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REVIEW

Local anti-inflammatory effect and immunomodulatory activity of chitosan-based dressing in skin wound healing: a systematic review

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Abstract

Background and aim: Wound healing is a complex process comprised of several distinct phases. An imbalance in any of the stages creates a chronic wound with the potential to cause life-threatening complications for patients. Chitosan is a biopolymer that has shown to positively impact the different healing phases. This systematic review aimed to evaluate the anti-inflammatory and immunomodulatory properties of chitosan-based wound therapy for the skin healing process after an injury.

Methods: A systematic review was conducted in November 2021 following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. The PubMed, Embase, Google Scholar, and Cochrane online databases were queried to capture all publications in the last ten years that investigated the chitosan effects on inflammation and immune reaction.

Results: A total of 234 studies were screened after removing duplicates, and fourteen articles fulfilled our inclusion and exclusion criteria. In the studies, chitosan was combined with a wide range of products. One clinical trial was found that treated patients with diabetic foot ulcers. All animal models in the studies used a full-thickness skin wound to test the effectiveness of chitosan in the healing process. Decreased pro-inflammatory cytokine levels, a shortened inflammatory phase and accelerated wound closure was observed in all of the studies.

Conclusions: Chitosan proved to be a feasible, versatile, and multifaceted biomaterial that enhances the biological response to a skin injury. When combined with other products, its potential to boost the healing process through regulation of the inflammatory and cellular activity is increased.

Relevance for patients: Although few clinical trials have been completed, Chitosan has become an excellent alternative to modulate the local inflammatory response promoting wound healing. Especially in patients with associated comorbidities that affect the typical resolution of skin healing, such as diabetes and vascular insufficiency. Therefore, using bioactive wound dressings based on Chitosan combined with nanoparticles, growth factors, lived cells, or medications, released in a controlled manner positively impacts patient life by shorting the wound healing process.

Keywords: deacetylated chitin, biocompatible materials, tissue engineering, wound healing, inflammation, skin regeneration

1. Introduction

The skin is the largest organ in the body and has the capacity to regenerate itself.[1] After an injury, the skin heals through several phases called hemostasis, inflammation, proliferation, and remodeling.[2, 3] (**Figure 1**). Although the skin can return to a normal state and regain its regular function, a series of factors, such as infection, foreign body presence, tissue necrosis, and patient comorbidities, can disrupt its healing.[4] [5] Therefore, a delay in wound closure increases the risk of bacterial colonization, invasion, and septicemia instauration leading patients to a life-threatening conditions.[6, 7]

In the United States alone, the healthcare system spends more than $70 billion on wound care each year.[8-10] Thus, it is necessary to find an effective treatment option that boosts the natural skin healing process by regulating the cytokines and growth factors that play a crucial role in modulating the cellular activity at the injury site.[11] Chitosan (CS) is a naturally derived biopolymer from the crustaceous cytoskeleton,[12-14] and it has previously shown to improve different phases of the skin healing process.[15-18] Important properties for tissue regeneration associated with this biomaterial include antimicrobial and anti-inflammatory activity, angiogenesis promotion, and collagen synthesis stimulation.[19-23] These properties have been potentiated by adding substances, chemicals components, and active biomolecules.[24-26] Accordingly, antioxidant properties have been found by combining chitosan with hyaluronan and phosphatidylcholine dihydroquercetin. Considering that the excessive production of free radicals during the inflammatory phase of healing exerts a negative effect on the cellular performance during the immune response, enhancing the elimination of the free radicals from the wound will decrease inflammation and promote skin regeneration.[27]

Immune response modulation at the skin injury site shortens the healing course by decreasing pro-inflammatory cytokine production, neutrophil infiltration, and number of reactive oxygen species while increasing specific growth factors that favor skin regeneration.[2, 28-30] Furthermore, finding the perfect balance between cytokines and chemokines production in the inflammatory stage of skin healing will orchestrate the interaction between platelets, neutrophils, macrophages, and monocytes essential for a successful recovery.[3, 31]

We hypothesize that CS can regulate the immune response and decrease inflammation at the wound site, thus enhancing the skin healing process. This systematic review evaluates the anti-inflammatory and immunomodulatory properties of different forms of CS for skin regeneration after an injury.

2. Methods

2.1 Study selection

A systematic review of articles illustrating skin wound healing using CS in *in-vivo* models was performed. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed for article identification and final selection.[32] Papers written in English that reported the utilization of the CS biomaterial, alone or in combination, to treat wounds were included. Additionally, assessment of the CS's anti-inflammatory activity through histological or molecular analysis, or gene expression quantification associated with skin healing process was required for inclusion. On the other hand, studies were excluded if they described *in vitro* or *in vivo* models with only macroscopy or gross morphological evaluation of the wounds.

2.2 Data source and search strategy

The search was conducted on November 18th, 2021, by querying the electronic databases MEDLINE (PubMed), EMBASE, Google Scholar and Cochrane Central Register of Controlled Trials. We used a combination of the following Medical Subject Headings terms: "anti-inflammatory activity" AND "chitosan" AND "skin wound healing". The inquiry was limited to articles published in the last ten years. Assessment of the studies' titles and abstracts constituted the initial screening. For the final selection, a comprehensive literature review referring to the inclusion and exclusion criteria described above was completed. Two authors independently performed the search and an agreement among them was attained.

2.3 Risk of bias

The Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool (Cochrane Library) was used to evaluate studies' risk of bias.[33] Descriptions of individualized bias and cross-sectional studies bias are shown in **figures 2** and **3,** respectively.

3. Results and discussion

3.1 Study selection and principal characteristics

The study selection process is outlined in the PRISMA flow diagram (**Figure 4**). Out of the 234 articles identified initially, a total of 14 articles fulfilled the inclusion criteria. A summary of the important characteristics of each article are presented in **tables 1** and **2**.

Thirteen of the studies utilized animal models with more than 50% of them examining rats as the main species,[26, 34-40] followed by mice.[41-45] A single paper reported a double-blind, randomized clinical trial with humans to assess the use of CS combined with isosorbide dinitrate to treat diabetic foot ulcers.[46]

A variety of CS forms were described, including hydrogels,[41-44, 46] sponges,[34] membranes,[26] dressings,[39, 40] patches,[38] nanoparticles,[37] and scaffolds.[35, 36, 45] No application of CS alone was reported. Collagen,[35, 36, 41] alginate,[39] hyaluronan,[26] curcumin,[36, 37] platelet-rich plasma,[40] metal ions,[44] human dermal fibroblast,[34] and growth factors,[42, 43] were combined with the CS to improve the healing process. A full-thickness cutaneous wound on the dorsum of the animal was created as the injury for all the animal models. Additionally, some studies assessed the association of comorbidities in the skin regeneration by producing skin radiation lesions,[34] inducing diabetes,[37, 41, 42] and co-infecting the wound.[40] Similarly, the benefits of CS on chronic wounds were evaluated in diabetic patients with foot ulcers. Totsuka et al.[46] achieved complete foot ulcer closure in 68 diabetic patients in fewer days when CS was combined with isosorbide nitrate compared to CS alone or a placebo.

3.2 Inflammatory and angiogenesis mediators in the skin healing process

Hemostasis and inflammation are the initial steps of skin healing and occur within the first three days after an injury.[47],[31] Neutrophils are the most abundant white blood cells at the wound site during the first 24 hours.[47] Their recruitment is mediated by pro-inflammatory cytokines, such as IL-1, IL-6, INF-α, TNF-α, TGF-β, macrophage inflammatory protein-1α and anaphylatoxins (C3a, C5a). However, IL-6 secreted mainly by macrophages, endothelial cells and fibroblasts has been described as one of the principal regulators in neutrophil recruitment.[48] High levels of IL-6 are related to poor wound healing and scarring.

Li, F et al.[37] analyzed the performance of the Curcumin (Cur) and CS nanoparticles (NPs) in an *in vitro* model, an lower expression of NF-κB and downregulation of TNF-α and IL-6 were observed. This demonstrated that Cur-CS-NPs inhibited the macrophage-induced inflammatory response. To test these results, wounds of diabetic rats were treated with Cur-CS-NPs. Thus, less inflammatory cellular infiltration, increased the production of new blood vessels, and a superior collagen distribution was found in the experimental group.

Comparable results were reported by Hu B, et al.[42] They found a decrease in levels of IL-6 when wounds of streptozotocin-induced diabetic mice were treated with CS hydrogel combined with Cur-NPs, aldehyde hyaluronic acid, carboxymethyl and epithelial growth factor compared to the control group. Furthermore, the implementation of this hydrogel allowed a controlled release of the components throughout the healing process. Thus, it was demonstrated that CNPs assuaged oxidative stress and inflammation during the early stage, while the late secretion of EGF drove the ECM remodeling. In addition, the histopathological analysis of the wound tissue showed a faster granulation tissue formation, reepithelization, and hair follicle regeneration in the experimental group.

Anti-inflammatory properties of Cur-CS were as well tested by Rezaii et al.[36] A Cur-CS-collagen scaffold was used to treat wounds in a rat model, and the team focused their investigation on TGF-β1 as this peptide has a broad range of effects on growth factors implicated in wound healing.[31]

The histological analysis illustrated that the upregulation of TGF-β1 at the early and late stages of skin healing was associated with quicker wound closure, neovascularization, greater collagen content, and reepithelization.

In the presence of infection, neutrophils assist in combating bacterial invasion by releasing reactive oxygen species (ROS), cytotoxic granules, and increasing pro-inflammatory macrophages and T cell chemotaxis.[50] Hence, an increase in the quantity of neutrophils will prolong the inflammatory state.[51] Zhou et al,[40] created an infected wound through inoculation of *Staphylococcus aureus* and *Escherichia coli* in a full-thickness skin lesion in rats. The group tested the healing properties of a nano silver-doped carboxymethyl CS grafted polyamide amine cationic polymer dressing mixed with platelet-rich plasma and sodium alginate (Alg/Ag@CMC-PAMAM/PRP). The downregulation of IL-6 and IL-1β and the up-regulation of TGF-β, CD31, and α-SMA in the rats treated with the dressing demonstrated that Alg/Ag@CMC-PAMAM/PRP supports healing of infected wounds by increasing angiogenesis and decreasing inflammation.

Similarly, You et al,[35] evaluated the *in vitro* anti-microbial properties of a collagen-CS-based scaffold combined with silver nanoparticles (NAg-CCs). Different concentrations of NAg were tested against *E. coli* and *S. aureus*, and the minimal inhibitory concentration was found to be ≤ 10 ppm. Therefore, they fabricated NAg-CCs scaffolds with this concentration, and the *in vivo* performance was studied. Afterward, real-time quantitative polymerase chain reaction (RT-qPCR) was used to assess the anti-inflammatory activity. A significantly lower mRNA expression of IL-6, TNF-α, TGF-β in the NAg-CCs group was detected compared to the control group that contained CS alone. Additionally, overexpression of IL-10 and IFN-γ was found at 1, 2 and 4 weeks after scaffold implantation. A western blot analysis supported these results at each time point.

Metal ions constitute another essential component of the wound microenvironment, and their presence is crucial for cellular biochemistry reactions. Some of the most studied metal ions and their function on skin healing include silver (Ag^{+}) which is recognized as an excellent option to treat infected

wounds; calcium (Ca^{2+}) related to improving reepithelization;[53] zinc (Zn^{2+}) associated with immune response, cell growth and migration;[54] and copper (Cu^{2+}) distinguished as a collagen stabilizer and angiogenesis promoter.[55] Based on these promising metals properties, Xiao et al,[44] created a gauze base dressing with CS hydrogel to load metal ions (Ag^{+}, Ca^{2+}, Zn^{2+}, Cu^{2+}) to treat *S. aureus* infected wounds. The freeze-thawing was the method used to create multiple ions co-loaded gauze (MD), layer-by-layer gauze (LBL), and timely dosing gauze (TD). LBL and TD can release multiple metal ions on demand in an *in vitro* model. The dressings' anti-inflammatory properties were assessed on day seven through immunofluorescence staining of the tissue. This showed a down-regulation of the TNF-α proinflammatory cytokine in all groups except in CS group. Furthermore, the growth pattern of bacterial colonies was documented. The MD and LBL groups showed significant colony reduction in the first two days compared to the TD group, which had the best anti-bacterial activity on day seven. Therefore, metal ions integrated into a gauze delayed bacterial growth *in vitro* and inhibited inflammation *in vivo* through TNF-α regulation and macrophage polarization. This ion delivery system proved to have an excellent anti-bacterial action and can be an option for infected wound treatment.

In addition to the angiogenic properties of CS proven by Zhou et al,[40] using Alg/Ag@CMC-PAMAM/PRP on infected wounds, Zhao et al.[39] observed an increase in neo-vascularization molecules related to VEFG and CD31 in an animal model. They fabricated fibrous spongiform calcium alginate (CAD) dressing mixed with CS (CCAD) to treat full-thickness wounds. More blood vessels and faster healing were reported in the CCAD group compared to the CAD group. The anti-inflammatory activity was additionally evaluated through the IHC staining of IL-6, and the CCAD group had lower IL-6 levels.

Li, Q et al,[43] demonstrated the capability of CS hydrogel to carry growth factors to promote wound healing. Angiogenesis was increased after applying a CS-IGF-1C hydrogel in *vegfr2*-transgenic mice. Bioluminescence confirmed the activation of the VEGF/VEGFR2 pathway. In addition, the levels of the proangiogenic genes (VEGF-A, HIF-1α, ANG1, and PDGF) were determined, and they were significantly higher in mice treated with the CS-IGF-1C hydrogel. Moreover, Wang et al.[56] proved the

practicality of this hydrogel by combining it with hyaluronic acid and adipose-derived stromal cells. The co-transplantation of the hydrogel and stem cells into the ischemic hind limbs of mice effectively increased blood perfusion and decreased inflammation.

3.3 The importance of balancing macrophage activation in the skin inflammatory response

Macrophages are widely described as a necessary for skin regeneration. Its depletion results in a reduction of extracellular matrix (ECM) formation and angiogenesis leading to delayed wound closure.[57] Two identified subsets of skin macrophages exist. One is known as the resident macrophages, and the other is the circulating monocyte-derived macrophages. The latter plays the central role in skin healing as the resident macrophages only provide a short-term response to an injury.[58]

Once monocytes arrive at the wound site, they differentiate into two critical types of monocyte-derived macrophages, M1 (proinflammatory) and M2 (reparative). (**Figure 5**). In normal conditions, 80-85% of the M1 macrophages transition into M2 macrophages around day five to seven post-injury. This is characterized by the production of cytokines (IL-4, IL-10, IL-13) and growth factors (VEGF, TGF-β, PDGF, IGF-1) that promote proliferation of keratinocytes, fibroblasts, and endothelial cells that lead to reepithelization and generate ECM components.[59] Therefore, dysregulation of these subtypes of macrophages results in non-healed wounds or tissue fibrosis.[57] Shen et al.[41] demonstrated that the macrophage immune response modulation occurred when using a sulfated CS doped collagen type I (Col I/SCS) hydrogel for diabetic mice wound treatment. Induction of polarization towards the M2 subpopulation ($CD206^-$) enhanced the macrophages' trans-differentiation into fibroblasts. Similarly, the use of NAg-CCs scaffold produced regulation of fibroblast migration and macrophage activation in the injury site demonstrated by low expression of CD68, TGF-β, and IL-6 and a high expression of IL-10 and IFN-γ. Importantly, IFN-γ is a known inducer of the M2 macrophages. [35,[57]]Comparably, CNs were incorporated into a CCs scaffold. Modulation of TGF-β1 and Smad7 levels was observed. The up-regulation of TGF-β1 mRNA on days three and 15 and Smad7 mRNA on day five post-treatment demonstrated negative feedback between these two molecules. This relationship

contributed to rapid wound closure due to accelerated progression from the inflammatory to the proliferative healing phase.[36]

On the other hand, a higher M2/M1 ratio was observed in wounds treated with CS metal ion-loaded gauzes. An increase in M2 phenotype demonstrated that the host's immune response could be modulated using drugs, preventing pathological macrophage activation.[44] The association of dysfunctional macrophages with the development of chronic wounds has been observed in diabetic patients.[57, 59] Targeting macrophages directly through a therapeutic intervention may enhance wound healing. Using Col I/SCS hydrogel on diabetic mice, Shen et al.[41] observed an up-regulation of IL-4 promoting macrophage polarization into the M2 phenotype. These results are comparable to those reported by Bonito et al,[57] and Schirmer et al,[60] which highlight the role of IL-4 in the modulation of macrophage activation. Additionally, Col I/SCS increased trans-differentiation of macrophages into fibroblasts, expanding the synthesis of ECM components and improving wound healing.[41]

3.5 Histopathological analysis

The therapeutic effect of CS in combination with several products was tested in 13 of the 14 studies by performing a morphological tissue evaluation using hematoxylin and eosin staining. A decrease in the inflammatory cells infiltration,[37, 38, 40, 45, 59] and the balance in granulation tissue formation,[26, 36, 38, 42, 44] was reported by the majority of the authors. Additionally described was an increase in reepithelization,[26, 34, 36, 38, 40-42] and neovascularization[36, 37, 39, 41-43, 45, 46].

Hydroxyproline content in the wounds was determined by R, N et al.[38] to evaluate the ECM formation capacity of polyvinyl alcohol/CS/curcumin patches. Higher content was observed in the group treated with the patches compared to the group managed with a commercial ointment. Additionally, the skin samples assessed collagen deposition by Masson Trichrome staining. On the 16th day post-treatment, the tissue samples showed thickness, dense, and uniform collagen fibers. These results were similar to those reported by studies using CS combined with collagen/silver,[35] collagen/curcumin,[36] calcium alginate,[39] sodium alginate/PRP/silver/PAMAM,[40] and hyaluronic acid/curcumin/EGF.[42]

The surge of tissue-engineered materials has allowed the creation of bioactive wound dressings to boost lesion healing.[12] CS has demonstrated versatility in this field due to its use in various forms, such as sponges, patches, membranes, scaffolds, and hydrogels. Moreover, the inclusion of nanoparticles, growth factors, lived cells or medications, released in a controlled manner further increases its usefulness for skin healing.

Conclusions

The skin begins a cascade of events following an injury, with inflammation being one of the most determining factors in healing. Hence, several therapeutic options focus on controlling this stage to accelerate wound closure. CS has shown to decrease inflammation, promote neovascularization, reepithelization, and prevent bacterial infiltration at the wound site in *in vivo* models. Its capacity to be combined with a variety of products known to promote wound healing, exerting a synergistic effect, demonstrates its versatility as a biomaterial. Therefore, with CS being inexpensive and accessible, it can provide a feasible alternative as a therapeutic option for wound treatment.

Limitation

The main limitation of this study was the discrepancies found between studies discussing CS in combination with several components as it was challenging to compare them. Additionally, the diversity of the protocols employed in the reviewed studies can contribute to the potential misinterpretation of data and results. Finally, the study selection process of systematic reviews may be a source of bias.

Conflict of interest

None.

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Figures

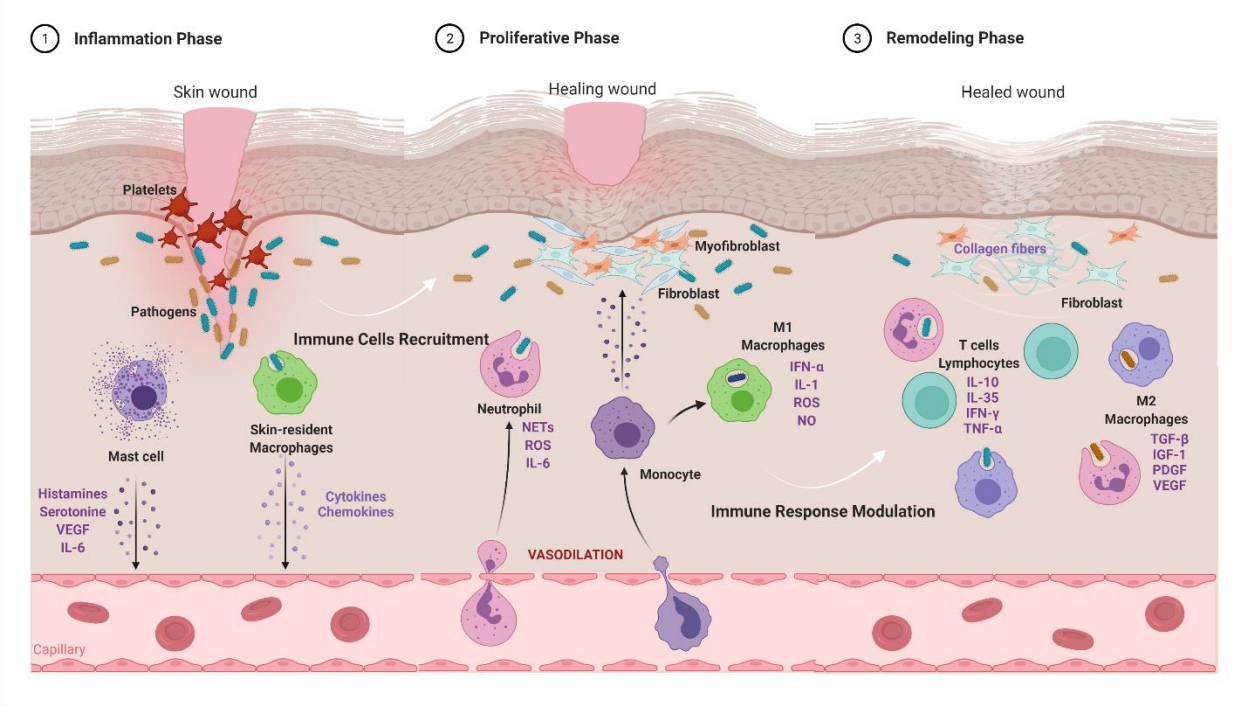

Figure 1. Graphic representation of immune response in the skin wound healing process. Cells, cytokines, chemokines, and growth factors are responsible for the modulation of skin healing phases. 1. Inflammation, 2. Proliferation and 3. Remodeling. Abbreviations, IL, interleukin; IFN, interferon; NETs, neutrophils extracellular traps; NO, nitric oxide; ROS, reactive oxygen species; TNF, tumor necrosis factor. VEGF, vascular endothelial growth factor. Created with BioRender.

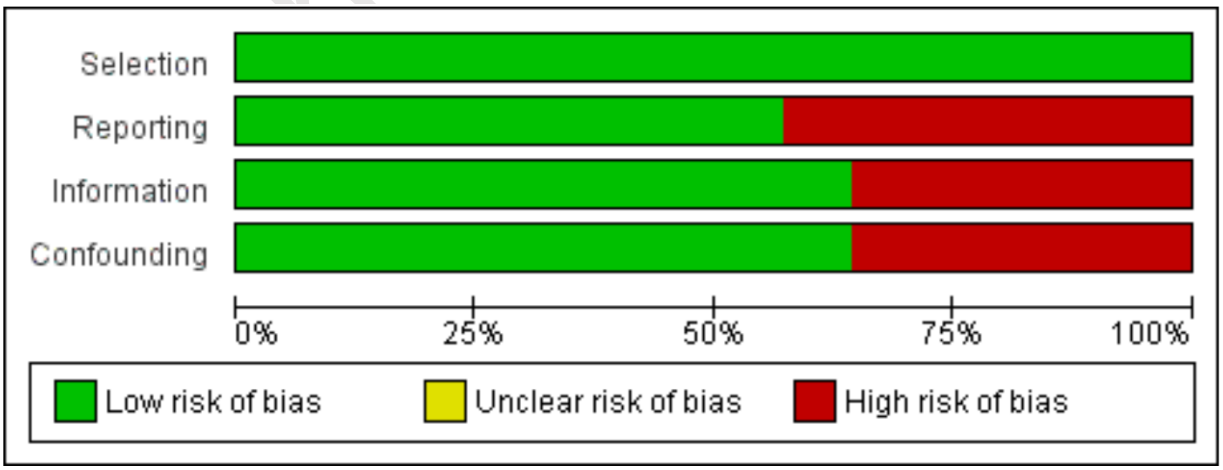

Figure 2. Individualized risk of bias. The green color represents low risk, and the red color represents a high risk of bias.

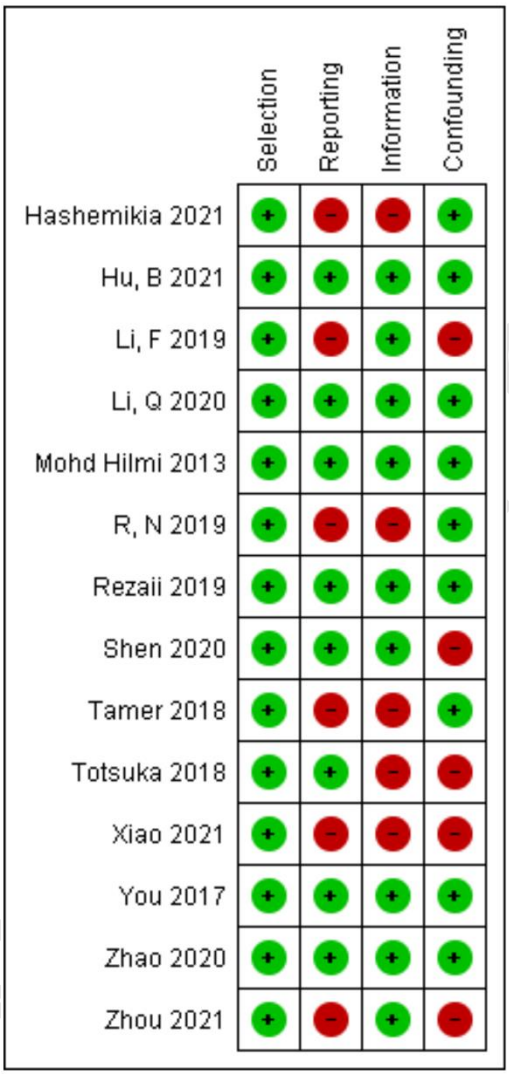

Figure 3. Summary of Risk of Bias all Included Studies. (+) indicates absence, and (-) indicates the presence of bias.

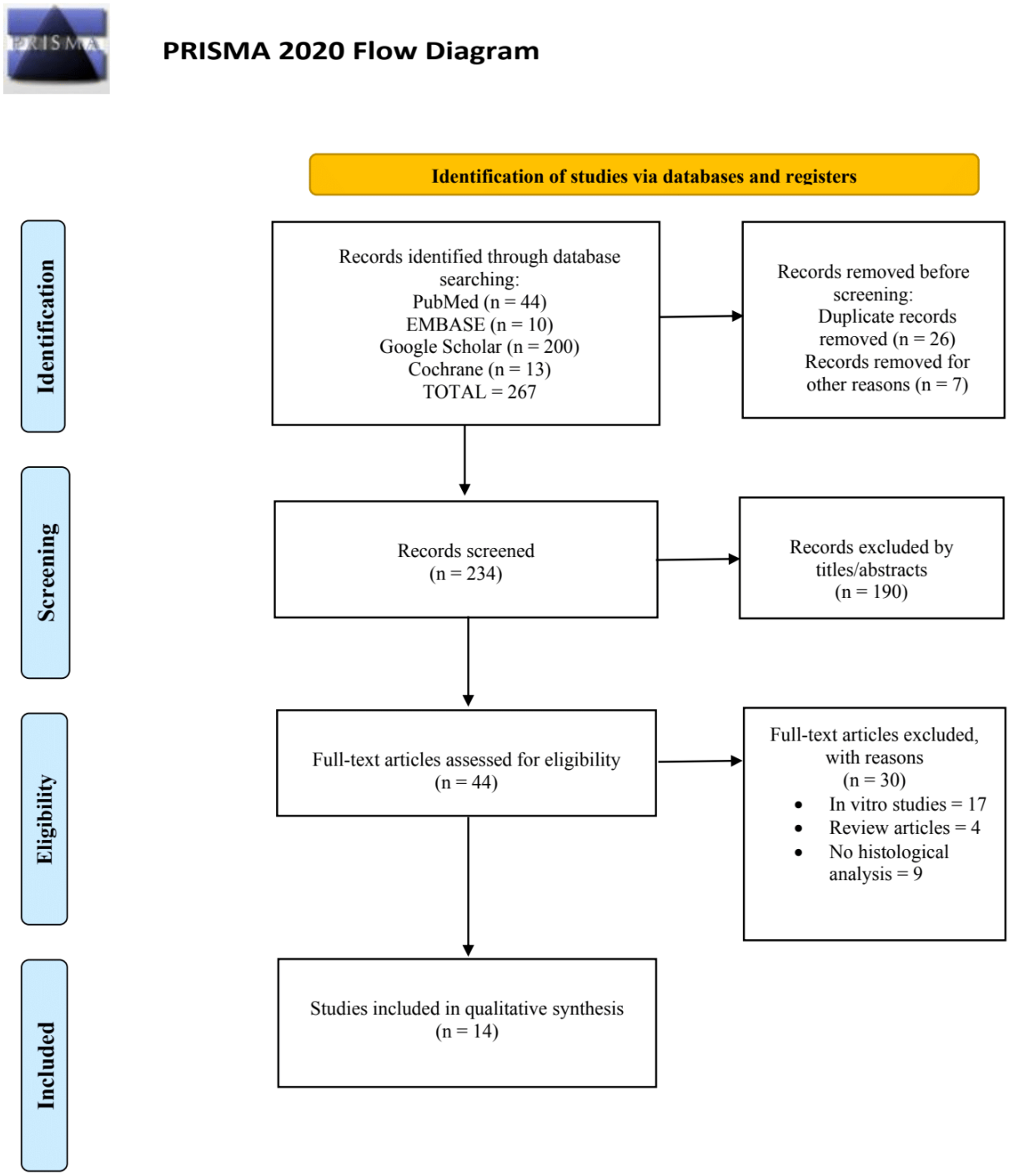

Figure 4. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram. Included and excluded studies.

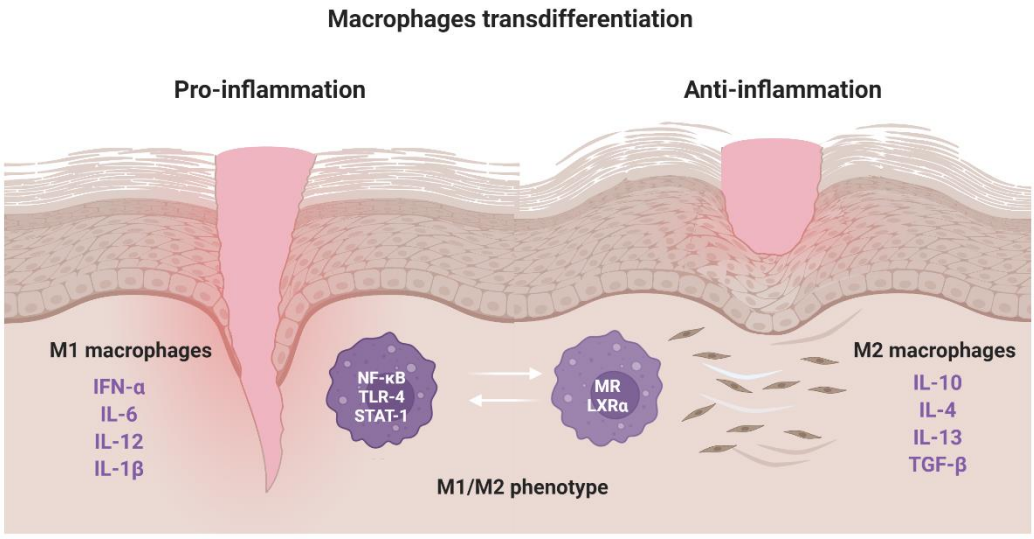

Figure 5. Schematic representation of macrophages phenotypes transdifferentiation**.** M1 macrophages regulate proinflammatory cytokines release. Conversely, M2 macrophages are responsible for anti-inflammatory cytokines regulation. Abbreviations, IL, interleukin; IFN, interferon; LXR, liver X receptor; MR, mineralocorticoid receptor, NF-kB, nuclear factor kappa light chain enhancer of activated B cells; STAT, signal transducer and activator of transcription, TGF, tumor growth factor; TLR, toll-like receptor. Created with BioRender.com

Table 1. Characteristics of included clinical trials.

Author/Year/Location	Number of Patients	Type of study	Chitosan form	Combination Loaded	Control group	Type of injury	Time of healing	Histology	IHC
Totsuka Sutto, S. E., et al. (2018) Mexico	68	Randomized Placebo-controlled Double-blinded Clinical trial	Gel	Isosorbide Dinitrate	Placebo	DFU	30 days TG 45 days Placebo	• Neo-angiogenesis • Fibroplasia	vWF Desmin VEGF-A α-SMA

Abbreviations: α-SMA, α-smooth muscle actin; DFU, Diabetic foot ulcers; IHC, immunohistochemistry; TG, Treated group; VEGF-A, Vascular endothelial growth factor-A; vWF, Von Willebrand Factor.

Table 2. Characteristics of included studies in animal models.

Author/Year/Location	Type of Animal	Chitosan form	Combination Loaded	Control group	Type of injury	Endpoint (Days)	Histology	IHC/IF/RT-qPCR/WBT
Mohd Hilmi, A. B., et al. (2013) Malaysia	Sprague Dawley rats	Sponge (Dermal substitute) Sponge plus hDF (Skin substitute)	Fibroblast	Duoderm CGF	Post-radiation injury (2 months) Full thickness cutaneous wounds 1cm x 1 cm	7, 14, 21	H&E MT distance ET length Re-epit	**IF** HLA K10
You, C., et al. (2017) China	Sprague-Dawley rats	Scaffold (NAg-CCS)	BT-1C MNAg 20–40nm in diameter	CCS	Full thickness skin defects in	7, 14, 28	H&E • Fibroblast's migration • ECM	**IHC** CD68 TGF-β1

					2 cm Ø		MTS	**RT-qPCR** TNF-α TGF-β IL-10 IL-6 IFN-γ
Tamer, T. M., et al. (2018) Egypt	Wistar rats	Membrane	Hyaluronan Edaravone	Cotton gauze	Full thickness skin defects 1.5cm x 1.5 cm	7, 14, 21	H&E • Epithelialization area • Granulation tissue • Connective tissue	N/A
Rezaii, M., et al. (2019) Tehran	Wistar rats	Scaffold	CNs Collagen	(-) Untreated (+) CCs	Full thickness cutaneous wounds 1 cm Ø	3, 7, 15	H&E • Epidermal thickness • Granulation tissue	**RT-qPCR** TGF-β1 Smad7

							• Vascular density MTS	
Li, F., et al. (2019) China	Sprague Dawley rats Streptozotocin-induced diabetic rat models	Nanoparticles	CNs	PBS	Full thickness skin defects 2 cm Ø	3, 7, 14	H&E • Inflammatory infiltrates • Neo-vascularization	**WBT** TLR-4 NF-κB p65 TNF-α IL-6
R, N., et al. (2019) India	Wistar rats	Patch	CNs PVA	(-) Untreated (+) Commercial ointment (mupirocin)	Full thickness skin defects 1.5 cm × 1.5 cm		H&E • Inflammatory infiltrates • Re-epi • Granulation tissue	N/A

							MTS HPA	
Zhao, W. Y., et al. (2020) **China**	Sprague-Dawley rats	Dressing	CA	CAD	Full thickness skin wound model 2 cm Ø	3, 7, 14	H&E • Inflammatory infiltrates • Blood vessels • Fibroblasts MTS	**IHC** IL-6 VEGF CD34 TGF-β1, Phospho-Smad2 Phospho-Smad3
Shen, T., et al. (2020) **China**	C57BL/6 mice Streptozotocin-induced diabetic	Hydrogel scaffold	Col I	(-) Tegaderm (+) Col I	Full thickness wounds 8 mm Ø	3, 7, 14, 18	H&E • Re-epi. • Collagen deposition, • Epithelial thickness	**IF** Collagen I CD31 CD68 F4/80

	mice models						• Neo-vascularizatio n	**ELISA** TNF-α IL-6 IL-10 IL-4 IL-1β **RT-qPCR** Peritoneal macrophages
Hu, B., et al. (2021). **USA**	C57BL/6 mice Streptozotocin induced diabetic	Hydrogel	OHA CNs EGF	Blank hydrogel OHA-CMC OHA-CMC/CNs	Full thickness skin 8 mm Ø	5, 10, 15	H&E • Re-epi. • Granulation tissue • Neo-vascularizatio n	**IHC/IF** IL-6 Collagen-I MMP9 CD31

				OHA-CMC/EGF OHA-CMC/CNs/EGF			MTS	
Li, Q., et al. (2020). **China**	*Vegfr2-luc* transgenic mice	Hydrogel	IGF-1	PBS CS hydrogel CS–IGF-1C	Full-thickness wound 1 cm Ø	3, 7, 14	H&E • Neo-vascularization • ECM remodeling	**IF** CD31 Ki-67 **RT-qPCR** VEGFA HIF-1α PDGF ANG1
Xiao, J., et al. (2021). **China**	C57BL/6 mice	Hydrogel	Metal Ions Ag+, Zn2+, and Cu2+	Untreated	Full thickness cutaneous	7, 17	H&E • Granulation tissue	**IHC** Cytokeratine

				CS alone coated gauze MD gauze	wounds 6 mm Ø		MTS	**IF** CD31 α-SMA TNF- α
Hashemikia, S., et al. (2021). **Iran**	Balb/C mice	Scaffold	PEO Silica Ciprofloxacin nanofibers	Untreated CS/PEO CSPEO/SiO2 Chit/ PEO/SiO2/Cip)	Full thickness excisional wound 6 mm Ø	4, 7, 14	H&E • Angiogenesis • Inflammatory infiltrates	N/A
Zhou, M., et al. (2021). **China**	Sprague-Dawley rats	Dressing	Platelet rich plasma (PRP)	Gauze (-) Aquacel Ag (+)	Full thickness excisional wound E.	7, 14	H&E • Inflammatory infiltrates • Re-epi.	**IHC** CD31 α-SMA, TNF-α

			Sodium alginate (Alg) Silver (Ag)- PAMAM		coli and S. aureus co-infected		MTS	IL-1β IL-6 TGF-β1 **RT-qPCR** CD31 α-SMA TGF-β TNF-a IL-6 IL-1β

Abbreviations: Ag^{+}; silver; Alg, sodium alginate; ANG1, angiopoietin 1; BT-1C, bovine type 1 collagen; CA, calcium alginate; CAD, Calcium Alginate Dressing; CS, chitosan; CNs, Curcumin nanoparticles; CMC, carboxymethyl chitosan; Cu^{2+}, copper; EGF, epidermal growth factor; ECM, extracellular matrix; ET, Epithelial tongue; HIF-1α, hypoxia inducible factor 1 alpha; H&E, hematoxylin and eosin; hDF, human dermis fibroblast; IFN-γ, interferon gamma; IGF-1C, insulin growth factor 1C; IHC, immunohistochemistry; IF, immunofluorescence; IL, interleukin; MD, multi XX; MMP9; metallopeptidase 9; MNAg, metallic nano silver particles; MT, migratory tongue; MTS, Masson's trichrome staining; N/A, not applicable; OH, aldehyde hyaluronic acid; PAMAM, Polyamide amine; PBS; phosphate buffer saline; PDGF, platelet derived growth factor; POE, Polyethylene oxide; Re-epi, re-epithelization; RT-qPCR, real time quantitative polymerase reaction chain; Sio2, silica; TGF-β, transforming growth factor beta; TNF-a, tumor necrosis factor alpha; WBT, western blood test; Zn^{2+}, zing.