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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

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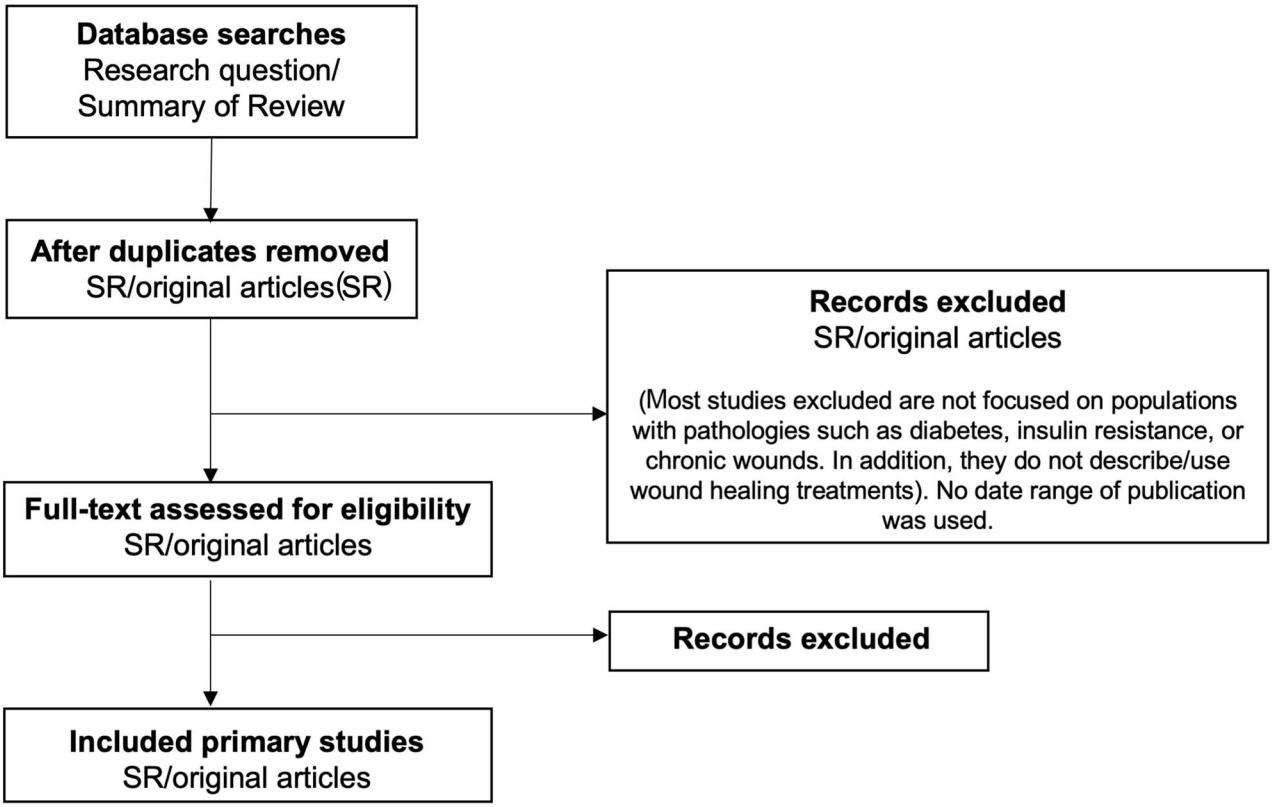


Figure 1. Flow diagram for the review process

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

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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

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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].

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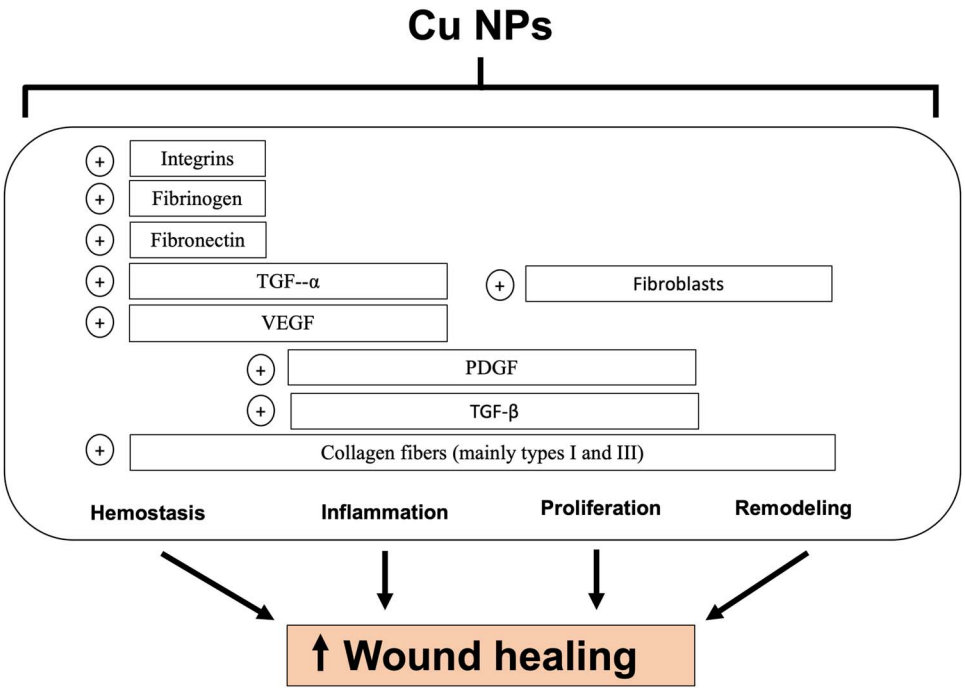


Figure 2. Copper nanoparticles action in wound healing. *NPs* nanoparticles, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor, *PDGF* platelet derived growth factor

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].

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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.

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