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Review

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Review

Role of copper nanoparticles in wound healing for chronic wounds: literature review

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Abstract

Chronic wounds are defined as wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner. The most common and inevitable impairment to wound healing is the installation of an infection, usually in the case of chronic wounds. Therefore, the objective of the present review was to identify the importance of copper nanoparticles in dressings for wound healing. Nanoparticles such as silver, gold and copper combat infectious processes through the inhibition of protein synthesis, peroxidation of the cell membrane and destroying the nucleic acids of bacteria and viruses. Among bioactive nanoparticles, copper plays a complex role in various cells, it modulates several cytokines and growth factor mechanisms of action and is essentially involved in all stages of the wound healing process. More importantly, copper plays a key role in skin regeneration and angiogenesis and accelerates the healing process through induction of vascular endothelial growth factor (VEGF) and angiogenesis by hypoxia-induced factor-1-alpha (HIF-1α) action where copper enhances HIF-1α expression and HIF-1α binding to the critical motifs in the promoter and putative enhancer regions of HIF-1-regulated genes.

Key words: Angiogenesis, Antimicrobial, Nanoparticles, Regeneration, Wound healing, Chronic wound, VEGF, HIF-1α

Highlights

- In this article, we review the physiology of wound healing and its relationship with epigenetics.
- The role of nanomaterials in the chronic wound healing process is discussed.
- The clinical evidence for the use of copper in treating chronic wound healing is reviewed.

Background

A chronic wound can be defined as one that has been unsuccessful in proceeding through a well-ordered and opportune reparative process to generate anatomic and functional integrity within a period of 3 months or that has continued through the repair process without achieving a sustained, anatomic and functional result [1,2]. Based on the causative etiologies, chronic wounds are classified into four categories: pressure ulcers, diabetic ulcers, venous ulcers and arterial insufficiency ulcers [3]. These chronic wounds are important in the health care system due to their increasing prevalence and their treatment costs. In effect, a retrospective analysis

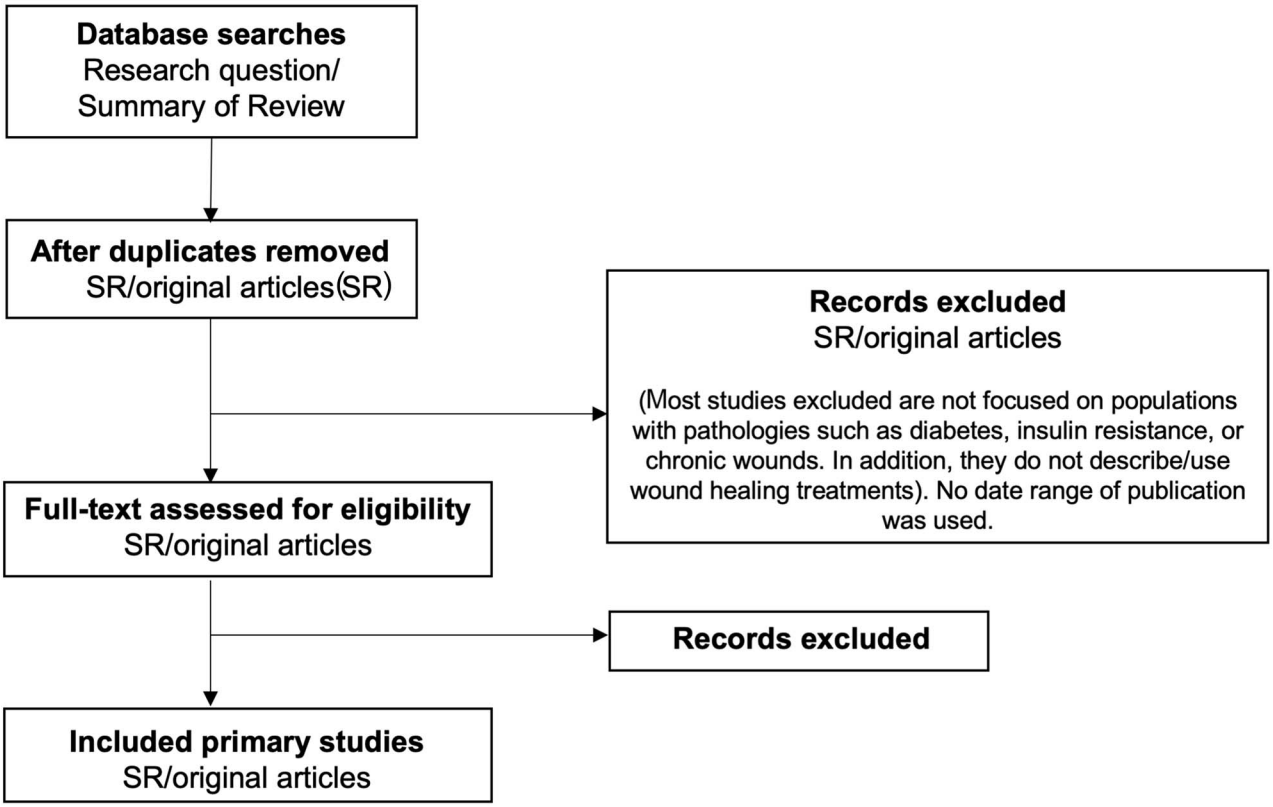

Figure 1. Flow diagram for the review process

of impact, cost and medicare policy implications of chronic nonhealing wounds concludes that they impact about 8.2 million Medicare beneficiaries in the United States [4]. In addition, often disguised as a comorbid condition, chronic wounds represent a silent epidemic that affects a large fraction of the world population [5], where the dramatic increase in the aging population will increase these numbers as wound closure is negatively associated with age [6,7]. Additionally, nowadays wound dressings have been enhanced using impregnated dressings for wound closure in animal and cell culture models [8–10]. Also, interest has grown in the use of metal nanoparticles (NPs) such as silver, gold and copper to combat infectious processes through the inhibition of protein synthesis, peroxidation of the cell membrane and destroying the nucleic acids of bacteria and viruses [11,12]. Figure 1 illustrates the flow chart for the study selection process.

Review

Physiologic wound healing

Physiologic wound healing is a highly organized process initiated by tissue injury and resolved by the restoration of tissue integrity. This process involves several overlapping phases, such as hemostasis, inflammation, proliferation and remodeling [13]. Hemostasis occurs immediately after injury to prevent exsanguination, where vasoconstriction takes place with platelet activation, adhesion and aggregation at the site of injury. Platelets become activated when exposed to extravascular collagen (such as type I collagen), which they detect via specific integrin receptors. Once in contact with collagen, platelets release soluble mediators (growth factors and cyclic adenosine monophosphate) and adhesive glycoproteins. These glycoproteins released from platelet alpha granules include fibrinogen, fibronectin, thrombospondin and von Willebrand factor. As platelet aggregation proceeds, clotting factors are released resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and the aggregated platelets become trapped in the fibrin web and provide the bulk of the clot [14]. Therefore, their matrix provides a surface on which inactive clotting enzyme proteases are bound, become activated and accelerate the clotting cascade [15].

The inflammatory phase involves activation of the innate immune system; neutrophils and monocytes are the major cells that migrate rapidly into the wound site upon injury. In this phase, the recruited neutrophils begin the phagocytosis of infectious agents by releasing a large variety of highly active antimicrobial substances like reactive oxygen species (ROS), cationic peptides, eicosanoids, proteases and myeloperoxidase [16], which also lead to the debridement of devitalized tissue by secreting enzymes such as matrix metalloproteinases (MMPs). Approximately after 3 days of injury, monocytes

differentiate into macrophages and they infiltrate into the wound site regulated by gradients of chemotactic factors, including growth factors, proinflammatory cytokines and chemokines [17]. In the normal wound healing process the inflammation phase usually lasts for 2–5 days.

As the inflammatory phase finishes angiogenesis starts, which includes endothelial cell proliferation, migration and branching to produce new blood vessels. Simultaneous with the proliferation of endothelial cells (ECs), pericytes attach to the basal membrane are activated [18], and supplies structural integrity to the ECs [19]. In addition to the local cells, circulating progenitor cells from the bone marrow are also found to support new blood vessel formation during wound healing [20–23].

The remodeling phase restores the morphology and the function of the tissue [24]. This is tightly connected with the inflammatory response and plays an important role in resolving inflammation. As the inflammation subsides, proliferation focuses on the re-epithelialization process, restoring the vascular network and forming granulation tissue. The remodeling phase starts at the end of the granulation tissue development, where mechanical tension and cytokines drive fibroblasts to differentiate into myofibroblasts, which express α-smooth muscle actin and contract the wound [25]. In this phase, the quickly produced collagen III in the extracellular matrix is replaced by collagen I, the number of new blood vessels and the blood flow decline and a mature avascular and acellular environment is formed [26,27]. Some skin components cannot be recovered after serious injury and the healed skin can only achieve a maximum of ~80% of the original tensile strength [28,29].

An inadequate repair process can cause severe damage, like the loss of skin or the beginning of an infection, with consequent injuries to the subjacent tissues and even systemic effects [30]. The most common and inevitable impairment to wound healing is the installation of an infection, as regularly occurs in the case of chronic wounds.

Epigenetic mechanisms

Epigenetics studies heritable gene expression changes, resulting in phenotype changes without modifications of the original DNA sequence [31]. Epigenetic regulatory mechanisms are fundamental to epidermic homeostasis and the pathogenesis of several skin illnesses, including skin cancer and psoriasis [31–33]. In effect, epigenetics plays an important role in the behavior and activity of different cell types during skin repair.

Wound healing is a complex process being divided into four distinct phases: hemostasis, inflammation, proliferation and remodeling [13]. These phases are not simple linear events but are overlapping and involve the transient activation and repression of up to a 1000 genes to achieve skin closure [34].

Previous studies have demonstrated that epigenetic modulators show contrasting expression patterns in intact and healing skin, where gene silencing is done by Polycomb group (PcG) proteins and involves the sequential action of two repressor complexes, PRC2 and PRC1 that function through modification of histones to change chromatin structure and modulate gene expression and cell behavior [35]. It is interesting that three major components of the PRC2 complex, Eed, Ezh2 and Suz12, are reduced in the epidermis during wound healing [36].

The PcG proteins' reexpression may be implicated in silencing the repair genes after completion of the healing process. Here, PcG protein loss is related to reduced levels of Lys27 of histone H3 (H3K27me3) in the wound healing of epidermis [36], due to trimethylation of H3K27me3 by Ezh2. Then, the PRC1 complex binds to H3K27me3 through CBX protein of to anchor the PRC1 complex at this site, while the PRC1 Ring1B protein catalyzes ubiquitylation of histone H2A at lysine K119 [37,38]. These events lead to chromatin compaction which subsequently leads to suppression of transcription. The transcription repression mechanism is not well defined, but the chromatin compaction could inhibit transcript elongation. In effect, previous findings suggest that inhibition of transcript elongation may be a crucial mechanism [39,40]. The combination of PRC2/PRC1 action results in the constant suppression of gene expression and the resulting gene silencing is linked with increased cell proliferation/survival and decreased senescence and differentiation [41,42].

Additionally, immunohistochemical expression patterns have shown a paucity of Eed and Ezh2 at the wound margin, while it was abundant further away from the wound [36]. In contrast, the expression of H3K27 histone demethylases JMJD3 and Utx was upregulated. However, levels of both Eed and Ezh2 were restored once re-epithelialization was complete, suggesting that there is a transient activation of repair genes via loss of PcG-protein-mediated silencing to permit epithelial closure, which indicates their significant involvement during skin regeneration.

Wound infections

Injuries that have not improved through the normal process of recovery and are exposed for more than 1 month are classified as chronic wounds [43]. Notwithstanding etiology, chronic wounds have high levels of ROS, proinflammatory cytokines, proteases and senescent cells, as well as the existence of stubborn infection and decreased levels of stem cells [44,45]. Although wound evaluation begins with wound appearance, morbid obesity or a very thin patient is a clue to the nutritional status that will have a bearing on treatment protocols as well as possibly on outcomes [46]. A visual examination is important for any type of chronic wound and it should considerer the depth, extent (size), location, general appearance, odor and notation of exudates since the baseline [47]. In addition, visual inspection of the wound looking for important features such as necrosis, gangrene, erythema or granular appearance will guide ulterior assessment and management [48].

While microorganisms are a common part of the intact skin microbiota and wounds, a critical onset of existing

bacteria and the formation of a biofilm may impede wound recovery [49]. Nevertheless, despite recent advances in the management of wounds, bacterial infections as *Staphylococcus aureus*, methicillin-resistant *S. aureus* and *Pseudomonas aeruginosa* are still diagnosed and considered as painful states in patients with infected wounds [50].

Due to wound have a non-sterile environment, effective treatments are still needed. Therefore, current research is looking for more efficient therapeutics for wound infections [51]. In chronic wounds, a fully dissolvable, non-replaceable or non-adherent wound dressing that distributes treatment to the wound site in a precise manner should be used to improve therapeutic and drug responses [52]. Dressings are used to remove excess fluid from the wound and protect it from infection, and they are usually left on the wound for several days. Antibiotic-embedded wound dressings can be used which are valuable in the management of infections where high concentrations of antibiotics are needed [53]. However, antibiotic-resistant bacteria have considerably increased due, among other reasons, to the overuse and misuse of antibiotics [54]. Given current problems posed by these infections, non-antibiotic treatments have been investigated, such as essential oils [55,56] and honey [27,57], in wound-healing. Nowadays, nanotechnology represents an emerging therapy for wound treatment through materials in nanometer size, displaying new applications in regenerative medicine and preventing various diseases [58].

Zhou *et al*. [59], indicated that copper sulfide NPs-incorporated hyaluronic acid hydrogel (CuS/HA) upregulated the expression of vascular endothelial growth factor (VEGF) in the wound area at the incipient stage of healing to promote angiogenesis. In addition, increased collagen deposition was observed.

The Cu ions interact with the carrier hydrogel by electrostatic or Van der Waals forces. When the volume of the hydrogel shrinks, electrostatic repulsion between ions encourages ion release and so there is evidence that shrinkage of the hydrogel increases Cu ion release [60]. Moreover, the temperature increased with near-infrared (NIR) light irradiation gradually makes the hydrogel a special carrier in drug delivery systems [59,60]. Therefore, Cu ions released from hydrogels stimulate proliferation and angiogenesis of cells to accelerate wound healing [59–62]. The NP-hydrogel is demonstrated to have the ability to kill bacteria while promoting healing of wounds. The excellent performance stems from the combined effects of hyperthermia, radical oxygen species and released copper ions produced during NIR irradiation of nanocomposite hydrogels (NP-hydrogels), where NP-hydrogel has been demonstrated to have the ability to kill bacteria while promoting healing of wounds. This excellent performance stems from the combined effects of hyperthermia, radical oxygen species and released copper ions produced during NIR irradiation of NP-hydrogels [59–62].

Likewise, hydrogels have been used on diabetic ulcer treatment, a type of chronic wound. In this process, a smart black phosphorus-based gel which serves for chronic wounds, impaired angiogenesis, persistent pain and bacterial infection, and exacerbated inflammation, suggesting the potential for significant improvements to the treatment of diabetic patients with ulcers. Superior bacterial inhibition of germanene-based hydrogel was also confirmed in antibacterial ring tests [63]. Moreover, these studies also allow the development of nanotechnology in medical applications and greatly expand research areas for hydrogel-based materials [63–66].

Nanomaterials in wound healing

The most usual preventable challenge to wound healing is an infection, where antimicrobials have been empirically used in topical form to attempt to prevent wound infection. Topical treatments are the classic procedure for wound management. This technique uses antiseptics, antibacterial and/or colloidal agents to prevent infections. However, to meet the challenges of infection, scientists are looking for new strategies of wound care. Nanotechnology, through the application of nanomaterials, has opened a new chapter in wound treatment, proposing solutions for the acceleration of healing as well as presenting distinctive properties as bactericidal agents [67,68]. Among nanomaterials, bioactive NPs have been considered for the clinic because of their low cost, high surface-to-volume ratio, high stability and safety. In effect, scientists have recently investigated several types of procedures to produce organic NPs or to synthesise inorganic NPs [68,69]. Due to their antibacterial properties and low toxicity profile, metal NPs such as copper, silver, gold and zinc represent ideal candidates for integration in wound dressings [70]. Among bioactive NPs, copper (Cu) plays a complex role in various cells, it modulates several cytokines and growth factor mechanisms of action and is essentially involved in all stages of the wound healing process [71].

Copper is an essential metal and is required in small quantities in many metabolic processes [8,72]. In fact, under controlled conditions, copper plays an important role in healing by enhancing the expression of extracellular matrix molecules such as fibrinogen, collagen formation and integrins, the main mediators of cell attachment to the extracellular matrix [8,72–74]. However, excessive use of copper is toxic, as it generates free radicals, which may lead to lipid peroxidation and cell death [75,76]. For example, in breast epithelial cells cultured with doses of 10 mg of copper in nanofiber, only 3% survived, which suggests the levels of copper released from the nanofibers are highly toxic to cells in tissue culture [77]. However, other studies have shown that copper concentrations at these levels cause no adverse reaction when applied to human skin [78].

Under physiological conditions, the level of intracellular free copper is regulated by its uptake, transport and excretion [79–81]. This uptake is primarily mediated by the CTR1 copper importer [82,83]. When copper drives into the cell via CTR1, it can be transported into various cellular compartments via the ATP7A copper transporting ATPase through the Atox1 copper chaperone [84,85].

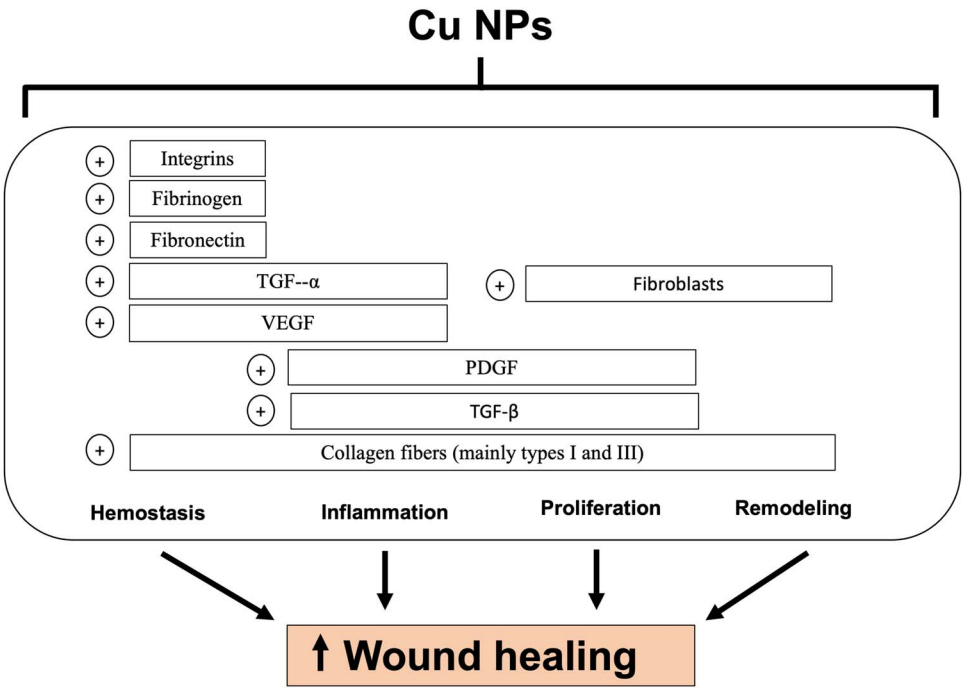

Figure 2. Copper nanoparticles action in wound healing. *NPs* nanoparticles, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor, *PDGF* platelet derived growth factor

Copper-transporting ATPases, such as ATP7A and ATP7B, maintain homeostasis and copper excretion across the intestine, liver and mammary glands [86]. However, deactivation of their transport activity is linked with reduced copper outflow from cells and, in some tissues, substantial copper excess [86,87].

The ATPase transporter ATP7A is a crucial regulator of secretory enzymes and of intracellular levels of Cu [85,88]. At basal conditions, ATP7A is localized at the trans-Golgi network (TGN), where ATP7A carries Cu to the secretory Cu enzymes, such as extracellular superoxide dismutase (SOD3) or proenzyme of lysyl oxidase (Pro-LOX), required for lysyl oxidase (LOX) activation [85,88], which stimulates tumorigenesis and metastasis [89]. In addition, ATP7A is partly implicated in VEGF- or ischemia-induced angiogenesis in ECs [80,90]. In pathological conditions, where cellular Cu is elevated, ATP7A is translocated from the TGN to the plasma membrane to export excess Cu. Also, it has been shown that relocalization of ATP7A from the TGN is triggered not just by increased cytoplasmic Cu but also by non-metal stimulants such as insulin, *N*-methyl-D-aspartic acid or *N*-methyl-D-aspartate, platelet-derived growth factor and hypoxia [84,91]. Today, the use of copper-based antimicrobial wound dressings is increasing. In effect, they are widely displacing silver-containing dressings for wound healing due to cellular silver toxicity [92]. Copper toxicity has been attributed to the Fenton-like reaction, which results in ROS formation in close spatial proximity to copper ions [93], which are responsible for both lipid and protein damage [93]. Moreover, sustained copper activity has been also observed in anoxic conditions in a ROS-independent process, which is sufficient to competitively disrupt cytoplasmic iron–sulfur enzymes (e.g. intracellular dehydratases) [65,94,95]. However, mammalian cells are partially protected by cytoplasmic metallothioneins, glutathione and Cu/Zn superoxide dismutase [96,97]. Figure 2 shows Cu sulfide NPs' (Cu NPs) action in wound healing.

All forms of copper may cause biotoxicity at high exposure concentrations [98]. Here, Cu NPs have shown cytotoxic and genotoxic effects on human skin epidermal cells, which were mediated by mitochondrial pathways triggered by ROS [99]. However, different concentrations (200, 100, 50, 20 and 10 μg/mL) of Cu NPs showed hardly any toxicity to the cells and in fact promoted the proliferation of cells [59], which could be due to the size of Cu NPs, where the colon removes most of the unabsorbed particles [62]. In effect, high levels of Cu in feces indicated that unabsorbed Cu NPs or absorbed Cu ions were predominantly eliminated through liver/feces [62]. In addition, cell viability assays have shown significant Cu NP concentration dependency, e.g. with a higher NP concentration, an increased response in growth factor stimulus to promote the proliferation of the cells has been shown [61].

In addition, copper has potent biocidal properties, but in contrast to silver, copper is well metabolized by the human body [100]. More importantly, copper plays a key role in skin regeneration and angiogenesis [101,102] and has been described to accelerate the healing process in animal models through induction of VEGF and angiogenesis [103] by hypoxia-induced factor-1-alpha (HIF-1α) action where copper enhances HIF-1α expression [8]. Also, HIF-1α binding to the critical motifs in the promoter and putative enhancer regions of HIF-1 regulated genes [104].

HIF-1 has been recognized as a critical helper factor in wound healing [105] induced by copper. Its action is important in wound healing because individuals with compromised peripheral blood supply (e.g. with vascular diseases or diabetes) do not have the ability to heal effectively due to low levels of copper in the wound site [106]. Several case reports described by Melamed *et al*. [107] have shown that copper oxide-containing wound dressings not only confer protection to the wound and the dressing from microbial contamination but in addition, and more importantly, stimulate skin regeneration and wound healing. In addition, sleeping on a copper oxide-impregnated pillowcase has resulted in a significant reduction of wrinkles and crow's-feet and resulted in an overall improved facial appearance compared to sleeping on a normal pillowcase [108,109].

MMPs and serine proteases are the major groups of proteases involved in the wound healing process [110,111]. Low copper concentrations (0.3–3 μM) have been found to stimulate the activity of MMPs, whereas high concentrations (1–100 μM) stimulate the expression of MMPs in fibroblasts [112]. Other studies have reported that both MMP2 and MMP3 can be upregulated by copper, although excess free metal can also inhibit MMP activity [112,113]. In effect, copper ions could stimulate angiogenesis by secretion of VEGF and thus promote wound healing. Nano-formed copper such as CuS NPs may also be capable of photothermal therapy induced by NIR light irradiation which would be effective in killing bacteria in a non-resistant and minimally invasive process [114,115]. As a result, CuS NPs may offer both angiogenesis and antibacterial ability, both of which are beneficial to accelerate wound healing [95,116,117]. Moreover, NPs with a concentration of 200 μg/ml could significantly promote cell proliferation in *in vitro* and *in vivo* models [59].

Limitations

The goal of this study was to identify the importance of copper NPs in dressings for wound healing. However, the lack of data linking wound infection and wound healing with Cu NPs remains a limitation of this study and needs to be addressed in future original studies. Unfortunately, the results provide little support for this notion. At best, we could only discern a trend towards enhanced skin regeneration and angiogenesis in most of the studies, with significant improvement in those who had copper NP exposure. However considering these data, it appears that copper exposure did significantly improve VEGF and HIF-1α expression, and HIF-1 binding to the critical motifs in the promoter and putative enhancer regions of HIF-1 regulated genes.

Conclusions

Copper is an essential mineral that plays a significant role in various physiological and metabolic processes, including angiogenesis, skin generation and expression and stabilization of extracellular skin proteins; and it also has potent antimicrobial properties. The combination of these two qualities makes copper an attractive material for the improvement of skin wellness. In addition, it has been demonstrated that pillowcases containing copper oxide reduce fine lines and wrinkles. Our review suggests that wound dressings containing copper oxide enhance wound healing through their angiogenesis, regeneration and antimicrobial properties. Thus, the introduction of copper oxide into regular products transforms them into enhanced products.

Abbreviations

ECs: Endothelial cells; MMP: Matrix metalloproteinases; NP: Nanoparticles; PcG: Polycomb group; ROS: Reactive oxygen species; VEGF: Vascular endothelial growth factor; HIF: Hypoxia-induced factor.

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Authors' contributions

JS and CS wrote the paper and edited the paper. CS supervised the project.

Conflicts of interests

None declared.

References

1. Lazarus GS, Cooper DM, Knighton DR, Margolis DJ, Pecoraro RE, Rodeheaver G, *et al*. Definitions and guidelines for assessment of wounds and evaluation of healing. *Wound Repair Regen*. 1994;2:165–70.
2. Werdin F, Tennenhaus M, Schaller HE, Rennekampff HO. Evidence-based management strategies for treatment of chronic wounds. *Eplasty*. 2009;9:e19.
3. Kirsner RS. The wound healing society chronic wound ulcer healing guidelines update of the 2006 guidelines–blending old with new. *Wound Repair Regen*. 2016;24:110–1.
4. Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, Nusgart M, *et al*. An economic evaluation of the impact, cost, and Medicare policy implications of chronic nonhealing wounds. *Value Health*. 2018;21:27–32.
5. Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, *et al*. Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen*. 2009;17:763–71.
6. Wicke C, Bachinger A, Coerper S, Beckert S, Witte MB, Königsrainer A. Aging influences wound healing in patients with chronic lower extremity wounds treated in a specialized wound care Center. *Wound Repair Regen*. 2009;17:25–33.
7. Järbrink K, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, *et al*. Prevalence and incidence of chronic wounds

and related complications: a protocol for a systematic review. *Syst Rev*. 2016;5:152.
8. Borkow G, Gabbay J, Dardik R, Eidelman AI, Lavie Y, Grunfeld Y, *et al*. Molecular mechanisms of enhanced wound healing by copper oxide-impregnated dressings. *Wound Repair Regen*. 2010;18:266–75.
9. Abdollahi Z, Zare EN, Salimi F, Goudarzi I, Tay FR, Makvandi P. Bioactive Carboxymethyl starch-based hydrogels decorated with CuO nanoparticles: antioxidant and antimicrobial properties and accelerated wound healing in vivo. *Int J Mol Sci*. 2021;22:2531.
10. Ghasemian Lemraski E, Jahangirian H, Dashti M, Khajehali E, Sharafinia S, Rafiee-Moghaddam R, *et al*. Antimicrobial double-layer wound dressing based on chitosan/polyvinyl alcohol/copper: in vitro and in vivo assessment. *Int J Nanomedicine*. 2021;16:223–35.
11. Faúndez G, Troncoso M, Navarrete P, Figueroa G. Antimicrobial activity of copper surfaces against suspensions of salmonella enterica and campylobacter jejuni. *BMC Microbiol*. 2004;4:19.
12. Perelshtein I, Applerot G, Perkas N, Wehrschuetz-Sigl E, Hasmann A, Guebitz G, *et al*. CuO–cotton nanocomposite: formation, morphology, and antibacterial activity. *Surf Coat Technol*. 2009;204:54–7.
13. Mathieu D, Linke JC, Wattel F. Non-healing wounds. In: Mathieu D (ed). *Handbook on hyperbaric medicine*. Netherlands: Springer, 2006, 401–28.
14. Gailit J, Clark RAF. Wound repair in context of extracellular matrix. *Curr Opin Cell Biol*. 1994;6:717–25.
15. Schultz GS, Chin GA, Moldawer L, Diegelmann RF. Principles of Wound Healing. In: Fitridge R, Thompson M (eds). *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists*. Australia: University of Adelaide Press, 2011.
16. Landen NX, Dongqing L, Stahle M. Transition from inflammation to proliferation: a critical step during wound healing. *Cell Mol Life Sci*. 2016;73:3861–85.
17. Serra MB, Barroso WA, da Silva NN, Silva S, Borges A, Abreu IC, *et al*. From inflammation to current and alternative therapies involved in wound healing. *Int J Inflamm*. 2017;2017:3406215.
18. Ansell DM, Izeta A. Pericytes in wound healing: friend or foe? *Exp Dermatol*. 2015;24:833–4.
19. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell*. 2011;21:193–215.
20. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, *et al*. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275:964–7.
21. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, *et al*. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med*. 2004;10:858–64.
22. Kosaraju R, Rennert RC, Maan ZN, Duscher D, Barrera J, Whittam AJ, *et al*. Adipose-derived stem cell-seeded hydrogels increase endogenous progenitor cell recruitment and neovascularization in wounds. *Tissue Eng Part A*. 2016;22:295–305.
23. Tepper OM, Capla JM, Galiano RD, Ceradini DJ, Callaghan MJ, Kleinman M, *et al*. Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. *Blood*. 2005;105:1068–77.
24. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. *Clin Dermatol*. 2007;25:9–18.
25. Hinz B. Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol*. 2007;127:526–37.
26. Greenhalgh DG. The role of apoptosis in wound healing. *Int J Biochem Cell Biol*. 1998;30:1019–30.
27. Schencke C, Vasconcellos A, Sandoval C, Torres P, Acevedo F, del Sol M. Morphometric evaluation of wound healing in burns treated with Ulmo (*Eucryphia cordifolia*) honey alone and supplemented with ascorbic acid in Guinea pig (*Cavia porcellus*). *Burns Trauma*. 2016;4:25.
28. Schencke C, Vásquez B, Sandoval C, del Sol M. El Rol de la Miel en los Procesos Morfofisiológicos de Reparación de Heridas. *Int J Morphol*. 2016;34:385–95.
29. Schilling JA. Wound healing. *Surg Clin North Am*. 1976;56:859–74.
30. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. Skin wound healing: an update on the current knowledge and concepts. *Eur Surg Res*. 2017;58:81–94.
31. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. *Genes Dev*. 2009;23:781–3.
32. Chen M, Chen ZQ, Cui PG, Yao X, Li YM, Li AS, *et al*. The methylation pattern of p16INK4a gene promoter in psoriatic epidermis and its clinical significance. *Br J Dermatol*. 2008;158:987–93.
33. Botchkarev VA, Gdula MR, Mardaryev AN, Sharov AA, Fessing MY. Epigenetic regulation of gene expression in keratinocytes. *J Invest Dermatol*. 2012;132:2505–21.
34. Cooper L, Johnson C, Burslem F, Martin P. Wound healing and inflammation genes revealed by array analysis of 'macrophageless' PU.1 null mice. *Genome Biol*. 2005;6:R5.
35. Eckert RL, Adhikary G, Rorke EA, Chew YC, Balasubramanian S. Polycomb group proteins are key regulators of keratinocyte function. *J Invest Dermatol*. 2011;131:295–301.
36. Shaw T, Martin P. Epigenetic reprogramming during wound healing: loss of polycomb-mediated silencing may enable upregulation of repair genes. *EMBO Rep*. 2009;10:881–6.
37. Fischle W, Wang Y, Jacobs SA, Kim Y, Allis CD, Khorasanizadeh S. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev*. 2003;17:1870–81.
38. Cao R, Tsukada Y, Zhang Y. Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. *Mol Cell*. 2005;20:845–54.
39. Stock JK, Giadrossi S, Casanova M, Brookes E, Vidal M, Koseki H, *et al*. Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. *Nat Cell Biol*. 2007;9:1428–35.
40. Zhou W, Zhu P, Wang J, Pascual G, Ohgi KA, Lozach J, *et al*. Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation. *Mol Cell*. 2008;29:69–80.
41. Jacobs JJ, van Lohuizen M. Polycomb repression: from cellular memory to cellular proliferation and cancer. *Biochim Biophys Acta*. 2002;1602:151–61.
42. Orlando V. Polycomb, epigenomes, and control of cell identity. *Cell*. 2003;112:599–606.
43. Neligan PC. Wound Healing. In: Sen CK, Roy S, Gordillo G (eds). *Plastic Surgery: Volume One*. Netherlands: Elsevier, 2017.

44. Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. *Adv Wound Care (New Rochelle)*. 2015;4:560–82.
45. Wang Z, Shi C. Cellular senescence is a promising target for chronic wounds: a comprehensive review. *Burns Trauma*. 2020;8:tkaa021.
46. Pierpont YN, Dinh TP, Salas RE, Johnson EL, Wright TG, Robson MC, *et al*. Obesity and surgical wound healing: a current review. *ISRN Obes*. 2014;2014:638936.
47. Frykberg RG, Zgonis T, Armstrong DG, Driver VR, Giurini JM, Kravitz SR, *et al*. Diabetic foot disorders. A clinical practice guideline (2006 revision). *J Foot Ankle Surg*. 2006;45:S1–66.
48. Grey JE, Enoch S, Harding KG. Wound assessment. *BMJ*. 2006;332:285–8.
49. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev*. 2001;14:244–69.
50. Cardona AF, Wilson SE. Skin and soft-tissue infections: a critical review and the role of telavancin in their treatment. *Clin Infect Dis*. 2015;61:S69–78.
51. Vyas KS, Vasconez HC. Wound healing: biologics, skin substitutes, biomembranes and scaffolds. *Healthcare (Basel)*. 2014;2:356–400.
52. Kopecki Z, Cowin AJ. Fighting chronic wound infection-one model at a time. *Wound Pract Res J Aust Wound Manag Assoc*. 2017;25:6–13.
53. Ramasubbu DA, Smith V, Hayden F, Cronin P. Systemic antibiotics for treating malignant wounds. *Cochrane Database Syst Rev*. 2017;8:CD011609.
54. Das P, Horton R. Antibiotics: achieving the balance between access and excess. *Lancet*. 2016;387:102–4.
55. Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally M. Chemical composition, antimicrobial and antibiotic potentiating activity of essential oils from 10 tropical medicinal plants from Mauritius. *J Herb Med*. 2016;6:88–95.
56. Aumeeruddy-Elalfi Z, Mahomoodally M. Extraction techniques and pharmacological potential of essential oils from medicinal and aromatic plants of Mauritius. In: Peters M (ed). *Essential Oils: Historical Significance, Chemical Composition and Medicinal Uses and Benefits*. United States of America: Nova Science Publishers, 2016.
57. Schencke C, Sandoval C, Vásquez B, del Sol M. Quantitative analysis of dermal scars in deep skin burns treated with Ulmo honey supplemented with ascorbic acid. *Int J Clin Exp Med*. 2018;11:2422–9.
58. Zarrintaj P, Moghaddam AS, Manouchehri S, Atoufi Z, Amiri A, Amirkhani MA, *et al*. Can regenerative medicine and nanotechnology combine to heal wounds? The search for the ideal wound dressing. *Nanomedicine (Lond)*. 2017;12:2403–22.
59. Zhou W, Zi L, Cen Y, You C, Tian M. Copper Sulfide nanoparticles-incorporated hyaluronic acid injectable hydrogel with enhanced angiogenesis to promote wound healing. *Front Bioeng Biotechnol*. 2020;8:417.
60. Li M, Liu X, Tan L, Cui Z, Yang X, Li Z, *et al*. Noninvasive rapid bacteria-killing and acceleration of wound healing through photothermal/photodynamic/copper ion synergistic action of a hybrid hydrogel. *Biomater Sci*. 2018;6:2110–21.
61. Gopal A, Kant V, Gopalakrishnan A, Tandan SK, Kumar D. Chitosan-based copper nanocomposite accelerates healing in excision wound model in rats. *Eur J Pharmacol*. 2014;731:8–19.
62. Lee IC, Ko JW, Park SH, Lim JO, Shin IS, Moon C, *et al*. Comparative toxicity and biodistribution of copper nanoparticles and cupric ions in rats. *Int J Nanomedicine*. 2016;11:2883–900.
63. Feng C, Ouyang J, Tang Z, Kong N, Liu Y, Fu L, *et al*. Germanene-based Theranostic materials for surgical adjuvant treatment: inhibiting tumor recurrence and wound infection. *Matter*. 2020;3:127–44.
64. Ouyang J, Ji X, Zhang X, Feng C, Tang Z, Kong N, *et al*. In situ sprayed NIR-responsive, analgesic black phosphorus-based gel for diabetic ulcer treatment. *Proc Natl Acad Sci U S A*. 2020;117:28667–77.
65. Liu Y, Wang J, Xiong Q, Hornburg D, Tao W, Farokhzad OC. Nano–bio interactions in cancer: from therapeutics delivery to early detection. *Acc Chem Res*. 2021;54:291–301.
66. Liu T, Xiao B, Xiang F, Tan J, Chen Z, Zhang X, *et al*. Ultrasmall copper-based nanoparticles for reactive oxygen species scavenging and alleviation of inflammation related diseases. *Nat Commun*. 2020;11:2788.
67. Kalashnikova I, Das S, Seal S. Nanomaterials for wound healing: scope and advancement. *Nanomedicine*. 2015;10:2593–612.
68. Barroso A, Mestre H, Ascenso A, Simões S, Reis C. Nanomaterials in wound healing: from material sciences to wound healing applications. *Nano Select*. 2020;1:443–60.
69. Wang X, Chang J, Wu C. Bioactive inorganic/organic nanocomposites for wound healing. *Appl Mater Today*. 2018;11:308–19.
70. Negut I, Grumezescu V, Grumezescu AM. Treatment strategies for infected wounds. *Molecules*. 2018;23:2392.
71. Kornblatt AP, Nicoletti VG, Travaglia A. The neglected role of copper ions in wound healing. *J Inorg Biochem*. 2016;161:1e8.
72. Uauy R, Olivares M, Gonzalez M. Essentiality of copper in humans. *Am J Clin Nutr*. 1998;67:952S–9.
73. Tenaud I, Sainte-Marie I, Jumbou O, Litoux P, Dreno B. In vitro modulation of keratinocyte wound healing integrins by zinc, copper and manganese. *Brit J Dermatol*. 1999;140:26–34.
74. Sen CK, Khanna S, Venojarvi M, Trikha P, Ellison EC, Hunt TK, *et al*. Copper-induced vascular endothelial growth factor expression and wound healing. *Am J Physiol Heart Circ Physiol*. 2002;282:H1821–7.
75. Quaranta D, Krans T, Espírito Santo C, Elowsky CG, Domaille DW, Chang CJ, *et al*. Mechanisms of contact-mediated killing of yeast cells on dry metallic copper surfaces. *Appl Environ Microbiol*. 2011;77:416–26.
76. Palza H. Antimicrobial polymers with metal nanoparticles. *Int J Mol Sci*. 2015;16:2099–116.
77. Ahire JJ, Hattingh M, Neveling DP, Dicks LM. Copper-containing anti-biofilm nanofiber scaffolds as a wound dressing material. *PLoS One*. 2016;11:e0152755.
78. Hostynek JJ, Maibach HI. Copper hypersensitivity: dermatologic aspects-an overview. *Rev Environ Health*. 2003;18:153–83.
79. Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, O'Halloran TV. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science*. 1999;284:805–8.
80. Kim HW, Lin A, Guldberg RE, Ushio-Fukai M, Fukai T. Essential role of extracellular SOD in reparative neovascularization induced by hindlimb ischemia. *Circ Res*. 2007;101:409–19.

81. Schlief ML, Gitlin JD. Copper homeostasis in the CNS: a novel link between the NMDA receptor and copper homeostasis in the hippocampus. *Mol Neurobiol*. 2006;33:81–90.
82. Kim BE, Nevitt T, Thiele DJ. Mechanisms for copper acquisition, distribution and regulation. *Nat Chem Biol*. 2008;4:176–85.
83. Kaplan JH, Lutsenko S. Copper transport in mammalian cells: special care for a metal with special needs. *J Biol Chem*. 2009;284:25461–5.
84. La Fontaine S, Mercer JF. Trafficking of the copper-ATPases, ATP7A and ATP7B: role in copper homeostasis. *Arch Biochem Biophys*. 2007;463:149–67.
85. Lutsenko S, Barnes NL, Bartee MY, Dmitriev OY. Function and regulation of human copper-transporting ATPases. *Physiol Rev*. 2007;87:1011–46.
86. Gupta A, Lutsenko S. Human copper transporters: mechanism, role in human diseases and therapeutic potential. *Future Med Chem*. 2009;1:1125–42.
87. Wee NK, Weinstein DC, Fraser ST, Assinder SJ. The mammalian copper transporters CTR1 and CTR2 and their roles in development and disease. *Int J Biochem Cell Biol*. 2013;45:960–3.
88. Fukai T, Ushio-Fukai M, Kaplan JH. Copper transporters and copper chaperones: roles in cardiovascular physiology and disease. *Am J Physiol Cell Physiol*. 2018;315:C186–201.
89. Shanbhag V, Jasmer-McDonald K, Zhu S, Martin AL, Gudekar N, Khan A, *et al*. ATP7A delivers copper to the lysyl oxidase family of enzymes and promotes tumorigenesis and metastasis. *Proc Natl Acad Sci U S A*. 2019;116:6836–41.
90. Chen GF, Sudhahar V, Youn SW, Das A, Cho J, Kamiya T, *et al*. Copper transport protein antioxidant-1 promotes inflammatory neovascularization via chaperone and transcription factor function. *Sci Rep*. 2015;5:14780.
91. Ash D, Sudhahar V, Youn SW, Okur MN, Das A, O'Bryan JP, *et al*. The P-type ATPase transporter ATP7A promotes angiogenesis by limiting autophagic degradation of VEGFR2. *Nat Commun*. 2021;12:3091.
92. Chambers H, Dumville JC, Cullum N. Silver treatments for leg ulcers: a systematic review. *Wound Repair Regen*. 2007;15:165–73.
93. Grass G, Rensing C, Solioz M. Metallic copper as an antimicrobial surface. *Appl Environ Microbiol*. 2011;77:1541–7.
94. Macomber L, Imlay JA. The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. *Proc Natl Acad Sci U S A*. 2009;106:8344–9.
95. Peng Y, He D, Ge X, Lu Y, Chai Y, Zhang Y, *et al*. Construction of heparin-based hydrogel incorporated with Cu5.4O ultrasmall nanozymes for wound healing and inflammation inhibition. *Bioact Mater*. 2021;6:3109–24.
96. Hatori Y, Clasen S, Hasan NM, Barry AN, Lutsenko S. Functional partnership of the copper export machinery and glutathione balance in human cells. *J Biol Chem*. 2012;287:26678–87.
97. Babula P, Masarik M, Adam V, Eckschlager T, Stiborova M, Trnkova L, *et al*. Mammalian metallothioneins: properties and functions. *Metallomics*. 2012;4:739–50.
98. Ameh T, Sayes CM. The potential exposure and hazards of copper nanoparticles: a review. *Environ Toxicol Pharmacol*. 2019;71:103220.
99. Alarifi S, Ali D, Verma A, Alakhtani S, Ali BA. Cytotoxicity and genotoxicity of copper oxide nanoparticles in human skin keratinocytes cells. *Int J Toxicol*. 2016;32:296–307.
100. Borkow G, Gabbay J. Copper as a biocidal tool. *Curr Med Chem*. 2005;12:2163–75.
101. Alizadeh S, Seyedalipour B, Shafieyan S, Kheime A, Mohammadi P, Aghdami N. Copper nanoparticles promote rapid wound healing in acute full thickness defect via acceleration of skin cell migration, proliferation, and neovascularization. *Biochem Biophys Res Commun*. 2019;517:684–90.
102. Chen M, Li R, Yin W, Wang T, Kang YJ. Copper promotes migration of adipose-derived stem cells by enhancing vimentin-Ser39 phosphorylation. *Exp Cell Res*. 2020;388:111859.
103. Das A, Sudhahar V, Chen GF, Kim HW, Youn SW, Finney L, *et al*. Endothelial Antioxidant-1: a key mediator of copper-dependent wound healing in vivo. *Sci Rep*. 2016;6:33783.
104. Wu Z, Zhang W, Kang YJ. Copper affects the binding of HIF-1alpha to the critical motifs of its target genes. *Metallomics*. 2019;11:429–38.
105. Ruthenborg RJ, Ban JJ, Wazir A, Takeda N, Kim JW. Regulation of wound healing and fibrosis by hypoxia and hypoxia-inducible factor-1. *Mol Cells*. 2014;37:637–43.
106. Borkow G, Gabbay J, Zatcoff RC. Could chronic wounds not heal due to too low local copper levels? *Med Hypotheses*. 2008;70:610–3.
107. Melamed E, Kiambi P, Okoth D, Honigber I, Tamir E, Borkow G. Healing of chronic wounds by copper oxide-impregnated wound dressings-case series. *Medicina (Kaunas)*. 2021;57:296.
108. Baek JH, Yoo MA, Koh JS, Borkow G. Reduction of facial wrinkles depth by sleeping on copper oxide-containing pillowcases: a double blind, placebo controlled, parallel, randomized clinical study. *J Cosmet Dermatol*. 2012;11:193–200.
109. Dykes P. Increase in skin surface elasticity in normal volunteer subjects following the use of copper oxide impregnated socks. *Skin Res Technol*. 2015;21:272–7.
110. Visse R, Nagase H. Matrix Metalloproteinases and tissue inhibitors of Metalloproteinases. *Circ Res*. 2003;92:827–39.
111. Michopoulou A, Rousselle P. How do epidermal matrix metalloproteinases support re-epithelialization during skin healing? *Eur J Dermatol*. 2015;25:33–42.
112. Philips N, Hwang H, Chauhan S, Leonardi D, Gonzalez S. Stimulation of cell proliferation and expression of matrixmetalloproteinase-1 and interluekin-8 genes in dermal fibroblasts by copper. *Connect Tissue Res*. 2010;51:224–9.
113. Adamson IY, Vincent R, Bakowska J. Differential production of metalloproteinases after instilling various urban air particle samples to rat lung. *Exp Lung Res*. 2003;29:375–88.
114. Zhou M, Li J, Liang S, Sood AK, Liang D, Li C. CuS nanodots with ultrahigh efficient renal clearance for positron emission tomography imaging and image-guided photothermal therapy. *ACS Nano*. 2015;9:7085–96.
115. Feng X, Xu W, Li Z, Song W, Ding J, Chen X. Immunomodulatory nanosystems. *Adv Sci*. 2019;6:1900101.
116. Li Z, Zhou F, Li Z, Lin S, Chen L. Hydrogel cross-linked with dynamic covalent bonding and micellization for promoting burn wound healing. *ACS Appl Mater Interfaces*. 2018;10:25194–202.
117. Shanmugapriya K, Kang HW. Engineering pharmaceutical nanocarriers for photodynamic therapy on wound healing: review. *Mater Sci Eng C*. 2019;105:110110.