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Molecular mechanisms of action of negative pressure wound therapy: a systematic review

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Abstract

Negative pressure wound therapy (NPWT) has significantly advanced wound care and continues to find new applications. Its effects at a molecular level however, remain a subject of debate. The aim of this systematic review is to summarize the current evidence regarding the molecular mechanisms of action of NPWT. Medline, Embase, EBSCO databases and clinical trial registries were searched from inception to January 2023. Clinical studies, animal models or in-vitro studies that quantitatively or semi-quantitatively evaluated the influence of NPWT on growth factors, cytokine or gene-expression in the circulation or wound-bed were included. Risk of Bias assessment was performed using the RoBANS tool for non-randomized studies, the COCHRANE's Risk of Bias 2(ROB-2) tool for randomized clinical studies, OHAT tool for in-vitro studies or the SYRCLE tool for animal model studies. A descriptive summary was collated and the aggregated data is presented as a narrative synthesis. This review included 19 clinical studies, 11 animal studies and 3 in-vitro studies. The effects of NPWT on 43 biomarkers and 17 gene expressions were studied across included studies. NPWT stimulates modulation of numerous local and circulating cytokines and growth factor expressions to promote an anti-inflammatory profile. This is most likely achieved by down-regulation of TNFα, upregulation of VEGF, TGF-β and fibronectin.

Introduction

Open surgical wounds or surgical wounds healing by secondary intention are a common and complex problem. These wounds frequently take a long time to heal, require regular dressing changes and present a significant morbidity to the patient and a significant financial burden to healthcare systems. They may need many modalities of treatment, are susceptible to secondary infection, and may also require prolonged hospitalization and/or further operations (Ref. 1). The requirement to manage exudate and avoid repeated wound dressing changes has led to a significant increase in the use of newer modalities of wound management such as negative pressure wound therapy (NPWT) (Ref. 2).

NPWT is currently used widely in many aspects of wound care and has been strongly promoted for use on complex wounds (Refs 3, 4). NPWT involves the application of an airtight wound dressing through which a negative pressure is applied, often with any wound and tissue fluid drawn away from the area being collected into a canister. The amount of pressure applied using the therapy can vary and there is no universally agreed protocol for its use (Ref. 5). A number of surgical and non-surgical specialties prescribe NPWT leading to its widespread implementation in both primary/community care and in tertiary care (Refs 6, 7).

NPWT is postulated to facilitate wound healing via several primary and secondary mechanisms. The proposed primary mechanisms of action include macro-deformation or wound shrinkage, micro-deformation at the foam-wound interface, fluid removal whilst maintaining a moist environment and stabilization of the wound environment. The proposed secondary mechanisms include alteration of the mechanotransduction pathways and alteration of the wound healing microenvironment including cellular proliferation, differentiation, cell migration, angiogenesis and neurogenesis. Many theories have been proposed to support these primary and secondary mechanisms at a molecular level and the aim of this systematic review is to summarize the currently available evidence regarding the molecular mechanisms of action of NPWT (Refs 8, 9, 10, 11, 12, 13, 14, 15).

Methods

Search strategy

Medline, Embase and Elton Bryson Stephens Company (EBSCO) databases, and Clinical trial registries were searched from inception to January 2023 using pre-specified key words (Supplementary file 1). Article screening and extraction was performed by two authors (BR and NS) using the Rayyan online screening and data tool (Ref. 16). The reference lists of the retrieved articles and similar review articles in the field were also searched to identify additional papers. Studies that examined the mechanism of action of NPWT in patients or in

animal models in preclinical studies or in-vitro studies were included. We included studies that evaluated the effect of any form of NPWT on open surgical wounds including diabetic foot ulcers, pressure ulcers, surgical site infections (SSI), traumatic wounds and post-operative wounds. Studies which focussed on the effects of NPWT on primarily closed wounds or stoma creation were excluded. Case reports, non-English papers, editorials/commentaries, reviews, letters and papers with limited data on methodology were excluded. The study was registered in the PROSPERO database (CRD42022303088) and was performed according to Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines (Ref. 17).

Data extraction

The key details regarding the method and results were recorded on a bespoke data extraction sheet. Data extraction was conducted by two independent reviewers (BR and NS). Discrepancies were resolved by discussion amongst the authors and a tie-breaking vote from the authors not involved in the screening process. Data elements extracted included study name and year of publication, country, immune cell/mediator(s) described in the study, model (clinical studies, animal wound models or in-vitro),type of wound, specific device with control intervention, duration and time points of analysis, quantitative/qualitative outcomes, duration of follow-up, publication status, funding and conflict of interest.

Assessment of risk of bias (RoB)

Risk of Bias assessment was performed using the RoBANS tool (Ref. 18) for non-randomized studies, the COCHRANE's Risk of Bias 2(ROB-2) tool (Ref. 19) for randomized clinical studies, Office of Health Assessment and Translation (OHAT) tool (Ref. 20) for in-vitro studies or the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool (Ref. 21) for animal model studies. The risk of bias assessment and quality assessment figures were produced with the help of the interactive online web application, 'robvis' (Ref. 22).

Data synthesis and analysis

Due to the diversity of the variables and immune markers being evaluated and the heterogeneity of the studies being reviewed, it was not possible to pool data and present findings as a meta-analysis. Instead, a descriptive summary was performed with aggregated data presented as a narrative synthesis. The narrative synthesis includes elements such as the immune cell or biomarker of interest, its context and the impact of NPWT on it and the relationship between the immune cell/biomarker and wound healing and the concordance between studies with respect to these findings. Also, each study's methodological and summary characteristics are presented in a separate table to include the author(s), institution, year of publication, sample size, study model, biomarkers/cell markers under review and key findings reported by authors.

Results

Out of 6397 potential studies, 33 studies were included in the systematic review. This included 19 clinical studies, 11 animal studies and 3 in-vitro studies. The exclusion of all the other studies has been outlined in Figure 1 in accordance with the PRISMA reporting guidelines. Out of the 11 animal models, 1 study was conducted in a rabbit model, 5 studies were conducted in murine models and 5 studies in porcine models. Thirteen studies had a high risk of bias and 3 studies had some concerns of bias. Ten clinical studies and 10 animal studies analysed tissue samples from wounds while 5 clinical studies analysed the wound effluent. Five clinical studies and one animal study also used serum samples to correlate the effect of NPWT on wounds. Twenty-eight studies focussed on the effect of NPWT on molecular and cellular biomarkers, while 5 focussed on the effect of NPWT on differential gene expression in wound or serum samples. Substrate analysis was carried out by a combination of quantitative and semiquantitative methods including enzyme-linked immunosorbent assay(ELISA), immunohistochemical(IHC) staining or Western blot analysis. Analysis of gene expression was predominantly carried out by RNA sequencing and/or reverse transcription-quantitative polymerase chain reaction(RT-qPCR). These findings are elaborated in Table 1.

Vascular Endothelial Growth Factor (VEGF) was the most frequently studied growth factor in relation to NPWT with 7 papers identified (Refs 27, 28, 34, 38, 41, 45, 49, 50). Results from clinical studies were reported in 4 studies (Refs 27, 28, 34, 38). A significant increase in the local VEGF concentration was seen in clinical wounds treated with NPWT, and reports from animal studies concurred with these findings (Refs 41, 45, 49, 50). This increase in VEGF has been postulated to contribute to the increased neovascularization and granulation tissue formation in patients treated with NPWT. VEGF was elevated in all 7 studies which studied its effects. Tumour necrosis Factor alpha(TNF α) was downregulated in 5 out of 8 studies and was the next most common biomarker that was studied (Refs 25, 26, 34, 37, 44, 55). TNF α is considered as a pro-inflammatory cytokine and a potent inducing agent for the upregulation of cytokines, reactive oxygen species and apoptosis. Elevated levels of TNF α in the wound bed have been associated with chronic non healing wounds with reduced granulation tissue production. Transforming Growth Factor Beta (TGF β) was upregulated in 5 out of 7 studies that studied its effects. The data from the in vitro models included in this paper (Refs 53, 54, 55) suggest that it leads to increased granulation tissue production. NPWT induces the production of TGF-β1, which is crucial for the initiation of the proliferation phase of wound healing. The effect of NPWT on wound healing is mediated through various signals, including TGF-β-Smad, which further underscores the importance of TGF-β in this context. Fibronectin was upregulated in both studies which evaluated its effects (Refs 23, 39). Equivocal results were obtained across all studies with respect to Interleukins (IL) and Matrix Metalloproteinases (MMP) including IL1β, IL 6, IL8, IL8, MMP 2, 3 and 9. The effects of NPWT on 43 other molecular biomarkers and 13 different gene expressions were analysed across included studies (Table 1).

Clinical/human studies

Nineteen clinical studies were conducted to assess the MOA of NPWT from 2003 to 2022 with study numbers varying from three to172 patients. Out of these, 12 studies compared the mechanisms of action between NPWT and standard dressings and other studies studied the MOA of NPWT alone. Eleven studies used granulation tissue samples from wound beds, 5 studies studied samples from wound effluents and 5 studies analysed peripheral blood samples. Ten studies had a high risk of bias, one study had some concerns of bias and eight studies had a low risk of bias. (Fig. 2a, 2b) The main cytokines of interest in these studies were VEGF,TNF α, Interleukin(IL)-6, IL – 8, IL 1B, and the family of matrix metalloproteinases (MMP) MMP-1, MMP-2, MMP-9, MMP-13. VEGF was upregulated in all four studies which studies it's effects TNF α was downregulated in four out of four studies, Fibronectin and TGF B1 were

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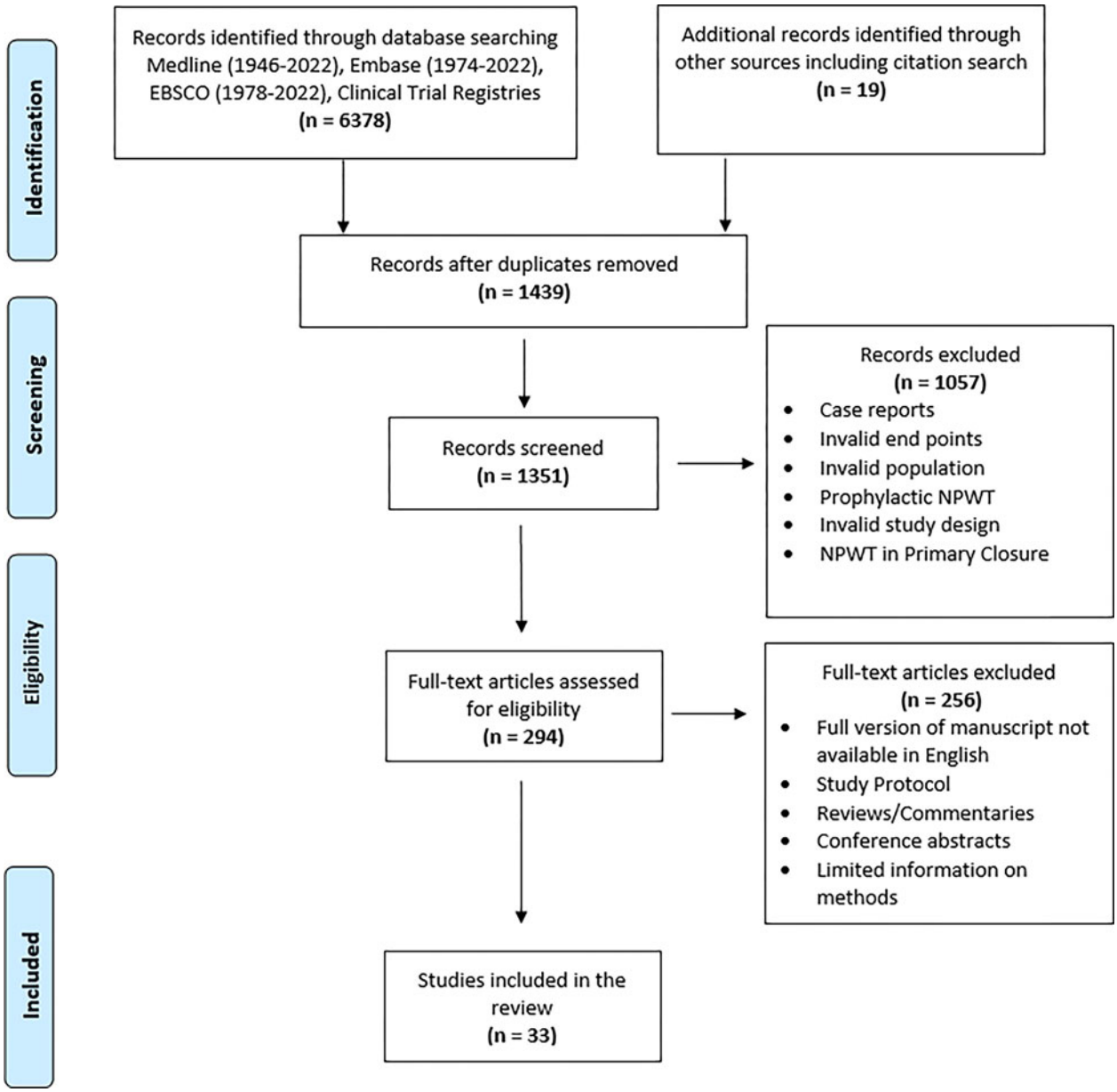

Figure 1. Literature search and study selection.

upregulated in both studies which studied their effects. There was no concordance regarding the impact of NPWT on the other cytokines, biomarkers and/or genes.

Animal studies

Eleven animal studies were included in this review out of which five studies used murine models, five studies used porcine models and one study used rabbit models. The sample size ranged from six to 56 animals. Three studies had a high risk of bias, two studies had some concerns and six studies had a low risk of bias. (Fig. 2c) All studies used tissue samples and two studies also used serum samples in addition for analysis. The main cytokines of interest in these studies were TNF α, FGF-2, TGFB1, PDGF and VEGF. Three out of three studies reported the upregulation of VEGF following NPWT. Two studies reported the upregulation of TNF α while one study reported its upregulation following NPWT. The results of most of the included animal studies suggest that many of the cytokines/chemokines and genes are upregulated following the upregulation of NPWT.

In vitro studies

Three studies studied the mechanisms of action of NPWT using in vitro models using murine fibroblasts (Ref. 53), human fibroblasts in a 3D fibrin matrix (Ref. 56) and a combination of PMNs, HL 60 cell lines and Macrophages (Ref. 55) respectively. Each study examined a completely different set of biomarkers (Table 1). Two studies conducted their experiments in a cell culture under negative pressure. Two studies also reported the upregulation of TGF-B under the effect of NPWT (Refs 53, 56). The risk of bias assessment using the OHAT tool revealed a low risk of bias for one study, some concerns of bias and high risk of bias for the other two studies.

Gene expression changes

The effect of NPWT on 17 different gene expressions was assessed in this systematic review (Table 1). Since no two studies evaluated the effects of similar gene expressions, it was not possible to collate these findings. The results of the included studies have suggested that the genes induced by NPWT were associated with

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Table 1. Characteristics of the included studies

Study	Participants	*N*	ROB	Randomization	Comparator	Substrate	Focus	Markers under study
Arslan (Ref. 23)	Humans	11	H	N	None	Tissue	Biomarkers	Increased Fibronectin levels
Borys (Ref. 24)	Human	29	L	N	Standard dressings	Tissue	Differential gene expression	GA2 downregulated C1QBP upregulated RAB35 downregulated SYNJ1 downregulated
Stechmiller (Ref. 25)	Human	8	H	N	None	Wound effluent	Biomarkers	TNF alpha downregulated IL 1B upregulated MMp-2:downregulated MMP3: upregulated TIMP-1: upregulated
Eisenhardt (Ref. 26)	Humans	30	L	Y	Petroleum gauze dressing	Tissue	Biomarkers	TNF alpha: downregulated IL1 B: downregulated CD68: downregulated
Labler (Ref. 27)	Humans	21	H	N	Epigard	Wound effluent	Biomarkers	IL6: increased IL8: increased 1L10: no change VEGF: increased FGF2: no change
Labler (Ref. 28)	Humans	32	S	N	Epigard	Wound effluent	Biomarkers	IL6: no stat diff IL8: increased VEGF: increased FGF2: no stat diff Increased vWF and CD31
Greene (Ref. 29)	Human	3	H	N	Foam filler	Tissue	Biomarkers	MMP-2 : reduced MMP-9/NGAL complex: Reduced
Frear (Ref. 30)	Humans	8	H	N	Standard dressing	Wound effluent	Proteomics	Increased: MMP Arginase 1 Low affinity IgGFc IIIA FilaminA Alpha 2 Macroglobulin Hemoglobin alpha
Hohendroff (Ref. 31)	Humans	69	H	N	Standard dressing	Blood sample	Plasma Biomarkers	Angiopoeitin-2: reduced Overall Microvesicles: reduced
Jia (Ref. 32)	Humans	3	H	N	None	Tissue	Proteomics	Wound Serum CTSS : Decreased decreased ITIH4: Increased increased PROS1: increased increased PRDX2: Increased increased
Kapusta (Ref. 33)	Humans	35	H	N	Standard dressing	Venous blood	Micro RNA levels	Let 7-2-3p miRNA upregulation
Karam (Ref. 34)	Humans	40	L	Y	Moist dressing	Tissue	mRNA levels	**Downregulated:** TNF alpha IL 1B MMP1/9 **Upregulated:**

								TGF B1 VEGF TIMP1
Ludwig- Slomczynska (Ref. 35)	Humans	36	H	N	Standard dressing	Tissue and blood samples	DNA hybridization	DNA repair and autocrine signalling via retinoic acid receptor: Chr6p21 Chr20p13 Delacoix Morf Hypermethylation of c2,c3,c4 C1QBP upregulated
Moues (Ref. 36)	Humans	33	L	Y	Standard dressing	Wound effluent	Biomarkers	Lower pro MMP Lower total MMP-9/TIMP-1
Wang (Ref. 37)	Humans	26	L	Y	Standard dressing	Tissue	Biomarkers	**Downregulated:** TNF A IL 6 PC Jun Nterm kinase NO difference P38; Ex signal regulated kinase 1 or 2
Mu (Ref. 38)	human	84	L	Y	None	Peripheral blood	EPCs	Increased CD 34,CD 133, KDR, VEGF, SDF-1a in the serum and wound
Yang (Ref. 39)	Human	40	L	Y	None	Tissue	Biomarkers	Increased cFN, increased TGF-B1
Liu (Ref. 40)	Human	172	H	N	None	Blood and tissue	HSA-miR levels	Decreased levels of HSA-miR-203 (p-miR-203 and T-miR-203), p63
Yang (Ref. 41)	Human	30	L	Y	None	Tissue	Biomarkers	Upregulation of bFGF and phosphorylated (ERK)1/2
Kilpadi (Ref. 42)	Porcine	12	H	N	Saline soaked dressing	Serum	Biomarkers	TGF B : No diff IL 6: no peak IL 8: no diff IL 10: early peaking
Norbury (Ref. 43)	Porcine	10	H	N	Duoderm	Serum and Tissue	Biomarkers	IL6 decreased No difference in IL 1b,4,8,TGF,B or TNFA
Brownhill (Ref. 44)	Porcine	12	H	N	Single use NPWT	Tissue	Biomarkers	CXC11 : Higher in tNPWT CSF2: Higher in tNPWT IL6: Higher in tNPWT Il1a: Higher in tNPWT Il 1B Higher in tNPWT CCL2 Higher in tNPWT IL10 Higher in tNPWT TNF Higher in tNPWT COL1A2 Higher in sNPWT CoL3A1 Higher in sNPWT CTGF Higher in sNPWT DCN Higher in sNPWT MMP3 Higher in tNPWT MMP 9: Higher in tNPWT
Zhou (Ref. 45)	Porcine	6	S	N	High Pressure NPWT	Tissue	Biomarkers	VEGF: Upregulated best at 150 mm FGF2: Upregulated best at 150 mm

(*Continued*)

Table 1. (*Continued.*)

Study	Participants	*N*	ROB	Randomization	Comparator	Substrate	Focus	Markers under study
Li (Ref. 46)	Porcine	56	L	Y	Standard dressing	Tissue	Biomarkers	MPO: increased IL 1B: increased TNFA: Increased IL 10: Increased ICAM: CD54 increased
Aydin (Ref. 47)	Rabbit	30	S	N	Control	Tissue	Biomarkers	No change in CD34/CD31
Younan (Ref. 48)	Murine	40	L	N	Cyclical NPWT; Occlusive dressings	Tissue	Biomarkers	CGRP : increased substance P: increased NGF : increased Highest for cyclical > continuous
Erba (Ref. 49)	Murine	50	L	N	Continuous vs cyclical NPWT	Tissue	Biomarkers-	VEGF dimers higher in VAC VEGF higher at surface of wound HIF 1alpha higher in control
Jacobs (Ref. 50)	Murine	-	L	N	Standard dressing	Tissue	Biomarkers	VEGF 40% upregulation FGF- 2 140% upregulation CD31: increased expression
Scherer (Ref. 51)	Murine	20	L	N	Duoderm	Tissue	Biomarkers:	PECAM-1 Increased Ki 67 – increased
Qiu (Ref. 52)	Murine	48	L	Y	None	Tissue	Biomarkers	CD31: Increased CD68 : Reduced MDA: Reduced SOD: reduced CAT: reduced Raftlin: increased
Lu (Ref. 53)	In vitro	–	L	N	PU Foam	Murine fibroblast cultured	Biomarkers	FGF-2 upregulated B FGF – upregulated TGFB1 upregulated Alpha SMA upregulated Type 1 collagen alpha 1 upregulated
McNulty (Ref. 54)	In vitro	–	L	N	None	Human fibroblasts in 3d fibrin matrix	Biomarkers	PDGF: Increased by 53% TGF-B increased by 80%
Dong (Ref. 55)	In vitro	–	L	N	None	PMNs HL 60 Macrophages	Biomarkers	Flow cytometry Decreased apoptosis by PMN/macrophages ELISA TNF alpha downregulated IFN Gamma upregulated EGF upregulated EGFR upregulated IL17 upregulated Western blot CDC42 increased

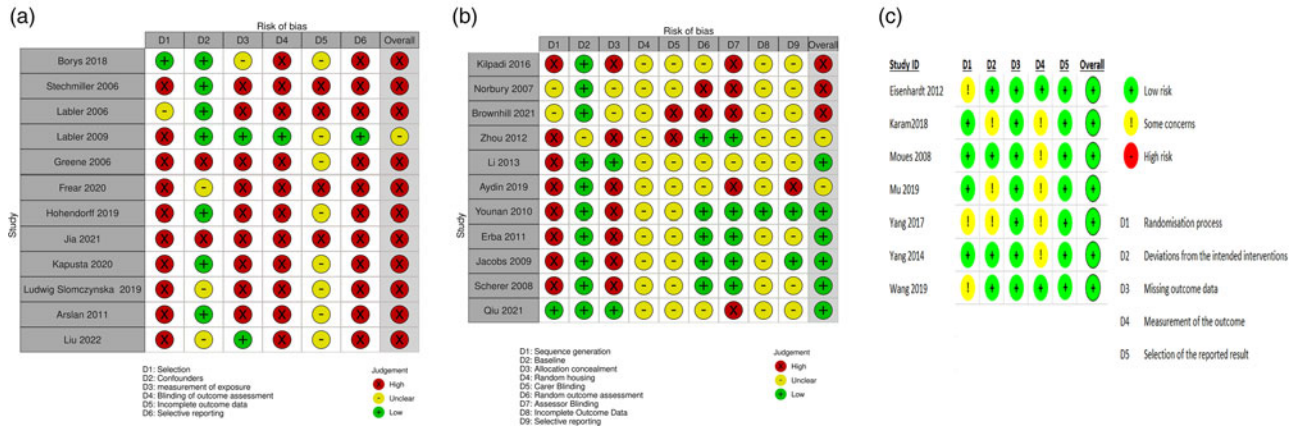

Figure 2. Risk of Bias Assessment of the included studies: (a) RoBANS for non-randomized studies, (b) SYRCLE tool for animal studies, (c) RoB-2 tool for randomized studies.

cell proliferation and inflammation, and the most down-regulated genes were linked to epidermal differentiation. NPWT has also been postulated to aid differential gene expression to influence re-epithelialization and angiogenesis (Ref. 30). NPWT was also observed to alter multiple proteins in the granulation tissue to aid antioxidant pathways and detoxification (Ref. 57) The gene ontology enrichment analysis performed in one of the studies was consistent with a number of previous studies showing that the wound healing process was associated with altered extracellular matrix deposition (Ref. 58), cytoskeletal deregulation (Ref. 59), dyslipidemia (Ref. 60) and prolonged inflammation response (Ref. 61). They also unexpectedly found some signalling pathways that seemed weakly relevant to the curative effect of wounds in the enrichment analysis of Kyoto Encyclopaedia of Genes and Genomes (KEGG) signalling pathways, such as thyroid hormone synthesis, thyroid hormone signalling pathway, human T-cell leukaemia virus 1 infection and African trypanosomiasis (Refs 62, 63, 64).

Discussion

This systematic review summarizes the current understanding of the mechanism of action of NPWT based on studies published over the last 20 years. The effect of NPWT was assessed in 33 studies which included 19 clinical studies, 11 animal models and 3 in-vitro studies. Given that more than 43 different molecular biomarkers and 17 different gene expressions were analysed across all studies, there was some clear concordance in actions on several markers studied and variation between studies with respect to the effects on other biomarkers/genes following NPWT (Table 2).

It has been postulated that NPWT produces hypoxia driven immunomodulation, local and/or systemic attenuation of the acute inflammatory response, angiogenesis and cell recruitment which combine to produce the clinical effects of NPWT (Refs 15, 51). However, the specific mechanisms of action by which these are achieved continue to be controversial. This is mainly because of the limited concordance among these studies to enable conclusions with regard to the specific mechanisms involved. The previous systematic review in this topic (Ref. 65) suggested that human studies supported angiogenesis via VEGF, cell recruitment predominantly via IL-8 and reduced MMP expression, animal models suggested an anti-inflammatory response via IL-10, VEGF, FGF-2, CGRP and substance P and in vitro models suggested increased granulation tissue formation. They also reported that human studies predominantly studied cytokine and MMP data while growth factor data were predominantly derived from animal studies and in vitro models. However,

Table 2. Variation in outcomes following NPWT on common biomarkers of interest

Biomarker of interest	Studies suggesting upregulation	Studies suggesting downregulation	Studies suggesting no change
Vascular endothelial growth factors(VEGF)	Zhou (Ref. 45), Erba (Ref. 49), Jacobs (Ref. 50), Labler (Ref. 27), Labler (Ref. 28), Karam (Ref. 34), Mu (Ref. 38)		
Tumour Necrosis Factor-alpha	Brownhill (Ref. 44), Li (Ref. 46)	Stechmiller (Ref. 25), Eisenhardt (Ref. 26), Karam (Ref. 34), Wang (Ref. 37), Dong (Ref. 55)	Norbury (Ref. 43)
Transforming Growth Factor Beta	Karam (Ref. 34), Yang (Ref. 39), Lu (Ref. 53), McNulty (Ref. 54), Brownhill (Ref. 44)		Kilpadi (Ref. 42), Norbury (Ref. 43)
Interleukins(IL) IL6 IL8 IL-1B	Labler (Ref. 27), Labler (Ref. 28) Stechmiller (Ref. 25), Brownhill (Ref. 44), Li (Ref. 46)	Wang (Ref. 37)	Kilpadi (Ref. 42), Labler (Ref. 28) Kilpadi (Ref. 42)
MatrixMetalloproteinases (MMP) MMP 2 MMP 3 MMP 9	Stechmiller (Ref. 25), Greene (Ref. 29), Stechmiller (Ref. 25), Brownhill (Ref. 44) Stechmiller (Ref. 25), Brownhill (Ref. 44)	Karam (Ref. 34), Greene (Ref. 29)	

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the effect of NPWT on the differential gene expressions was not explored in this review. First insights into the molecular mechanisms behind NPWT suggested that NPWT also induces gene expression changes at the wound bed. These changes were postulated to range from 10-fold induction to 27-fold suppression (Refs 41, 66, 67).

Since this previous systematic review, more than 20 newer biomarkers, cytokines and genes have been studied across 19 more recent studies, the summary of which has been collated in this paper. The data summarized in this review confirms that NPWT-induced strain promotes a pro-angiogenic and pro-mitogenic phenotype in subjacent cell proliferation. NPWT induced cell deformation leads to proliferation as a consequence of cytoskeletal tension. Integrins, adhesive contacts within the cell matrix, act as strain gauges, triggering mechanoreceptor signalling pathways (Refs 68, 69). Application of NPWT results in positive pressure at the wound bed and hence reduced blood flow in the tissue immediately adjacent to the filler material (Ref. 70). NPWT enhances specific inflammatory gene expression at the acute phase associated with epithelial migration and wound healing. However, its continued use may inhibit epithelial differentiation (Ref. 66). NPWT is also associated with an up-regulation of basic fibroblast growth factor (bFGF) and extracellular signal-regulated kinase (ERK) 1/2 signalling, which may be involved in promoting the NPWT-mediated wound healing response (Ref. 41).

This systematic review has a few limitations. The inherent heterogeneity of the included studies makes the data unsuitable for meta-analysis. The clinical studies were mostly underpowered and were opportunistic as reported in the previous review. There was a significant variation in terms of the methodology, mainly concerning sample collection/storage, time interval from collection to analysis and techniques utilized to extract and study the biomarkers of interest. The data from a majority of human studies do not take into account extrinsic factors such as collection and storage of samples which do not account for degradation of biomarkers. Moreover, important clinical information including the use of antibiotics, immunosuppressants including corticosteroids or anti-biologicals was not included. Given the extensive number of biomarkers and genes analysed in the included studies, there was limited concordance to suggest a strong correlation between NPWT and regulation of many biomarkers. The time-points at which these biomarkers were studied also varied significantly among studies. It has also been suggested that the magnitude of negative pressure employed is likely to influence blood flow, which in turn influences the degree of hypoxia and reperfusion. This has been shown to alter the expression of mechanosensitive genes (Refs 10, 71).

There were some discrepancies between animal and human studies especially with respect to the regulation of MMP and IL-6. Although the animal studies address most of these issues, the extrapolation of this data to predict clinical biological response is not appropriate. Although in-vitro studies using human cell lines have the potential to circumvent these concerns, only three studies have been conducted over the last 10 years. Only two out of three studies studied the effects of NPWT on human derived cell lines and analysed a completely different set of biomarkers via different methodologies. Although we have a better understanding of the primary and secondary mechanisms of action of NPWT, namely: macrodeformation, cellular proliferation, differentiation, cell migration, angiogenesis and neurogenesis, a comprehensive temporal expression profile of most biomarker changes with NPWT remains elusive. However, VEGF was elevated in all 7 reports which had studied its effects. Tumour necrosis Factor alpha (TNF α) was downregulated in 5 out of 8 studies, Transforming Growth Factor Beta (TGF β) was upregulated in 4 out of 7 studies, and Fibronectin was upregulated in both studies which evaluated its effects.

In conclusion, NPWT stimulates modulation of numerous local and circulating cytokines and growth factor expressions to promote an anti-inflammatory profile. This is most likely achieved by downregulation of TNFα, upregulation of VEGF, TGF-β and fibronectin. This review has also identified many other biomarkers and gene expressions of interest with regard NPWT actions which may help to direct future research in this field.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/erm.2023.24.

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Competing interest. None.

Data availability statement. The data that support the findings of this study are available from the corresponding author,[BR], upon reasonable request. This paper was shortlisted for the ESVS Prize poster session at the Annual Meeting of the European Society of Vascular Surgery at Rome, Italy in September 2022.

Ethical standards. None.

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