Copper nanoparticles in wound healing: a review

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Abstract
Wound healing is a complex and dynamic process that occurs in the human body which has four precise and highly programmed stages: haemostasis, inflammation, proliferation and remodelling. Many factors can interfere with this one or more phases, thus causing impaired wound healing. This article reviews the recent studies on the copper nanoparticles that aid in wound healing process and also the potential biomedical applications involved. The biomedical potential of copper nanoparticles discussed include antioxidant, anti-inflammatory, anticancer, neuroprotective activities. A better understanding of the potent effects of copper nanoparticles on wound repair may lead to therapeutics that leads to rapid wound healing process with low dose, side-effects than conventional wound treatments

Keywords: Wound healing, copper nanoparticles, impaired wounds, biomedical applications.

Introduction

Nanotechnology is a relatively new field of study that deals with nanoparticles that play critical roles in research and application. Nanotechnology is cumulative knowledge from disciplines such as chemistry, biology, physics, engineering, materials science, and health sciences [1]. Recent years have seen a surge in interest in the incorporation of functionalized and modified nanostructures in a variety of biomedical applications. Nanotechnology has a broad range of applications in medicine and biomedical engineering, including implant and tissue engineering, diagnosis, and therapy. The present situation necessitates the development of nanotools capable of responding to biological challenges and facilitating the development of more efficient biomedical techniques [2].

Quantum effects become apparent when matter is reduced to tens of nano meters or less, and these can drastically alter a material's optical, magnetic, or electrical properties [3]. Size-dependent features have been exploited for centuries in some circumstances. Since the tenth century, for example, gold and silver nanoparticles have been utilized as colourful pigments in stained glass and ceramics [4]. Gold particles might seem red, blue, or gold in colour depending on their size. The ancient chemists' task was to make all nanoparticles the same size (and thus the same hue), and producing nanoparticles with a single size is still a challenge today [5, 6].

Copper nanoparticles and its different applications

Copper (Cu) and copper-based nanoparticles have gained considerable interest in recent years due to their earth-abundant and affordable copper metal. Cu is a 3d transition metal with intriguing physico-chemical characteristics. Cu has a wide spectrum of oxidation states (Cu^0, Cu^I, Cu^{II}, and Cu^{III}) that allow reactivity via one- and two-electron routes. Cu-based Nano catalysts have various uses in nanotechnology, including catalytic organic transformations, electrocatalysis, and photocatalysis [7].

For thousands of years, copper has been a crucial metal in society due to its excellent electrical conductivity, thermal conductivity, corrosion resistance, ductility and malleability, and moderate tensile strength. These qualities have made copper the preferred metal for most car radiators and air conditioners [8]. A high surface-to-volume ratio makes Cu NPs extremely reactive and easy to interact with other particles, increasing antibacterial efficiency [9,10].

Copper is one of the most extensively used metals, yet its high oxidation tendency makes nanoscale production difficult. The oxide phases of copper are more thermodynamically stable than gold or silver [11]. Many businesses, including electronics, do not want copper oxide on the surface of nanoparticles because copper is a cheaper alternative to existing pricey metals. Copper nanoparticles (Cu NPs) lose electrical conductivity when exposed to oxide phases [12, 13]. Rarely found in the literature are methods that create pure Cu NPs unless done in an inert atmosphere [14].

Nanoparticles and its applications in various biomedical applications

Antimicrobial activity

Das et al ., 2020 synthesized *M. oleifera* leaf extract mediated copper nanoparticles had significant antioxidant activity and also antibacterial action against *E. coli, Klebsiella pneumoniae, Staphylococcus aureus, and Enterococcus faecalis* (MIC values for extract: 500, 250, and 250 g/mL, respectively). Toxic activity against *Aspergillus niger*, *Aspergillus flavus*,*Candida albicans*, and *Candida glabrata* is comparable between *M. oleifera* leaf extract and copper nanoparticles (MIC values for extract: 62.5, 125, and 250 g/mL; for copper nanoparticles: 125, 125, and 31.2 g/mL). The green production of copper nanoparticles employing *M. oleifera* leaf hydroalcoholic extract was successful and it can also be used to treat microbial diseases due to their antioxidant, antibacterial, and antifungal properties [15].

Amin et al., 2021 synthesized copper nanoparticles using *Aervajavanica* leaf extract and examined its antibacterial efficacy against *Escherichia coli*, *P. aeruginosa*, *Staphylococcus aureus*, *A. baumannii*and antifungal efficacy against *C. albicans, C. krusei,* and *C. tropicalis* at different concentrations. The antimicrobial activity was compared to Norfloxacin and amphotericin B. Copper oxide nanoparticles had MIC and MBC of 128 µg/mL against all tested bacterial pathogens. CuO-NPs MFC and MIC of fungus were 160 µg/mL.CuO-NPs can thus be used as a broad-spectrum antibacterial agent. It was concluded that CuO-NPs were safe below 60 µg/mL based on their cytotoxic activities against Neuro2A cells [16].

Neuroprotective agent:

Zhang et al., 2020 supported the use of *Crocus sativus* leaf extract mediated copper nanoparticles as neuroprotective supplements against methadone-induced cell death in PC12. The study indicated that copper nanoparticles were manufactured in the perfect way feasible using FE-SEM, FT-IR, XRD, EDX, TEM, and UV–Vis spectroscopy. They were found to be 27.5 nm in size and round in form in TEM and FE-SEM pictures. The Rat inflammatory cytokine assay kit was used to quantify inflammatory cytokine concentrations in the present study. An assay kit for caspase-3 activity was used, as was Rhodamine123 fluorescent dye for mitochondrial membrane potential. Trypan blue test was used to assess PC12 cell viability. Copper nanoparticles reduced inflammatory cytokine levels, caspase-3 activity, and DNA fragmentation while increasing cell survival and mitochondrial membrane potential in methadone-treated PC12 cells (p 0.01). The highest dose of copper nanoparticles (4µ g) showed the best neuroprotective benefits. Following the aforesaid findings, copper nanoparticles containing *Crocus sativus* leaf aqueous extract can be employed in peripheral and central nervous system treatments as neuroprotective promoters [17].

Anticancer agent:

The elevation of endoplasmic reticulum -stress-related mRNA and protein expression was recently revealed to cause commercial CuO-NP-induced apoptosis [18]. In the HCT-116 colon cancer cell line, lipidomic was used to detect changes in lipid profile after exposure to commercial CuO NP [19]. This therapy stimulates autophagy but not apoptosis, which may contribute to cell death. In fact, inhibiting autophagy, either pharmacologically or genetically, successfully induces death in CuO-NP-treated cells [20].

High intracellular ROS levels can cause DNA damage in cells. DNA breakage causes oxidative stress in cancer cells generated by *A. indica* leaf CuO NPs [21]. However, CuO NPs from *M.chamomilla* flower extract react immediately with the plasmid, implicated in DNA breakage [22]. The overexpression of tumour suppressor genes (p53 and p21) causes cell cycle arrest and apoptosis [23].

Recently, shahriary et al., 2022 investigated the anticancer effects of copper nanoparticles mediated by *Artemisia deserti*aqueous leaf extract on A2780-CP cisplatin-resistant ovarian cancer cells. MTT, Annexin/PI staining, qRT-PCR, cell cycle analysis, and measurement of produced cellular ROS were used to determine apoptosis in A2780-CP cells. Additionally, the results indicated that the biosynthesized CuO NPs were cytotoxic to A2780-CP cells but negligible to human foreskin fibroblasts (HFF). These two features make biogenic synthesis copper oxide nanoparticles an attractive candidate for cancer treatment investigations [24].

Antioxidant activity

Recently, Liu et al. evaluated the antioxidant capacity of copper nanoparticles using an aqueous extract of *Cinnamomum zelanicum*. Butylated hydroxytoluene (BHT) was employed as a positive control in the DPPH test. DPPH was used to assess the antioxidant potential of *Cinnamomum zelanicum* aqueous extract, CuNPs, and butylated hydroxytoluene. The IC50 values were 429, 227, and 209 µg/mL for *C. zeylanicum* leaf aqueous extract, BHT, and copper nanoparticles, respectively [25]. Green or biosynthesized nanoparticles may possess antioxidant properties due to metabolites [26–29]. Numerous studies discovered that phenolic and flavonoid nanoparticles possessed antioxidant properties.

Wu et al., 2020 synthesized *Cissus vitiginea* mediated copper nanoparticles and tested its antioxidant activity by performing DPPH assay. Antioxidants naturally safeguard humans against a variety of degenerative diseases [30]. The antioxidant activity of green synthesized copper nanoparticles was compared to that of ascorbic acid. The percentage of green synthesis mediated copper nanoparticles inhibited varied. The change in colour from violet to yellow indicates that hydrogen atom donation decreased DPPH. Copper nanoparticles and plant extract both inhibited at a maximum of 21% and 19%, respectively. The leaf extract of *C. vitiginea* contains polyphenols, flavonoids, and nanoparticles. These phytochemicals may provide a novel strategy for enhancing traditional therapy and curing a variety of incurable diseases [31].

Anti-inflammatory activity

Green synthesis of nanoparticles involves the usage of a variety of organisms such as bacteria, fungi, microalgae, seaweeds and diverse types of plants. The copper nanoparticles synthesized using plant extracts are found to be more safe and effective than chemical and mechanical methods. AI-Jubouri et al (2022) reported the anti-inflammatory activity of copper nanoparticles which was synthesized using Myrtus communis leaves extract. The author studied the anti-inflammatory activity of synthesized copper nanoparticles in in vitro methods such as albumin denaturation assay, Membrane stabilization and Proteinase inhibitory activity. The in vitro anti-inflammatory activity results showed significant effect of the green synthesized copper nanoparticles which was equal to that of standard drug. The copper nanoparticles effectively inhibit the neutrophil lysosomes which can cause additional tissue inflammation and damage [32].

Recently, Rajeshkumar et al (2022) reported the anti-inflammatory activity of *Aeromonas hydrophila* mediated copper nanoparticles. The anti-inflammatory activity of biosynthesized copper nanoparticles was tested using an in vitro membrane stabilization method. The copper nanoparticles were found to exhibit 93.71% more anti-inflammatory activity than the standard drug [33].

Faisal et al (2022) synthesized copper nanoparticles using Bacopa monnieri leaf extract and the UV-Visible spectroscopy results showed its maximum absorption peak at 535 nm which symbolized the existence of monoclinic-shaped nanoparticles. The SEM and TEM results revealed the size of copper nanoparticles to be 34.4 nm. The rat paw edema assay was performed in this study to evaluate the anti-inflammatory activity of biosynthesized copper nanoparticles. The treatment group was treated with copper nanoparticles up to 48 h and compared to that of standard diclofenac sodium (100 mg/ kg). The in vivo anti-inflammatory results revealed that copper nanoparticles inhibited edema up to 74 % whereas standard inhibited only 24%. Treating edema with 21 days with copper nanoparticles exhibited 82 % inhibition and standard up to 73 %. From the above mentioned research works, we can easily conclude that biosynthesized copper nanoparticles will act as a potential anti-inflammatory agent in both in vivo and in vitro basis [34].

Wound healing activity of various nanoparticles

SILVER NANOPARTICLES

Infectious wounds pose a major threat to the health of humans and create massive challenges for the clinical field. The routine use of antibiotics leads to drug resistant wound pathogens and often alternative treatment strategies are required. Recently, Joughi et al (2022) investigated the antibacterial and wound healing activities of silver/ laterite/ chitosan nanocomposites. The synthesized silver/ laterite nanocomposite was coated with chitosan for enhanced wound healing activities. The TEM results revealed the shape of the nanocomposite to be spherical and size to be 12- 26 nm. The antibacterial activity of the nanocomposite both in vitro and in vivo exhibited potential effects such as increase in collagen content, re-epithelialization. These effects adversely helped in the accelerating wound healing process through the expression of pro-inflammatory TGF- β1, bFGF genes [35].

Silver nanoparticles synthesized using plant extract have gained interest among researchers worldwide. The reason behind this is because of their environmentally friendly and cost effective technique nature. To extend its application in the biomedical field, recently, Lubis et al (2022) utilized*Persicaria odorata* leaves extract to reduce the silver ions to silver nanoparticles. The *Persicaria odorata* leaves mediated silver nanoparticles (PO-AgNPs) exhibited its UV-Visible absorption peak at 440 nm. The high resolution Transmission Electron Microscopy (HR-TEM) images showed the nanosphere shape of the silver nanoparticles with diameters of 11±3 nm. The (PO-AgNPs) was tested for its antimicrobial activities against wound pathogens such as *Staphylococcus epidermidis* and *Methicillin Resistant Staphylococcus aureus* (MRSA). The above mentioned studies were also corroborated with Time kill kinetic assay. The antimicrobial activity showed that the (PO-AgNPs) are bactericidal against both strains after 24 h. The in vitro wound healing activity of (PO-AgNPs) was performed using human fibroblast cells-HSF 1184 and the results proved that these silver nanoparticles are non-toxic to normal cells which also enhanced cell migration ability than non-treated cells [36].

Nowadays, researchers have gained great attention towards electrospun nanofibers due to their effective properties such as strength, flexibility and environment friendly characteristics. Recently, Polyvinyl alcohol (PVA) nanofibers were loaded with Chocolate band snail mucus (*Eobaniavermiculata*) mediated silver nanoparticles (PVA/AgNPs-SM) to develop into a wound healing dressing material. The wound healing in vivo studies of the bio nanofiber resulted in a wound healing acceleration process along with inhibition of wound pathogens at the infected site [37].

Gold Nanoparticles:

Generally, gold nanoparticles are known to have good biocompatibility, enhanced antimicrobial and wound healing potential [38]. Currently, Battool et al (2022) used hydrogel extracted from *Cydonia oblonga* seeds as a reducing agent to synthesize gold nanoparticles. The biosynthesized gold nanoparticles exhibited surface plasmon resonance at 560 nm and its morphology was found to be cubic and rectangle shape. The biosynthesized gold nanoparticles inhibited the microbial growth upto 12 mm in diameter. In in vivo studies, the wounds treated with gold nanoparticles in murine models showed 99 % wound closure within 5 days. Gold nanoparticles also exhibited the increase in the expression levels of NANOG and CD-34 proteins in quantitative PCR analysis results which directly indicates the potent effect of the biosynthesized nanoparticles in enhanced wound healing process [39].

The use of gold nanoparticles as nano delivery systems in treating pathogenic bacterial infection provides an alternative option to improve efficacy than conventional therapeutic agents [40]. Recently, a hybrid gold nanoparticlewith a vehicle free antimicrobial polymer polyhexamethylene biguanide (PHMB@ Au Nps) was developed to inhibit the *Staphylococcus aureus* growth at the wound site and also to promote enhanced wound healing by photothermal bactericidal effect under near infra-red radiation. The in vivo study results revealed that hybrid gold nanoparticles directly inhibited the biofilm formation and through the photothermal therapy the bacteria were quickly removed. The hybrid gold nanoparticles also accelerated angiogenesis and mediated the transition of macrophages from M1 TO M2 type [41]. Gold nanoparticles synthesized using *Limoniaacidissima*aqueous leaf extract showed maximum antibacterial activity against *Escherichia coli*, a major wound pathogen with a high zone of inhibition around 19 mm. The cytotoxic effect was studied using L929 cell lines in which a significant effect was observed and also cell migration assay revealed no toxicity [42].

ZINC NANOPARTICLES

Skin is considered as the largest mechanical barrier in preventing the invasion of pathogens. The colonization of pathogenic bacteria at wound site leads to fever and sepsis which leads to delayed wound healing process [43]. Therefore, researchers are aimed to develop a novel pharmaceutical yet wound healing material. Generally, zinc oxide nanoparticles possess many therapeutic effects such as anti-cancer, antibacterial, antioxidant and can be used as an adjuvant drug to evacuate from the toxic side effects caused by chemotherapeutic drugs [44]. A novel azithromycin- loaded zinc oxide nanoparticles (AZM-ZnOnps) was synthesized to study its efficacy in treating infected wounds.

The synthesized zinc nanoparticles size was found to be 34 and 39 nm and with a maximum load capacity of 160.4 mg/g azithromycin was adsorbed on the zinc nanoparticles surface. The antibacterial efficacy of zinc nanoparticles was tested in

Staphylococcus aureus and E. coli. The results exhibited excellent antimicrobial activities than the control group. The (AZM-ZnOnps) were impregnated into hydroxypropyl methyl cellulose (HPMC) gel to study excisional wounds in rat models. The wound healing study results revealed that zinc nanoparticles effectively cleared bacteria at the infected site and also increased epidermal regeneration and stimulated tissue formation which sets a future platform for rapid wound healing [45].

Recently, Irfan et al (2022) used Gum *Acacia modesta* as reducing agent to synthesize zinc oxide nanoparticles and also impregnated on surgical sutures to enhance the wound healing process. The synthesized zinc nanoparticles are found to be rod shaped with size of about 70 ± 03 nm. The XRD results confirmed the crystalline nature of the zinc oxide nanoparticle. The Acacia modesta mediated zinc nanoparticles showed an effective zone of inhibition against wound pathogens such as Methicillin resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*. The zinc nanoparticles coated suture material exhibited good tensile strength and the wound healing potential was studied using Sprague Dawley rats through an incision wound model. The coated suture materials resulted in enhancing rapid wound healing process through increased epithelialization, wound contraction, and no infection on wounded site were recorded. The coated zinc nano sutures resulted in increased collagen fibers, rapid angiogenesis at healed tissues than standard surgical sutures [46].

Zhang et al (2022) studied the wound healing potential of spindle-like zinc silicate nanoparticles in treating skin burn wound healing. The neurovascular network damage is the major cause in skin burn wound healing. Therefore, there is a need for biomaterials which can promote both wound healing and reconstruct cutaneous neurovascular damage. Zinc silicate nanoparticles incorporated bioactive nanofibrous scaffolds are made to specifically treat innervated and vascularized skin burn wound healing. The zinc silicate nanoparticles with spindle morphology were synthesized through facile hydrothermal methods. The incorporation of zinc nanoparticles with scaffolds resulted in excellent neurogenic and angiogenic in vitro activities. The release of bioactive like Zn and Si ions from zinc silicate nanoparticles resulted in rapid wound healing with newly formed blood vessels and nerve fibres. Conclusively, the zinc nanoparticle based bioactive scaffolds can be used in future biomedical applications for multifunctional tissue regeneration [47].

STRONTIUM NANOPARTICLES

Strontium nanoparticles are widely used for its bone regeneration and growth stimulant properties. Strontium based nanoparticles gained huge attention among researchers due its functional similarity with calcium [48]. Strontium nanoparticles can also aid in wound healing applications. An injectable strontium ion cross linked starch hydrogel (SSH) based wound dressing was easy to be delivered onto the wound surface. It also protected the wound through its good adhesive nature and exhibited its strong antibacterial inhibition against Staphylococcus aureus. The in vivo studies of strontium based wound dressing resulted in proliferation of NIH/3T3 fibroblasts. This proliferation leads to the migration of human umbilical vein endothelial cells (HUVECs). This demonstrates that strontium based wound dressings can be used in future biomedical applications for an effective and rapid wound healing process [49].

Copper nanoparticles and its wound healing activity

Copper nanoparticles are recently developed as polyfunctional gel along with wound healing properties. The prepared copper nanoparticle based polyfunctional gel increased the wound regeneration rate up to 30-40 % compared to the control. The active re-epithelialization and deep formation of collagen fibers and connective tissue with an ordered structure are observed in the gel with copper nanoparticles treated animal groups rather than in control groups. The in vitro cell line studies exhibited fibroblast proliferation which indicated the good biocompatibility nature of the copper nanoparticles. The copper nanoparticles are known to activate the wound healing signalling pathway by increasing nitric oxide in the wound tissue. In this study, the nitric oxide level in the wound tissue was confirmed by using Electron Paramagnetic resonance [50].

Diabetic wounds and ulcers remain a major global concern which often leads to fatal conditions and pose challenges to medical and health care sectors. Li et al (2022) developed a sodium alginate hydrogel with both deferoxamine and copper nanoparticles through calcium ion cross linking method. The in vivo diabetic wound mouse model study resulted in exhibiting increased angiogenesis and reduced long lasting inflammation which can be used for clinical application in refractory wounds [51].

Development of side effects has become more normal nowadays and also a problematic issue in medical science research. Hydrogel is often used for its tissue compatibility, proper moisture but it lacks antimicrobial resistance. The copper nanoparticles are studied for its excellent antimicrobial properties and incorporation of copper nanoparticles into hydrogel will lead to an effective wound dressing material. Lemraski et al (2021) developed chitosan/ polyvinyl alcohol / copper nanoparticles and characterized using SEM, TEM, EDS, FT-IR, XRD. The copper nanoparticles-basedelectro spun nanofibers were evaluated for its wound healing process in rat open wounds. The antimicrobial activity of the hydrogel exhibited its inhibition against both gram positive and gram-negative wound pathogens, Therefore, the copper-based hydrogel can be used for treating and inhibiting the prolonged nature of open wounds [52].

Multi- species biofilms pose a major threat due to their strong resistance mechanisms. A novel chitosan based metal composite was prepared through green synthesis route without adding any toxic reducing agents. The novel chitosan based copper nanocomposite was found to have spherical shape with size of about 10-30 nm. In vivo studies, the novel metal nanocomposite ointment increased the levels of wound contracture, vessel, hexuronic acid, hydroxyproline, fibrocyte, hexosamine and fibroblasts. It also decreased wound area, total cells, neutrophil count in comparison with other groups in rats [53]. Copper nanoparticles with optimal size and low molecular weight chitosan derivatives open new horizons for wound management. Copper nanoparticles showed high synergistic effect in the combination of 0.002% copper nanoparticles and 0.05% chitosan derivative. This synergistic effect resulted in increasing the wound closure rate up to 37 %-39 %. This coppernanoparticles-based hydrogel combination showed effective angiogenesis, collagen deposition in the wound site and cell biocompatibility [54].

Wound healing is a complex and dynamic process. Conventional pharmaceutical agents often hasten the wound healing process due to its high dose usage and side effects [55,56]. Saddik et al (2020) prepared phenytoin loaded copper nanoparticles which were synthesized using liquorice aqueous extract. Through the adsorption method, copper nanoparticles were loaded with phenytoin and evaluated for its wound healing efficacy. Phenytoin loaded copper nanoparticles impregnated to hydroxypropyl methylcellulose gel for in vivo wound healing studies. The in vivo animal study was carried out using an excisional wound model in rats. The results revealed that Phenytoin loaded copper nanoparticles stimulated granulation and tissue formation and also accelerated tissue regeneration. An increase in the expression of dermal procollagen type I and decrease in the inflammatory JAK3 are observed in quantitative real time PCR results [57,58].*Allium saralicum* was used as a reducing agent to synthesize copper nanoparticles and its cutaneous wound healing effects in vivo and in vitro studies were evaluated. The biosynthesized copper nanoparticles showed higher antibacterial and antifungal effects [59]. The green synthesized copper nanoparticle based ointment was used in the treatment groups which substantially reduced the wound area, total cells and neutrophil count. The in vitro cell line studies on the HUVEC cell line revealed that synthesized copper nanoparticles showed dose dependent high cell viability [60,61]. *Falcaria vulgaris* leaf extract conjugated copper nanoparticles increased wound contracture by inhibiting growth of all fungi at 2-4 mg/mL concentration and growth of bacteria at 2-8 mg/mL concentration. The biosynthesized copper nanoparticles are reported to have a notable amount of antioxidant, antifungal, antibacterial and wound healing activities without causing any cytotoxic effects to normal cells in wound healing studies [62, 63].

Conclusion

Nanotechnology based platform is a new emerging field and its wound healing applications have gained numerous investigators attention in the last decade. Copper nanoparticles have been studied and shown great benefits in treating various wounds with low dose usage rather than conventional drugs. The copper nanoparticles possess different therapeutic and biomedical applications such as reduced cytotoxicity, higher stability, deep skin penetration, controlled drug delivery, reduced tissue inflammation. The copper nanoparticles' morphology, biodegradability nature make them take part in all stages of the wound healing process. The physicochemical properties such as magnetic, optical, electronic properties and biological properties involving antimicrobial, angiogenesis will aid in the tissue repair and cell regeneration which leads to rapid wound healing process.

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