INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Review Article

Silver Nanoparticles Against Bacterial Biofilms In Chronic Wounds: Mechanisms, Nano–Biofilm Interactions, And Translational Perspectives

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ARTICLE INFO

Published: 29 Sept. 2026
Keywords:
Chronic wound healing, silver nanoparticles (AgNPs), bacterial biofilm, antimicrobial resistance, reactive oxygen species (ROS), and biofilm disruption.
DOI:
10.5281/zenodo.23038974

ABSTRACT

Chronic wounds present a significant health challenge in the global as they lack time, recur often and are prone to infections. Delayed healing of wound is attributed to one of the most important factors, bacterial biofilms which are more susceptible to the conventional antibiotics and host immune reactions. The significant advancements of nanotechnology and nanoparticles in particular, particularly silver nanoparticles (AgNPs) have gained a greater focus as an emerging treatment approach for disruption of biofilms and enhancing wounds. AgNPs have strong potent antimicrobial and antibiofilm effects by a variety of mechanisms, such as disruption of cell membranes, production of reactive oxygen species, release of silver ion and quorum sensing inactivation. Moreover, they have peculiar physicochemical properties, like high surface area and more reactivity, which are allowing them to interact with the microbial cells and biofilm matrices. Some of the synthesis methods like chemical, physical and green methods have been developed with an objective of maximizing the biomedical application of these methods. Recently, new representations for wound dressings, such as hydrogels and nanofibers in smart wound dressings are also being developed using AgNPs for providing prolonged treatment of wounds and targeting them to a specific area. Despite their great potential, the clinical translation has to be concerned with issues associated with toxicity, pharmacokinetics, and regulatory issues. All these silver nanoparticle-based treatments could be regarded as the latest promising method in wound infections control and the healing of chronic wounds

INTRODUCTION

Chronic wounds are one of the most important health challenges in the world and include the most common DLFUs (diabetic foot ulcers), ulcers of

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

the lower legs and pressure ulcers. Unlike direct wounds, chronic wounds are chronic inflammatory and not going through normal stages of healing and decreases the rate of repair of tissues, inviting infection [1, 2]. Microbial biofilms are among the leading factors that are associated with delayed healing of chronic wounds. Biofilms are micro-organisms formations that are enclosed by an extracellular polymeric substance (EPS) in order to cushion bacteria against the host immune as well as antimicrobial agents [3]. The most prevalent images of biofilm forming pathogens on chronic wounds that is largely involved in the formation of chronic infections and slow healing of the wound are Staphylococcus aureus and Pseudomonas aeruginosa [3, 4]. The biofilm bacteria are less susceptible to the antibiotics than are planktonic bacteria and more antibiotics would be required to eradicate them. Biofilm mediated antimicrobial resistance is a major problem in the management of chronic wounds and has resulted in the global rise of resistance to many antimicrobial agents, a condition known as “multi-drug resistance” [5]. These infections do not tend to be eliminated by the traditional antimicrobial treatments since the biofilm matrix is not readily accessible, as well as the active, metabolically dormant types of bacterial cells [4, 6]. In the recent years, though, nanotechnology has emerged as one of the possible solutions to fill in those gaps in the therapeutic successes of wound management infections. Some of the physicochemical characteristics of nanomaterials include high surface area, high reactivity (enhanced) and better cell interaction in microorganisms [7]. Silver nanoparticles (AgNPs) are one of the nanomaterials that exerted great apprehension due to its vast usage in antimicrobial and antibiofilm factors. The antibacterial activities of AgNPs might be related to several activities including destabilization of bacterial cell membrane, generation of reactive oxygen species (ROS), and effects on the bacteria's DNA replication and protein synthesis [8, 9]. These multi-target properties render silver nanoparticles very active in multi-drugresistant bacteria, biofilm associated infection, and this is what offers the silver nanoparticles an opportunity as new therapeutic formulations in the management of chronic wounds.

Epidemiology and Burden of Chronic Wounds

Chronic wounds that are related to delay in healing and a high degree of reoccurrence are one of the prevailing world health concerns. The low prevalence of chronic wounds globally is estimated to be approximately 2.21 per 1000 population and prevalence rate of chronic leg ulcer is approximated as approximately 1.51 per 1000 population, and thus they dominate the globe [10]. A prevalence of 1.89 per 1000 population is reported by the recently completed community-based studies in India and the prevalence in the rural population exceeded that of the urban population [11]. The epidemiology of non-healing wounds is closely related to aging, diabetes mellitus, vascular diseases, and obesity, all of which are becoming epidemic in the world; thus, the epidemic of non-healing wounds is rising [12]. The cost of chronic wound burden is huge to patients and system. The injuries result in profound diminution of quality of life due to pain, infection, the high level of immobility, and social exclusion [11, 12]. Chronic wounds are costly in terms of economy as they cause long-term treatment, repeated visits to a hospital, and long-term care. To explain, chronic wounds are associated with billions of healthcare expenditures, and their incidence also involves millions of individuals in the United States alone [13]. Besides, infection and amputation are most often common and associated particularly with diabetic patients and therefore lead to morbidity and high medical costs. Cumulatively, chronic wounds have been dubbed as a silent epidemic due

to the elevated prevalence, under-reporting as well as high clinical and economic cost [13].

Bacterial Biofilms in Chronic Wounds

3.1 Structure and Composition

Biofilms are highly complicated communities of microbes that are enclosed by self-produced extracellular polymeric substance (EPS) network. The polysaccharides, proteins, lipids, and extracellular DNA are the main components in this matrix; these components provide the bacteria cells with structural stability and protection. The EPS matrix facilitates the adhesion of bacteria to the tissues of the wound and shields them against environmental forces, immune reactions of the host and anti-microbial therapy [14,15]. In the biofilm structure, bacteria have varying metabolic conditions depending on the location in the biofilm. The cells in the outer layers are active and exposed to oxygen and nutrients, besides, the bacteria in the deeper layers are usually in a dormant state with a low metabolic rate. This structural heterogeneity helps to raise the levels of antibiotic resistance and reinfection [15,16]. Also, biofilms can harbour several bacteria species that constitute polymicrobial communities, which can synergistically survive and be more virulent in chronic wounds [17].

3.2 Common Pathogens

Chronic wound biofilms are commonly polymicrobial, that is, they consist of aerobic and anaerobic bacteria. Staphylococcus aureus and Pseudomonas aeruginosa are the most common microorganisms that are isolated in the chronically infected wounds [14,17]. These bacteria are characterized by high-capacity to create biofilms, their resistance to a large number of antimicrobial agents. Other organisms that are commonly detected are Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli, Proteus species and coagulase negative staphylococci [17,18]. The existence of several species of bacteria present and interact within the biofilm milieu in most chronic wounds leads to increase *in virulence* and antimicrobial resistance. The coexistence of S. aureus and P. aeruginosa is of special interest, where the interaction of the two can lead to the development and maintenance of biofilm in chronic wounds [18].

3.3 Impact on Wound Healing

Bacterial biofilm presence has an extensive influence during wound healing. Biofilms disrupt normal wound healing by perpetuating chronic inflammation, suppressing epithelial cell migration, and suppressing tissue regeneration [15, 19]. The EPS matrix is an obstacle that restricts the entry of antimicrobial agents, and the infections are difficult to treat with the usual antibiotics. More so, the bacteria in biofilms may also be 100–1000 times more resistant to antibiotics than planktonic bacteria [16, 19]. This heightened resistance is caused by a number of factors, such as decreased metabolic activity of bacteria in the deeper layers of biofilms, presence of per sister cells, and a protective EPS matrix. As a result, biofilm-associated infections tend to cause chronic inflammation and slow tissue and wound healing. Biofilms have thus been targeted as one of the most important mechanisms in enhancing chronic wound management and therapeutic outcomes [19].

Silver Nanoparticle: Characteristics and Preparation.

4.1 Physicochemical Properties

Silver nanoparticles (AgNPs) are multifunctional NANPs into which special physicochemical properties are given, which help in obtaining a very high antimicrobial activity of AgNPs. Due to their small dimensions, large surface to volume ratio, and their surface energy, their interaction with the microbial cells and biological systems gets increased. The size decrease causes an increase in the surface area of the antimicrobial nanoparticles and increases their antimicrobial activity, i.e., effect on the bacterial membranes

[20, 21]. One of the most important properties of the AgNPs is the ability to release silver ions (Ag ions) slowly. These ions disrupt the DNA, enzymes, and protein of microorganisms and cause the disruption of cellular functions that eventually leads to cell death. The released silver ions also have the potential to promote the generation of Reactive Oxygen Species (ROS) resulting in oxidative stress and hence some damages to proteins, lipids and nucleic acid contents of cells [21,22]. In addition, the physicochemical properties of AgNPs (size, shape, surface charge, crystallinity and morphology) also play significant roles in influencing biological and biochemical activity and stability. The smaller the nanosize the more antimicrobial, due to higher reactivity and increase in penetration to the bacterial cell [22,23]

4.2 Methods of Synthesis

There are various methods of synthesizing silver nanoparticles, which are broadly categorized as chemical, physical and biological (green) synthesis methods [24].

Chemical Methods

Among the most popular ways of AgNPs production is chemical synthesis. Typically, silver salts such as silver nitrate (AgNO3) are reduced through chemical reducing agents such as sodium borohydride, citrate or hydrazine in this method. To control the size, aggregation and stability of the nanoparticles, usually stabilizing agents or surfactants are added to them. The chemical methods are suitable in the strict control of the size and morphology of the particles, but can use toxic chemicals limiting biomedical applications [24,25].

Physical Methods

Methods in physical synthesis are synthetic techniques such as evaporation-condensation, laser ablation and ball milling process. Such procedures tend to give very pure nanoparticles without any chemical contaminants. However, they have high operating cost and require a high amount of energy and particular operating conditions to be less competitive in large-scale production [24].

Green (Biological) Synthesis

A method of silver nanoparticles manufacturing that attracts interest due to its environmental friendly and sustainable approach is green synthesis. This is a method that uses any biological material such as plant extracts, bacteria, fungi or algae as reducing and stabilizing agents. The extracts from the plants contain phytochemicals which act as reducing and stabilizing agents simultaneously, by converting the silver ions to nanoparticles. It is a beneficial approach because of its eco-friendliness, low cost and use in the field of biomedicine by green synthesis [26, 27].

Mechanisms of Antibiofilm Activity.

1.Destruction of the Bacterial Cell Membrane

The mode of action of silver nanoparticles (AgNPs) comprises potent antibacterial impacts of bacteria, purposeful to assault bacterial cell membrane that is underlying to cell structure and physiological functions. This allows the surface of the bacterial cells to be readily destabilized by the interaction of AgNPs with the constituents of the membrane which have negative charges like phospholipids, lipopolysaccharides, teichoic acids, leading to leakage of the ions, proteins, ATP, and nucleic acids in the cells, which causes osmotic imbalance, resulting in cell lysis. Additionally, AgNPs generate oxidative stress and cause lipid peroxidation, and inhibit the activity of membrane-bound enzymes in respiration, leading to chaos in the electron transport chain and the production of ATP is disrupted [31, 33]. This is a multi-targeted membrane damage that is fast and effective against Gram-positives and Gram-negatives as well as inhibiting initial adhesion and biofilm formation [29,34].

2. Silver Ion (Ag^+) Release

AgNPs are used as the second messenger of the continual release of bioactive silver ions (Ag+) which have antimicrobial effects. Environmental conditions like the size of a nanoparticle, small particles release ions more efficiently, the pH, and the level of oxygen for the release of Ag + also depend [28,32]. Upon release Ag + and binds ATP denaturation of enzymes and inhibition of key metabolic functions such as cellular respiration by binding tightly to thiol (-SH) groups within proteins and enzymes [31,33]. Besides, an interaction between the silver ions and the nucleic acids and other mediators causes a disruption in the replication and transcription of DNA, disturbs mechanisms of the membrane permeability and ion transport that cause an imbalance in the cell [30, 32]. The release continuous to the antimicrobial activity is ensured in the case of infections related to biofilms, which requires long-term activity throughout the infection [32, 37].

3. Generation of Reactive Oxygen Species (ROS)

Silver nanoparticles result in reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, which are central to the action in antibacterial effect. Such ROS induce oxidative stress of bacterial cells that damages biomolecules which are extremely important to this cell such as lipids, proteins and nucleic acids [30, 33]. The damage to membrane integrity occurs due to the process of lipid peroxidation and enzyme and metabolic dysfunction as a result of the oxidation of proteins. Damage to DNA by ROS (strand breaks, base damage) upsets replication and transcription processes and accretion of oxidative stress is ultimately lethal to the bacterium [32, 33]. ROS also add to the destruction of extracellular polymeric matrix in biofilms which increases antimicrobial penetration and biofilm disruption [34, 37].

4. Inhibition of Quorum Sensing

Quorum sensing (QS) is a bacterial biofilm-forming and its communication system that controls virulence, adhesion and biofilm-forming genes expression; via autoinducer signalling molecules. AgNPs inhibit QS pathways through disruption of synthesis and activity of this signaling and disrupting the receptor binding and signal transduction pathways [29,35]. This inhibits the expression of the genes controlled by the QS and the coordinated activity of the bacteria necessary to develop into biofilms. In addition, AgNPs may interfere with signaling molecules, such as acyl-homoserine lactones, that cause insufficiency in signaling approaches between bacterial cells and the outputs of virulence factors [34, 35]. As a result of such interaction, antimicrobial resistance is promoted and bacterial pathogenicity is reduced particularly in chronic biofilm associated infections [32, 37].

5. EPS Matrix Degradation

The structural component of biofilms is referred to as the extracellular polymeric substance (EPS) matrix that gives resistance to the environmental stress and antimicrobials. The mechanism by which AgNPs cause this matrix is through inhibition of its major constituents such as polysaccharides, proteins and extracellular DNA and enhancing it to degrade by oxidative means [34, 35]. The consequence of such a process is a fragmentation of the biofilm structure and massive permeability and penetrability of antimicrobial agents. This makes the embedded bacteria to experience greater exposure to environmental stress and immune functions that can be eliminated and enhance the outcome of the treatment of chronic infections [32, 37].

6. DNA Damage and Inhibition of Replication

Silver nanoparticles enter the bacteria cells and interact directly with the DNA molecules that are unable to resist the structure and condense on the surfaces containing pieces of them [28, 30]. The consequences of such changes interfere with the

replication process and transcription process of DNA and hence prevent cell division and reproduction in bacteria. In addition, AgNPs produce ROS, which induces the oxidative stress of DNA, as well as resulting in genetic instability and cell dysfunction [31, 33]. This genotoxic impact adds a significant contribution to killing of bacterial cells and preventing the expression of virulence and resistance genes that prevents the biofilm sustenance [29, 34].

7. Disruption of Protein Synthesis

AgNPs disrupt bacterial protein synthesis by binding with ribosomal subunits and interrupting the translation process which does not produce necessary proteins necessary to maintain cellular metabolism and survival [30, 31]. Silver ions are also known to react with enzymes and structural proteins and denature them and lead to functional loss; this further interferes again with cellular metabolism [32, 33]. Due to this, there is a prevention of bacterial growth, a decrease in biofilm growth, and an increase in susceptibility to antimicrobial agents [34, 37].

8. Modulation of Signal Transduction Pathways

Silver nanoparticles can also modify bacterial signaling through interruption of the phosphorylation mechanism and control proteins related to cellular communication and response to stress [33, 36]. This disruption disturbs the patterns of gene expression, such as more virulence and biofilm formation patterns, and compromises bacterial adaptation to environmental changes [29,36]. As a result, the survival and pathogenicity of bacteria are minimized, and the broad-spectrum intracellular action of AgNPs is emphasized [33, 36].

9. Synergistic Interaction with Antibiotics

AgNPs have powerful synergistic actions in combination with traditional antibiotics via enhancing the level of membrane permeability and simplifying the entry of drugs to bacteria cells. They also prevent resistance mechanisms including efflux pumps and enzymatic degradation which results in elevation of intracellular concentration of antibiotics and increase of antibacterial activity [36, 37]. This combination method especially works well against the multidrug-resistant bacteria and the biofilm-related infections, lowering the dose of antibiotics administered and increasing treatment outcomes [32, 37].

10. Anti-Adhesion Properties

Silver nanoparticles also inhibit the adhesion of bacteria by modulating the properties of the surface, including charge, hydrophobicity and roughness which prevents the initial sticking of the bacteria to the surfaces as well [29,35]. They also disrupt the adhesion-related proteins and receptor interactions and inhibit colonization and early biofilm formation [34, 35]. This antistickiness is especially in use in a medical device and wound care, where inhibiting bacteria to attach to the surface provides major benefits by reducing the likelihood of infection and better healing [37].

Pharmacokinetics and Biodistribution of Silver Nanoparticles (AgNPs)

1.Pharmacokinetics of Silver Nanoparticles

The absorption and distribution (ADME) are the basis of pharmacokinetics of silver nanoparticles (AgNPs) and highly depend on the physicochemical properties of the nanoparticles i.e. size of the particles, their morphology and surface functionalisation. AgNPs can enter the body through a range of different routes, i.e. oral, inhalational, dermal, and intravenous exposures with absorption efficacy depending on route and nanoparticle characteristics. Smaller nanoparticles are also more absorbent and bioavailable because they possess more surface area and are able to found more penetration to biological membranes and cellular barriers [38, 39]. After entry into the systemic circulation, AgNPs quickly react with plasma proteins to create a “protein corona in the

surface that can have an important effect on the biological identity, their stability, and uptake by cells [39,40]. Nevertheless, as opposed to classical medicaments, AgNPs do not undergo drug metabolism but, instead, undergo physico-chemical reactions, such as oxidative dissolution, which in turn releases silver ions (Ag3), a source of treatment and toxicity [40, 41]. It is largely excreted via the hepatobiliary and renal routes although the incomplete clearance and sluggish elimination Kinetics can result in systemic retention and tissue accumulation especially when it comes to a repeated or chronic exposure [39, 41].

2. Biodistribution of Silver Nanoparticles

Following systemic absorption, AgNPs are widely distributed in the body with a vectors toward the reticuloendothelial system (RES)-related organs in the liver and the spleen most prominently due to their action in the elimination of the circulating nanoparticles [39,41]. Liver is the best location where deposition can occur because of the good blood circulation and detoxification capacity of the liver and the spleen is another filtration organ that is secondary. It has also been found out that AgNPs can also be deposited to the lungs, kidney, brain and even in the gastrointestinal tract depending on the route of exposure and the nature of the nanoparticles [38, 42]. Physiological barriers such as the blood-brain barrier and the placental barrier are overcome by smaller nanoparticles (smaller than 1020 nm). This is questionable with respect to neurotoxicity and developmental outcome. Recently, *in vivo* studies (2023) have shown that oral/ING (oral dextose) administration of AgNPs could reach intestinal tissue, and activate local immune-inflammatory responses, reflecting engagement of other organ systems [42]. Another important modification in many cases is the biodistribution, as PEGylated or coated nanoparticles exhibit a longer circulation time, and the biodistribution can differ upon the modification as well as uptake by macrophages, which is lower for PEGylated nanoparticles than for non-coated and coated SNPs [40, 43].

3. Metabolism and Biotransformation

The oxidative dissolution is considered as the primary method for bio transforming the AgNPs in the formation of a slow elution process of the Ag + ions, which are regarded as the primary active species resulting in biological properties. Such ion release can be done under extra-cellular biological fluids and intracellularly after the incorporation of the nanoparticles [40, 41]. The AgNPs are able to accumulate in endosomes and lysosomes in the cells. Dissolution and release ions is boosted by the acidic quality. Silver ions released can bind to bio molecules such as proteins, enzymes, and nucleic acid and alter the cellular functions thereby causing antimicrobial and cytotoxic effects [41, 43]. Besides, the AgNPs can also be complexed with the sulphur- or selenium-containing biomolecule to affect its persistence and toxicity in tissues. These processes of transformation are critical issues in the description of the pharmacological and toxicological nomenclature of AgNPs [39,41].

4. Excretion and Clearance

The excretion of the AgNPs and its ionic forms occurs mainly through hepatobiliary excretion through faces and to a smaller extent through excretion through renal excretion through urine. The clearance rate is however comparatively slow relative to traditional drugs resulting in long biological half-life and bioaccumulation [38, 41]. In clearing kinetics, size of the particle, coating and aggregation condition of the particle play a critical role. The filter is small nanoparticles that are filtered by the kidneys, and large or aggregates that remain in the rest of the organs, like liver and spleen [39,43]. The prolonged exposure studies have proven that overall clearance may fail to capture tissue accumulation and may raise the issue of long-term toxicity and systemic effects

[38, 42]. 5. Factors effecting pharmacokinetics and biodistribution.

5. Factors effecting pharmacokinetics and biodistribution.

Some of the factors that influence critically the pharmacokinetics and biodistribution of AgNPs include particle size, shape, surface charge, coating materials, dose and route of administration among others. Cellular size, size penetration, penetration and dissemination or large particle size reasons for easier entrapment in the Reticuloendothelial system can be used to describe the small sizes of nanoparticles [38, 39]. The positively charged nanoparticles are more effective in interacting with biological membranes due to surface charge, which is vital since positively charged nanoparticles have high uptake with may also result in high levels of cytotoxicity [29,43]. Use of a polymer like polyethylene glycol (PEG) can help to stabilize nanoparticles, extend blood circulation times, and minimize immunogenicity. Here, state of aggregation, solubility and rate of release of silver ions are also extremely significant in affecting the biological interactions and toxicity. Knowledge of these factors will be indispensable for the development of the AgNP based drug delivery systems that can be effective and reliable in clinical applications [40, 41].

Advanced Wound Dressings

1. Hydrogel Dressings

Hydrogel dressings consist of hydrophillyl networks of polymer that has the ability to absorb as well as hold massive quantities of water thus providing a moist wound condition which is very vital in healing. They promote autolytic debridement by softening necrotic tissue and enzymatic soft tissue degradation which eliminates the necessity of surgical intervention. Hydrogels also offer cooling effect which helps in pain management and inflammation as well hence suitability in burn wounds and dry necrotic wounds. Also, these dressings may be used to deliver bioactive agents like antibiotics, growth factors, and nanoparticles, and provide a controlled delivery of drugs to the wound site [44, 45].

2. Hydrocolloid Dressings

Hydrocolloid dressings are made out of gel-forming substances that include carboxymethyl cellulose, gelatin, and pectin, which react with the presence of exudate secreted into the wound to produce a viscous gel layer over the wound. This gel can keep the environment wet and this will increase the rate of migration of epithelial cells and their proliferation thus making the wounds mend faster. Hydrocolloids are also occlusive in nature which gives them a protective effect against bacterial contamination and external irritants. These dressings are especially useful in low and moderately exuding wounds and they are associated with increased wear time which eliminates the frequency of dressing changes and enhances patient compliance [45, 46].

3. Foam Dressings

Foam dressings are normally manufactured using polyurethane products and they are meant to absorb moderate to high exudates and at the same time a moist healing environment. They offer thermal insulation and mechanical cushioning that offer protection of the wound against external trauma and pain alleviation effects. The foams dressings also prevent the maceration of the surrounding tissues as the surplus moisture is controlled. Their high absorbency and protection make them great in chronic wounds which include pressure ulcers and diabetic foot ulcers [44, 47].

4. Alginate Dressings

Alginate dressings consist of natural polysaccharides in the brown seaweed, and they are constituted of calcium or sodium alginate fibers. These fibers further exchange ions with the wound exudate to create a gel-like form which helps to preserve a moist environment and

haemostasis. This introduces the option of using alginate dressings especially in bleeding wounds and wounds undergoing excessive exudates. They also aid in cellular functioning and tissue recuperation and aid in accelerated healing of wounds [46, 48].

5. Film Dressings

Film dressings consist of thin transparent semi-permeable polyurethane membranes through which oxygen and water vapor may pass but not bacteria and other contaminants. These dressings are fitting to superficial wounds, and in most cases they are used as a secondary dressing. Their transparency allows monitoring the wound constantly without causing any disruption to the healing process, which minimizes the risk of infecting and increases clinical results [44, 45].

6. Nanoparticle-Based Dressings

Wound dressing made of nanoparticles include metallic or polymeric nanoparticles including silver, gold, and zinc oxide to offer greater antimicrobial and antibiofilm functionality. AgNPs have a specific advantage because of the possibility to destroy the cell membranes of the bacteria, to produce the reactive oxygen species and block the formation of biofilms. Such dressings also facilitate the sustained and regulated delivery of therapeutic agents thereby enhancing the efficacy of treatment in chronic and infected wounds. The nanotechnology-based systems are already a big breakthrough in wound care because they are multifunctional in nature [47, 49].

7. Bioactive and Smart Dressings.

Bioactive dressings are used as active forms of wound healing, with growth factors, collagen, chitosan, and extracellular matrix components being included in the dressings. These materials promote tissue regeneration, angiogenesis and proliferation of cells. A higher technology is smart dressings, which are able to react to variations of the wound conditions (pH, temperature, or infection) and deliver drugs in a controlled fashion. Such responsive systems make it possible to treat a wound in a personalized way and enhance the healing process of chronic wounds [48, 50].

8. Antimicrobial Dressings

Dressings impregnated with either silver, iodine, honey or antibiotics are used to inhibit or manage the wound site. They are especially useful in chronic wounds wherein the development of biofilm and colonization by bacteria prevents healing. The popularity of silver based dressings is explained by their wide spectrum of antimicrobial action, and the lack of resistance that might occur. Such dressings are used to reduce the microbial load, prevent the spread of infection, and achieve a faster recovery [49, 51].

Comparative Nanotechnology Approaches in Wound Healing

1.Metallic Nanoparticles (AgNPs, AuNPs, ZnO NPs)

One of the most-studied ways of nanotechnology to heal wounds antimicrobial and bioactive, is the use of nanoparticles of metals. Because of these mechanisms, silver nanoparticle (AgNP) has a great potential to affect multidrug-resistant pathogens (MDRPs) such as membrane disruption and release of silver ions (Ag^{+}) and reactive oxygen species (ROS) [52, 53]. Furthermore, the AgNPs are permeable to biofilms and break the skeleton of the extracellular polymeric substance (EPS), thereby further improving the penetration and activity of the biofilms [52, 54]. The AuNPs, on the other hand, have a low toxic effect and promote the healing of wounds because of their ability to inhibit the proliferation of fibroblasts, collagen deposition and angiogenesis by regulating growth factor signaling pathways [53, 55]. Zinc oxide nan particles (ZnO NPs) are proven to possess antimicrobial activity, safety profile, advantage in epithelialization and anti-inflammatory response, at the same time exhibiting ROS mediated bacterial inhibition balance which is beneficial for antimicrobial

activity in cosmetics [54, 56]. Depending on the size, concentration, and duration of the exposure period the metallic nanoparticles might have beneficial properties or turn out to be cytotoxic, oxidatively stressed and accumulated in tissue [52, 56].

2. Polymeric Nanoparticles

The polymers of the nanoparticles are mostly chitosan, PLGA and alginate, which are used in the controlled and targeted delivery of drugs in wound healing. This results in improved stability of drugs or inhibit the enzymatic desiccation of the bioactive agents in the vicinity of the wound or to give a sustained drug delivery at the wound site [53, 57]. Another type of nanoparticles includes those made from chitosan that have intrinsic antimicrobial and haemostatic properties and contribute to the healing process as well [57, 58]. Polymers are less toxic and biocompatible than metals, so polymer nanoparticles can be used to treat chronic wounds over an extended period of time [53, 57]. Moreover, they can also be engineered to release the drug in response to environmental signals (pH or temperature) in the vicinity, which causes an increased therapeutic effect in the treatment of the target organ or disease [57].

3. Lipid-Based Nanocarriers (Liposomes, Solid Lipid Nanoparticles)

Among the lipid based nanocarriers, which showed a high percentage of the two most popular categories (liposomes for biocompatibility and encapsulation ability and solid lipid nanoparticles for encapsulation ability), the compounds to be mentioned are liposomes and solid lipid nanoparticles. They are more effective in penetrating the drug into the wound tissue due to interactions with the biological membranes, and their intracellular transport [57, 59]. The potential fusion on the cell membrane and the additional stabilization of the physical environment and of changes of the release properties achieved by SLNs [59] are also beneficial. Although the systems based on lipids are more versatile (in drug encapsulating) than polymeric nanoparticles, they may have a range of disadvantages such as instability, leakage of the encapsulated drug, and short shelf life [57, 59].

4. Nanofibers (Electrospun Systems)

Electrospun nanofibers are similar to the extracellular matrix (ECM) that provides a structure that aids in cell adhesion, cell growth, or tissue repair. They possess high surface area-volume ratios, porosity; are easily exchangeable for oxygen, nutrients and exudates; and effectively manage them [52,60]. Nanofibers can be functionalized to deliver sustainably and locally antimicrobial agents, nanoparticles and growth factors [60]. Nanofibers also offer better protection and stimulation of the biology as compared to other nanotechnology methods, hence being extremely useful in chronic wound management and tissue engineering fields [60].

5. Dendrimers

Dendrimers are specially-designed polymers with many active functional groups and are highly branched, allowing drugs to be easily packed into them and delivered to targeted sites. The design has enabled the efficient control of mass loading, mass release, and precision targeting [57, 61]. There is also the possibility that dendrimers can react with bacterial membranes with a combination of therapeutic agents and enhance antimicrobial activity [61]. However, these are not yet clinically relevant, largely as a result of their complicated synthetic processes [57, 61] and high costs of production, as well as the potential to become cytotoxic following the presence of positively charged surfaces.

6. Carbon Based Nanomaterials (Graphene, Carbon Nanotubes)

The antimicrobial activity of carbon nanomaterials (carbon nanotubes (CNTs) and graphene oxide (GO)) is high and so is also the mechanical

properties. These materials have a direct effect on the membranes of bacteria and also create oxidative stress for bacteria resulting in bacterial cell death [59, 62]. They also improve mechanical strength and durability of the wound dressing which leads to wound dressing's ability to be used on load-bearing or highly movable wound site [62]. The toxic and biodegradation issues, and the potential inflammatory response with long-term use, however, restrict their use during clinical practice [59, 62]

7. Biohybrid and Smart Nanomaterials

The biohybrid and smart nanomaterials have recently been developed on the basis of incorporating the biological components with synthetic nanostructures in order to foster therapeutic activity. These platforms are able to identify local signs on the wounds, such as variation in pH level and temperature and infection, and deliver certain drugs localized in an on-demand mode [52, 53]. The nanomaterials are smart and can enhance pharmacokinetics, diminish the system toxicity and increase the targeted delivery of medicines and treatment changes [53, 63] Visualised in comparison with the traditional approach of Nanotechnology, the systems can be optimized to provide a custom approach and the future of wound healing technologies [63].

***In Vivo and In Vitro* Studies**

The therapeutic evaluation of the formulations of silver nanoparticles (AgNP) for wound healing is well established in wound animal models, but more frequently *in vitro* biofilm models. Through the use of *in vitro* studies, antimicrobial (antibiofilm) activity of AgNPs against some wound pathogens such as S. aureus and P. aeruginosa has been demonstrated, which is attributed to its influence on the behaviours of the cell membrane, formation of ROS, and inhibition of biofilm formation. These types of systems yield huge reductions in the microplanktons zooplankton, in the growth and biofilm biomass in the laboratory models [64]. *In vivo* found studies using animal models of wounds (like rat and mouse excision wounds) showed that topical AgNP formulations had a marked positive impact on the contraction of wounds, deposition of collagen and the re-epithelialization of the wound compared to the untreated control animals. Besides, AgNP-based dressings result in reduced levels of bacterial load and promote tissue regeneration, hence could be useful for infected and chronic wounds treatment [65, 66].

Safety and Toxicity considerations

Although silver is known to have a strong antimicrobial effect and nanoparticles (AgNPs) are also capable of killing microorganisms, both of these factors could result in the development of skin cell cytotoxicity since silver can be present in high amount. The dose-dependency of AgNPs toxicity was established and it was shown that their ability to reduce the cell viability decreases with increasing doses. Hence the controlled-release systems and the optimized doses are available to improve the biocompatibility and cellular toxicity of the wound-healing systems [67, 68].

Clinical Translation and Regulatory Aspects

1.Clinical Translation of Nanotechnology-Based Wound Therapies

The development of an innovative product that is safe and effective therapeutic product from the lab to final human use is a clinical translation of nanotechnology based wound healing systems. *In vivo* research studies have also demonstrated the antimicrobial, anti-inflammatory and regenerative properties of nanomaterials, such as silver nanoparticles (SNPs), polymeric nanoparticles and nanofiber-based dressings [69, 70]. The issues, however, that have to be addressed to be able to translate successfully are scalability, repeatability and longterm safety. One of its drawbacks is the lack of homology between the *in vitro* and *in vivo* performance, including the behavior of nanoparticles in the coronas of proteins, the

immune response and in the microenvironment of a wound [70, 71]. Additionally, the clinical efficacy would have to be proven by a properly-conducted randomized controlled trial to demonstrate the therapeutic superiority over the use of the conventional wound care agent [71].

2. Considerations for pharmacokinetics and biodistribution

To be able to translate nanomaterials to clinical applications, it is important to have an understanding of pharmacokinetics and biodistribution. Resistance of nanoparticles against penetration in the intracellular space via the skin barrier may be local or systemic depending on the size, surface charge and composition of the nanoparticles [72]. It has been demonstrated that even if the nanoparticles are smaller they are able to enter into systemic circulation and can lodge in other organs like liver, spleen, kidney, raising long term toxicity concerns [72, 73]. Surface modification, such as PEGylation and functionalization can reduce circulation time and decrease immune wipe out which can improve therapy performance [73]. Assessments should then be made on Absorption, Distribution, Metabolism and Excretion (ADME) to make the regimens safe and as effective as possible [72].

3. Safety and toxicological issues

Safety evaluation is one of the most critical process in wound products which enable nanotechnologies to be marketed. Depending on the physicochemical properties, some of the effects that nanoparticles can produce are cytotoxic, oxidative stress, inflammatory, and genotoxic effects [73, 74]. The toxicity and accumulation of silver nanoparticles and other metal based nanoparticles have been found to be dose dependent with respect to the tissues [73]. As a result, it needs to be the subject of extensive animal toxicity tests before it can be clinically used, including dermal, acute and chronic toxicity tests. One of the challenges placed on the toxicity tests is that there is no consistent protocol for performing these tests yet as there are many different types of nanomaterials, there are no universal standardized guidelines [74].

4. Regulatory frameworks and guidelines

Clinically approved wound dressings would be approved by the United States Food and Drug Administration (FDA), the European Medicines Agency (EMA) and others with their own regulatory agency. The products made with nanos are categorized based on the end use purpose, for instance, drug, medical device or combination product [75]. The medical devices world in terms of "nanoparticle dressings" is regulated from the medical equipment world in terms of "wound healing setting" as the dressings should meet the standard of safety, effectiveness and quality of the preparation. Regulators are interested in the consistency and safety measures of products are called by Excellent Manufacturing Practice (GMP), quality-by-design (QbD), and the measurement of risk assessment of the product [75, 76].

5. Challenges in Standardization and Scale-Up

The difficulty in synthesizing, characterizing and evaluating the efficacy of nanoparticles in a standardizable method is one of the obstacles to clinical applications of translation. Particle size, shape and surface modification are factors that can be key particle parameters that influence the biological performance and reproducibility [70, 76]. The other major concern is to increase the scale of production from industry to laboratory without affecting the quality and functions. High tech manufacturing processes and controls to simplify processing to achieve homogeneity and assurance of batch to batch compliance as well [76] are required.

6.Clinical Trials and Marketed Products

A number of nanotechnologies wound care products are also newly available commercially, such as silver nanoparticle dressings which are

extensively used in practice. The antimicrobial activities of the products are greater than that of the conventional dressings, and the time to heal wounds is less than that of the conventional dressings [69, 71]. Unfortunately, the amount of large-scale clinical trials in which novel nanomaterials are tested is small. Long term clinical data are required for establishing long-term safety, cost efficacy as well as comparative efficacy [71].

7. Ethical and environmental considerations

Further worries are raised on the moral and environmental aspect of using nanotechnology in clinical practice. The possibility of accumulating nanoparticles in the organism and releasing them into the environment as well as the effects of ecological consequences over time [74, 76]. Ethically patient safety is related to informed consent and equitable access to high tech treatment. Some of the concerns addressed by the regulatory bodies involve the sustainable development and environmental risk assessment in order to minimize the involved risks of the nanomaterials [76].

Challenges and Limitations

Wound healing – Using nanotechnology has a high therapeutic value, but there are several challenges and limitations that hinder its application as a general wound therapeutic approach. The major concerns include toxicity and biocompatibility since nanoparticles, particularly metallic nanoparticles like silver nanoparticles, can induce oxidative stress, inflammation or due to less biocompatibility and cytotoxic effects due to their size, concentration, and exposure time [77, 78]. In addition, there is insufficient information on the bioaccumulation of nanomaterials in critical organs such as liver or kidney; this makes recommending the safety of the long-term use of nanomaterials questionable, because of the potential for chronic toxicity [78, 79]. The second one is the lack of universal protocols in the synthesis and the characterization of the nanoparticles itself resulting in variability in the physicochemical properties of different nanoparticles and different biological response from different research [79, 80]. There are also a number of other challenges associated with inserting into this laboratory to the industry production process, with the need for batch-to-batch consistency to meet regulatory requirements making the industrial process even more costly and challenging [80, 81]. Furthermore, there are no well-defined regulations/approval protocols for nanomaterials and clinical translation of nanomaterials has been delayed due to the requirement of submitting extensive data related to the safety and effectiveness of nanomaterials [81]. Finally, any factor that could cause nanomaterials to undergo degradation in storage, such as aggregation, can impact the performance of the nanomaterials. Furthermore, it can take a longer time for the immune system to clear the infection if only a small amount of tissue is penetrated into deep wound.Also, slow clearance of the infection and a slower treatment efficacy can result in a small penetration of deep wound tissue and quick clearance by the immune system [79, 81]. Despite these advantages, their use today is restricted to daily clinical practice due to lack of large clinical studies, high production costs, limited availability, and ethical/environmental concerns regarding disposal of the nanoparticles along with potential long term effects [78, 80].

Future Perspectives

The future potential for nanotechnology in wound healing is that it will develop into multifunctional, smart and customized treatment systems which are capable of treating the complexity of chronic wounds. Various novel nano constructor materials are being designed to respond to the stimuli of some wound conditions, such as pH, temperature and infection, which they can release drugs upon request and controlled release to obtain better

therapeutic efficiency and minimize the side effects [82, 83]. It is expected that when nanotechnology is integrated with biosensors and wearable devices, the real-time physical wound conditions, such as infection state and healing process, will be monitored, which will enable precision medicine approaches [83, 84]. Additionally, emerging interest exists with regards to biohybrid nanomaterials that combine synthetic nanoparticles with biological molecules such as growth factors, peptides, stem cells among others, which can stimulate tissue regeneration, angiogenesis and accelerated wound healing [84, 85]. New technologies and advances in nanofabrication, including 3D printing and electrospinning would make it possible to produce patient-specific wound dressings in the future. Additionally, the safety and biocompatibility and biodegradability of nanomaterials are being enhanced to overcome the existing clinical constraints and regulation challenges [83, 84]. Although these developments are promising, effective clinical validation, cost-efficiency, and standard regulatory systems will be needed in order to successfully translate these developed nanotechnologies into routine clinical application [85, 86].

CONCLUSION

Nanotechnology is a novel technology that emerged as an exciting and a revolutionary approach to heal wounds as it gives a solution to wound control in a more advanced way, it helps to safely and quickly supply the drugs, and regenerates tissue. The ability of nanomaterials such as metallic nanoparticles, polymeric systems, lipid-based carriers, nanofibers, and smart nanomaterials has shown that in many ways they could address the shortcomings of traditional wound therapies due to superior antimicrobial action, controlled release of drugs, and targeted action [86, 87]. Such systems are vital in the treatment of chronic wounds since they interrupt biofilms, enhance angiogenesis, and effect quicker healing responses [87, 88]. However, their clinical application is still limited due to toxicity, lack of long-term safety data, production cost and regulatory issues [88, 89]. Despite these drawbacks, advances in recent years in nanotechnology and biocompatible and stimulus-responsive systems have persisted and would enhance therapeutic effects and the safety of the clinical translation in the future [86, 89]. Consequently, through further research, uniform regulatory guidelines and solid clinical validation, nanotechnology-based wound healing systems have a huge potential of transforming how wounds are treated in modern times and how patient outcomes can also be enhanced [86,89]

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HOW TO CITE: Thirumurugan P., Bharath Vishnu V. S., Pavinesh Kumar K., Srimathi R., Gayathri R.* , Silver Nanoparticles against Bacterial Biofilms in Chronic Wounds: Mechanisms, Nano–Biofilm Interactions, and Translational Perspectives, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 9, 3978-3996. https://doi.org/ 10.5281/zenodo.23038974