figure 3A). The modelling further incorporated two simulated alternative NPWT treatment technologies, namely, by means of a CB versus a CL suNPWT system, both with absorptive dressings. Epidermis, dermis, adipose, and underlying skeletal muscle tissue layers were included in the modelling, with thicknesses of 1, 2, 8 and 12 mm, respectively[41] (Figure 3A).

Selection of the appropriate dressing size for the NPWT is always based on clinical judgement, considering the individual patient, the anatomical location, and the aetiology of the wound to be treated. Either a full-size dressing (FSD) can be used, where the wound pad covers the wound and a larger portion of the peri-wound area, modelled here as margins of 4 cm between the wound boundaries and the borders of the dressing (Figure 3B), or the choice could be a dressing with a narrower fit, that is, a reduced-size dressing (RSD) modelled here to leave margins of 1.3 cm between the wound edges and the dressing borders. The size of the FSD was determined to be 200 × 155 mm, for both the CB- and CL-suNPWT systems, where the longest aspect of the dressing aligned with the widest dimension of the wound, thereby covering the entire wound-bed and the peri-wound skin so that margins of 4 cm existed between the boundaries of the wound and the borders of the dressing (Figure 3B). In addition, for the purpose of sensitivity analyses of the model outcomes (specified further below) to the size of the applied dressing, an RSD was also modelled for each suNPWT system type, with a size of 145 × 95 mm, leaving margins of 1.3 cm between the wound edges and the dressing borders. The overall dimensions of the geometrical wound model domain were 500 × 500 × 23 mm (length × width × height), which leaves sufficient intact tissue volume around the simulated cavity wound site to avoid potential boundary effects, according to the Saint-Venant's principle. The above-described geometry of the model was created using the Scan-IP module of the Simpleware® (Synopsis, Exeter, UK) software package.[42]

2.2 | Computational simulations and outcome measures

Each of the aforementioned soft tissue types was assumed to behave as a viscoelastic solid. The hyperelastic component of this viscoelastic behaviour was considered to be Neo-Hookean,[43-45] with a strain energy density function W:

$$W = \frac{\mu}{2}(I_1 - 3) - \mu \ln J + \frac{\lambda}{2}(\ln J)^2 \quad (1)$$

where λ and μ are Lame's first and second parameters, respectively, I_1 is the first invariant of the right Cauchy-Green deformation tensor and J is the determinant of the deformation gradient tensor. The viscous, stress relaxation component of the viscoelastic behaviour of each of the individual tissue layers was represented using a Prony-series:

$$G(t) = 1 + \sum_{i=1}^{N} \gamma_i e^{-t/\tau_i} \quad (2)$$

where γ_i, τ_i, and $i = 1, 2, \ldots N$, are the tissue-type-specific material constants. For efficiency of the model formulation and computations, we set N = 2 for all tissues, which yields short-term and long-term viscoelastic relaxation time constants for each soft tissue type, τ_1 and τ_2, respectively. The λ and μ, τ_1 and τ_1, and γ_1 and γ_2 parameter values for each tissue type were selected based on published literature, as detailed in Table 1. The suNPWT

TABLE 1 Mechanical properties of the model components

Model component	Lamé constants		Viscoelastic parameters				Numbers of elements
	λ [MPa]	μ [MPa]	γ_1	τ_1 [s]	γ_2	τ_2 [s]	
Epidermis[a,b,c]	827	34.45	0.0864	0.212	0.214	4.68	133 157
Dermis[a,b,c]	82.7	3.44	0.0864	0.212	0.214	4.68	174 710
Adipose[a,b,c]	0.0827	0.0034	0.3988	2.04	0.12381	76.96	140 173
Muscle[c]	0.659	0.071	4.836	0.016	0.423	8.59	69 918
Dressing[d] (full-size)	0.051	0.0057	–	–	–	–	109 770
Dressing[d] (reduced-size)	0.051	0.0057	–	–	–	–	77 079

[a]Hendricks et al., 2006.[44]
[b]Xu and Lu, 2009.[46]
[c]Katzengold et al., 2018.[45]
[d]Measured in the current study.

dressing properties were determined experimentally, by subjecting commercially available dressing specimens to compression testing using an electromechanical testing machine (Instron® Series 5944, Instron Co., MA, USA) based on the relevant testing standard ASTM D3574-11,[47] where the deformation rate was set to 50 ± 5 mm/min. Considering the dressing test specimens as homogenous, isotropic and hyperelastic materials, we fed the empirical stress–strain curves to the ABAQUS FE software suite (Dassault Systems, Vélizy-Villacoublay, France) to determine their λ and μ using the reverse engineering technique (Table 1).

The action of the suNPWT systems was simulated as static negative pressure, delivered through the applied dressing at nominal pressures of -80 mmHg and -125 mmHg for the CL- and CB-suNPWT systems, respectively (abbreviated below as CL-80 and CB-125). For the CL-suNPWT system, we also considered drops in the intended negative pressure delivered to the open wound, indicative of when the dressing of this system approaches saturation.[15] These losses of intended negative pressure were simulated to occur at $|20|$ mmHg intervals, down to a minimum absolute value of $|-20|$ mmHg, as reported in our published work.[15]

Meshing of the model into tetrahedral elements was performed using the Scan-IP module of the Simpleware® software,[42] first using the automated algorithm, and then, by manually refining the mesh near the borders of the wound-bed and the dressing. The resulting element numbers for each tissue type and the dressings are further listed in Table 1. All the FE analyses were conducted using the FEBio Software suite (University of Utah, Salt Lake City, UT, USA).[43]

The model variants with the CB- and CL-suNPWT systems and the FSDs versus RSDs were analysed for the levels of the Green-Lagrange effective strains in a 2 cm-diameter disk-shaped region of interest (ROI) located at the peri-wound skin, 7 cm laterally to the centre of the wound-bed, on the axis of the narrower wound width (ie, the shorter axis of the ellipse-shape wound) (Figure 4A). We analysed the strain levels separately for the epidermal and dermal layers, as well as collectively (for the whole 'peri-wound skin') (Figure 4), and plotted the histograms of the volumetric tissue exposures to strains in the ROI as function of the suNPWT system type and dressing size (Figure 5). We then further calculated the percentage of tissue volume above the $\varepsilon = 1.75\%$ sensitivity threshold level for fibroblast migration for each suNPWT system type, and compared these between the CB- and CL-suNPWT treatment conditions and for the FSDs versus the RSDs, per each skin layer and for peri-wound skin altogether (Figure 6). Possible occurrences of pressure drops in the CL-suNPWT system (ie, pressures $<$ the nominal 80 mmHg) due to near-saturation of its dressing[15] were considered in all these simulations.

2.3 | Animal study

The animal study described here was conducted under approval no. APAFIS#23070-2 019 111 311 173 309.v2 of the NAMSA Medical Device Testing Ethical Committee registered by the French Department of Agriculture (Chasse-sur-Rhône, France), to compare between the wound healing performance of the CB-125 and CL-80 suNPWT systems when applied to open wounds.

The animal study included 6 female domestic pigs (of the Landrace-Large White cross breed), weighing 80 to 91 kg at the study start. In each animal, three circular full-thickness dermo-epidermic wounds with diameter of 3 cm and depths of 5 to 9 mm, extending to the deep fascia, were induced contra-laterally on the back (Figure 7A). The cranial wounds (L1 and R1; Figure 7A) were in general mildly deeper than the caudal wounds

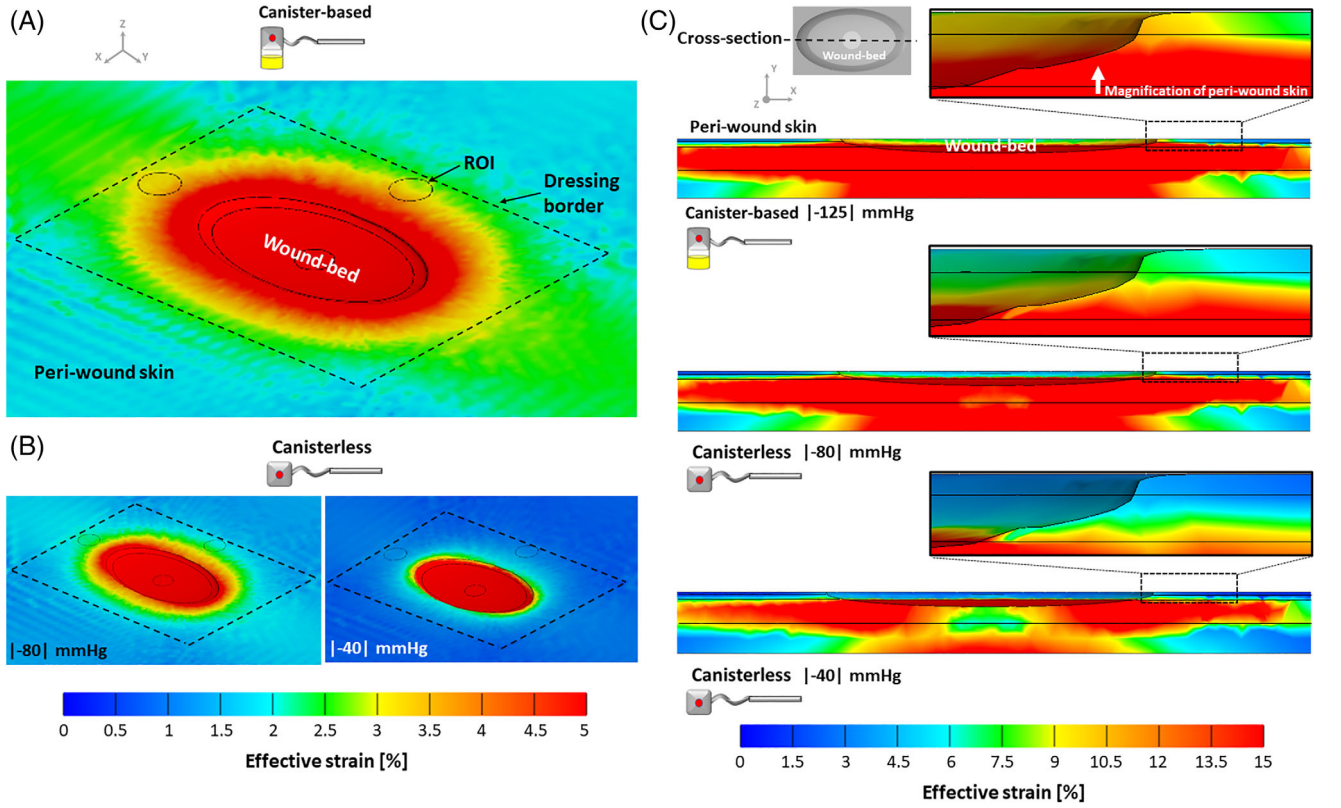

FIGURE 4 Distributions of the effective strains in the wound-bed and peri-wound skin under the dressing from A,B, top and C, cross-sectional views. The strain state in the wound-bed and peri-wound skin induced by a canister-based (CB) single-use negative pressure wound therapy (suNPWT) system operating at a negative pressure level of |−125| mmHg A, is characterised by greater strain magnitudes and farther reach of the strain field with respect to a canisterless (CL) suNPWT system operating at a pressure level of |−80| mmHg (B; lefthand side panel), which visualises the different influence zones (IZs) of these two distinct suNPWT system designs (the dressings were hidden for clarity). Loss of negative pressure in the CL suNPWT system, causing it to deliver only |−40| mmHg to the wound-bed, leads to a substantial decrease in the magnitude and area of the IZ (B; righthand side panel). C, Comparison of the peri-wound skin strain states (magnified in the upper right panels) between a CB suNPWT system operating at a negative pressure level of |−125| mmHg and delivering this pressure level de facto (top frame), a CL suNPWT system delivering |−80| mmHg to the wound-bed (centre frame), and where that CL system suffers loss of negative pressure down to a level of |−40|mmHg (bottom frame). The reductions in the magnitudes and reach of peri-wound tissue strains for the CL suNPWT system, which becomes even more pronounced with the simulated pressure drop, is clearly shown (centre versus bottom frames). Of note, the shrinkage of the IZ with the loss of negative pressure in the CL system applies both with regards to the spread of the strains over the surface of the peri-wound skin, and across the depth of the skin (through the epidermis and dermis). ROI, region of interest (for the further analyses of volumetric tissue exposures to strains)

(L3 and R3; Figure 7A) due to the differences in the anatomical sites. The CB-125 and CL-80 suNPWT systems were then applied to the 3 wounds on each side of the back in all the animals. All sites of application were randomised (equally) for the locations of the suNPWT systems of each type, that is, a suNPWT system of a certain type was placed on either the right (R1, R2, R3) or the left back sides (L1, L2, L3), respectively (Figure 7A), so that the animal body-side did not affect the acquired histomorphometric data reported below. From the start of the study and at each dressing change session, the wounds were subjected to quantitative wound healing morphometry, as well as to macroscopic examinations to assess signs of infection and maceration, and macrophotographs of the wounds were acquired. Where maceration was noticed, it was classified by an expert veterinarian as "slight", "moderate", "marked" or "very marked".

The fluid management capacity of the suNPWT systems was evaluated by weighing the used dressings from both suNPWT system types, and for the CB-125 system, the fluid contents in the canisters were also measured. At the day of study termination and after euthanasia, a total of 18 full wound tissue samples (9 for each suNPWT system type) were collected and stored in 10% neutral buffered formalin solution for a maximum of 72 h, prior to the histopathological analyses. In preparation for these histological studies, the wound samples were dehydrated in alcohol solutions of increasing concentrations, cleared

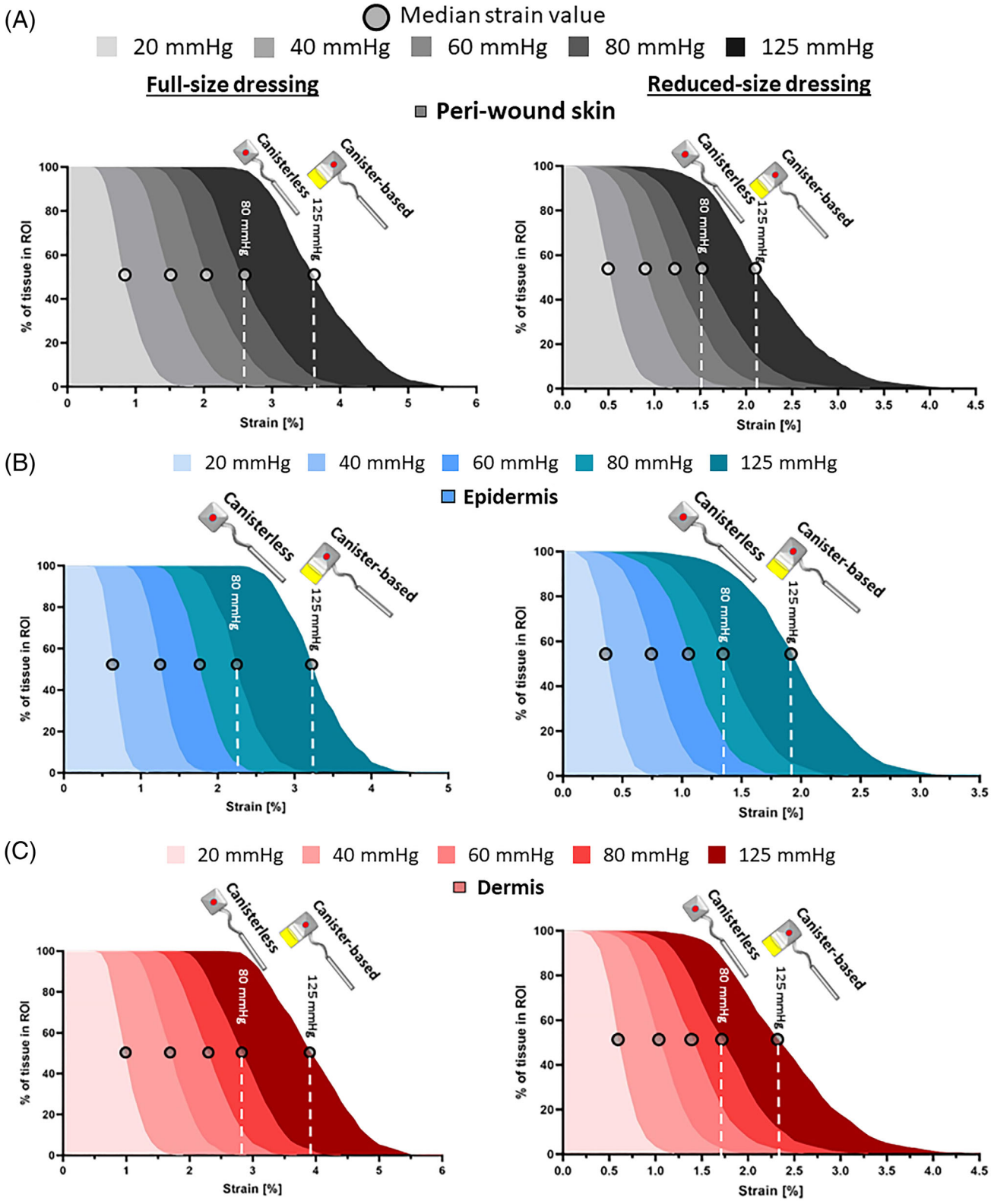

FIGURE 5 Comparisons of the volumetric peri-wound tissue exposures to effective strains, between treatment conditions where a canisterless, versus a canister-based single-use negative pressure wound therapy (suNPWT) system were applied, and when a full-size (left column) versus reduced-size (right column) dressings were simulated, for the whole peri-wound skin, that is, the epidermis and dermis layers combined together A, for the epidermis alone B, and for the dermis only C. Possible occurrences of pressure loss in the canisterless suNPWT system (ie, pressures < the nominal 80 mmHg) due to near-saturation of its dressing were considered in these simulations

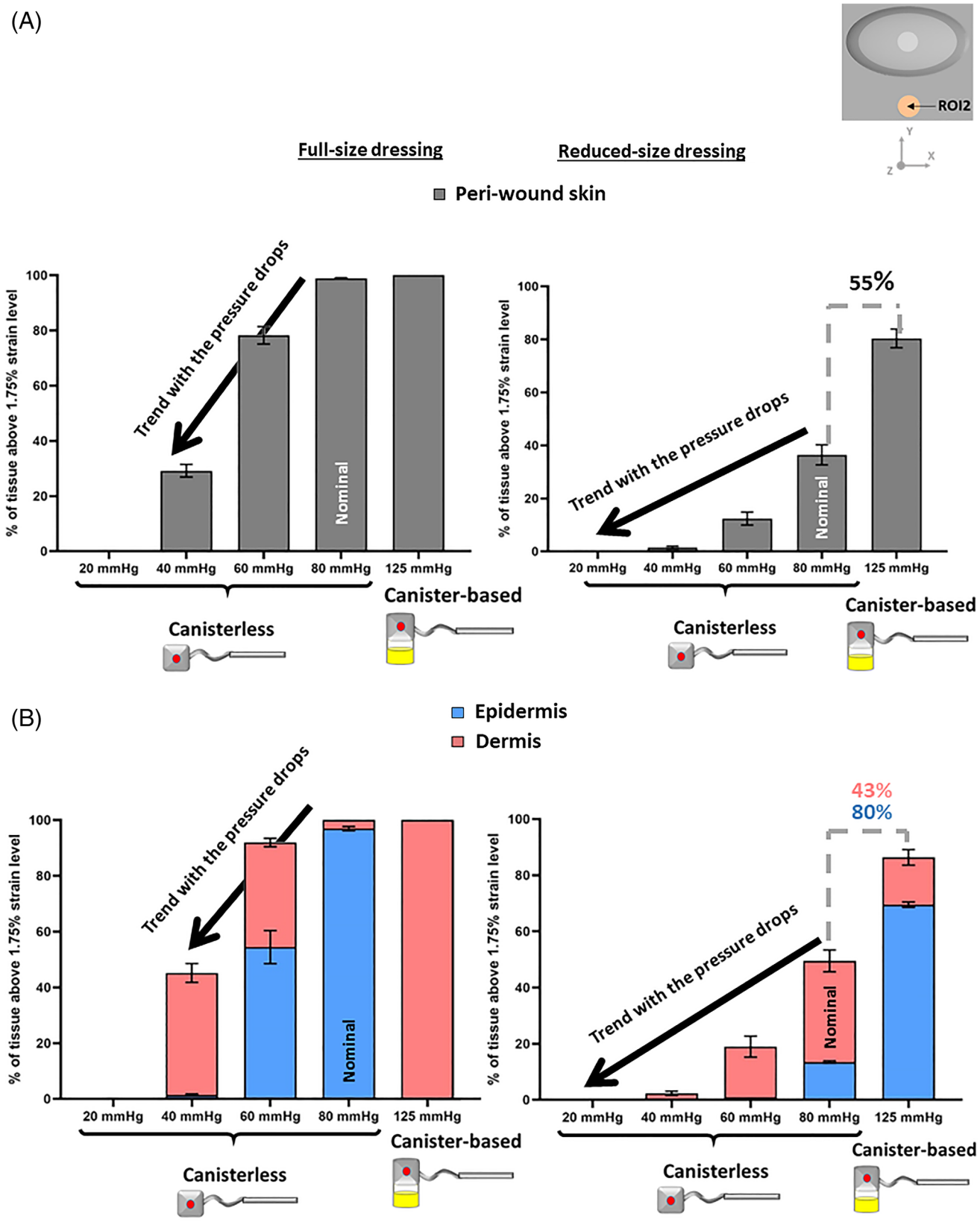

FIGURE 6 Legend on next page.

in xylene and embedded in paraffin. Central serial sections of 4.5-μm thickness each were then cut from every wound sample using a microtome, and stained by means of either Feulgin & Rossenbeck stain (FRS) for DNA material, or using Picrosirius red stain (PRS) for collagen. The stained histological slices were then digitised for respective histomorphometric evaluations of cell ingrowth (CI) and collagen remodelling index (CRI), as follows.

The digitised images of the FRS-stained slices were processed for the staining intensity (Figure 7B), which is proportional to the DNA concentration, and hence, is positively correlated with the number of the infiltrating dermal fibroblasts that migrate into and proliferate within the wound-bed, and which make the majority of the infiltrating cells at the granulation stage (though other cells including keratinocytes, endothelial cells and inflammatory cells may concurrently migrate to the wound-bed as well).[48] Accordingly, a cell ingrowth (CI) percentage measure was obtained from each digitised FRS-stained slice through image processing, by automated calculation of the total stained area (where presence of DNA was detected) over the total area of the micrograph under observation.

The digitised images of the sections stained by PRS were processed separately under polarised light for collagen polymorphism, that is, to determine the collagen remodelling ratio (CRI) = amount of mature collagen-type-I fibres (which are thick and densely-packed) over immature collagen-type-III fibres (that are thin and loosely-arranged).[49] The cross-polarisation of light causes the highly bi-refringent collagen fibres to display different shades, depending on the amounts of the type-I versus type-III collagen (which differ in thickness, packing and molecular organisation). The mature collagen-type-I fibres appear in red-orange colour whereas the immature collagen-type-III appears green (Figure 7C). The CRI can hence be calculated through image processing that automatically counts the red-orange versus green pixels in the polarised micrograph, and then calculates the red-orange/green ratio. As the CRI expresses the extent of collagen fibrillogenesis (ie, new collagen synthesis by fibroblasts), it is a specific marker for the intensity of fibroblastic activity.[50] To summarise the above histomorphometric analyses, the fibroblast infiltration and biological activity response to the application of a suNPWT system were quantified here by the corresponding CI and CRI values, whereas greater CI and CRI indicate the presence of a larger number of cells and more collagen synthesis by fibroblasts in the wound-bed, respectively. Therefore, unpaired, two-tailed *t*-tests were used to compare the CI and CRI at the study termination day between the CB-125 and CL-80 suNPWT systems for a statistical significance level of $P < 0.05$.

3 | RESULTS

3.1 | Computational finite element modelling

The applied negative pressure is uniformly distributed under the dressing. However, as shown by the computational FE modelling, the resulting mechanical strains in the wound and peri-wound tissues (associated with the different stiffnesses of the distorted tissue types and the geometrical discontinuities formed by the wound cavity) decay with the distance from the centre of the wound (Figure 4). The sharpest decline in tissue strain levels occurred at around the midpoint of the ROI, that is, at a distance corresponding to approximately 3.5 cm from the wound edge in the studied cases (Figure 4). The extent of the decay in the peri-wound skin strain level was approximately 1.5% from the ROI point that is nearest to the wound, to the farthest point (Figure 4A), that is, the strain gradient across the ROI (along a vector directed orthogonally away from the wound) was 0.75%/cm. Importantly, as the selected ROI captured the steepest change in peri-wound skin strains, we considered it to be appropriately located for assessing the peri-wound skin strain environment for potential stimulation of migration (mechanotaxis) of fibroblasts that reside at, or arrive into the peri-wound, towards the wound-bed (as described in the Introduction section and the literature cited therein). Strains were also generally greater in the dermis with respect to the epidermis (Figure 4B), as could be expected given that

FIGURE 6 Comparisons of the percentage [%] of peri-wound tissue exposures to effective strains above the $\varepsilon = 1.75\%$ sensitivity threshold level, between treatment conditions where a canisterless, versus a canister-based single-use negative pressure wound therapy (suNPWT) system were applied, and when a full-size (left column) versus a reduced-size (right column) dressings were simulated, for the whole peri-wound skin A, and for the epidermis and dermis as separate layers B. Possible occurrences of pressure losses in the canisterless suNPWT system (ie, pressures < the nominal 80 mmHg) were considered here. The percentage of peri-wound exposures to strains above the 1.75% level for the canister-based suNPWT system (bars on the right-hand side of each panel), which does not suffer the dressing saturation problem as exudate fluid is constantly transferred out of the dressing to its canister, are used as reference values for these systematic comparisons

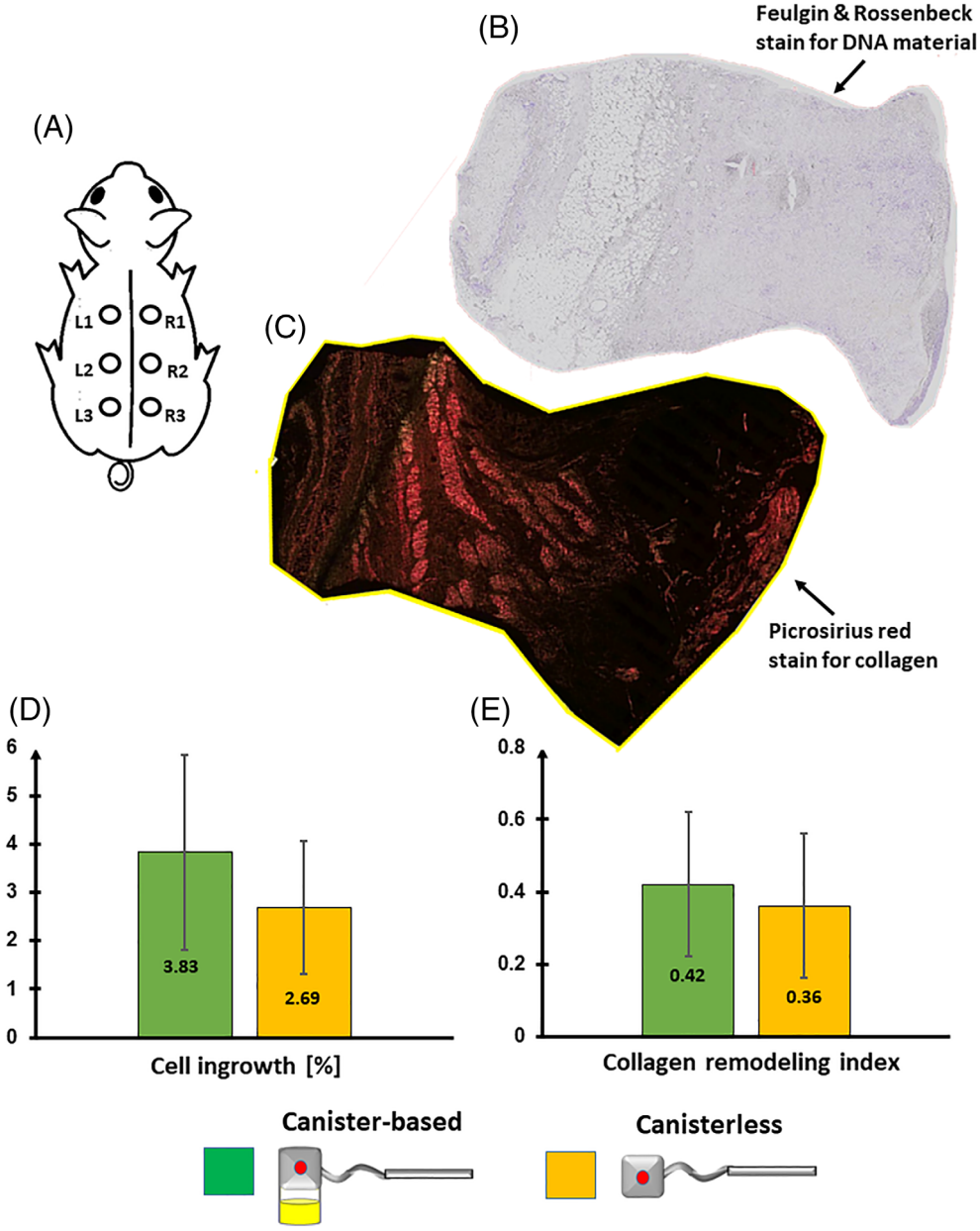

FIGURE 7 The animal study used to compare the biological outcome measures of the wound healing experiments conducted at 6 sites on the back of each pig A, revealed consistent trends of effects of cell ingrowth B, and collagen remodelling index C, measures that were 1.4-times and 1.2-times greater for the canister-based single-use negative pressure wound therapy (suNPWT) system, respectively D,E, however, without statistical significance with respect to the corresponding values of the canisterless suNPWT system

the dermal stiffness is lower by an order-of-magnitude than that of the epidermis (Table 1). In addition, and as expected, the size of the IZ in the peri-wound, namely, both the area of the IZ and the magnitude of tissue strains therein, decreased when there was loss of the (absolute) pressure level delivered by the CL-80 suNPWT system (Figures 1B, 2 and 4C).

An investigation of the strains in the ROI following the application of both types of suNPWT systems revealed that the median strain values in the ROI decreased substantially as a function of the level of the negative pressure as well as with the dressing size (Figure 5). For an FSD, the median strain was consistently greater, and within the 3% to 4% range for the CB-125 suNPWT system as compared to the median strains for the CL-80 suNPWT system that were at the 2% to 3% range, regardless of the skin depth. In other words, the difference in nominal pressure values between the CB-125 and CL-80 suNPWT systems, where each system is used with an FSD, translates to approximately 1%-greater peri-wound skin strains induced by the CB-125 suNPWT system, which is a mild difference, pending that each suNPWT system actually delivers the nominal negative pressure level as defined by the manufacturer, to the wound-bed (Figure 5; left column**)**.

Nevertheless, when pressure drops were simulated to occur in the CL-80 suNPWT system, the median strain fell below $\varepsilon = 1.75\%$ for this type of suNPWT system if the pressure delivered to the wound-bed de facto was half the nominal (80 mmHg) value (Figure 5; left column**)**.

For an RSD, this phenomenon further intensified for the CL-80 suNPWT system, such that the median strain in the ROI fell below the 1.75% threshold already when a pressure drop of 20 mmHg was simulated to occur (Figure 5; right column). Noteworthy is that for the CB-125 suNPWT system, the median strain in the ROI always remained above the $\varepsilon = 1.75\%$ threshold, irrespectively of the dressing size, or the depth into skin tissue within the ROI (Figure 5). In contrast, for the CL-80 suNPWT system, pressure drops were shown to lower the peri-wound skin strain from the aforementioned 2% to 3% range down to 0.5% to 1% (Figure 5), which is substantially below the $\varepsilon = 1.75\%$ fibroblast sensitivity threshold for migratory activity. Ultimately, under the influence of severe pressure drops in the CL-80 suNPWT system, the peri-wound skin strains can be as much as 2.5%-lower for this system with respect to the CB-125 suNPWT system if both systems are utilising an FSD (Figure 5; left column). This is a substantial strain difference that is induced by the two suNPWT system types in terms of the mechano-activation of fibroblasts in the peri-wound skin, resulting in potential inferiority of the CL-80 suNPWT system in promoting a migratory fibroblast response if the pressure delivery by this system is inconsistent over the time course of the treatment.

The analyses of percentage of skin volume exposure in the ROI to mechanical strains above the $\varepsilon = 1.75\%$ threshold level yielded consistent findings for the effects of the pressure magnitude and the occurrence of pressure drops in the CL-80 suNPWT system (Figure 6). Specifically, for an FSD, the whole skin and each skin layer were above the $\varepsilon = 1.75\%$ fibroblast sensitivity threshold level for both suNPWT system types as long as the nominal 80 mmHg pressure was indeed delivered by the CL-80 suNWPT system (Figure 6A). However, for the CL-80 suNWPT system, it was demonstrated that a pressure drop of 20 mmHg would result in that approximately 20% of the peri-wound skin becomes underexposed to stimulating strain levels, with the majority of the strain reduction occurring in the epidermis (Figure 6A; left column). These underexposure conditions escalated considerably for a pressure drop of 40 mmHg, and, if the pressure dropped further by additional 20 mmHg, the entire volume of peri-wound skin was underexposed to stimulating mechanical strains (Figure 6A; left column).

For the cases of RSDs, the peri-wound skin volume exposed to stimulating strains induced by the CL-80 suNPWT system was already lower by 55% with respect to the tissue volume stimulated by the CB-125 suNPWT system when the CL-80 suNPWT system was operating at its nominal pressure value (Figure 6A; right column). Moreover, sharper drops in the strain exposure of peri-wound skin to stimulating strains were demonstrated with each pressure drop increment occurring in the CL-80 suNPWT system, with respect to the FSD cases. That is, when the CL-80 suNPWT system delivered 40 mmHg through an RSD, the peri-wound skin exposure to strains above the 1.75% threshold became negligible (Figure 6A; right column). Overall, with the occurrence of pressure drops, the CL-80 suNPWT system exhibited the development of considerable underexposure of peri-wound skin to stimulating mechanical strains, which were more dominant for RSDs but substantially present for FSDs as well, particularly if the actually delivered pressure levels were half or below-half the 80 mmHg nominal pressure level (Figure 6).

3.2 | Animal study

Histomorphology analysis of tissues sampled from the animal study revealed that the CB-125 suNPWT system promoted greater cellular activity than the CL-80 suNPWT system, which manifested as 1.42-times and 1.17-times greater (though nonsignificant) CI and CRI values, respectively, for the CB-125 suNPWT system (Figure 7D,E). These consistently higher CI and CRI values indicated that the larger number of present fibroblasts indeed contributed to comparatively more collagen fibrillogenesis in the wounds treated by the CB-125 suNPWT system relative to the CL-80 suNPWT system.

A continuous, consistent negative pressure level is also critically important for effective fluid management, to avoid infections and maceration. In this regard, it was observed that the collected and removed exudate volumes were overall considerably greater for the CB-125 mmHg suNPWT system. Specifically, the weights of the exudate in the dressings of the CL-80 suNPWT system were 3.8, 4.6, 2.4 and 1.2 g at study days 3, 6, 10 and 14, respectively. In contrast, the weights of the exudate in the CB-125 suNPWT system (dressing + canister) were 4.2 + 1.8, 6.7 + 1.2, 3.1 + 0.6 and 0.8 + 0.1 g at the corresponding study days 3, 6, 10 and 14, respectively. That is, the cumulative (dressing + canister) fluid collection by the CB-125 suNPWT system was on average 1.4 ± 0.4 greater than that of the CL-80 system throughout the study period. At the peak of exudation, at days 3 and 6, the fluid collection by the CB-125 suNPWT system was 1.6-fold and 1.7-fold greater, respectively, than that for the CL-80 system, which is expected due to the nominally greater negative pressure level delivered by the CB-125 system, but may also reflect episodes of pressure loss in the CL-80 suNPWT system compromising a consistent exudate removal, as discussed above.

Related to the latter point which indicates possible episodes of inferior exudate removal by the CL suNPWT

system (and the potential consequences of wound and peri-wound overhydration caused by a saturated CL dressing), macroscopic wound evaluations and scaled macrophotographs acquired at study day 14 revealed potentially important differences between the wound healing processes induced by the two suNPWT system types. Specifically, the wounds treated with the CL suNPWT system developed more protruding granulation tissue which is often a sign of excessive moisture (the mean surface area of the hypergranulation tissue was 14.70 mm^2 versus 1.88 mm^2 for the CL and CB suNPWT systems, respectively; $P < 0.05$). In addition, the observed maceration counts were greater (n = 11) for the wounds treated by means of the CL than for those treated by the CB (n = 9) systems, and whilst all the observed maceration cases for the CB system were classified as "slight" by the expert veterinarian, the maceration cases for the CL system varied over the 'slight' to "marked" range. Yellowish slough, likely indicating the presence of infection, was observed for two wounds treated by the CL system at day 10 and for an additional wound treated by CL at day 14, whereas for the CB system there was just one such observation for a single wound at day 14. The infection signs were not always associated with maceration, and overall, the maceration cases and potential infections did not considerably affect the progress of healing, as the histopathology of the macerated/infected wounds was homogeneous throughout and similar to the wounds not showing these signs.

4 | DISCUSSION

It is a consensus that suNPWT is able to enhance the healing of open wounds as well as of closed (surgical) incisions, and thereby, reduce the cost of wound care,[12,51-56] however, suNPWT systems are not all the same. It is therefore imperative to understand the different modes of action of commercially available suNPWT technologies and systems, and their resulting performance metrics, primarily with respect to the CB versus CL design concepts, but also regarding the mechanobiological effects of the magnitude of the intended negative pressure. In our published work focusing on the above questions in the context of NPWT of surgical incision wounds, we developed and utilised a computational modelling framework of closed incisions integrated with laboratory bench-test work for simulated clinical use of suNPWT systems.[15] In addition, and similarly to the current study, we further conducted and reported a preclinical study in a porcine model for closed incision.[15] In this recently published study, we specifically focussed on the impact of effective fluid management for continuous delivery of the intended negative pressure, and the consequences of potential losses of the pressure level over the therapy period. We found that a greater (absolute) negative pressure level and continuous, consistent delivery of the intended negative pressure through a controlled fluid management technology, constantly removing excess fluid from the dressing, provides superior biomechanical performances of the suNPWT system, which are more likely to result in better quality of the repaired tissues.[15]

The conclusions from the results presented here are consistent with our published research, and furthermore, extend the previous conclusions (concerning closed incision wounds[15]) to the various clinical scenarios of open wounds. Specifically, in the current work, we focused on the mechanobiological role of the negative pressure in stimulating fibroblasts to migrate from the peri-wound into the wound-bed, and on the importance of consistent therapy for that mechano-stimulation to both: (i) provide an IZ with magnitude to reach into a sufficient peri-wound tissue volume, to capitalise on the reservoir of neighbouring fibroblasts; and, (ii) sustain the therapeutic impact of the IZ over the entire therapy time. The results from the FE simulations of the IZ as a function of the applied negative pressure clearly demonstrate the importance of continuous delivery of the intended negative pressure.

Already at baseline conditions, that is, for constant and continuous delivery of the intended negative pressure, the peri-wound skin strain magnitudes associated with the IZ for the CB-125 suNPWT system at the ROI (approximate strain range: 2.5% to 3.5%) are greater compared to those for the CL-80 suNPWT system (approximate strain range: 1.5% to 2%) (Figure 4A,B). However, at occurrences of loss of the intended negative pressure in the CL −80 suNPWT system due to saturation of its dressing, the stimulation level at the peri-wound drops remarkably, and may even approach zero if the pressure losses only allow |−20| mmHg to be actually delivered by the CL system to the wound-bed (Figures 4B,C and 6A, B). At 50% loss of the intended negative pressure for the CL-80 suNPWT system, the effective strain delivered to the actual wound is also impaired considerably, and surface skin strains at the ROI drop below the 1.5% level (Figure 4B, right frame). This effect of shrinkage of the IZ with the loss of negative pressure in the CL system applies both with regards to the spread of the strains over the surface of the peri-wound skin, and across the depth of the skin (Figure 4C). Of note, if an open wound is situated at a more challenging anatomic location, where there is no space for an FSD, and thereby, an RSD must be applied, then the above effect of reduced stimulation of the peri-wound with the loss of pressure escalates, and already approaches zero when |−40| mmHg (ie, half the

intended negative pressure) is actually delivered by the CL system to the wound-bed (Figure 6C,D). Overall, the current computational results demonstrate that the stability of the negative pressure delivered to the wound-bed is critical regardless of the dressing size, but the impact on the IZ as a function of the pressure loss is exacerbated for a smaller dressing (Figure 6).

Importantly, given that for all wounds (eg, surgical, traumatic, and chronic) the peri-wound skin is the main biological reservoir for immediately available tissue-repairing cells, and thereby, for adequate wound healing, effective and consistent mechano-stimulation of the peri-wound over time is fundamental to a successful clinical outcome. This underpins the inherent advantage of a CB suNPWT technology in achieving such consistent peri-wound mechano-stimulation. Indeed, the biological measures of CI and CRI acquired in the current animal study provided experimental support in demonstrating that, and, albeit statistically nonsignificant, greater cellular activity and more de novo collagen synthesis by fibroblasts were demonstrated for the CB-125 suNPWT system (Figure 7D,E). This is further consistent with previous results reported by Jeong and colleagues,[57] who applied stationary (as opposed to single-use) NPWT at a 125 mmHg pressure level, and a foam-film dressing (ie, not an absorptive dressing) to chronically infected wounds of 3 patients. Although appreciating that their work differed from the current study in the stationary delivery mode of the negative pressure and the dressing type that was used, based on immunostaining of biopsies of debrided tissues from the treated wounds, this research group likewise concluded that a consistent therapy utilising a $|-125|$ mmHg pressure level boosts the collagen synthesis resulted by the enhanced infiltration of tissue-repairing cells.

Ideally, a suNPWT system with an absorptive dressing should equally distribute the negative pressure under the entire wound pad area, and, also, deliver it consistently over the time of the intended treatment. Here, we investigated both the spatial distribution of peri-wound skin strains and their potential variation over time if a pressure loss occurs in a suNPWT system.

The ability of a suNPWT system to deform the peri-wound and thereby, stimulate tissue-repairing cells residing in the peri-wound to migrate into the wound-bed depends on: (a) The size, shape and mechanical properties of the wound and peri-wound tissues which are influenced by the stage of the healing process, for example, as closure progresses and scarring forms, and; (b) The magnitude of the IZ, which in turn depends on the level and continuity of the applied negative pressure. Nonetheless, if loss of pressure occurs during the intended therapy period, the impact of this on the peri-wound skin strain environment can be rapid and intense, as demonstrated in the current work (Figures 4-6), with potential consequences on the biological activity and the healing process (Figure 7). It is expected, based on the current results (Figures 4-6), that the loss of negative pressure has a prompter effect than natural healing on the peri-wound skin strain environment, since substantial pressure drops have an immediate effect on the strain state (eg, see Figure 4C), as opposed to the progress of healing that has a slow, gradual influence on the peri-wound strains. Clearly, pressure losses, both instant and over time, are unwarranted, not only because they divert the course of the NPWT from the intended care, but also since their occurrence dramatically changes the cell environment at the critical time of recruitment of tissue-repairing cells.

To summarise the computational results obtained in this study, important differences were demonstrated between the two studied suNPWT system technologies with regards to the induction of consistent peri-wound skin strains for the stimulation of fibroblast migratory behaviour. These differences in suNPWT system performance originate from the following factors: (*i*) The nominal pressure level delivered by the suNPWT system; (*ii*) The fluid management technology of the suNPWT system, which in turn, has implications on the ability of the CL design type to deliver consistent negative pressures over time; and (*iii*) The dressing size used for the application of the NPWT. Importantly, a continuous, consistent negative pressure level is vital for effective mechano-stimulation of the peri-wound skin and of the fibroblasts within, to trigger their migratory activity (Figures 4-6). Accordingly, and in excellent agreement with the above computational results, the histomorphology analyses of tissues sampled from the animal study revealed that the CB-125 suNPWT system promoted greater (though non-significant) cellular activity, indicated by higher CI and CRI values compared to the CL-80 suNPWT system (Figure 7D,E). These consistently higher CI and CRI values suggest that the larger number of present fibroblasts indeed contributed to comparatively more collagen fibrillogenesis in the wounds treated by the CB-125 suNPWT system, relative to the CL-80 suNPWT system. The current animal study provided further indication for the likelihood of better healing outcomes using a CB suNPWT system, as there was less maceration and infection associated with the CB technology.

Some limitations existed in our study which should be discussed. First, we assumed uniform thicknesses of the soft tissue layers below a flat skin. This simplifies the anatomical conditions on the one hand, but on the other hand, allows to methodologically study the IZ and reach of the strain stimulation induced by the suNPWT systems without the bias caused by random tissue surface waviness, potential skin or subdermal lesions, or any other

patient- and/or wound-specific geometrical or mechanical stiffness irregularities that may influence the strain state of skin. We further aimed at representing a generic anatomical domain, rather than limiting the scope of the current work to a specific anatomical site. Second, we did not consider spatial or temporal changes in the stiffness of skin over the course of the NPWT, which may, in the real-world, affect the strain state of skin, and thereby, the IZ induced by the suNPWT and its extent of penetration into the peri-wound skin. Sustained exposure to wound fluids, for example, may locally soften certain peri-wound skin regions, and thereby, alter the skin strain state in space and time. Likewise, scarring of a certain region in the wound which changes the stiffness of a portion of the wound-bed will change the strain state of the wound and peri-wound. Again, the motivation to create a generic environment for the study of the fundamental, suNPWT-related biomechanical factors affecting the IZ required the assumptions that the peri-wound skin stiffness does not change in space or time, that skin is not wrinkled or folded and is not otherwise compromised. Third, our animal study included a relatively small number of animals, which likely reduced the statistical power, however, the nature of these experiments inherently restricted the number of test animals to the necessary minimum, for ethical reasons. Lastly, for the computational modelling work, some of the tissue mechanical properties used for the modelling were adopted from published animal research where fresh tissue specimens were studied (Table 1), in the absence of relevant test data from fresh human tissues.

We conclude that in the treatment of open wounds by means of suNPWT, and similarly to suNPWT treatment of closed incisions,[15] consistent delivery of the negative pressure to the wound-bed is crucial. A CB suNPWT technology offers intrinsic stability of the delivered intended negative pressure, since exudate is constantly being transferred away from the wound, into the canister, which prevents the dressing from approaching saturation, rendering this technology superior for steady stimulation of the peri-wound to maximise the attraction of peri-wound fibroblasts for tissue repair. Although the CL suNPWT system appears to induce similar stimulation in the peri-wound when it operates at its nominal pressure level, the major concern that is raised on the basis of the current study is that loss of pressure (associated with the inherent CL design) will adversely lower the peri-wound skin stimulation to an extent equivalent to a complete lack of NPWT in the aspect of peri-wound stimulation.

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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

* This concept of the IZ was adopted from the orthopaedic mechanobiology literature where similar terminology is often used to describe the mechano-responsiveness of bone cells (osteocytes).

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