Expert Reviews in Molecular Medicine

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

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Corresponding Author:	Bharadhwaj Ravindhran, MRCS Hull York Medical School Hull, East Riding of Yorkshire UNITED KINGDOM
First Author:	Bharadhwaj Ravindhran, MRCS
Order of Authors:	Bharadhwaj Ravindhran, MRCS
	Nicole Schafer
	Annabel Howitt
	Daniel Carradice, MD
	George Smith, MD
	Ian Clifford Chetter, MD
Keywords:	Negative-pressure Wound therapy; wound healing; biomarkers; wound care; molecular mechanisms
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Author Comments:	Dear editorial team, Many thanks for your kind comments and suggestions. Please find attached the updated manuscript and AM file for your perusal.
Response to Reviewers:	Response to reviewers: Reviewer #1: Dear authors, The work is really interesting about the several studies at different stages about negative pressure wound therapy. However, I have some suggestions to improve the quality of the manuscript. Response: Dear reviewer, many thanks for your kind comments and key suggestions. They have been incorporated as follows. Comment 1. The references should be revised because I found one of them in red. Response: Many thanks for pointing this out, this has duly been rectified. Comment 2. The authors should discuss more in deep the subtype of TGF-beta is involved in the negative pressure wound therapy? Response: Many thanks for this suggestion, they have duly been added to lines 171-176. Comment 3. I think the authors should include a graphical abstract about the work

	Response: Many thanks for this suggestion. The graphical abstract has been added as an additional file. Reviewer #2: Dear Editor, This is a very interesting review on the topic. It is well-written and the purpose is important. I think it will contribute to future prospective trials on the use of NPWT. Response: Dear reviewer, many thanks for your kind comments.

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Authors: Bharadhwaj Ravindhran[1,2]

Nicole Schafer[1]

Annabel Howitt[1]

Daniel Carradice[1]

George Smith[1]

Ian Chetter[1]

[1] Academic Vascular Surgical Unit, Hull Royal Infirmary, Hull, UK.

[2] Department of Health Sciences, University of York, UK.

Corresponding author: Bharadhwaj Ravindhran

Academic Vascular Surgical Unit

Hull Royal Infirmary

Hull HU32JZ

Bharadhwaj.Ravindhran@nhs.net

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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 downregulation of TNFα, upregulation of VEGF, TGF-β and fibronectin.

Key words: Negative-pressure Wound therapy, wound healing, biomarkers, wound care , molecular mechanisms

Molecular Mechanisms of Action of Negative-Pressure Wound Therapy: A Systematic Review

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 hospitalisation and/or further operations.[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).[2]

Negative pressure wound therapy (NPWT) is currently used widely in many aspects of wound care and has been strongly promoted for use on complex wounds.[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.[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.[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 stabilisation 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.[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[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[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[18] for non-randomized studies, the COCHRANE's Risk of Bias 2(ROB-2) tool[19] for randomized clinical studies, Office of Health Assessment and Translation (OHAT) tool[20] for in-vitro studies or the Systematic Review Centre for

Laboratory Animal Experimentation (SYRCLE) tool[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”[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. 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 have 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. 13 studies had a high risk of bias and 3 studies had some concerns of bias. 10 clinical studies and 10 animal studies analysed tissue samples from wounds while 5 clinical studies analysed the wound effluent. 5 clinical studies and one animal study also used serum samples to correlate the effect of NPWT on wounds. 28 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[23][24][25][26][27][28,29][30]. Results from clinical studies were reported in 4 studies[23–26]. 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[27–30]. 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[25,31,32][33–35]. 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 has 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[34,36,37] 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[38,39]. 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:

19 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.(figure 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 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:

11 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.(figure 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[40], human fibroblasts in a 3D fibrin matrix[41] and a combination of PMNs, HL 60 cell lines and Macrophages[34] 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[40,41]. 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 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 [42].NPWT was also observed to alter multiple proteins in the granulation tissue to aid antioxidant pathways and detoxification.[43] 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[44], cytoskeletal deregulation [45], dyslipidemia [46] and prolonged inflammation response [47]. 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.[48][49][50].

Discussion:

This systematic review summarises 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.[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 [52] 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, 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.[53][27][54]

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. [55][56]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.[57] 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.[53] 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.[27]

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 were 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[10,58]

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 has 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 (Vascular Endothelial Growth Factor) 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.

1. Chetter IC, Oswald A V., Fletcher M, Dumville JC, Cullum NA(2017). A survey of patients with surgical wounds healing by secondary intention; an assessment of prevalence, aetiology, duration and management. J Tissue Viability [Internet]. [cited 2022 Aug 27];26(2):103–7. Available from: https://pubmed.ncbi.nlm.nih.gov/28049612/
2. Chetter I, Arundel C, Bell K, Buckley H, Claxton K, Corbacho Martin B, et al.(2019) Surgical wounds healing by secondary intention: characterising and quantifying the problem, identifying effective treatments, and assessing the feasibility of conducting a randomised controlled trial of negative pressure wound therapy versus usual care.
3. Armstrong DG, Andros G. (2012) Use of negative pressure wound therapy to help facilitate limb preservation. Int Wound J [Internet]. [cited 2022 Aug 27];9(SUPPL.1):1–7. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1742-481X.2012.01015.x
4. Using negative pressure therapy in wound healing | Nursing Times [Internet]. [cited 2022 Aug 27]. Available from: https://www.nursingtimes.net/clinical-archive/tissue-viability/using-negative-pressure-therapy-in-wound-healing-31-08-2012/
5. Peinemann F, Sauerland S.(2011) Negative-pressure wound therapy: systematic review of randomized controlled trials. Dtsch Arztebl Int [Internet]. [cited 2022 Aug 27];108(22):381–9. Available from: https://pubmed.ncbi.nlm.nih.gov/21712971/
6. Liu Z, Dumville JC, Hinchliffe RJ, Cullum N, Game F, Stubbs N, et al.(2018) Negative pressure wound therapy for treating foot wounds in people with diabetes mellitus. Cochrane database Syst Rev [Internet][cited 2022 Aug 27];10(10). Available from: https://pubmed.ncbi.nlm.nih.gov/30328611/
7. Gao J, Wang Y, Song J, Li Z, Ren J, Wang P.(2021) Negative pressure wound therapy for surgical site infections: A systematic review and meta-analysis. J Adv Nurs [Internet][cited 2022 Aug 27];77(10):3980–90. Available from: https://pubmed.ncbi.nlm.nih.gov/33905552/
8. Borgquist O, Ingemansson R, Malmsjö M (2011). The influence of low and high pressure levels during negative-pressure wound therapy on wound contraction and fluid evacuation. Plast Reconstr Surg. 127(2):551–9.
9. Kairinos N, Solomons M, Hudson DA. (2010) The paradox of negative pressure wound therapy--in vitro studies. J Plast Reconstr Aesthet Surg [Internet][cited 2021 Oct 28];63(1):174–9. Available from: https://pubmed.ncbi.nlm.nih.gov/19036656/
10. Saxena V, Hwang CW, Huang S,Eichbaum Q, Ingber D, Orgil DP.(2004) Vacuum-assisted

closure: microdeformations of wounds and cell proliferation. Plast Reconstr Surg [Internet].[cited 2021 Oct 28];114(5):1086–96. Available from: https://pubmed.ncbi.nlm.nih.gov/15457017/

11. Yang CC, Chang DS, Webb LX.(2006) Vacuum-assisted closure for fasciotomy wounds following compartment syndrome of the leg. J Surg Orthop Adv. 15(1):19–23.

12. Adámková M, Tymonová J, Zámečníková I, Kadlčík M, Klosová H.(2005) First experience with the use of vacuum assisted closure in the treatment of skin defects at the Burn Center. Acta Chir Plast;47(1):24–7.

13. Kim PJ, Attinger CE, Steinberg JS, Evans KK, Lehner B, Willy C, et al.(2013) Negative-pressure wound therapy with instillation: International consensus guidelines. Plast Reconstr Surg. 132(6):1569–79.

14. Gerry R, Kwei S, Bayer L, Breuing KH.(2007) Silver-impregnated vacuum-assisted closure in the treatment of recalcitrant venous stasis ulcers. Ann Plast Surg. 59(1):58–62.

15. Orgill DP, Manders EK, Sumpio BE, Lee RC, Attinger CE, Gurtner GC, et al.(2009) The mechanisms of action of vacuum assisted closure: More to learn. Surgery;146(1):40–51.

16. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A(2016). Rayyan—a web and mobile app for systematic reviews. Syst Rev [Internet]. [cited 2022 Apr 30];5(1). Available from: https://link.springer.com/epdf/10.1186/s13643-016-0384-4

17. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al.(2016) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Rev Esp Nutr Humana y Diet [Internet][cited 2022 Apr 30];20(2):148–60. Available from: https://systematicreviewsjournal.biomedcentral.com/articles/10.1186/2046-4053-4-1

18. Risk of Bias Assessment tool for Non-randomized Studies (RoBANS): Development and validation of a new instrument | Colloquium Abstracts [Internet]. [cited 2022 Apr 30]. Available from: https://abstracts.cochrane.org/2011-madrid/risk-bias-assessment-tool-non-randomized-studies-robans-development-and-validation-new

19. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al.(2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ [Internet]. 2019 [cited 2022 Apr 30];366. Available from: https://pubmed.ncbi.nlm.nih.gov/31462531/

20. Office of Health Assessment and Translation (OHAT). Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration: National Institute of Environmental Health Sciences; 2019 [Available from: https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookmarch2019_508.pdf

21. Hooijmans, C.R., Rovers, M.M., de Vries, R.B. et al. SYRCLE's risk of bias tool for animal

400 studies. BMC Med Res Methodol 14, 43 (2014). https://doi.org/10.1186/1471-2288-14-43
401 22. McGuinness LA, Higgins JPT.(2021) Risk-of-bias VISualization (robvis): An R package and Shiny
402 web app for visualizing risk-of-bias assessments. Res Synth Methods [Internet].[cited 2022
403 May 8];12(1):55–61. Available from:
404 https://onlinelibrary.wiley.com/doi/full/10.1002/jrsm.1411
405 23. Labler L, Rancan M, Mica L, Härter L, Mihic-Probst D, Keel M.(2009) Vacuum-assisted closure
406 therapy increases local interleukin-8 and vascular endothelial growth factor levels in
407 traumatic wounds. J Trauma [Internet]. [cited 2021 Nov 16];66(3):749–57. Available from:
408 https://pubmed.ncbi.nlm.nih.gov/19276749/
409 24. Labler L, Keel M, Trentz O, Heinzelmann M (2006). Wound conditioning by vacuum assisted
410 closure (V.A.C.) in postoperative infections after dorsal spine surgery. Eur Spine J [Internet].
411 [cited 2022 Sep 7];15(9):1388–96. Available from:
412 https://pubmed.ncbi.nlm.nih.gov/16835734/
413 25. Karam RA, Rezk NA, Abdel Rahman TM, Al Saeed M.(2018) Effect of negative pressure wound
414 therapy on molecular markers in diabetic foot ulcers. Gene;667:56–61.
415 26. Mu S, Hua Q, Jia Y, Chen MW, Tang Y, Deng D, et al.(2019) Effect of negative-pressure wound
416 therapy on the circulating number of peripheral endothelial progenitor cells in diabetic
417 patients with mild to moderate degrees of ischaemic foot ulcer.
418 https://doi.org/101177/1708538119836360 [Internet][cited 2022 Sep 28];27(4):381–9.
419 Available from: https://journals.sagepub.com/doi/abs/10.1177/1708538119836360
420 27. Yang SL, Han R, Liu Y, Hu LY, Li XL, Zhu LY.(2014) Negative pressure wound therapy is
421 associated with up-regulation of bFGF and ERK1/2 in human diabetic foot wounds. Wound
422 Repair Regen [Internet]. [cited 2022 Aug 27];22(4):548–54. Available from:
423 https://pubmed.ncbi.nlm.nih.gov/24809625/
424 28. Zhou M, Yu A, Wu G, Xia C, Hu X, Qi B.(2012) Role of different negative pressure values in the
425 process of infected wounds treated by vacuum-assisted closure: an experimental study. Int
426 Wound J [Internet]. [cited 2022 Sep 28];10(5):508–15. Available from:
427 https://pubmed.ncbi.nlm.nih.gov/22640026/
428 29. Erba P, Ogawa R, Ackermann M, Adini A, Miele LF, Dastouri P, et al.(2011) Angiogenesis in
429 wounds treated by microdeformational wound therapy. Ann Surg [Internet]. 2011 Feb [cited
430 2021 Nov 16];253(2):402–9. Available from: https://pubmed.ncbi.nlm.nih.gov/21217515/
431 30. Jacobs S, Simhaee DA, Marsano A, Fomovsky GM, Niedt G, Wu JK.(2009) Efficacy and
432 mechanisms of vacuum-assisted closure (VAC) therapy in promoting wound healing: a rodent
433 model. J Plast Reconstr Aesthet Surg [Internet].[cited 2022 Sep 28];62(10):1331–8. Available

from: https://pubmed.ncbi.nlm.nih.gov/18617451/

31. Stechmiller JK, Kilpadi D V., Childress B, Schultz GS.(2006) Effect of Vacuum-Assisted Closure Therapy on the expression of cytokines and proteases in wound fluid of adults with pressure ulcers. Wound Repair Regen [Internet]. 2006 [cited 2021 Nov 16];14(3):371–3. Available from: https://pubmed.ncbi.nlm.nih.gov/16808818/

32. Eisenhardt SU, Schmidt Y, Thiele JR, Iblher N, Penna V, Torio-Padron N, et al.(2012) Negative pressure wound therapy reduces the ischaemia/reperfusion-associated inflammatory response in free muscle flaps. J Plast Reconstr Aesthet Surg [Internet][cited 2021 Nov 16];65(5):640–9. Available from: https://pubmed.ncbi.nlm.nih.gov/22137686/

33. Wang T, Li X, Fan L, Chen B, Liu J, Tao Y, et al.(2019) Negative pressure wound therapy promoted wound healing by suppressing inflammation via down-regulating MAPK-JNK signaling pathway in diabetic foot patients. Diabetes Res Clin Pract [Internet]. [cited 2022 Sep 28];150:81–9. Available from: http://www.diabetesresearchclinicalpractice.com/article/S0168822718312919/fulltext

34. Dong J, Qing C, Song F, Wang X, Lu S, Tian M.(2020) Potential molecular mechanisms of negative pressure in promoting wound healing. Int Wound J [Internet]. [cited 2022 Sep 28];17(5):1428–38. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/iwj.13423

35. Brownhill VR, Huddleston E, Bell A, Hart J, Webster I, Hardman MJ, et al.(2021) Pre-Clinical Assessment of Single-Use Negative Pressure Wound Therapy During In Vivo Porcine Wound Healing. Adv wound care [Internet]. [cited 2022 Sep 28];10(7):345–56. Available from: https://pubmed.ncbi.nlm.nih.gov/32633639/

36. Lu F, Ogawa R, Nguyen DT, Chen B, Guo D, Helm DL, et al.(2011) Microdeformation of three-dimensional cultured fibroblasts induces gene expression and morphological changes. Ann Plast Surg [Internet] [cited 2022 Sep 28];66(3):296–300. Available from: https://pubmed.ncbi.nlm.nih.gov/21233699/

37. McNulty AK, Schmidt M, Feeley T, Villanueva P, Kieswetter K.(2009) Effects of negative pressure wound therapy on cellular energetics in fibroblasts grown in a provisional wound (fibrin) matrix. Wound Repair Regen [Internet]. [cited 2021 Nov 16];17(2):192–9. Available from: https://pubmed.ncbi.nlm.nih.gov/19320887/

38. Arslan E, Ozturk OG, Aksoy A, Polat G.(2011) Vacuum-assisted closure therapy leads to an increase in plasma fibronectin level. Int Wound J [Internet].[cited 2023 Feb 18];8(3):224–8. Available from: https://pubmed.ncbi.nlm.nih.gov/21401882/

39. Yang SL, Zhu LY, Han R, Sun LL, Dou JT.(2017) Effect of Negative Pressure Wound Therapy on

Cellular Fibronectin and Transforming Growth Factor-β1 Expression in Diabetic Foot Wounds. Foot ankle Int [Internet]. [cited 2023 Feb 22];38(8):893–900. Available from: https://pubmed.ncbi.nlm.nih.gov/28459181/

40. Lu F, Ogawa R, Nguyen DT, Chen B, Guo D, Helm DL, et al.(2011) Microdeformation of three-dimensional cultured fibroblasts induces gene expression and morphological changes. Ann Plast Surg [Internet].[cited 2021 Nov 16];66(3):296–300. Available from: https://pubmed.ncbi.nlm.nih.gov/21233699/

41. McNulty AK, Schmidt M, Feeley T, Kieswetter K.(2007) Effects of negative pressure wound therapy on fibroblast viability, chemotactic signaling, and proliferation in a provisional wound (fibrin) matrix. Wound Repair Regen [Internet].Nov [cited 2022 Sep 28];15(6):838–46. Available from: https://pubmed.ncbi.nlm.nih.gov/18028132/

42. Frear CC, Zang T, Griffin BR, McPhail SM, Parker TJ, Kimble RM, et al.(2020) The modulation of the burn wound environment by negative pressure wound therapy: Insights from the proteome. Wound Repair Regen [Internet]. [cited 2022 Aug 27];29(2):288–97. Available from: https://pubmed.ncbi.nlm.nih.gov/33374033/

43. Liu X, Zheng N, Shi YN, Yuan J, Li L.(2014) Thyroid hormone induced angiogenesis through the integrin αvβ3/protein kinase D/histone deacetylase 5 signaling pathway. J Mol Endocrinol [Internet].[cited 2022 Aug 27];52(3):245–54. Available from: https://pubmed.ncbi.nlm.nih.gov/24532656/

44. Maione AG, Smith A, Kashpur O, Yanez V, Knight E, Mooney DJ, et al.(2016) Altered ECM deposition by diabetic foot ulcer-derived fibroblasts implicates fibronectin in chronic wound repair. Wound Repair Regen [Internet] [cited 2022 Aug 27];24(4):630–43. Available from: https://pubmed.ncbi.nlm.nih.gov/27102877/

45. Jozic I, Vukelic S, Stojadinovic O, Liang L, Ramirez HA, Pastar I, et al.(2016) Stress Signals, Mediated by Membranous Glucocorticoid Receptor, Activate PLC/PKC/GSK-3β/β-catenin Pathway to Inhibit Wound Closure. J Invest Dermatol [Internet].[cited 2022 Aug 27];137(5):1144–54. Available from: https://europepmc.org/articles/PMC7540219

46. Yazdanpanah L, Shahbazian H, Nazari I, Hesam S, Ahmadi F, Cheraghian B, et al.(2018) Risk factors associated with diabetic foot ulcer-free survival in patients with diabetes. Diabetes Metab Syndr [Internet][cited 2022 Aug 27];12(6):1039–43. Available from: https://pubmed.ncbi.nlm.nih.gov/30168426/

47. Gouin JP, Kiecolt-Glaser JK.(2011) The Impact of Psychological Stress on Wound Healing: Methods and Mechanisms. Immunol Allergy Clin North Am [Internet].[cited 2022 Aug 27];31(1):81. Available from: /pmc/articles/PMC3052954/

48. Capewell P, Cren-Travaillé C, Marchesi F, Johnston P, Clucas C, Benson RA, et al.(2016) The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. Elife. 2016 Sep 22;5(September2016).
49. McGill NK, Vyas J, Shimauchi T, Tokura Y, Piguet V. (2012) HTLV-1-associated infective dermatitis: updates on the pathogenesis. Exp Dermatol [Internet] [cited 2022 Aug 27];21(11):815–21. Available from: https://pubmed.ncbi.nlm.nih.gov/23163646/
50. Shimauchi T, Piguet V. (2015) DC-T cell virological synapses and the skin: novel perspectives in dermatology. Exp Dermatol [Internet]. Jan 1 [cited 2022 Aug 27];24(1):1–4. Available from: https://pubmed.ncbi.nlm.nih.gov/25039899/
51. Scherer SS, Pietramaggiori G, Mathews JC, Prsa MJ, Huang S, Orgill DP.(2008)The mechanism of action of the vacuum-assisted closure device. Plast Reconstr Surg [Internet][cited 2021 Nov 16];122(3):786–97. Available from: https://pubmed.ncbi.nlm.nih.gov/18766042/
52. Glass GE, Murphy GF, Esmaeili A, Lai LM, Nanchahal J.(2014) Systematic review of molecular mechanism of action of negative-pressure wound therapy. Br J Surg [Internet][cited 2022 Aug 27];101(13):1627–36. Available from: https://pubmed.ncbi.nlm.nih.gov/25294112/
53. Nuutila K, Siltanen A, Peura M, Harjula A, Nieminen T, Vuola J, et al.(2013) Gene expression profiling of negative-pressure-treated skin graft donor site wounds. Burns [Internet][cited 2022 Aug 27];39(4):687–93. Available from: https://pubmed.ncbi.nlm.nih.gov/23141686/
54. Liu D, Zhang L, Li T, Wang G, Du H, Hou H, et al.(2014) Negative-pressure wound therapy enhances local inflammatory responses in acute infected soft-tissue wound. Cell Biochem Biophys [Internet].[cited 2022 Aug 27];70(1):539–47. Available from: https://pubmed.ncbi.nlm.nih.gov/24748178/
55. Shyy JYJ, Chien S. (1997)Role of integrins in cellular responses to mechanical stress and adhesion. Curr Opin Cell Biol [Internet] [cited 2022 Aug 27];9(5):707–13. Available from: https://pubmed.ncbi.nlm.nih.gov/9330875/
56. Wilkes R, Zhao Y, Kieswetter K, Haridas B.(2009) Effects of dressing type on 3D tissue microdeformations during negative pressure wound therapy: a computational study. J Biomech Eng [Internet][cited 2022 Aug 27];131(3). Available from: https://pubmed.ncbi.nlm.nih.gov/19154071/
57. Wilkes R, Zhao Y, Cunningham K, Kieswetter K, Haridas B.(2009) 3D strain measurement in soft tissue: demonstration of a novel inverse finite element model algorithm on MicroCT images of a tissue phantom exposed to negative pressure wound therapy. J Mech Behav Biomed Mater [Internet]. [cited 2021 Nov 16];2(3):272–87. Available from: https://pubmed.ncbi.nlm.nih.gov/19627832/

536 58. Glass GE, Nanchahal J.(2012) The methodology of negative pressure wound therapy:
537 separating fact from fiction. J Plast Reconstr Aesthet Surg [Internet].[cited 2022 Sep
538 7];65(8):989–1001. Available from: https://pubmed.ncbi.nlm.nih.gov/22236476/
539 59. Borys S, Hohendorff J, Koblik T, Witek P, Ludwig-Slomczynska A, Frankfurter C, et al.(2018)
540 Negative-pressure wound therapy for management of chronic neuropathic noninfected
541 diabetic foot ulcerations - short-term efficacy and long-term outcomes. Endocrine [Internet].
542 [cited 2023 Feb 18];62(3):611–6. Available from:
543 https://pubmed.ncbi.nlm.nih.gov/30099674/
544 60. Greene AK, Puder M, Roy R, Arsenault D, Kwei S, Moses MA, et al.(2006) Microdeformational
545 wound therapy: effects on angiogenesis and matrix metalloproteinases in chronic wounds of
546 3 debilitated patients. Ann Plast Surg [Internet][cited 2021 Nov 16];56(4):418–22. Available
547 from: https://pubmed.ncbi.nlm.nih.gov/16557076/
548 61. Hohendorff J, Drozdz A, Borys S, Ludwig-Slomczynska AH, Kiec-Wilk B, Stepien EL, et al. (2019)
549 Effects of Negative Pressure Wound Therapy on Levels of Angiopoetin-2 and Other Selected
550 Circulating Signaling Molecules in Patients with Diabetic Foot Ulcer. J Diabetes Res.
551 62. Jia Z, Liu L, Zhang S, Zhao X, Luo L, Tang Y, et al.(2021) Proteomics changes after negative
552 pressure wound therapy in diabetic foot ulcers. Mol Med Rep [Internet].[cited 2022 Aug
553 27];24(6). Available from: https://pubmed.ncbi.nlm.nih.gov/34608502/
554 63. Kapusta P, Konieczny PS, Hohendorff J, Borys S, Totoń-Żurańska J, Kieć-Wilk BM, et al. (2020)
555 Negative pressure wound therapy affects circulating plasma microRNAs in patients with
556 diabetic foot ulceration. Diabetes Res Clin Pract [Internet][cited 2023 Feb 19];165. Available
557 from: http://www.diabetesresearchclinicalpractice.com/article/S0168822720305015/fulltext
558 64. Ludwig-Slomczynska AH, Borys S, Seweryn MT, Hohendorff J, Kapusta P, Kiec-Wilk B, et
559 al.(2019) DNA methylation analysis of negative pressure therapy effect in diabetic foot ulcers.
560 Endocr Connect [Internet].[cited 2023 Feb 19];8(11):1474–82. Available from:
561 https://pubmed.ncbi.nlm.nih.gov/31634866/
562 65. Mouës CM, Van Toorenenbergen AW, Heule F, Hop WC, Hovius SER.(2008) The role of topical
563 negative pressure in wound repair: expression of biochemical markers in wound fluid during
564 wound healing. Wound Repair Regen [Internet][cited 2023 Feb 22];16(4):488–94. Available
565 from: https://pubmed.ncbi.nlm.nih.gov/18638266/
566 66. Mu S, Hua Q, Jia Y, Chen M-W, Tang Y, Deng D, et al.(2019) Effect of negative-pressure wound
567 therapy on the circulating number of peripheral endothelial progenitor cells in diabetic
568 patients with mild to moderate degrees of ischaemic foot ulcer.
569 https://doi.org/101177/1708538119836360 [Internet][cited 2023 Feb 22];27(4):381–9.

570 Available from: https://journals.sagepub.com/doi/10.1177/1708538119836360
571 67. Liu L, Chen R, Jia Z, Li X, Tang Y, Zhao X, et al.(2022) Downregulation of hsa-miR-203 in
572 peripheral blood and wound margin tissue by negative pressure wound therapy contributes
573 to wound healing of diabetic foot ulcers. Microvasc Res. ;139:104275.
574 68. Kilpadi D V., Bower CE, Reade CC, Robinson PJ, Sun YS, Zeri R, et al.(2006) Effect of Vacuum
575 Assisted Closure Therapy on early systemic cytokine levels in a swine model. Wound Repair
576 Regen [Internet][cited 2023 Feb 22];14(2):210–5. Available from:
577 https://pubmed.ncbi.nlm.nih.gov/16630111/
578 69. Norbury K, Kieswetter K (2007) Vacuum-assisted Closure Therapy Attenuates the
579 Inflammatory Response in a Porcine Acute Wound Healing Model - PubMed [Internet]. [cited
580 2023 Mar 14]. Available from: https://pubmed.ncbi.nlm.nih.gov/26110258/
581 70. Brownhill VR, Huddleston E, Bell A, Hart J, Webster I, Hardman MJ, et al.(2021) Pre-Clinical
582 Assessment of Single-Use Negative Pressure Wound Therapy During In Vivo Porcine Wound
583 Healing. Adv wound care [Internet][cited 2023 Feb 22];10(7):345–56. Available from:
584 https://pubmed.ncbi.nlm.nih.gov/32633639/
585 71. Zhou M, Yu A, Wu G, Xia C, Hu X, Qi B.(2013) Role of different negative pressure values in the
586 process of infected wounds treated by vacuum-assisted closure: an experimental study. Int
587 Wound J [Internet][cited 2023 Feb 22];10(5):508–15. Available from:
588 https://onlinelibrary.wiley.com/doi/full/10.1111/j.1742-481X.2012.01008.x
589 72. Li J, Topaz M, Tan H, Li Y, Li W, Xun W, et al. (2013)Treatment of infected soft tissue blast
590 injury in swine by regulated negative pressure wound therapy. Ann Surg [Internet][cited 2023
591 Mar 14];257(2):335–44. Available from: https://pubmed.ncbi.nlm.nih.gov/23108116/
592 73. Aydin OE, Algan S, Tan O, Demirci E, Keles ON, Kantarci A. (2019) A novel method for flap
593 delay vacuum assisted flap delay: an experimental study in rabbits. J Plast Surg Hand Surg
594 [Internet][cited 2023 Feb 22];53(4):208–15. Available from:
595 https://pubmed.ncbi.nlm.nih.gov/30929553/
596 74. Younan G, Ogawa R, Ramirez M, Helm D, Dastouri P, Orgill DP.(2010) Analysis of nerve and
597 neuropeptide patterns in vacuum-assisted closure-treated diabetic murine wounds. Plast
598 Reconstr Surg [Internet][cited 2021 Nov 16];126(1):87–96. Available from:
599 https://pubmed.ncbi.nlm.nih.gov/20595860/
600 75. Jacobs S, Simhaee DA, Marsano A, Fomovsky GM, Niedt G, Wu JK.(2009) Efficacy and
601 mechanisms of vacuum-assisted closure (VAC) therapy in promoting wound healing: a rodent
602 model. J Plast Reconstr Aesthetic Surg;62(10):1331–8.
603 76. SS S, G P, JC M, MJ P, S H, DP O.(2008) The mechanism of action of the vacuum-assisted

closure device. Plast Reconstr Surg [Internet][cited 2021 Oct 28];122(3):786–97. Available from: https://pubmed.ncbi.nlm.nih.gov/18766042/

77. Qiu X, Wu Y, Zhang D, Zhang H, Yu A, Li Z.(2021) Roles of Oxidative Stress and Raftlin in Wound Healing Under Negative-Pressure Wound Therapy. Clin Cosmet Investig Dermatol [Internet] [cited 2023 Feb 22];14:1745. Available from: /pmc/articles/PMC8612843/

78. McNulty AK, Schmidt M, Feeley T, Villanueva P, Kieswetter K.(2009) Effects of negative pressure wound therapy on cellular energetics in fibroblasts grown in a provisional wound (fibrin) matrix. Wound Repair Regen [Internet] [cited 2023 Feb 22];17(2):192–9. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1524-475X.2009.00460.x

Legends for figures:

Figure 1: Literature search and study selection

Fig 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

Table 1: Characteristics of the included studies

Study	Participants	N	ROB	Randomisation	Comparator	Substrate	Focus	Markers under study
Arslan 2011[38]	Humans	11	H	N	None	Tissue	Biomarkers	Increased Fibronectin levels
Borys 2018[59]	Human	29	L	N	Standard dressings	Tissue	Differential gene expression	GA2 downregulated C1QBP upregulated RAB35 downregulated SYNJ1 downregulated

Stechmiller 2006[31]	Human	8	H	N	None	Wound effluent	Biomarkers	TNF alpha downregulated IL 1B upregulated MMp-2:downregulated MMP3: upregulated TIMP-1: upregulated
Eisenhardt 2012[32]	Humans	30	L	Y	Petroleum gauze dressing	Tissue	Biomarkers	TNF alpha: downregulated IL1 B: downregulated CD68: downregulated
Labler 2006[24]	Humans	21	H	N	Epigard	Wound effluent	Biomarkers	IL6: increased IL8: increased 1L10: no change VEGF: increased FGF2: no change
Labler 2009[23]	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 2006[60]	Human	3	H	N	Foam filler	Tissue	Biomarkers	MMP-2 : reduced MMP-9/NGAL complex: Reduced
Frear 2020[42]	Humans	8	H	N	Standard dressing	Wound effluent	Proteomics	Increased: MMP Arginase 1 Low affinity IgGFc IIIA FilaminA Alpha 2 Macroglobulin Hemoglobin alpha
Hohendroff 2019[61]	Humans	69	H	N	Standard dressing	Blood sample	Plasma Biomarkers	Angiopoeitin-2 : reduced

								Overall Microvesicles: reduced
Jia 2021[62]	Humans	3	H	N	None	Tissue	Proteomics	Wound Serum CTSS : Decreased decreased ITIH4: Increased increased PROS1: increased increased PRDX2: Increased increased
Kapusta 2020[63]	Humans	35	H	N	Standard dressing	Venous blood	Micro RNA levels	Let 7-2-3p miRNA upregulation
Karam 2018[25]	Humans	40	L	Y	Moist dressing	Tissue	mRNA levels	**Downregulated:** TNF alpha IL 1B MMP1/9 **Upregulated:** TGF B1 VEGF TIMP1
LudwigSlomczynska 2019[64]	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 2008[65]	Humans	33	L	Y	Standard dressing	Wound effluent	Biomarkers	Lower pro MMP Lower total MMP-9/TIMP-1
Wang 2019[33]	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 2019[66]	human	84	L	Y	None	Peripheral blood	EPCs	Increased CD 34,CD 133, KDR, VEGF, SDF-1a in the serum and wound
Yang 2017[39]	Human	40	L	Y	None	Tissue	Biomarkers	Increased cFN, increased TGF-B1
Liu 2022[67]	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 2014[27]	Human	30	L	Y	None	Tissue	Biomarkers	Upregulation of bFGF and phosphorylated (ERK)1/2
Kilpadi 2006[68]	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 2007[69]	Porcine	10	H	N	Duoderm	Serum and Tissue	Biomarkers	IL6 decreased No difference in IL 1b,4,8,TGF,B or TNFA
Brownhill 2021[70]	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 2013[71]	Porcine	6	S	N	High Pressure NPWT	Tissue	Biomarkers	VEGF: Upregulated best at 150mm FGF2: Upregulated best at 150mm
Li 2013[72]	Porcine	56	L	Y	Standard dressing	Tissue	Biomarkers	MPO: increased IL 1B: increased TNFA: Increased IL 10: Increased ICAM: CD54 increased
Aydin 2019[73]	Rabbit	30	S	N	Control	Tissue	Biomarkers	No change in CD34/CD31
Younan 2010[74]	Murine	40	L	N	Cyclical NPWT; Occlusive dressings	Tissue	Biomarkers	CGRP : increased substance P: increased NGF : increased Highest for cyclical > continuous
Erba 2011[29]	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 2009[75]	Murine	-	L	N	Standard dressing	Tissue	Biomarkers	VEGF 40% upregulation FGF-2 140 % upregulation CD31: increased expression
Scherer 2008[76]	Murine	20	L	N	Duoderm	Tissue	Biomarkers:	PECAM-1 Increased Ki 67 - increased

Qiu 2021[77]	Murine	48	L	Y	None	Tissue	Biomarkers	CD31: Increased CD68 : Reduced MDA: Reduced SOD: reduced CAT: reduced Raftlin: increased
Lu 2011[40]	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 2009[78]	In vitro	-	L	N	None	Human fibroblasts in 3d fibrin matrix	Biomarkers	PDGF: Increased by 53% TGF-B increased by 80%
Dong 2020[34]	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

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

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Biomarker of interest	Studies suggesting upregulation	Studies suggesting downregulation	Studies suggesting no change
Vascular endothelial growth factors(VEGF)	Zhou 2012, Erba 2011, Jacobs 2009, Labler 2006, Labler 2009, Karam 2018,Mu 2019,		
Tumour Necrosis Factor-alpha	Brownhill 2021, Li 2013	Stechmiller 2006,Eisenhardt 2012, Karam 2018,Wang 2019, Dong 2020	Norbury 2007
Transforming Growth Factor Beta	Karam 2018,Yang 2017,Lu 2011,McNulty 2009, Brownhill 2021		Kilpadi 2016, Norbury 2007
Interleukins(IL) IL6 IL8 IL-1B	 Labler 2006 Labler 2006, Labler 2009 Stechmiller 2006, Brownhill 2021, Li 2013	Wang 2019	 Kilpadi 2016, Labler 2009 Kilpadi 2016
MatrixMetalloproteinases(MMP) MMP 2 MMP 3 MMP 9	 Stechmiller 2006,Greene 2006, Stechmiller 2006,Brownhill 2021 Stechmiller 2006, Brownhill 2021	 Karam 2018,Greene 2006	

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Figure 1: Literature search and study selection

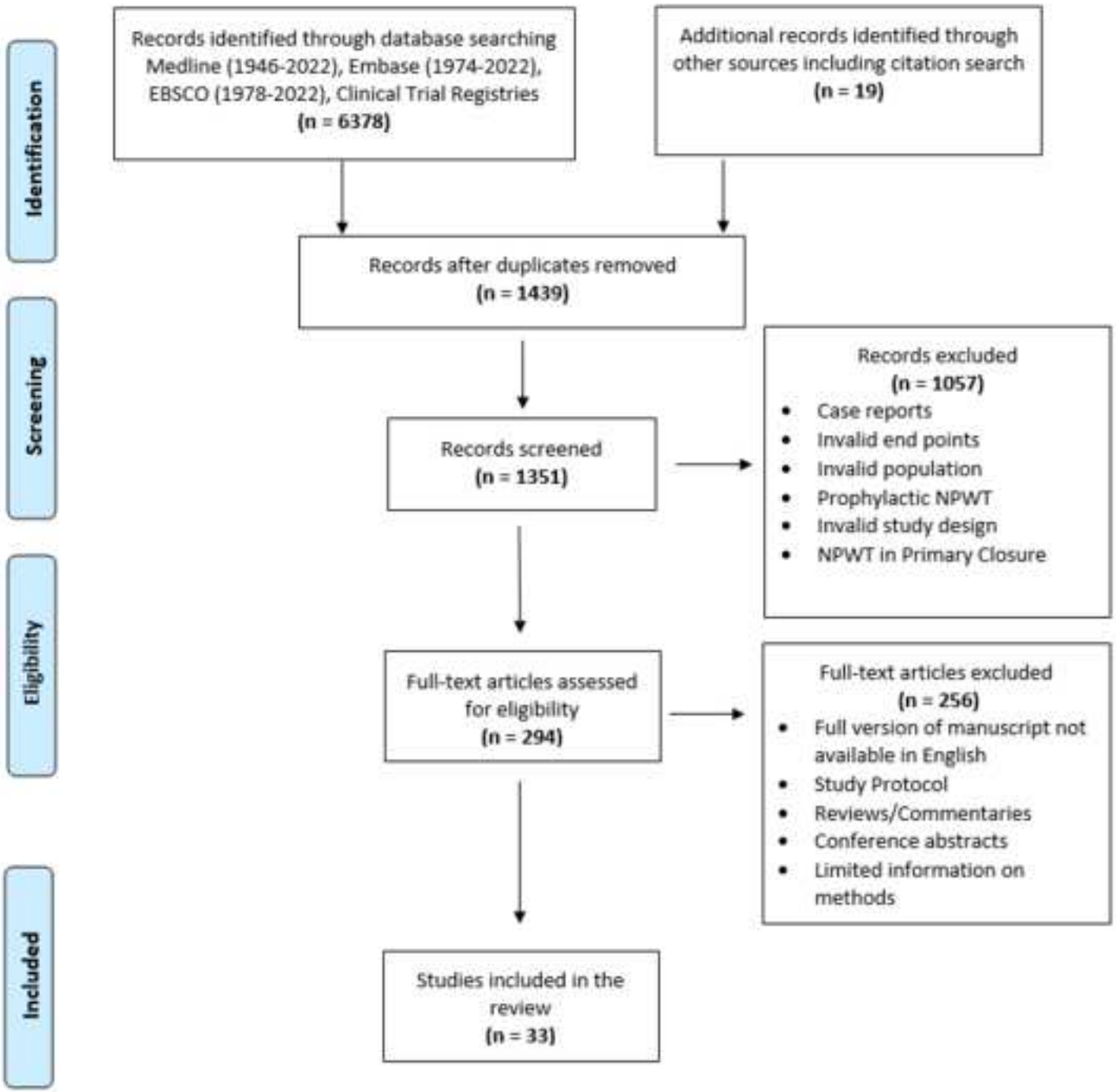
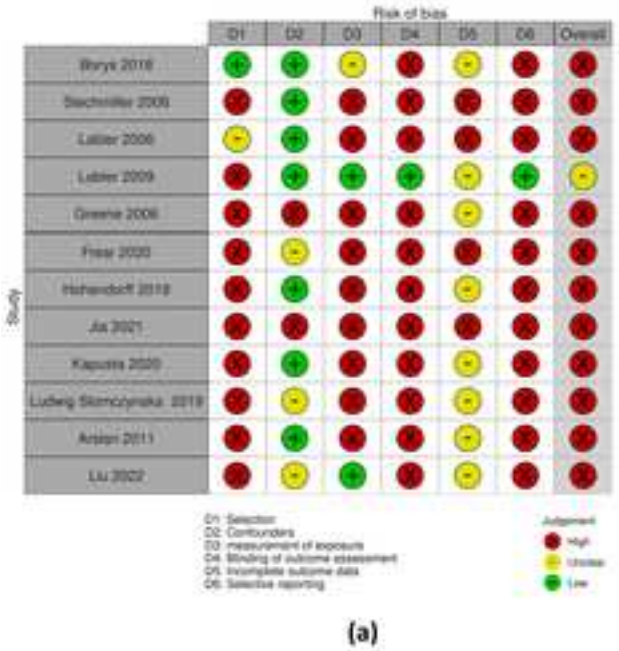
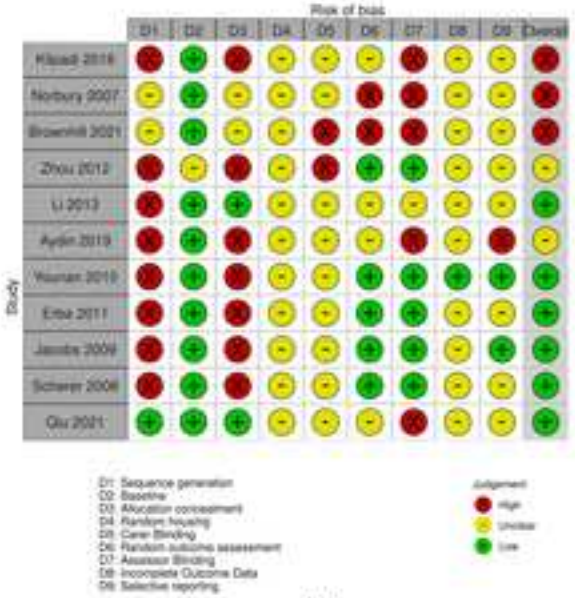


Fig 2: Risk of Bias Assessment of the included studies: (a) RoBANS for non-randomized studies,(b) SYRCLE tool for Animal studies, (c) RoB-2 tool for randomized

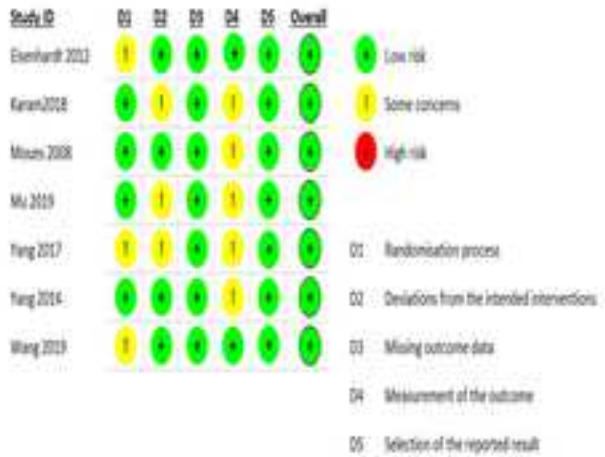
Click here to access/download;Figure;Figure 2.jpg



(a)



(b)



(c)

Molecular mechanisms of action of negative pressure wound therapy: A Systematic review

B Ravindhran et al, Academic Vascular Surgical Unit, Hull York Medical School, Hull, United Kingdom

PICO

Open surgical wounds including diabetic ulcers, pressure ulcers, surgical site infections, traumatic wounds

Any form of NPWT

Standard care or standard dressings

Mechanism of action at a molecular level

Medline, Embase, EBSCO databases and clinical trial registries from inception to January 2023

19 clinical studies
- 7 randomized studies
- 12 non randomized studies

11 animal studies
- 5 murine models
- 5 porcine models
- 1 rabbit models

3 in-vitro studies
- Murine fibroblasts
- Human fibroblasts
- PMNs and HeLa 60 cell lines

33 Studies included in the review

Agreement of 90%

43 biomarkers and 17 gene expressions studied

VEGF elevated in 7 out of 7 studies

TNF α downregulated in 5 out of 8 studies

TGF-B upregulated in 5 out of 7 studies

Fibronectin upregulated in 2 studies

NPWT stimulates wound healing by modulation of numerous local and circulating cytokines and growth factor expressions to promote an anti-inflammatory profile.

Response to reviewers:

Reviewer #1: Dear authors, The work is really interesting about the several studies at different stages about negative pressure wound therapy. However, I have some suggestions to improve the quality of the manuscript.

Response: Dear reviewer, many thanks for your kind comments and key suggestions. They have been incorporated as follows.

Comment 1. The references should be revised because I found one of them in red.

Response: Many thanks for pointing this out, this has duly been rectified.

Comment 2. The authors should discuss more in deep the subtype of TGF-beta is involved in the negative pressure wound therapy?

Response: Many thanks for this suggestion, they have duly been added to lines 171-176.

Comment 3. I think the authors should include a graphical abstract about the work

Response: Many thanks for this suggestion. The graphical abstract has been added as an additional file.

Reviewer #2: Dear Editor,

This is a very interesting review on the topic. It is well-written and the purpose is important. I think it will contribute to future prospective trials on the use of NPWT.

Response: Dear reviewer, many thanks for your kind comments.

The Editor in Chief, 15/3/23

Expert Reviews in Molecular Medicine

Dear Prof. Curtin,

We would greatly appreciate your consideration of the enclosed manuscript entitled “Molecular mechanisms of action of negative pressure wound therapy: A systematic review” for publication in The Expert Reviews in Molecular Medicine.

This systematic review summarises the current understanding of the mechanism of action of negative pressure wound therapy on open surgical wounds at a molecular level, based on studies published over the last 20 years. Although many theories have been proposed to support its primary and secondary mechanisms at a molecular level, the evidence has not been collated since 2014.

The wide-ranging readership of the Expert Reviews in Molecular Medicine undoubtedly offers the appropriate platform to disseminate this work at the interface between wound healing and molecular medicine. We confirm that this manuscript has not been published and is not under consideration for publication elsewhere and if the article is accepted it will not be published elsewhere in the same form without the consent of the publisher.

Yours Sincerely,

Bharadhwaj Ravindhran

NIHR Academic Clinical Fellow

Specialty Registrar in Vascular Surgery

Yorkshire and the Humber

(On behalf of all authors)

Supplementary material

Search strategy

1.	exp Negative-Pressure Wound Therapy/
2.	exp Suction/
3.	exp Vacuum/
4.	(negative pressure or negative-pressure or NPWT).tw.
5.	(sub-atmospheric or subatmospheric).tw.
6.	Topical Negative Pressure.tw.
7.	TNP.tw.
8.	Sub-atmospheric wound therapy.tw.
9.	Microdeformational wound therapy.tw.
10.	MDWT.tw.
11.	(wound adj3 suction).tw.
12.	(wound adj3 drainage).tw.
13.	((foam adj3 suction) or (suction adj dressing$)).tw.
14.	(vacuum assisted closure technique or VAC).tw.
15.	((vacuum adj therapy) or (vacuum adj dressing$) or (vacuum adj seal$) or (vacuum adj closure) or (suction$ adj drainage)).tw.
16.	or/1-15
17.	exp Surgical Site Infection/
18.	Surgical Site Dehiscence.tw.
19.	(wound* adj7 dehisc*).tw.
20.	(wound* adj7 infect*).tw.
21.	(wound adj7 disrupt*).tw.
22.	wound complication*.tw.
23.	(surg* adj7 infect*).tw.
24.	(surg* adj7 wound*).tw.
25.	(surg* adj7 site*).tw.
26.	(surg* adj7 incision*).tw.
27.	(surg* adj7 dehisc*).tw.
28.	or/17-27
29.	(intent* or second* or heal* or complic*).tw.
30.	((open* or clos*) adj5 wound*).tw.
31.	29 or 30

32. 31 and 28
33. randomised controlled trial.pt.
34. controlled clinical trial.pt.
35. randomi?ed.ab.
36. placeb*.ab.
37. clinical trials as topic.sh.
38. random*.ab.
39. trial.ti.
40. exp animals/ not humans.sh.
41. or/33-40
42. 32 and 41
43. cytokin*.tw.
44. chemokin*.tw.
45. Angio*.tw.
46. exp growth factors/
47. tumour necrosis factor-alpha.tw.
48. TNF.tw.
49. Interleukin.tw.
50. or/43-49
51. 42 and 50

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Line 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Lines 29 -46
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	lines 83-91
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	lines 89-91
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Lines 100 -109
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Lines 98-100
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Supplementary material 1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Lines 98-111
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Lines 113-122
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Lines 133-142
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Lines 133-142
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Lines 126-131
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	NA
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	NA
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	NA
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	NA
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	NA
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	NA
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	NA
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NA

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Figures 1
Study characteristics	17	Cite each included study and present its characteristics.	Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Table 1 ; Figure 2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	NA
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	145-228
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	NA
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NA
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Lines 230-252
	23b	Discuss any limitations of the evidence included in the review.	Lines 268-281
	23c	Discuss any limitations of the review processes used.	Lines 268-281
	23d	Discuss implications of the results for practice, policy, and future research.	Lines 283-296
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Prospero ID CRD42022303088
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	No protocol prepared
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Line 17
Competing interests	26	Declare any competing interests of review authors.	Line 18
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Line 19-20

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

PRISMA 2020 Checklist

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