

Review Article

Ahmad Yasser Hamdi Nor Azlan, Haliza Katas*, Mohd Fauzi Mh Busra, Nur Atiqah Mohamad Salleh, and Ali Smandri

Metal nanoparticles and biomaterials: The multipronged approach for potential diabetic wound therapy

<https://doi.org/10.1515/ntrev-2021-0046>
received April 2, 2021; accepted June 25, 2021

Abstract: Metal nanoparticles have been widely used in the treatment of diabetic wounds owing to their proven antibacterial activity and enhanced wound healing effects. Therefore, in this review, we discuss the use of metal nanoparticles in managing diabetic wounds, mainly silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), and zinc nanoparticles (ZnO nanoparticles), as well as their combination with biomaterials such as chitosan, bacterial cellulose, growth factors, *etc.* The combination of metal nanoparticles and biomaterials reportedly halts the growth and multiplication of bacterial strains commonly involved in diabetic wounds, including gram-positive (*Staphylococcus aureus* and *Acinetobacter calcoaceticus*) and gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*). Furthermore, these combinations have demonstrated enhanced wound healing of diabetic wounds during *in vitro* and *in vivo* studies. Additionally, we highlighted the barriers and challenges associated with the use of metal nanoparticles, including toxicities. Moreover, toxicities were mainly related to the method of synthesis employed, as well as the physical characteristics of nanoparticles, including size, shape, surface charge, and morphology. Collectively, dual-therapy composed of

metal nanoparticles and biomaterials has been shown to promote wound healing and can be developed as a promising future therapy for better outcomes in diabetic wound healing.

Keywords: diabetes mellitus, chronic wound, nanocomposites, antimicrobial activity, diabetic ulcer

Abbreviations

DAPT	4,6-diamino-2-pyrimidinethiol
BC	bacterial cellulose
CRT	calreticulin
CBV	capillary blood cell velocity
DM	diabetes mellitus
T1D	diabetes type 1
T2D	diabetes type 2
DFU	diabetic foot ulcer
DsiRNA	dicer substrate small interfering RNA
ER	endoplasmic reticulum
EGF	epidermal growth factor
ECM	extracellular matrix
bFGF	fibroblast growth factor-basic
FKDP	fur keratin-derived powder
GM3S	ganglioside-monosialic acid 3 synthase
GD	gestational diabetes
AuNPs	gold nanoparticles
GM-CSF	granulocyte-macrophage colony-stimulating factor
HDMECS	human dermal microvascular endothelial cells
HLA	human leukocyte antigen
Has	hyaluronic acids
IDDM	insulin-dependent diabetes mellitus
IGF1R	insulin growth factor 1 receptor
IL-1 α	interleukin 1 alpha
LDF	laser Doppler fluxmetry

* **Corresponding author: Haliza Katas**, Centre for Drug Delivery Technology, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia, e-mail: haliza.katas@ukm.edu.my

Ahmad Yasser Hamdi Nor Azlan: Centre for Drug Delivery Technology, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia; Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur (Royal College of Medicine Perak), 3, Jalan Greentown, 30450, Ipoh, Perak, Malaysia

Mohd Fauzi Mh Busra, Nur Atiqah Mohamad Salleh, Ali Smandri: Centre for Tissue Engineering and Regenerative Medicine, UKM Medical Centre, 56000, Cheras, Kuala Lumpur, Malaysia

KGF	keratinocyte growth factor
MPP	matrix metalloprotein
mRNA	messenger RNA
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MOL	<i>moringa oleifera</i> leaf
MDR	multidrug resistance
DMF	<i>N,N</i> -dimethylformamide
NHMS	national health and morbidity survey
Nic	nicotinamide
NIDDM	non-insulin-dependent diabetes mellitus
PAD	peripheral arterial disease
PDGF	platelet-derived growth factor
PDGF-BB	platelet-derived growth factor two beta subunit
PAA	poly(acrylic acid)
PEG	polyethylene glycol
PLGA	poly(lactic- <i>co</i> -glycolic acid)
PD	polydopamine
PGE2	prostaglandin E2
PGT	prostaglandin transporter
RCT	randomized control trial
ROS	reactive oxygen species
RISC	RNA-induced silencing complex
RNAi	RNA interference
AgNPs	silver nanoparticles
siRNA	small interfering RNA
TGF- β	transforming growth factor-beta
TNF- α	tumor necrosis factor-alpha
VRSA	vancomycin-resistant <i>S. aureus</i>
VEGF	vascular endothelial growth factor
ZnO	zinc oxide

nanoparticles (AgNPs), gold nanoparticles (AuNPs), zinc nanoparticles (ZnO nanoparticles), and titanium nanoparticles, is still attracting great interest among researchers for the development of diabetic wound therapies, mainly in treating infections. Several studies have demonstrated the ability of AgNPs, AuNPs, and ZnO nanoparticles to inhibit and kill various strains of gram-positive and gram-negative bacteria, including *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, common bacteria infecting diabetic wounds [4]. Nonetheless, killing bacteria is insufficient to successfully treat diabetic wounds as co-morbidities associated with the disease complicate the healing process, rendering it difficult to treat.

Moreover, biomaterial-based treatments have been developed to address the complexities of the wound healing process, including the overproduction of reactive oxygen species (ROS), poor re-epithelialization, decreased formation of granulation tissue, and stunted angiogenesis, additionally observed during the healing of diabetic wounds. Numerous therapies have combined metal nanoparticles with biomaterials as a strategy to enhance antibacterial activity and/or achieve dual-therapy action. Biomaterials are commonly used in these therapies owing to their unique, quantifiable, and adaptive properties for ameliorating the wound environment, as well as the ability of biomaterial-based systems to load various drugs [2]. This review summarizes the current developments in diabetic wound therapy involving the use of metal nanoparticles and biomaterials as nanocomposites. Furthermore, problems and challenges related to the application of metal nanoparticles and wound healing therapy are highlighted. To date, several therapeutic strategies consisting of metal nanoparticles and biomaterials have been reported; however, this review mainly focuses on *in vitro* and *in vivo* studies related to diabetic wounds.

1 Background

Diabetes mellitus (DM) remains a predominant global health problem as new cases continue rising owing to an increase in population growth rates, overweight individuals or obesity, sedentary lifestyles, and a lack of physical activity [1,2]. Diabetic patients are commonly associated with complications such as neuropathy, immune system deficiency, and infections that slow down the wound healing process [3]. Delayed wound healing and its associated complications may affect the patient's quality of life and increase the risk of amputation. Although many therapeutics have been proposed to improve healing in diabetic wounds, the use of metal nanoparticles, such as silver

2 Diabetes and its complications

Diabetes is a condition characterized by higher than normal blood glucose levels owing to irregularities in insulin production [5]. Diabetes can be divided into (1) Type 1, a condition in which the production of insulin is diminished, also known as insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes; (2) Type 2, a condition in which the response toward insulin is impaired owing to non-functional β -cells, also known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes; and (3) gestational diabetes that occurs

during pregnancy. All three types of diabetes present the same signs and symptoms, including increased blood glucose levels, excessive urine production, increased thirst, increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism [6]. The International Diabetes Federation has estimated that 8.3% (382 million) of adults worldwide suffered from diabetes in 2013 [7]. Furthermore, it is estimated that globally 422 million adults lived with DM in 2014 when compared with 108 million in 1980, demonstrating that the global prevalence of diabetes has increased from 4.7% in 1980 to 8.5% in 2014. Additionally, the number of deaths reported owing to diabetes was approximately 1.5 million in 2012 (according to WHO in 2016).

Notably, the highest prevalence of diabetes was observed in low-income countries (Bangladesh, India, Pakistan, and Zimbabwe) when compared with lower-middle-income countries (China, Colombia, and Iran), one occupied territory (Palestine), upper-middle-income countries (Argentina, Brazil, Chile, Malaysia, Poland, South Africa, and Turkey), and high-income countries (Canada, Sweden, and the United Arab Emirates) [7]. In Malaysia alone, the National Health and Morbidity Survey (NHMS volume II) reported that the overall prevalence of DM (known and undiagnosed) among adults 18 years and older was 17.5% in 2015. In general, an increased prevalence was observed with age, rising from 5.5% in the 18–19-year-old age group to 39.1% among the 70–74-year-old age group. Neuropathy (nerve damage) affecting 30–50% of diabetic patients is a common complication. Some patients may experience sensory problems, such as total loss of sensory or progressive build-up of unpleasant sensory symptoms such as tingling, hyperalgesia, and burning pain [8]. In the case of cuts or scratches (minor trauma), the wounds tend to develop into ulcers if proper treatment to prevent infection is not immediately provided, resulting in lower-extremity amputations.

In patients with diabetes, amputations are primarily caused by hyperglycemia-induced peripheral arterial diseases, accelerating direct damage to the nerves and blood vessels [9]. In these patients, the lifetime risk for developing diabetic foot ulcers ranges from 15 to 25%, mostly caused by the two predisposing factors, diabetic peripheral neuropathy and limb ischemia [10]. Moreover, it has been reported that diabetic individuals were eight times more likely to undergo a lower-limb amputation than non-diabetic individuals at the age of 45 years and older [11]. Therefore, preventive measures should be employed to avoid amputations, such as regular inspection of feet, identification of any foot at risk, wearing appropriate footwear, and patient education [12], as well as the effective treatment of wounds.

2.1 Diabetic wounds

Wounds can be categorized into acute and chronic, depending on the duration of the healing process. If a wound fails to heal after 90 days, it is classified as chronic. Wound healing can be divided into four phases: hemostasis, inflammation, proliferation, and remodeling [13]. When an injury occurs, platelets in the blood will adhere to the injury site and release chemical signals to promote clotting, resulting in the activation of fibrin. Fibrin forms a glue-like material to bind platelets, preventing further bleeding, a process known as hemostasis. Inflammation involves phagocytosis and the release of platelet-derived growth factors (transforming growth factor-beta (TGF- β) and tumor necrosis factor-alpha (TNF- α)) and cytokines such as interleukin 1 alpha (IL-1 α). The release of growth factors and cytokines leads to proliferation, resulting in new tissue formation, angiogenesis, collagen deposition, and epithelization. Remodeling is the last phase of the wound healing process, during which collagen is realigned and excess cells are removed by apoptosis [14,15].

Diabetic wounds are a pathological condition caused by various factors, including mechanical changes in the conformation of the bony architecture of the foot, peripheral neuropathy, and atherosclerotic peripheral arterial disease, all of which occur with higher frequency and intensity in the diabetic population. The risk factors for diabetic ulcers include poorly fitted or poor-quality shoes, poor hygiene (not washing the feet regularly or thoroughly), improper trimming of toenails, alcohol consumption, ocular diseases associated with diabetes, heart disease, kidney disease, obesity, and tobacco use (inhibits blood circulation).

The delayed healing of diabetic wounds is caused by several factors. First, the increased blood sugar level stiffens arteries and narrows blood vessels to restrict the delivery of oxygen and nutrients essential for the natural healing process [6]. Second, patients with diabetes experience decreased or poor blood circulation as these patients are at risk for peripheral arterial disease (PAD), a condition restricting blood flow to the feet and legs. PAD usually affects those with chronic wounds, particularly diabetic foot ulcers. Third, peripheral neuropathy, also known as nerve damage, is caused by a lack of blood circulation. In the extremities, this condition leads to a reduced supply of oxygen and nutrients to tissues and nerves, damaging nerves around the area and reducing sensation to pain, temperature, and touch. Finally, diabetes also weakens the immune system, affecting the body's ability to send white blood cells to

fight bacteria, rendering the wounds more susceptible to infection. Therefore, diabetic wounds commonly develop into infections such as non-healing diabetic foot ulcers [6,16].

S. aureus and *P. aeruginosa* are the common bacteria infecting diabetic wounds and can form biofilms, a major contributor to the failure of antibiotic treatment [13]. *S. aureus* is the most common single isolate (76%) in diabetic wounds and foot ulcers, resulting in alterations in wound healing. Moreover, wound infections can result in more serious conditions, including bacteremia or sepsis, causing morbidity and mortality. Over the last 40 years, MRSA infections have become endemic in hospitals in the United States and worldwide. In 2002, the first clinical isolate of vancomycin-resistant *S. aureus* (VRSA) was found in diabetic foot ulcers [17]. Bacterial infections can lengthen the increased release of proinflammatory cytokines, such as IL-1 and TNF- α , and therefore extend the inflammatory phase. If this persists, the wound may enter a chronic state and fail to heal. Therefore, the optimal management of infections in diabetic wounds is crucial to reduce the rate of morbidity and mortality. In patients with diabetes, the common complication inducing morbidity and mortality is impaired skin wound healing [18] owing to decreased blood flow and nerve damage, both of which are more likely to occur in patients with high blood glucose levels [19].

Furthermore, wound healing is affected by local and systemic factors. Local factors such as oxygenation and infection influence the healing process directly. Oxygen is important in the metabolism of cells as it synthesizes energy and increases angiogenesis needed during the process of wound healing. In contrast, systemic factors concerning the disease state or overall health of patients affect the healing process, including factors such as age, stress levels, hormone production, diabetes, medications, and obesity [13].

3 Current development of diabetic wound therapy

Current diabetic wound care therapies aim to protect wounds from external agents (such as bacterial infections and mechanical stress) and enhance wound closure by maintaining moisture, removing necrotic tissue and bacterial biofilm [15,20]. Furthermore, these therapies should be able to modulate and unlock the inflammatory phase and boost the reparative phase of healing (*e.g.*, epithelial migration, granular tissue formation through collagen

deposition and extracellular matrix (ECM) remodeling, angiogenesis, tissue blood perfusion, and lymphangiogenesis), as well as ensure adequate perfusion and pressure mitigation [15,20]. In general, diabetic wounds can be prevented by regular inspection of the feet, protection from infection, and control of DM by monitoring blood glucose and cholesterol levels, and blood pressure [6].

Previously, a high rate of wound healing was reported for a maximum period of 4 weeks following the application of Graftskin without any significant side effects [21]. In patients with diabetes, lack of angiogenesis and vascularization are biological factors attributed to delayed wound healing. By rectifying these abnormalities, the rate of amputation can be reduced; hence, several studies have attempted to achieve this goal by targeting different pathophysiological pathways. With the advancement of technology, novel therapies for diabetic wounds have been developed, including skin substitutes, negative pressure wound therapy, hyperbaric oxygen, and wound dressings with the inclusion of growth factors, as well as bioengineered tissues [20]. For example, topical vascular endothelial growth factor (VEGF) was used to treat diabetic wounds owing to its ability to enhance epithelization, matrix deposition, proliferation, and release platelet-derived growth factor (PDGF)-B, as well as fibroblast growth factor-2 [22].

Growth factors play important roles in the wound healing process, influencing cellular activity and proliferation, triggering migration of inflammatory cells into the wound bed, and activating protein synthesis and turning off events, including the healing progress [23]. Notably, secreted growth factors mediate their actions through autocrine, paracrine, or endocrine mechanisms by binding to the membrane or cytoplasmic receptors. This binding results in a cascade of events activating the cellular machinery to stimulate wound healing, even at low concentrations [24]. VEGF, fibroblast growth factor-basic (bFGF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) are examples of growth factors evaluated clinically. One such growth factor approved by the US FDA, platelet-derived growth factor two beta subunit (PDGF-BB), is currently being used in the treatment of chronic ulcers [25]. VEGF can be classified into VEGF-A, -B, -C, -D, -E, and placental growth factor. However, VEGF-A is the only confirmed VEGF initiating angiogenesis by facilitating the proliferation and migration of endothelial cells. In the early phase of wound healing, VEGF-A is secreted by platelets and macrophages in response to tissue injury. Furthermore, VEGF-A demonstrated improved re-epithelization of diabetic foot wounds associated with the enhancement of vessel formation [24].

Previously, a diabetic mouse model treated with recombinant human VEGF165 protein was investigated. The mice received 5 doses of VEGF (20 g) or a vehicle (phosphate-buffered saline) every other day, applied to one wound. The VEGF-treated wounds presented accelerated repair, with an average healing time of 12 days when compared with untreated mice requiring 25 days to demonstrate similar healing [22]. In another study, patients presented 75% or greater ulcer reduction after treatment with bFGF for approximately 8 weeks [26].

In patients with diabetes, prostaglandin E2 (PGE2), which is known to enhance angiogenesis and vasodilation, is found to be diminished. This reduction is associated with the upregulation of the prostaglandin transporter (PGT) gene, induced by hyperglycemia. Reportedly, the expression of PGT, as well as its activity, increased by up to three-fold in human dermal microvascular endothelial cells (HDMECs) exposed to high concentrations of glucose, resulting in the decreased production of PGE2 [27]. Moreover, the upregulation of PGT mediates limitations of endothelial and peripheral vascular functions, resulting in slower healing of diabetic wounds (Figure 1) [28–30]. Therefore, PGT has been identified as a therapeutic target for promoting angiogenesis and vascularization in the treatment of diabetic wounds. In patients with diabetes, low levels of PGE2 were alleviated by injecting the PGT inhibitor, T26A. Additionally, PGT inhibitors increased the blood flow in ischemic hind limbs of non-diabetic and streptozocin-induced diabetic rats. Immunohistochemical examination revealed enhanced vascularization and re-epithelization of cutaneous wounds [31], demonstrating the potential use of PGT inhibitors in treating diabetic wounds.

Another approach involves the knockdown of the PGT gene to inhibit its activity on angiogenesis and vascularization. This can be achieved by applying RNA interference (RNAi)-based molecules such as small interfering RNA (siRNA, 21mer duplex RNA) and its newer version, Dicer substrate small interfering RNA (DsiRNA, 27mer duplex RNA), which have higher specificity and more potency [32]. RNAi can be defined as a natural defense mechanism of eukaryotic cells, mediated through the RNA-induced silencing complex (RISC), resulting in the sequence-specific degradation of messenger RNA (mRNA) [33]. The gene silencing effect can last for 3 to 7 days in rapidly dividing cells or several weeks in non-dividing cells [34]. The designs, mechanisms, and applications of siRNA, as well as DsiRNA, in treating various diseases through *in vitro* and *in vivo* studies have been reviewed by Raja *et al.* [35]. RNAi therapeutics are intrinsically unstable against various enzymes and are highly charged, presenting considerable difficulties in crossing cell membranes. Therefore, in an attempt to improve their transport, a delivery system is required to transfer and protect them. Furthermore, the delivery of RNAi therapeutics should be able to target the site of action to achieve high efficacy and minimal toxicity [36].

3.1 Biomaterials

Biomaterial-based wound dressings are being developed for treating diabetic wounds, mainly to accommodate the need to reduce the inflammatory phase during the healing process. Biomaterials accelerate wound healing

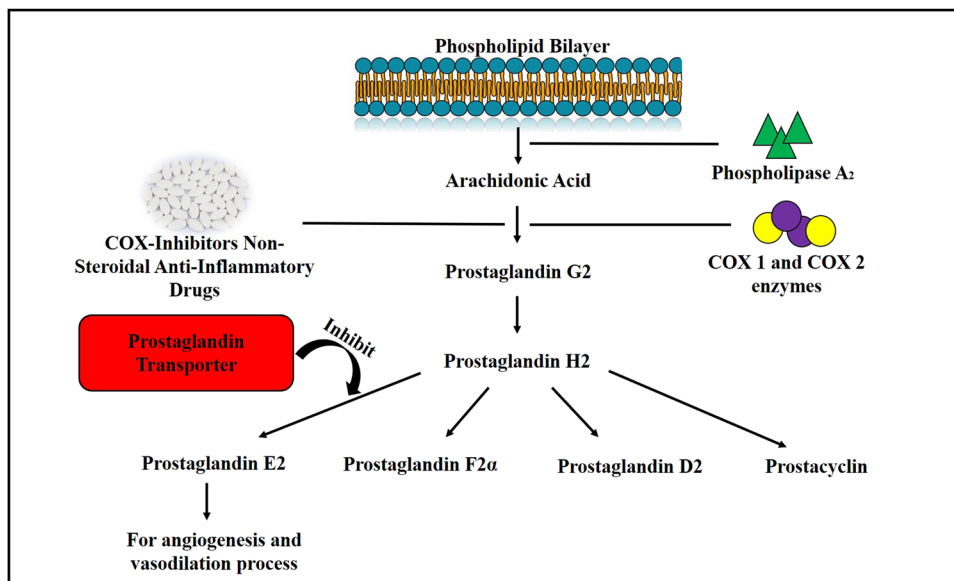


Figure 1: Mechanism of prostaglandin synthesis.

by maintaining moisture in the wound microenvironment, facilitating cell proliferation at the wound site [37]. Chitosan, hyaluronic acids (Has), poly(lactic-co-glycolic acid) (PLGA), collagen, and fibrin are several examples of biomaterials that have been developed for diabetic wound therapy to elicit healing through cell-material interactions and/or sustained delivery of drugs or active agents [2].

Integra Dermal Regeneration Template is a commercial biomaterial-based wound dressing initially designed by Yannas and Burke in 1980 [38]. Treatment with Integra involves a two-step procedure, where Integra is applied on the wound site and remains for at least 21–28 days before replacement with a skin graft [39]. Integra is an acellular bilayer matrix consisting of bovine tendon collagen type I and shark chondroitin-6-sulfate glycosaminoglycan, which bind to the silicone pseudo-epidermis. The bioscaffold is constructed to allow the integration of host fibroblasts into the dermal component, bovine collagen type I. When the cells populate the dermal layer of the bioscaffold, the dermal materials degrade as neodermis, leaving the pseudo-epidermal components at the wound site, preventing bacterial infection and acting as a water vapor barrier. In a clinical trial conducted in type I and type II diabetic patients with foot ulcers, the patients treated with Integra healed 5 weeks faster than the control group, with the average wound size reduction per week being 50% faster than the control group [40]. Despite the successful use of biomaterials as wound dressings, the main concern related to bioengineered products still exists, primarily related to a bacterial infection at the wound site. Hence, the addition of antimicrobial agents to biomaterial-based wound dressings such as metal nanoparticles is beneficial for improving their efficacy.

3.2 Metal nanoparticles and their synthesis

Nanoparticles are tiny materials ranging from 1 to 100 nm in size, which can be categorized into different classes based on their properties, shapes, or sizes. Nanoparticles can be divided into carbon-based, ceramic, metal, semiconductor, polymeric, and lipid-based nanoparticles [41]. Furthermore, they are unique owing to their optical and electronic conductivity, which greatly depends on the particle size and shape. They have achieved significant consideration for their potential applications in drug delivery systems and as antimicrobial agents. Nanoparticles, particularly metal nanoparticles, have been extensively evaluated as

antimicrobial agents owing to their ability to combat multi-drug-resistant pathogens, including the inhibition of biofilm formation through several mechanisms, mainly attributed to the diversity of the intrinsic, as well as modified chemical composition properties. Moreover, the physicochemical properties of nanoparticles, including particle size and shape, chemical modification, and surface coating, greatly influence their antibacterial activity, rendering them adaptable as antimicrobial agents [42]. Most antibiotic resistance mechanisms are unrelated to nanoparticles as their activity is attributed to direct contact with the bacterial cell wall, without the need for cell penetration. Therefore, nanoparticles are less likely to promote bacterial resistance than antibiotics [43]. Metal nanoparticles such as AgNPs, AuNPs, and ZnO nanoparticles have been widely studied for their antimicrobial activities.

Nanoparticles can be synthesized *via* physical and chemical methods. Evaporation-condensation and laser ablation are the most common physical methods [44]. ZnO, as small as 30 nm, can be obtained by physical methods such as high-energy ball milling. The milling time is one parameter influencing nanoparticle size, shape, and antibacterial activity [45]. Despite the advantages of physical methods such as the absence of solvent contamination and uniform nanoparticles produced, factors such as high energy, time consumption, and heat generation remain limitations. Furthermore, physical methods may require a large space, such as the synthesis of AgNPs using a tube furnace at atmospheric pressure [44].

For the chemical synthesis of metal nanoparticles, common reducing agents include sodium citrate (a well-known method developed by Turkevich and French), sodium borohydride [46,47], ascorbate, Tollens reagent, *N,N*-dimethylformamide (DMF), and poly(ethylene glycol)-block copolymers [44]. Notably, chemical methods are the best methods to obtain a small size (<100 nm) and different nanoparticle shapes, by varying the molar concentration of the reactant, dispersant, and feed rate of the reactant. Furthermore, the selection of appropriate reducing agents is a crucial factor because the size, shape, and size distribution of particles are strongly dependent on the nature of the reducing agent [48]. AgNPs synthesized using a water-soluble polymer, poly(acrylic acid) (PAA), formed stable AgNPs. Polyacrylate anions (PA^-) obtained from PAA with uncoordinated carboxylate groups bind with metallic cations (Ag^+) to form an intermediate charged cluster and AgNPs [49]. Although markedly small sizes of AgNPs can be obtained using sodium citrate (42–58 nm), the nanoparticles tend to agglomerate owing to instability [50]. Similarly, extremely fine AuNPs (4–13 nm) could be obtained by reducing gold ions using trisodium citrate

and sodium borohydride, but poor nanoparticle stability was reported [51]. A similar aggregation issue was reported with titanium nanoparticles synthesized using sodium borohydride [52]. Therefore, the selection of the reducing agent is important for the chemical synthesis of metal nanoparticles, in addition to specific catalysts and regulated reaction environments, including appropriate temperature, pressure, and pH.

Chemical reducing agents are generally expensive, limiting their application. Chemically synthesized metal nanoparticles may contribute to the harmful effects of chemical residues adhered to the nanoparticles. For example, the chemical synthesis of ZnO using hydrazine, a highly reactive alkali, and reducing agent requires protective equipment to avoid skin burns, severe damage to the respiratory tract, and death due to overexposure [53]. Therefore, synthesizing nanoparticles using green or biological methods remain preferable as the use of toxic chemicals can be avoided [54,55]. Green synthesis (biosynthesis) of metal nanoparticles uses natural sources as reducing agents, including microorganisms [56], fungi [57], plant extracts, and marine-derived compounds [58]. The biosynthesis of metal nanoparticles is shown in Figure 2.

Reducing agents from different sources present certain advantages and limitations. For example, microbes require more complex procedures (*e.g.*, elaborate purification process), and bacterial adherence on the particle surfaces may increase the potential danger to the environment, as well as human health [59]. The plant-mediated synthesis of nanoparticles demonstrates more advantages when compared with other sources owing to its suitability for large-scale production and cost-effectiveness.

3.3 Applications of metal nanoparticles in diabetic wound therapy

The escalation of microbial resistance has resulted in extensive research on metal nanoparticles as potential antibacterial agents owing to their proven effectiveness in inhibiting and killing bacteria. Compared with eukaryotes, prokaryotes are known to be more susceptible to the toxic effects of silver. Higher silver concentrations are required to kill eukaryotes owing to the larger cell size, as well as the redundancy of structure and function within

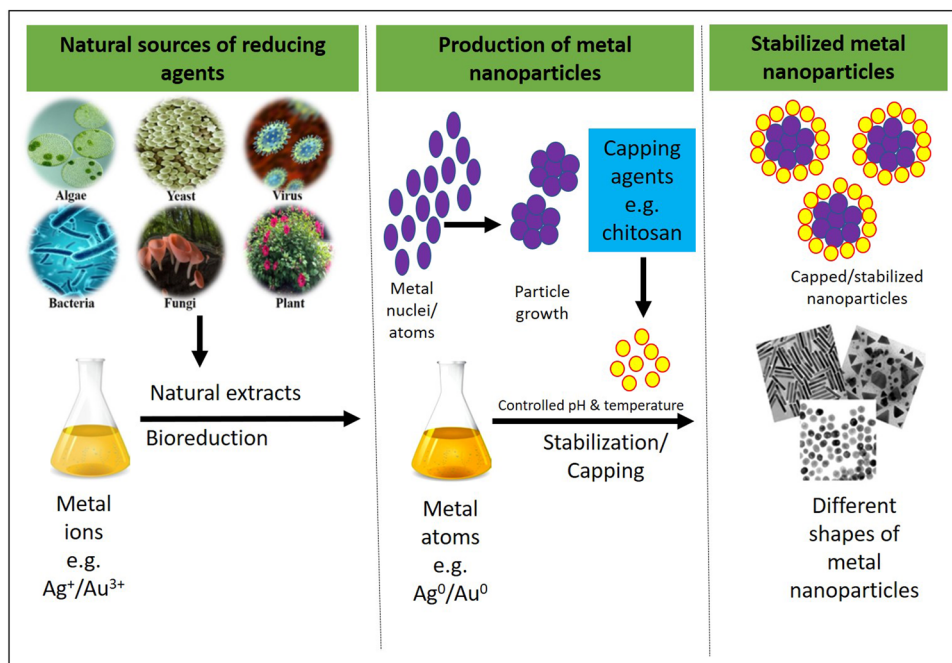


Figure 2: Mechanism of green synthesis (biosynthesis) of metal nanoparticles. Metal ions such as Ag⁺ and Au³⁺ are reduced into metal nuclei/atoms (Ag⁰ and Au⁰) using extracts of natural sources such as plants and microorganisms. The metal nuclei/atoms are then accumulated or growing, and therefore, stabilizing/capping agents (*e.g.*, chitosan) are added to produce stabilized metal nanoparticles with small particle sizes. Various shapes of metal nanoparticles can be produced by controlling parameters during the synthesis such as concentration of metal ions and reducing agents as well as pH and temperature.

the eukaryotic cell [60]. For promoting wound healing, metal nanoparticles have been tested alone or in combination with other materials, as a strategy to enhance antibacterial activity. Similar to antibiotics, bacteria may develop resistance toward metal nanoparticles *via* several mechanisms: (1) reduction of Ag^+ to its neutral oxidation state with lower toxicity and (2) active Ag^+ efflux from the cell by P-type adenosine triphosphatases or chemiosmotic Ag^+/H^+ antiporters [60]. Rapidly evolving resistance toward citrate-coated AgNPs was reported for *Escherichia coli* [61], while *Bacillus subtilis* developed resistance after prolonged pre-exposure to metallic nanosilver [62]. These findings have prompted scientists to combine metal nanoparticles with other antimicrobial agents to overcome resistance and enhance their activity in killing microorganisms.

3.3.1 Enhancement of antimicrobial activity of metal nanoparticles

3.3.1.1 AgNPs

Reportedly, AgNPs exhibit broad-spectrum antimicrobial activity; therefore, AgNPs have received great interest regarding their biomedical applications, including the management of chronic wounds [63,64]. For AgNPs, different mechanisms of antibacterial action have been reported, including the ability to increase the permeability of cell membranes, interfere with DNA replication, denature bacterial proteins, and release silver ions inside the bacterial cell, leading to bacterial cell death. Meanwhile, the increase in membrane permeability and release of ROS within the fungal cell were proposed as the antifungal activity of AgNPs, resulting in fungal cell death [65].

Over the years, AgNPs have been combined with various biomaterials to enhance their antimicrobial activity, particularly for diabetic wound treatment. Sponges containing chitosan, HA, and nanosilver have been shown to inhibit MRSA, promoting further research on the development of potential wound dressings for diabetic foot ulcers saturated with antibiotic-resistant bacteria [66]. The combination of AgNPs and chitin nanofibers as a wound dressing exhibited strong antimicrobial activity with the same toxicity as the AgNPs alone. However, an *in vivo* study demonstrated that the combination of AgNPs and nanofibers delayed wound healing, which could be mitigated by washing with saline [67]. Conversely, a combination of ϵ -polylysine (EPL-*g*-butyl) through one-pot synthesis demonstrated the synergistic antibacterial

effects of AgNPs against *P. aeruginosa* and *S. aureus*, revealing no evidence of bacterial resistance. The irreversible disruption of the bacterial cell wall following binding and subsequent penetration of bacterial cells, as well as potent inhibition of enzymatic activity, have been proposed as mechanisms of antibacterial action leading to bacterial apoptosis. The nanocomposites of EPL-*g*-butyl and AgNPs modulate inflammatory cells and thus promote wound healing without causing side effects on the dermal tissues of diabetes-induced rats [68].

Dressings containing AgNPs adsorbed onto matrices of cellulose nanocrystals inhibited the growth of *S. aureus* and *P. aeruginosa*. Moreover, the dressings containing smaller sizes of AgNPs (NC-1) presented the highest zone of inhibition, indicating that activity was influenced by particle size. Additionally, NC-1 demonstrated superior positive results in terms of early neo-vascularization, collagen deposition, and re-epithelialization, resulting in faster healing [69]. More recently, Abdalla *et al.* [70] reported that the combination of AgNPs with graphene oxide or lactoferrin as an antibiofilm agent contributed to greater antibacterial activity against *P. aeruginosa*, even though graphene oxide and lactoferrin failed to demonstrate any antibacterial activity.

3.3.1.2 AuNPs

A growing interest has been reported in the application of AuNPs for wound care management owing to their unique properties, including small size, large surface area, high reactivity to living cells, and good cellular penetration [71]. AuNPs exhibit antibacterial activity with strong bactericidal effects against pathogens, including drug-resistant bacteria [72]. Furthermore, the combination with ampicillin (AuNPs-Amp) inhibited ampicillin-resistant bacteria, including MRSA, *P. aeruginosa*, *Enterobacter aerogenes*, and the *E. coli* K-12 sub-strain, DH5- α . The killing effect was enhanced by overcoming the high concentration of expressed β -lactamase and inhibiting the transmembrane pumps catalyzing drug efflux from the bacterial cell.

Furthermore, the combination with other materials has been reported, including the integration of bacterial cellulose (BC) and 4,6-diamino-2-pyrimidinethiol (DAPT)-modified AuNPs (Au-DAPT NPs) to form BC-Au-DAPT. These nanoparticles suppressed the proliferation of *E. coli* and *P. aeruginosa*, as well as their multidrug resistance (MDR) strains, with better efficacy than the commercial antibiotics (cefazolin/sulfamethoxazole) by inhibiting energy metabolism and dissipating bacterial membranes. BC is an excellent wound dressing that facilitates cellular

respiration and keeps the wound wet for a long period. Further research on *E. coli*- or *P. aeruginosa*-infected full-thickness skin wounds in rats showed that BC-Au-DAPT nanocomposites inhibited bacterial growth and promoted wound repair [73]. Additionally, several studies combined AuNPs with other agents with antibiofilm activity, including certain enzymes and peptides. Lysozyme-coated AuNPs in combination with the β -lactam antibiotic ampicillin (AUNC-L-Amp) eradicated MRSA infections of diabetic wounds, leading to faster wound healing [74]. In a different study, the integration of peptide LL37 and AuNPs with enhanced bactericidal activity led to further investigation in a diabetic-induced animal model. The combined formulation inhibited bacterial infection in diabetic wounds and, hence, accelerated the wound closure rate, re-epithelization, and stimulated granular tissue formation, as well as enhanced the expression of VEGF with minimal toxicity [84].

3.3.1.3 ZnO nanoparticles

The antimicrobial activity of ZnO nanoparticles has been reported in the management of infected wounds. Chitin hydrogel containing ZnO nanoparticles exhibited antibacterial activity against *S. aureus* and *E. coli* [75]. The presence of a thick layer of peptidoglycans in the cell wall of *S. aureus* was attributed to the difficulty in bacterial cell wall penetration by particles. *In vivo* investigations in Sprague–Dawley rats demonstrated that the hydrogel promoted wound healing by increasing re-epithelialization and collagen deposition, showing its potential use in treating burn and chronic wounds, as well as diabetic foot ulcers [75].

3.3.2 Others

Moreover, some materials used as reducing and/or stabilizing agents in the synthesis of metal nanoparticles are known to enhance the antimicrobial activity of nanoparticles. Biosynthesized AgNPs using curcumin-cyclodextrins as reducing and stabilizing agents inhibited gram-positive (*S. aureus*) and gram-negative (*P. aeruginosa*) bacteria. Curcumin is a natural polyphenol extracted from turmeric and reportedly possesses wound healing properties owing to its antimicrobial, antioxidant, and anti-inflammatory effects. However, curcumin is hydrophobic, and this barrier was overcome by microencapsulation into cyclodextrins. These AgNPs were more effective in killing *P. aeruginosa* than *S. aureus* via different mechanisms of action. In *P. aeruginosa*, AgNPs separated the

cytoplasm from the bacterial cell wall (plasmolysis effect), leading to cell death. In contrast, AgNPs inhibited cell wall synthesis in *S. aureus* [65].

Furthermore, the high antimicrobial activity of biosynthesized AgNPs, synthesized using the rhizosphere soil bacterium *Brevibacillus brevis* KN8(2) cell-free culture filtrates, was reported. The filtrate contained the endotoxin neutralizing lipopeptide surfactin to provide anti-pseudomonal and anti-endotoxin activity, potentially healing wound infections. The nanoparticles were assessed in diabetic mice and were found to reduce matrix metalloproteinase2 (MMP-2) and MMP-9 mRNA levels, as well as their proteins, in wounded granular tissue samples, demonstrating early wound healing potentially beneficial for the management of diabetic foot ulcers [76]. More recently, Katas *et al.* [77] successfully biosynthesized AuNPs using a mushroom extract, *Lignosus rhinocerotis*, and chitosan as a reducing and stabilizing agent, respectively. The AuNPs inhibited gram-negative (*P. aeruginosa* and *E. coli*) bacteria more effectively than gram-positive bacteria (*S. aureus*) owing to synergistic effects in the presence of chitosan, which also possesses antibacterial activity.

3.3.3 Multi-action therapy of metal nanoparticles and biomaterials for diabetic wounds

Management of diabetic wounds by solely offering antibacterial effects is ineffective in achieving good clinical outcomes. The main reason is the complexity of diabetic wounds owing to multi-factorial etiology, including hyperglycemia, neuropathy, poor blood circulation, and weakened immune response. To improve the efficacy of diabetic wound treatment, biomaterials are used in combination with metal nanoparticles as multi-action therapy.

3.3.3.1 AgNPs

A hybrid hydrogel was developed by integrating AgNPs with an antifouling agent (zero charges) consisting of cationic chitosan and anionic dextran. The hydrogel accelerated diabetic wound repair and treated infections by providing slow and sustained release of AgNPs [78]. The wound healing efficacy of a dressing consisting of fur keratin-derived powder (FKDP) and AgNPs (FKDP-AgNPs) was evaluated for its potential use in treating diabetic wounds [79]. FKDP was used to enhance healing in impaired wounds (acute and chronic), while AgNPs acted

as an antibacterial agent. The FKDP-AgNP dressing did not inhibit the growth of fibroblasts or induce hemolysis during *in vitro* studies, demonstrating the non-cytotoxicity of the dressing. Conversely, *in vivo* investigations demonstrated the ability of the FKDP-AgNP dressing to significantly accelerate wound closure and epithelization at day 5 and 8 when compared with control groups.

Additionally, sodium alginate was used to synthesize AgNPs, which were later loaded into non-woven fabrics (AgNPS/ALG/non-woven fabrics) prior to the addition of nicotinamide (Nic), an anti-inflammatory drug. The Ag/ALG/Nic wound dressing effectively killed gram-negative (*E. coli*) and gram-positive bacteria (*S. aureus*). The wound area significantly decreased, from 1.5 cm² at the start of the experiment to 0.46 cm² on day 4, followed by complete healing on day 10. Other than the anti-inflammatory and antibacterial activities of Nic and AgNPs, the larger surface area and easily modifiable surface provided by the non-woven fabric dressing contributed to the healing of diabetic wounds [80].

Moreover, AgNPs were synthesized using a natural vitamin B6 complex, utilizing pyridoxine as a chelating ligand *via* a reverse microemulsion technique to form silver(II) pyridoxine nanoparticles (SPNs) with wound healing, moisturizing, and antibacterial properties. These SPNs presented dual therapeutic properties, promoting re-epithelialization, and acting as an antibacterial agent for wound healing. The multi-action SPNs promoted proliferation and migration rates of fibroblasts and keratinocyte cells. Further *in vivo* studies demonstrated that SPNs enhanced wound healing in diabetic mice [81].

3.3.3.2 AuNPs

The same approach was utilized for AuNPs by combining them with biomaterials as multi-action formulations or dressings. Calreticulin (CRT) – functionalized AuNPs were synthesized and evaluated for their efficacy in treating wounds *via in vitro* and *in vivo* studies. CRT is a calcium-binding resident protein of the endoplasmic reticulum (ER) directing the proper folding of proteins and homeostatic control of cytosolic and ER calcium levels. Topically applied CRT increased the wound re-epithelialization rate and formation of granulation tissues/neodermis. The CRT-functionalized nanoparticles promoted proliferation and migration, resulting in enhanced wound healing. Histological evaluation of diabetic mouse wound samples further confirmed re-epithelialization and formation of granular tissues, as well as increased collagen deposition [109].

The modified keratinocyte growth factor (KGF) plays an important role in keratinocyte proliferation *via* strong binding to KGF receptors, accelerating cutaneous wound healing. Therefore, KGF was conjugated with AuNPs using polyethylene glycol (PEG) thiolates (KGF-AuNPs), and the nanoparticles demonstrated higher efficacy than KGF alone in treating wounds [82]. Cell viability tests revealed the non-toxicity of KGF-AuNPs, as more than 95% of keratinocyte cells remained active 48 h after incubation, while the cell migration study showed enhanced cell migration, suggesting the wound healing property of KGF-AuNPs. The wound healing efficacy of KGF-AuNPs was further evaluated in a diabetic rat model, revealing that all wounds demonstrated approximately 80% closure within six days of therapy with KGF-AuNPs.

In diabetic mice, overexpression of ganglioside-mono-sialic acid 3 synthase (GM3S) was demonstrated, causing insulin resistance and slow wound healing. This abnormality was rectified by applying spherical nucleic acid (SNA)-AuNP conjugates, with siRNA against GM3S used to silence the target gene. The conjugates promoted keratinocyte migration into the wound bed, increased the level of insulin growth factor 1 receptor (IGF1R) and epidermal growth factor (EGF) receptor phosphorylation, and therefore, accelerated wound closure in a type II diabetic mouse model [83]. The combination of AuNPs with peptide LL37 and plasmid DNA (pDNA) revealed an excellent bactericidal effect owing to the synergetic antibacterial effect of cationic AuNPs and LL37 coated on nanoparticle surfaces. In comparison with the positive control, vancomycin, the nanoparticles were found to be more effective in killing MRSA. Moreover, keratinocytes treated with the nanoparticles expressed higher levels of VEGF (2235.28 ± 146.22 pg/mL) than the blank control (353.42 ± 31.88 pg/mL). Further investigation in a diabetic murine full-thickness, skin excisional acute wound model, presenting pathogenesis similar to impaired diabetic foot ulcer healing, showed that the nanoparticle treated group demonstrated faster wound healing (within 7 days) than the group treated with protein LL37 (within 9 days) and AuNPs-protein LL37 (within 9 days). The nanoparticles reportedly promoted angiogenesis and inhibited bacterial infection in diabetic wounds, resulting in accelerated wound closure rates and re-epithelialization, demonstrating improved granular tissue formation, and high VEGF expression [84]. It is well documented that AuNPs possess anti-inflammatory activities, and in combination with antioxidants, they can accelerate cutaneous wound healing [85]. A summary of the current development of formulations or dressings based on metal nanoparticles combined with biomaterials for diabetic wounds is shown in Table 1.

Table 1: Formulations or dressings consisting of metal nanoparticles and biomaterials as potential therapies for diabetic wounds

Metal nanoparticles	Biomaterials	Microbes	Potential applications/findings	Reference
Silver nanoparticles (AgNPs)	Chitosan and hyaluronic acid	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	Wound dressing in treating MRSA-infected diabetic infected wound	Anisha et al., 2013 [66]
	Chitin nanofiber	—	Causing delayed wound healing that could be mitigated by washing with saline	Kinoda et al., 2016 [67]
	ϵ -polylysine (EPL-g-butyl)	<i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i>	Modulating cell inflammation and promoting wound healing without significant side effects on dermal tissues	Dai et al., 2016 [68]
	Cellulose nanocrystal	<i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i>	Inducing early neo-vascularization, enhancing collagen deposition and re-epithelialization, leading to faster healing	Singla et al., 2017 [69]
	Graphene oxide and lactoferrin	<i>Pseudomonas aeruginosa</i>	Wound dressings for chronic wounds including diabetic wounds	Abdalla et al., 2020 [70]
	Antifouling agent (mixture of cationic chitosan and anionic dextran)	<i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i>	Improving immune system against infection through the slow sustained release of AgNPs and accelerating diabetic wound repair as the wounds almost completely healed after 7 days of treatment	Shi et al., 2019 [78]
	Fur keratin-derived powder (FKDP)	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	The dressing did not inhibit fibroblasts growth or induce hemolysis and significantly accelerating wound closure and epithelization at day 5 and 8	Konop et al., 2020 [79]
	Non-woven fabrics and nicotinamide	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	The bandage provided a large surface area and an easy modifiable surface. In combination with nicotinamide (anti-inflammatory) and AgNPs, the bandage promoted healing of the diabetic wound	Montaser et al., 2016 [80]
	Pyridoxine	<i>Acinetobacter calcoaceticus</i> , <i>Citrobacter freundii</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterococcus faecalis</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , and <i>Propionibacterium acnes</i>	The nanoparticles increased the proliferation and migration rate of fibroblasts and keratinocytes as well as enhanced wound healing in diabetic mice	Rangasamy et al., 2016 [81]
	Curcumin-cyclodextrins and bacterial cellulose	<i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i>	AgNPs were synthesized using aqueous curcumin: hydroxypropyl- β -cyclodextrin complex and loaded into bacterial cellulose hydrogels. The hydrogels showed moist wound-healing properties that enhanced wound healing, and therefore, suitable for chronic wound treatment	Gupta et al., 2020 [65]
	<i>Brevibacillus brevis</i> KN8(2)	<i>Pseudomonas aeruginosa</i>	AgNPs synthesized from <i>Brevibacillus brevis</i> KN8(2) possess high antimicrobial activity. In wound healing efficacy study of diabetic mice, AgNPs caused decreased mRNA and protein expression level of MMP-2 and MMP-9, leading to early wound healing	Krishnan et al., 2017 [76]

(continued)

Table 1: (continued)

Metal nanoparticles	Biomaterials	Microbes	Potential applications/findings	Reference
Gold nanoparticles (AuNPs)	Bacterial cellulose (BC) decorated by 4,6-diamino-2-pyrimidinethiol (DAPT)	<i>Escherichia coli</i> and multidrug-resistant (MDR) <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> and MDR <i>Pseudomonas aeruginosa</i>	The nanoparticles inhibited bacteria growth and promoted wound repair in <i>Escherichia coli</i> and <i>Pseudomonas aeruginosa</i> -infected full-thickness skin wounds of rats	Li <i>et al.</i> , 2017 [73]
	Lysozyme and β -lactam antibiotic ampicillin	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	Enhancing antimicrobial activity of AuNPs. Eradicating MRSA infections from diabetic wound, resulting in pronounced and faster wound healing	Kalita <i>et al.</i> , 2018 [74]
	Peptide LL37	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	Inhibiting bacterial infection of diabetic wounds, leading to increased wound closure rate and re-epithelization and stimulated granulation tissue formation as well as enhanced VEGF expression	Wang <i>et al.</i> , 2018 [84]
	<i>Lignosus rhinocerotis</i> and chitosan	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , and <i>Escherichia coli</i>	Potential antibacterial agent for possible use in chronic wounds including diabetic wound	Katas <i>et al.</i> , 2018 [64]
	Calreticulin		Promoting cell proliferation and migration <i>in vitro</i> and enhancing wound healing in the diabetic mice model. The histological evaluation showed increased re-epithelization, granular tissue formation, and collagen deposition	Hernández Martínez <i>et al.</i> , 2019 [109]
	Keratinocyte growth factor (KGF)		Enhancing cell migration <i>in vitro</i> and wound closed almost 80% in diabetic-rat model	Li <i>et al.</i> , 2019 [82]
	siRNA targeted GM3S		Promoting keratinocyte migration into the wound bed, increasing insulin growth factor 1 receptor (IGF1R), and EGF receptor phosphorylation, and accelerating wound closure in type-II diabetic mice model	Randeria <i>et al.</i> , 2015 [83]
	Peptide (LL37) and plasmid DNA (pDNA)	MRSA	Promoting angiogenesis and inhibiting bacterial infection of diabetic wounds, resulting in accelerated wound closure rate, faster re-epithelization, improved granulation tissue formation and high VEGF expression level	Wang <i>et al.</i> , 2018 [84]
ZnO nanoparticles	Chitin	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	The development of chitin hydrogel/nano ZnO composite bandages possesses antibacterial activity and proved to be non-toxic toward human dermal fibroblasts	Kumar <i>et al.</i> , 2012 [75]
	Chitosan	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	The nanocomposite bandages promoted wound healing, increased re-epithelialization and helps in collagen deposition, suitable for treating burn wounds, chronic wounds, and diabetic foot ulcers	Kumar <i>et al.</i> , 2012 [75]

4 Current limitations

4.1 Relationship between physicochemical characteristics and biological properties of metal nanoparticles

Reportedly, several factors are known to affect the biological properties of nanoparticles, including their antibacterial activity. These factors include the type of synthesis, particle size, shape, and surface charge. All these factors play vital roles in determining the effectiveness of metal nanoparticles as antibacterial agents in wound healing applications. In addition to nanoparticle properties, their behavior in biological systems is further influenced by the shape, size, and surface charge of the nanostructure. Moreover, factors related to the synthesis of nanoparticles, such as type, process parameters, and metal salts, have been shown to affect the size, shape, and morphology of nanomaterials [86].

Physical and chemical synthesis are more labor-intensive and hazardous than the biological synthesis of metal nanoparticles. Biological synthesis or biosynthesis is more attractive as it offers advantages such as high yield, solubility, and stability [87]. Therefore, the type of synthesis can indirectly affect the antibacterial properties of metal nanoparticles [88]. Several studies have shown the higher antibacterial activity of biosynthesized AgNPs than the chemically synthesized AgNPs. AgNPs biosynthesized using a guava leaf extract demonstrated significantly higher antibacterial activity, as well as stability against *E. coli*, when compared with the chemically synthesized counterpart, possibly owing to the adsorption of biomolecules on the surface of biosynthesized AgNPs. Similar findings were reported with biosynthesized ZnO nanoparticles using *C. halicacabum* leaf extract at various concentrations (0.2, 0.4, and 0.6 mL); higher antibacterial activity was observed than that with chemically synthesized ZnO nanoparticles [89]. Additionally, different methods of synthesizing metal nanoparticles impacted the size of the produced particles. The particle size of ZnO nanoparticles biosynthesized using different concentrations of *C. halicacabum* (0.2, 0.4, and 0.6 mL) was in the range of 48–62 nm with low zeta potential values. The particles were relatively smaller than those synthesized chemically using zinc nitrate and potassium hydroxide with the size of 65 nm [89]. In contrast, smaller particle sizes were obtained with chemically synthesized AuNPs using sodium citrate than those biologically synthesized using *L. rhinocerotis* extracts, locally known as Tiger Milk Mushroom [90]. Diverse findings have demonstrated that several factors contribute to

the physicochemical and biological properties of the final products, presenting a major challenge to the manufacture of metal nanoparticles, particularly for clinical use.

Monodisperse nanoparticles are an ideal characteristic for pharmaceutical and medical applications. Smaller sizes of metal nanoparticles are also desirable as they exhibit higher antibacterial activity than larger ones owing to their higher particle surface area, leading to better contact between particles and bacterial cells. Markedly small AgNPs (10–15 nm) were obtained by synthesizing them in an agar hydrogel matrix with the addition of fumaric acid. The hydrogel showed antibacterial effects against common bacterial strains causing wound infections, including *E. coli*, *S. aureus*, and *P. aeruginosa* [91]. However, smaller-sized particles can be more toxic, presenting another challenge for scientists to develop formulations or dressings selective to bacterial cells only, attempting to prevent adverse effects on human cells [88]. Additionally, particle shape plays an important role in the effectiveness of metal nanoparticles in killing bacteria or inhibiting bacterial growth. Metal nanoparticles with flower-shaped particles killed *S. aureus* more efficiently than star-shaped and spherical particles because of sharp edges presented on their surfaces, piercing and rupturing the bacterial membrane [64,92].

Chemical and/or biological coatings on the surface of metal nanoparticles could also be attributed to the cytotoxic effect. Biopolymers are commonly used to cap and stabilize metal nanoparticles. Biopolymers are polymeric biomolecules consisting of monomeric units covalently bonded to form larger molecules. The prefix “bio” refers to polymers that are synthesized from living organisms and are biodegradable [93]. For example, chitosan [94], gum arabic [95], and soluble starch [96] are used as reducing and stabilizing agents for the preparation of metal nanoparticles [97] to improve the stability and biocompatibility of metal nanoparticles [98].

4.2 Toxicity of metal nanoparticles

Despite the benefits of metal nanoparticles in wound healing, concerns regarding potential toxicity still arise, especially for long-term use. The toxicity of metal nanoparticles is influenced by numerous factors, including nanoparticle concentration, size, surface charge, surface coating, solubility, surface functionalization, distribution, mode of entry and action, growth media, exposure time, and cell type [85,88,99]. Silver dressings directly in contact with the damaged skin (a breached dermal barrier) are prone to systemic absorption, inducing toxicity [100].

In humans, a lethal dose of silver nitrate is approximately 10 g [101], and although different toxicity levels of AgNPs have been reported, the effects depend on the cell type as well as the particle size. AgNPs reportedly kill mammalian cells at concentrations as low as 2–5 µg/mL [102]. The cytotoxicity of AgNPs in human skin fibroblasts is significantly influenced by the size of nanoparticles and exposure time, with a smaller size and longer duration of exposure resulting in higher cytotoxicity [103]. Furthermore, the effect was dose-dependent, as a study showed that at the AgNP concentration range of 1–100 µM, the cytotoxicity was insignificant; at 200–300 µM, the effect gradually increased and became toxic at 1 and 2 µM after human dermal fibroblasts were exposed to AgNPs for 72 h [104]. The mechanism of AgNP cytotoxicity is still not fully understood. However, AgNP cytotoxicity is associated with the induction of mitochondrial dysfunction and the generation of ROS [105]. Smaller AgNPs generated more ROS than larger particles, killing more macrophages [106] and demonstrating the higher toxicity of smaller nanoparticles.

In general, AuNPs are less toxic than AgNPs. A study compared the cytotoxicity of AuNPs and AgNPs of the same particle size in human dermal fibroblasts. The cell proliferation rate with AuNPs was more than 90%, with no cytotoxicity observed. However, the cell proliferation rate decreased to 69% with AgNPs, with noted cytotoxicity [107]. Despite the lower toxicity of AuNPs, their cytotoxicity is dose-dependent, with lower concentrations (5 ppm) stimulating keratinocyte proliferation, but higher concentrations (>10 ppm) demonstrating toxic effects [107]. Furthermore, the particle size of AuNPs affected the secretion of proinflammatory cytokines and antibody production. Larger AuNPs (40 nm) stimulated high levels of proinflammatory cytokines, including IL-6, IL-12, and TNF-α, while smaller AuNPs (20 nm) failed to demonstrate any cytokine production. Furthermore, shape and surface charge may affect the cytotoxicity of metal nanoparticles. *In vitro*, rod-shaped AuNPs were found to be more toxic to culture cells when compared with spherical ones [108]. Conversely, the IC₅₀ of negatively charged AuNPs was >7.37 and 72 µmol/L for monkey fibroblasts (Cos-1) and red blood cells, respectively, while the IC₅₀ for positively charged AuNPs was 1 µmol/L for both cell lines, indicating the higher toxicity of positively charged AuNPs. The higher toxicity of positively charged AuNPs was associated with their ability to interact with the negatively charged cellular membrane. In another study, both charges of AuNPs were toxic to HaCaT (human keratinocytes) cell lines at a concentration of 10 µg/mL [85], demonstrating that the effect is multi-factorial. Therefore, cytotoxicity testing should be conducted for each type of metal nanoparticle, on all the cell types to be applied

clinically. A rapid approach without the use of any toxic chemicals such as the application of microwave irradiation in biosynthesizing metal nanoparticles could be a good strategy to minimize cytotoxicity of the particles. Furthermore, the combination of metal nanoparticles with certain biomaterials can be strategically utilized to reduce cytotoxicity.

5 Conclusion

This review summarizes the importance of metal nanoparticles and their combination with biomaterials. Metal nanoparticles possess superior bactericidal potential against both gram-positive and gram-negative bacteria; however, in treating diabetic wounds, solely killing the bacteria is ineffective in achieving good clinical outcomes. Complications in diabetic patients have been attributed to the delayed wound healing observed; therefore, a strategy that combines multiple modes of action in one formulation or dressing is crucial. The use of biomaterials in combination with metal nanoparticles is considered a good strategy for simultaneously combating infections and promoting diabetic wound healing. Despite strong evidence for the effectiveness of metal nanoparticle antibacterial activity, the development of bacterial resistance toward metal nanoparticles is increasing; hence, the need for combination with other antibacterial agents is paramount. Additionally, the challenges and barriers related to the manufacturing and cytotoxicity of metal nanoparticles need to be surmounted by applying advanced technology to ensure the safety and efficacy of the therapy.

Acknowledgments: The authors would like to acknowledge all the members of CDDR and CTERM for their advice and technical assistance.

Funding information: This study was supported by grants from Universiti Kebangsaan Malaysia (Research University Grant (GUP-2019-004).

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: All data generated or analysed during this study are included in this published article.

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