



REVIEW ARTICLE

Silver Nanoparticles as Real Topical Bullets for Wound Healing

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Abstract Nanotechnology is on the threshold of providing a host of new materials and approaches, revolutionizing the medical and pharmaceutical fields. Several areas of medical care are already profiting from the advantage that nanotechnology offers. Recently, silver nanoparticles are attracting interest for a clinical application because of its potential biological properties such as antibacterial activity, anti-inflammatory effects, and wound healing efficacy, which could be exploited in developing better dressings for wounds and ulcers. This article reviews the role of silver nanoparticles in wound healing. © 2012 Elsevier Inc. All rights reserved.

Introduction

Wound healing is a complicated procedure involving a combination of activities of different tissues and cell lineages and has been the subject of concentrated research for a long time.¹ The focus on chronic wounds in the past few years has improved because of many problems that occur with chronic wounds and because of the expected increase in their occurrence and prevalence, due to the increasing prevalence of chronic pathologies such as diabetes. Chronic wounds give rise to serious health problems, accompanied by a decrease in quality of life and by economic problems due to the treatment costs and the decrease in a patient's productivity.² The wound healing process, illustrated in Figure 1, illuminates the causes of chronic wounds and affords a clear view of wound management.

When skin is injured, bleeding occurs, which activates hemostasis, initiated by exudates with components such as clotting factors. Eventually hemostasis results in the formation of a clot in the wound, and the bleeding stops.³ During this process, the wound passes through 4 phases, homeostasis, inflammation, granulation tissue formation, and remodeling, which overlap in time.^{1,4,5} The process is affected by various factors that are specific to the individual, such as nutritional status, age, systemic disease, medication, and behavior, along with the size, depth, causation, and the etiology of the wound.⁶

The inflammatory phase starts within minutes after the skin injury has occurred, simultaneously with hemostasis. The first inflammatory response is performed by leukocytes, specifically neutrophils, which migrate through the endothelium of the local blood vessels to the wound. The later response is carried out by monocytes, which differentiate into macrophages in the tissues after entering by a mechanism similar to that of the neutrophils. These macrophages in their turn secrete cytokines and in this way initiate an inflammatory response, which results in more

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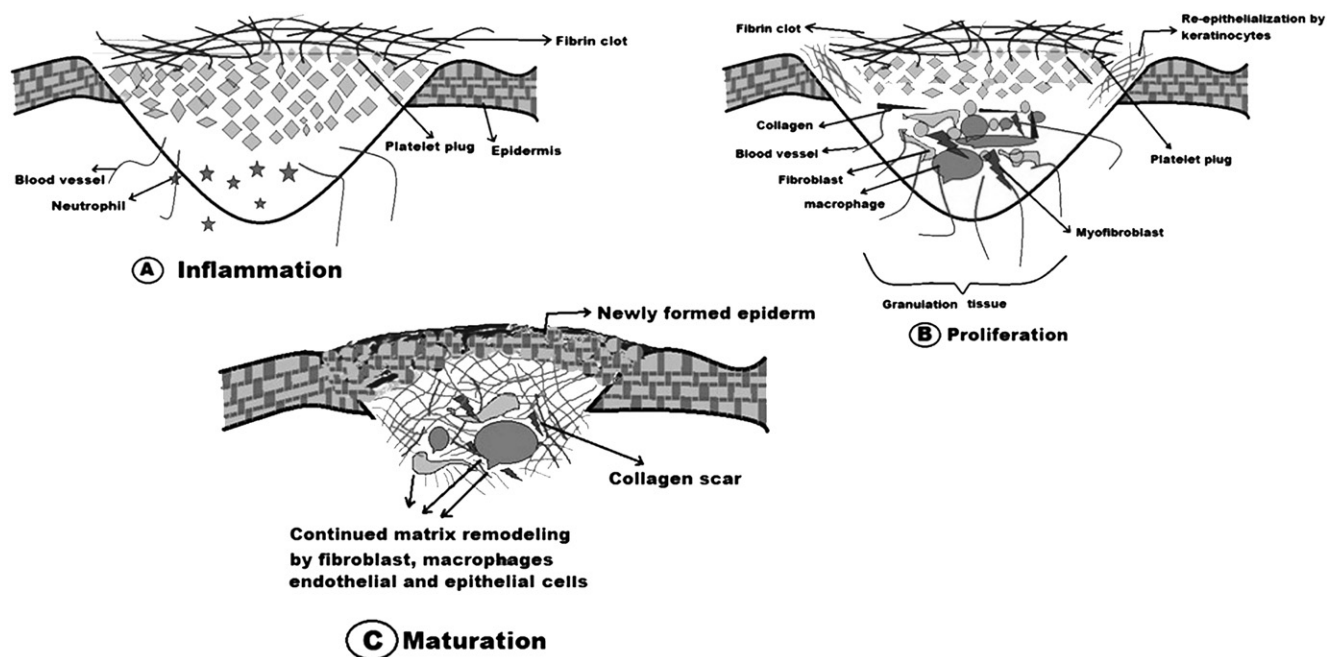


Figure 1 The Phases of Wound Healing Process. A, Inflammation; B, Proliferation; C, Scar Maturation.

cells of the immune system at the place of infection.⁴ The next 4 to 15 days are the proliferation phase, which includes the initial repair mechanisms of both the epidermis and the dermal layers of the skin. By the coordinated infiltration of fibroblasts, macrophages, and vascular tissue into the wound, a new dermal compound is developed named *granulation tissue*. This development is performed by the ingrowth of capillaries and lymphatic vessels into the wound and by the fibroblasts and myofibroblasts, which form collagen, responsible for the strength and form of the skin. Concurrently, keratinocytes migrate at the border of the wound over the granulation tissue in a process called reepithelialization. In this way the new outer layer of epidermis is differentiated.^{3,7} The last phase, the maturation phase, takes place when the wound is already healed and involves the further remodeling of the granulation tissue by its constituent cells. Synthesis of structural proteins, like collagen, continues for 6 to 12 months.⁷ A crucial process during the early stage of wound healing, reepithelialization, occurs, not only by the migration and proliferation of keratinocytes in the epidermal layer of the skin from the wound edge, but also by differentiation of stem cells residing in the bulge of the hair follicle.⁸ The vital goal for wound healing is rapid recovery with little scarring and maximal function. Rapid reepithelialization provides a more favorable environment, such as a scaffold of cells and various growth factors, which is essential in wound treatment. Wound contraction is another important process additional to reepithelialization in the early phase of wound healing. It minimizes the open area by pulling the neighboring tissue toward the wound center. In wound contraction, myofibroblasts generate alpha smooth muscle actin, which

plays a significant role. Myofibroblasts differentiated from fibroblasts produce the contractile force through which the wound area contracts during wound healing.^{9,10} This progression occurs more rapidly than reepithelialization because no cell proliferation is involved.¹¹

The present review describes currently available topical wound healing medications and their drawbacks. We describe the properties of silver nanoparticles to aid wound healing, such as antibacterial, anti-inflammatory effects; diminished resistance of bacteria to silver nanoparticles; and silver nanoparticles' mechanism of action. In addition, dressings impregnated with silver nanoparticles, the role of silver nanoparticles in impaired wound healing, and silver nanoparticles' mechanism of action in wound healing are discussed.

Topical Wound Healing Medications

In spite of the lack of standardized testing and formal evaluation, topical antimicrobials are still considered an essential component of wound care. Topical wound healing medications fall into 6 main categories, illustrated in [Figure 2](#) and [Table 1](#).

Antiseptics

Antiseptics are disinfectants that have a broad antimicrobial spectrum, but some are often toxic to host tissues.¹² There is a lot of discussion about the use of antiseptics on open wounds and their beneficial or detrimental outcomes on wound healing. One major advantage of antiseptics is

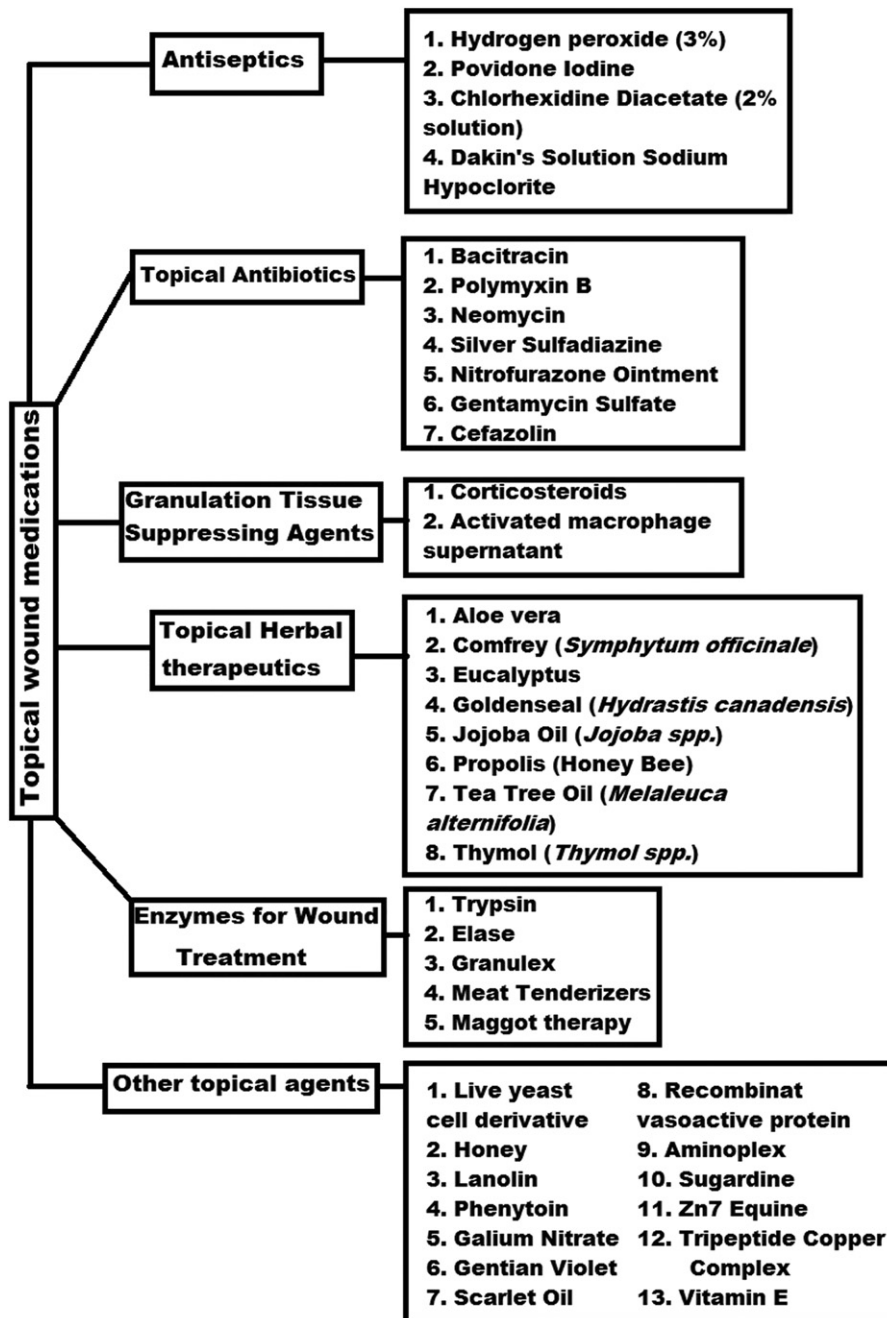


Figure 2 Commonly Available Topical Wound Healing Medications.

that they hardly ever select for resistant microbial strains, making them preferable to antibiotics with regard to the development of bacterial resistance. Some antiseptics have been found to be cytotoxic in vitro to both microorganisms and the host's cells.¹³

Hydrogen Peroxide (3%)

Hydrogen peroxide is an effective sporicide but has a narrow antimicrobial spectrum and is a widely used topical antiseptic that damages cellular components of bacteria on account of its highly reactive hydroxyl radical, but it must

be used in high concentration because of its catalase activity on many pathogenic bacteria. Hydrogen peroxide 3% solution has been shown to be cytotoxic to fibroblasts and to result in thrombosis of microvasculature.¹⁴ The cellular toxicity of hydrogen peroxide to fibroblasts exceeds its bacterial potency; therefore, it is unsuitable as a wound-cleansing solution.¹⁵

Povidone Iodine

Iodines have been shown to be efficient against methicillin-resistant *Staphylococcus aureus* in vitro and in

Table 1 Compilation of Topical Wound Healing Medications, Organisms Covered and Drawbacks

Medications	Organisms Covered	Drawbacks
Hydrogen peroxide	Sporocide	Narrow spectrum, unsuitable for wound cleaning solution because of cellular toxicity to fibroblast ¹⁵
Povidone iodine	G(+), G(-), Y, F	Causes hyperthyroidism, thyrotoxicosis, hypothyroidism, dermal hypersensitivity ¹⁹⁻²¹
Chlorhexidine	G(+), G(-), Y	Proteus, pseudomonas inherent resistance and no effect against fungi (or) Candida ²²
Dakin's solution	G(+), G(-), Y, F	Cytotoxic to fibroblast and narrow margin of safety ^{20,23,24}
Bacitracin	G(+)	Causes allergic contact dermatitis, ineffective against <i>Pseudomonas aeruginosa</i> and poor absorption ¹⁵
Polymyxin B	G(-)	Narrow spectrum and causes allergic contact dermatitis ²⁰
Neomycin	G(-)	Causes delayed hypersensitivity, IgE-mediated reaction, anaphylactic reaction, allergic contact dermatitis ¹⁹
Silver sulfadiazine	G(+), G(-), Y	Slow epithelialization and wound fragility ²⁵
Nitrofurazone	G(+), G(-)	Slow epithelialization and delayed wound contraction ¹⁶
Gentamycin	G(-)	Narrow spectrum, effective only against G(-) ²⁶
Cefazolin	G(+), some G(-)	The highest concentration is required (20 mg/kg) ²⁷
Mupirocin	G(+), MRSA	Narrow spectrum ²⁸

G(+), gram positive; G(-), gram negative; Y, yeast; F, fungus; MRSA, methicillin-resistant *Staphylococcus aureus*.

clinical studies and have been used for more than 100 years without producing bacterial resistance. Present formulations of iodophors, such as povidone iodine and cadexomer iodine, offer sustained release of low levels of free iodine, optimizing activity and reducing toxicity. Povidone iodine 10% solution has a broad range of antimicrobial activity that lasts for 4 to 6 hours following application. Solutions diluted to 0.1% to 0.2% (10-20 mL/1000 ml) are recommended in order to minimize cytotoxicity and increase the availability of free iodine for its antimicrobial action. At this concentration the solution kills bacteria within 15 seconds, and there is no known bacterial resistance to the product. Disadvantages of iodophors include skin irritation, allergy, and toxicity in vulnerable patients. Iodophors are capable of percutaneous and mucous membrane absorption, and as a result should not be used in pregnant women, newborns, or patients with thyroid disorders.^{16,17} In an ex vivo rat model, 10% povidone iodine ointment (Betadine) was shown to have a negative effect on microcirculation.¹⁸

Chlorhexidine (2% Solution)

Chlorhexidine is a useful topical therapeutic agent. It is a biguanide that exerts its antimicrobial effect by disrupting cytoplasmic membranes and has prolonged residual effect due to binding with protein in the stratum corneum. A potential drawback is that *Proteus* and *Pseudomonas* have developed resistance to this product, and it has no effect against fungi or *Candida*.²²

Dakin's Solution

Dakin's solution has broad-spectrum antimicrobial activity. Because of the release of chlorine and oxygen, it is more effective than povidone iodine or chlorhexidine in

killing *S aureus*.²⁹ It has been shown to be cytotoxic to fibroblasts and has a narrow margin of safety.²⁰

Topical Antibiotics

Antibiotics are chemicals, produced synthetically or naturally, that act on specific targets to kill microorganisms, resulting in a narrower spectrum of activity than antiseptics offer, and antibiotics are most effective when applied within 3 hours after wounding.¹⁴ Antibiotics are often less cytotoxic than are antiseptics; however, they are more likely to lose their efficacy to bacterial resistance.¹⁷ An additional known disadvantage of topical antibiotics is the occurrence of contact allergy.¹³ Contact allergy is sometimes secondary to the antibiotic, but it is more often a reaction to preservatives in the delivery vehicle. The ideal preservative, both effective and devoid of irritant or sensitizing potential, has yet to be discovered.

The most widely used antibiotics are bacitracin, polymyxin B, and neomycin as a triple antibiotic ointment. The triple combination is effective in a wide anti-microbial spectrum but ineffective against *Pseudomonas aeruginosa*.¹⁵ Silver sulfadiazine has a wide antimicrobial spectrum including *Pseudomonas* species and fungi, and gentamycin, nitrofurazone, and cefazolin are effective against both gram-positive and gram-negative organisms but have less effect against *Pseudomonas* species.¹⁵

Granulation Tissue Suppressing Agents

Corticosteroids may be applied to suppress the early formation of healthy exuberant granulation tissue, thus facilitating epithelialization and wound contraction,¹⁴ but they should not be applied to an infected wound.

Herbal Preparations

Herbal preparations are only one constituent of alternative medicine, which encompasses a wide multiplicity of approaches. A large number of herbal therapies and combinations of therapies currently exist for wound care. In general, these preparations consist of small amounts of the plant combined with a delivery substance (eg, ointment). From the scientific literature, the authors have attempted to compose a list readily available herbs; the source from which the herb is obtained is contained in parentheses in Figure 2. However, because of the underrepresentation of herbal therapies in scientific literature, this list is undoubtedly incomplete. Very few of these therapies have been tested scientifically in the horse for efficiency and/or toxicity. The authors highly advise further study by those interested in using herbal remedies.

Enzymes for Wound Debridement

Enzymes for wound debridement, trypsin, elase, and granulase are commonly used in the wound healing process. Nathan et al.³⁰ investigated the effect of trypsin and suggested that enzymes are a natural part of host defenses in the wound-healing process and that application of enzymes could potentially aid in the wound-healing process and the proteolytic activity of enzyme is supportive to digest the dressings in the burn wound. This study also concluded that wound enzyme activity and bacterial contamination are not related. Elase, or fibrinolysin and deoxyribonuclease, has been used in everything from treatment of monilial vulvovaginitis to chronic leg ulcers and burn wounds.³¹ In cases in which the use of elase has been reported to facilitate and extend the necrotic process, its use is highly contraindicated.³² Debriding preparations presently available must be used with caution as bacteremia has been reported in human patients after enzymatic debridement.³³

A live yeast cell derivative is a water-soluble extract of yeast reported to stimulate angiogenesis, epithelialization, and collagen formation.³⁴ It has been connected with improved wound healing in dogs. However, in horses, it prolonged wound healing by delaying wound contraction and resulted in excessive granulation tissue formation.³² Honey has many potentially useful properties, including broad-spectrum antimicrobial activity, anti-inflammatory action, and stimulation of new tissue growth.³⁵ Even though the exact mechanisms of honey's bacterial inhibition are unknown, possible mechanisms include osmotic action, low pH, its viscous nature, and production of hydrogen peroxide.³⁶ A review of randomized controlled trials involving honey in superficial burns and wounds concluded that confidence in honey as a useful treatment for superficial wounds and burns was low, although there appears to be some biological plausibility for its use.³⁷ See other topical agents in Figure 2.

How Do Silver Nanoparticles Benefit Wound Healing?

Silver therapy, in principle, has many benefits, such as (1) a multilevel antibacterial effect on cells, which considerably reduces the organism's chances of developing resistance; (2) effectiveness against multi-drug-resistant organisms; and (3) low systemic toxicity. However, silver compounds such as silver nitrate and silver sulfadiazine are used for topical applications because they may be neutralized by anions (chloride, bicarbonate, and protein) in body fluids or cause cosmetic abnormality (argyria, or blue-gray coloration) on prolonged use, and they can arrest the healing process via fibroblast and epithelial cell toxicity. Despite these shortcomings, silver sulfadiazine is the most popular topical antimicrobial silver delivery system in use because safer alternatives are unavailable.

Silver Nanoparticle Properties That Aid Wound Healing

Over the past few decades, silver nanoparticles, whose structures exhibit significantly novel and distinct physical, chemical, and biological properties and functionality due to their nanoscale size, have elicited much interest. Especially in the biological and pharmaceutical sectors, nanostructure materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity.³⁸ Decreasing the dimension of nanoparticles has a pronounced effect on their physical properties, which significantly differ from those of the bulk material. Moreover, there are several reasons for the use of silver nanoparticles in nanotechnology as well as in the medical and pharmaceutical fields, especially in wound healing. The properties that aid in wound healing are listed here and in Table 2. (1) Silver compounds have been used in medicine throughout the history of civilization.³⁹⁻⁴³ (2) It is easy to synthesize silver nanoparticles in large scale by several simple, inexpensive, safe, and reliable ways, including wet chemical, physical and biological methods.³⁸ (3) They can be synthesized in sizes from 2 to 500 nm by changing the reaction parameters. (4) They can be easily synthesized in different shapes (spheres, rods, tubes, wires, ribbons, plates, cubes, hexagons, triangles) by the selection of templates and reaction conditions.³⁸ (5) Because of the presence of a negative charge on their surface, they are highly reactive, which makes their surfaces modifiable by means of several biomolecules, a factor that aids in drug delivery.³⁸ Because of the strong interaction between the silver surface and molecules containing thiol or amine (organic molecules, DNA, proteins, enzymes, etc), the surface of silver nanoparticles can be easily modified.³⁸ (6) Silver nanoparticles exhibit antibacterial effects against a large number of bacterial species.⁴⁴ The antibacterial mechanism has not been fully elucidated, but observations from recent studies shed

Table 2 Properties of Silver Nanoparticles That Aid Wound Healing

Number	Properties	Reference
1	Silver compounds have been used in medicine throughout the history of civilization.	39,40,41
2	Silver nanoparticles are easy to synthesize by several simple, economical, safe, and reliable methods, such as wet chemical, physical, and biological.	38
3	They can be easily synthesized in different shapes (spheres, rods, tubes, wires, ribbons, plates, cubes, hexagons, triangles) and various sizes (2-100 nm) using templates and changing reaction conditions.	38
4	Because of the presence of a negative charge on the surface, they are highly reactive, which helps to modify the surface of silver nanoparticles with several biomolecules, which aids various drug delivery applications because of the strong interaction between the silver surface and thiol-containing or amine-containing molecules (organic molecules, DNA, proteins, enzymes, etc).	38
5	They exhibit powerful antibacterial effects against a large number of bacterial species.	44
6	Bacterial resistance to elemental silver is extremely rare, suggesting the presence of multiple bactericidal mechanisms acting in synergy.	45
7	They can be easily incorporated into cotton fabric and dressings, with no adverse effects observed for the dressing.	55
8	They possess efficient anti-inflammatory properties, is an another advantageous belongings to promote the wound healing by reducing cytokine release, decreasing lymphocyte and mast cell infiltration	56,57

light on the interactions involved.⁴⁵ It is believed that silver ions interact with 3 main components of the bacterial cell to produce a bactericidal effect: the peptidoglycan cell wall and the plasma membrane, bacterial (cytoplasmic) DNA^{46,47} and bacterial proteins,⁴⁶ and especially enzymes involved in vital cellular processes such as the electron transport chain. (7) Bacterial resistance to elemental silver is extremely rare,⁴⁵ emphasizing the presence of multiple bactericidal mechanisms acting in synergy. (8) Silver nanoparticles can be easily incorporated in cotton fabric and dressings and have significantly decreased wound-healing time by an average of 3.35 days and increased bacterial clearance from infected wounds, with no adverse effects observed for the dressing.⁴⁸⁻⁵⁵ (9) Anti-inflammatory properties of silver nanoparticles also promote wound healing by reducing cytokine release,⁵⁶ decreasing lymphocyte and mast cell infiltration.⁵⁷

Silver Nanoparticles as Antimicrobial Agents in Wound Healing

Because of the outbreak of infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, pharmaceutical companies and researchers are searching for new antibacterial agents that do not invite resistance and are low in cost. Silver nanoparticles have emerged as novel antimicrobial agents, owing to their high ratio of surface area to volume and their unique chemical and physical properties. Silver

nanoparticles can be used in various fields, particularly medicine and pharmaceuticals, because of their low toxicity to human cells, high thermal stability, and low volatility.⁴⁵ These attributes have resulted in a broad array of studies in which silver nanoparticles have played a role as drugs and as superior antimicrobial agents and have even been shown to prevent HIV binding to host cells.⁵⁸

Silver nanoparticles exhibit antibacterial effects against a large number of bacterial species (Table 3). The mechanisms of action and binding of silver nanoparticles to microbes remain unclear, but it is known that silver binds to the bacterial cell wall and cell membrane and inhibits the respiration process⁴⁰ by which the chemical energy of molecules is released and partially captured in the form of adenosine triphosphate. Silver nanoparticles interact with sulfur-containing proteins of the bacterial membrane, as well as with phosphorus-containing compounds such as DNA, to inhibit replication.⁴⁵ The bactericidal effect of silver has also been attributed to inactivation of the enzyme phosphomannose isomerase,⁵⁹ which catalyzes the conversion of mannose-6-phosphate to fructose-6-phosphate, an important intermediate of glycolysis, the most common pathway in bacteria for sugar catabolism.

Silver Nanoparticles and Antibiotic Resistance

Antibiotic resistance is a type of drug resistance in which a microorganism has developed the ability to survive

Table 3 Recent Studies on Antibacterial Activity of Silver Nanoparticles

Microbes Tested	Findings	References
<i>Enterococcus faecalis</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>S epidermidis</i> , <i>Enterococcus faecium</i> , <i>Klebsiella</i> <i>pneumonia</i>	Reducing saccharides were used to produce different sizes of silver nanoparticles (SNs); small SNs exhibited effective antibacterial activity against a range of different bacteria.	81,82
<i>E coli</i> , <i>Vibrio cholera</i> , <i>P aeruginosa</i> , <i>S typhi</i>	Effect of SNs against bacteria is size- and morphology dependent, small size and octahedral, decahedral particles having more reactive facets.	83
<i>E coli</i> , <i>S typhi</i> , <i>S aureus</i>	Effect of SNs was dose dependent; SNs more potent against G(-), eg, <i>E coli</i> , <i>S typhi</i> , and G(+), eg, <i>S aureus</i> , bacteria.	45
<i>P aeruginosa</i> , <i>S aureus</i> , <i>E coli</i> , MRSA	The SN-containing e-spin gelatin fiber specimens were investigated for antibacterial effect; they are more effective against <i>S aureus</i> and MRSA with glycine washing, and they were more effective in inhibiting <i>E coli</i> and <i>P aeruginosa</i> .	72
<i>S aureus</i> CCM 3953, <i>S aureus</i> MRSA, <i>E coli</i> CCM 3954, <i>P aeruginosa</i> CCM 3955	The 9-nm and 11-nm silver particles synthesized showed the highest activity against gram-positive and gram-negative bacteria.	84
<i>P aeruginosa</i> , <i>S aureus</i> , <i>E coli</i> , MRSA	The antibacterial activity of the nanoparticles varies when their size diminishes.	85
<i>S aureus</i> ATCC25923, methicillin-sensitive <i>S aureus</i> , and MRSA	SNs exhibit excellent bacteriostatic and bactericidal effect toward all clinical isolates tested regardless of their drug-resistant mechanisms.	86
<i>E coli</i> and <i>V cholera</i>	Alteration in membrane permeability and respiration of the silver nanoparticle-treated bacterial cells were evident from the activity of silver nanoparticles.	87

MRSA, methicillin-resistant *S aureus*.

exposure to an antibiotic. The volume of antibiotic prescribed, rather than compliance with antibiotics, is the major factor in increasing rates of bacterial resistance. The 4 main mechanisms by which microorganisms exhibit resistance to antimicrobials are (1) drug inactivation or modification (eg, enzymatic deactivation of penicillin G in some penicillin-resistant bacteria through the production of β -lactamases); (2) alteration of target site (eg, alteration of penicillin-binding proteins—the binding target site of penicillins—in methicillin-resistant *S aureus* and other penicillin-resistant bacteria); (3) alteration of metabolic pathway (eg, some sulfonamid-resistant bacteria do not require para-aminobenzoic acid, an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides; instead, like mammalian cells, they turn to using preformed folic acid); (4) reduced drug accumulation: by decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface (Figure 3).

Therefore, an alternative way to overcome the antibiotic and drug resistance of various microorganisms is needed desperately, especially in medical devices, pharmaceuticals, and so forth. The nano size allows expansion of the

contact surface of metal with the microorganisms, and this nano scale has applicability for medical devices and pharmaceuticals by means of surface-coating agents. Microbial resistance to silver itself has not been reported. However, clinically, silver-resistant strains of bacteria are a continuing problem in wound care despite many claims in the literature to the contrary. In fact, resistance to silver is rare, but not unknown. Kim et al.⁵⁸ studied the antimicrobial mechanism of silver nanoparticles for certain microbial species. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. Therefore, if the antibacterial effect of silver nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use silver nanoparticles as an antibacterial agent (Figure 4). Sondi and Salopek-Sondi⁶⁰ reported that the antimicrobial activity of silver nanoparticles on gram-negative bacteria was dependent on the concentration of silver nanoparticles and was closely associated with the formation of “pits” in the cell wall of bacteria. Silver nanoparticles that accumulated in the bacterial membrane caused permeability, resulting in cell death and degradation of the membrane structure. Kim et al.⁵⁸ suggested that the antimicrobial mechanism of

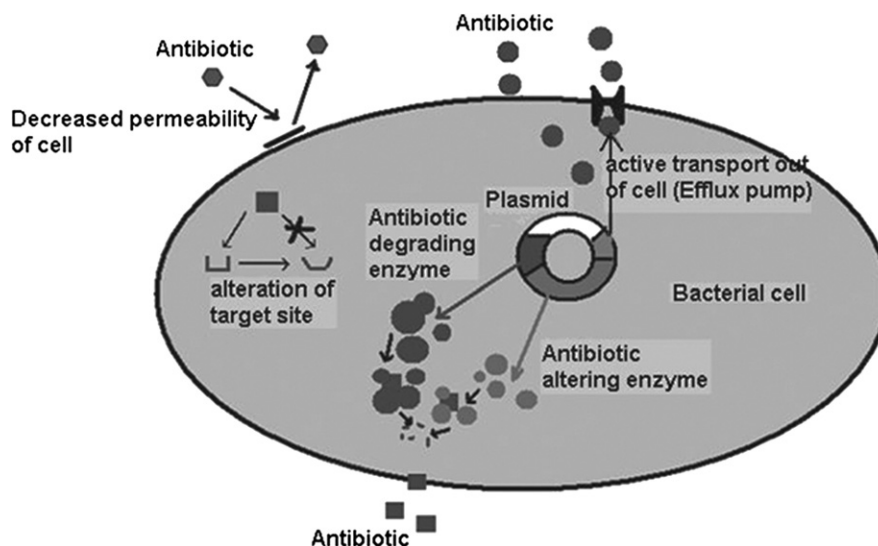


Figure 3 Various Mechanisms of Bacterial Resistance Against Antimicrobials.

silver nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane damage. The free radicals may be derived from the surface of silver nanoparticles and be responsible for the antimicrobial activity.⁵³ In proteomic and biochemical studies, nano molar concentrations of silver nanoparticles have killed *Escherichia coli* cells within minutes, possibly because of immediate dissipation of the proton motive force.⁶⁰ This action is similar to that found for antimicrobial activities of Ag⁺ ions.⁶¹ For example, low concentrations of Ag⁺ ions result in massive proton leakage through the *Vibrio cholerae* membrane.⁶² This proton leak might come either from any Ag⁺-modified membrane protein or any Ag⁺-modified phospholipid bilayer. The phenomenon causes deenergization of the membrane and consequently cell death.⁶² Shrivastava⁴⁵ studied the combined effect of silver nanoparticles with different antibiotics against *S aureus* and *E coli* using the disk diffusion methods.⁴⁵ In the presence of silver nanoparticles, the antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin increased against

both test strains. Similarly, Gajbhiye et al.⁶³ reported that the antifungal activity of fluconazole increased significantly in the presence of silver nanoparticles.⁶³ The maximum antifungal activity was observed against *C. albicans*, followed by *Trichoderma* species and *Phoma glomerata*.

Wound Dressings Impregnated With Silver Nanoparticles

Although wound healing takes place naturally on its own, some of its complications, such as sepsis, disruption of tissue and skin layer, maggot formation, and extension of infection to adjacent and interior organs, occur in major cases. To prevent extensive loss and damage to the tissue, skin grafting⁶⁴ and biological dressings⁶⁵ were developed. In order to heal the larger wound, the skin surface must be covered with sufficient dressing, even it is temporary. An ideal wound dressing should do the following: (1) Maintain a moist environment around the wound. (2) Permit diffusion of gases. (3) Remove excess exudates but

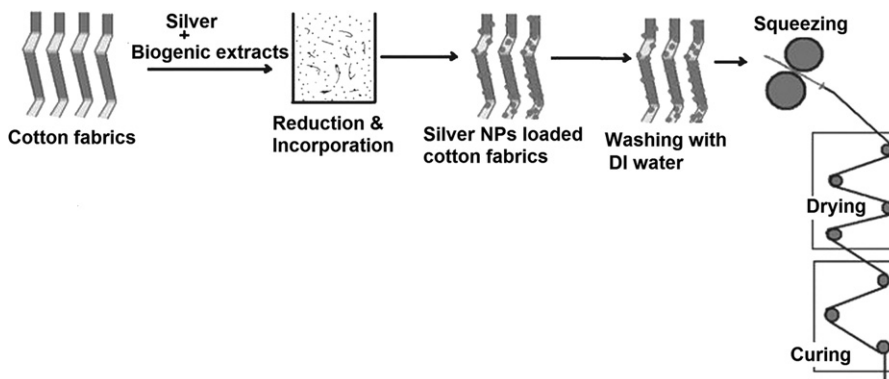


Figure 4 Incorporation of Silver Nanoparticles on Cotton Fabrics. NP, nanoparticles; DI water, deionised water.

prevent saturation of the dressing to its outer surface. (4) Protect wound from microbes and not contaminate the wound with foreign particles. (5) Provide mechanical protection. (6) Control local temperature and pH. (7) Be easy and comfortable to remove and change. (8) Minimize pain from the wound. (9) Be nontoxic. (10) Be cost-effective and cosmetically acceptable. (11) Prevent wound desiccation. (12) Stimulate the growth factors and be biocompatible and elastic. (13) Reduce malodor. (14) Conform to the site and shape of the wound. (15) Assist in wound bed preparation, such as debridement. (16) Satisfy patient and clinician expectations.^{66,67} Dressings made with biomaterials are becoming popular because of their many advantages. Impaired wound healing because of infections and other above-mentioned complications spurred the search for drug-loaded dressings.⁶⁸ Drug-loaded dressings are prepared by incorporating drugs such as antibacterials and antibiotics in the dressings. When applied to a wound, drug-loaded dressings act as a barrier to microorganisms and thus prevent secondary infections, while stimulating the wound-healing environment. Therefore, drug-loaded dressings are useful in preventing secondary infections on the wound and promoting fast wound healing. However, the ability of cotton fibers to absorb large amounts of moisture makes them more prone to microbial attack under certain conditions of humidity and temperature.³⁷ Moreover, cotton serves as a medium for the growth of bacteria and fungus.⁶⁹ For this reason, cotton fibers are treated with numerous chemicals to get better antimicrobial cotton textiles. Among the various antimicrobial agents, silver nanoparticles have shown strong inhibitory and antimicrobial activity and have no negative effect on the human body.⁷⁰ These particles can be incorporated into several kinds of materials, such as clothes. Clothes incorporating with silver nanoparticles are sterile and can be used to prevent or to minimize infection with pathogenic bacteria. Nowadays, metal-based topical dressings have been widely used as a treatment for infections in burns, open wounds, and chronic ulcers.⁷¹ Silver nanoparticles were incorporated by physical means; before being used, cotton fabrics were washed, sterilized, and dried, then submerged in an Erlenmeyer flask containing silver nanoparticles and agitated at 600 rpm for 24 hours and dried at 70°C, then cured at 150°C. The schematic representation of the formation of metal nanoparticles on cotton fabrics is presented in Figure 4.

Rujitanaroj et al.⁷² prepared mats of gelatin fibers containing silver nanoparticles by electrospinning to form a wound-dressing pad.⁷² These nanofiber webs have unique properties, such as a high ratio of surface area to volume, small pore size, and high porosity.^{73,74} These nanofibers impregnated with silver nanoparticles are very efficient for topical drug administration and wound healing because of their high ratio of surface area to volume.^{75,76} Maneerung et al.⁷⁷ has impregnated silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. Bacterial

cellulose is an interesting material for use as a wound dressing since it provides a moist environment to a wound, resulting in better wound healing. However, bacterial cellulose itself has no antimicrobial activity to prevent wound infection. To achieve antimicrobial activity, silver nanoparticles were impregnated into bacterial cellulose by immersing bacterial cellulose in a silver nitrate solution. The freeze-dried silver nanoparticle-impregnated bacterial cellulose exhibited strong antimicrobial activity against *E coli* (gram-negative) and *S aureus* (gram-positive). In a study by Miller et al.,⁷⁸ the effect of nano-crystalline silver on the healing of leg ulcers was studied. The silver dressing did not increase the overall healing rate, but it was associated with quicker healing in larger and older ulcers. An extensive metastudy by Storm-Versloot et al.⁷⁹ confirmed these findings in that most studies on silver dressings for nonhealing wounds did not show a significant reduction of infection when silver sulfadiazine cream or silver dressings were used. Wound healing was found to vary among the different studies reviewed, depending on the type of wounds included in the study and the exact dressing used.⁷⁹ A chitosan-nanocrystalline silver dressing showed superior healing rates (89%) compared with silver sulfadiazine dressings (68%) and chitosan film (74%).⁸⁰ In addition, the chitosan-nanocrystalline silver dressing deposited far less silver than did conventional silver sulfadiazine,⁸⁰ thus demonstrating that the use of silver nanoparticles may be safer in reducing the incidence of argyria and argyria (elevated silver concentration in the blood).

Silver Nanoparticles Suppress Both Local and Systemic Inflammation

The inflammatory response is an important part of wound healing. The various inflammatory mediators are secreted to adjust the healing process within wounds. In usual wound healing, the possibility of proinflammatory and anti-inflammatory cytokines is present, and the inflammatory response is totally appropriate. To achieve successful wound repair and tissue regeneration, the inflammatory response must be securely regulated in vivo. A vital mediator in this anti-inflammatory cascade appears to be interleukin 10 (IL-10), which can be produced by keratinocytes as well as inflammatory cells involved in the healing process, including T lymphocytes, macrophages, and B lymphocytes⁸⁸ (Figure 5). One of the unique actions of IL-10 is its ability to inhibit the synthesis of proinflammatory cytokines, which also include IL-6.^{89,90} IL-10 also inhibits leukocyte migration toward the site of inflammation, in part by inhibiting the synthesis of several chemokines, including monocyte chemoattractant protein-1 and macrophage inflammatory protein-1a.⁹¹ Both of these chemokines promote monocyte accumulation, and macrophage inflammatory protein-1a is also a potent neutrophil chemoattractant in mice.⁹² Tian et al.⁹³ investigated the

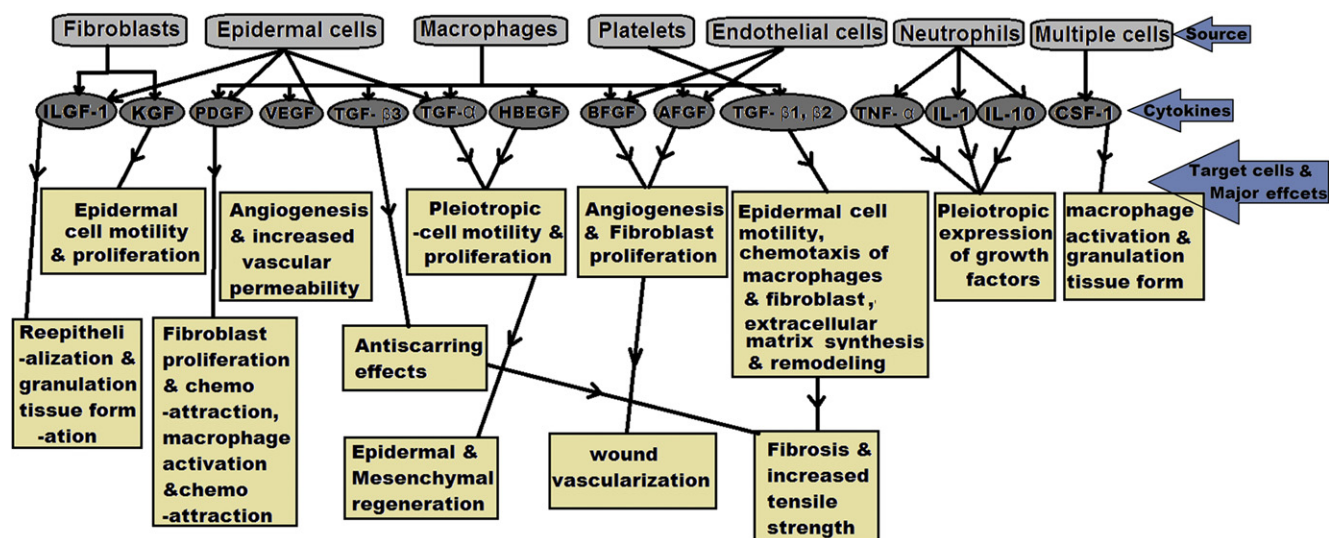


Figure 5 Role of the Different Cytokines and Mediators at the Various Stages of Normal Wound Healing. ILGF-1, insulin-like growth factor 1; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; TGF- β 3, transforming growth factor beta 3; TGF- α , transforming growth factor alpha; HBEGF, heparin-binding epidermal growth factor; BFGF, basic fibroblast growth factor; AFGF, acidic fibroblast growth factor; TGF- β 1, 2, transforming growth factor beta 1, 2; TNF- α , tumor necrosis factor alpha; IL-1, -10, interleukin 1, 10; CSF-1, colony stimulating factor 1.

effect of silver nanoparticles in the inflammatory response at the wound site and observed that low levels of expression of transforming growth factor β (TGF- β) coincided temporally with increased levels of interferon (IFN)- γ until wound closure in animals treated with silver nanoparticles. As IFN- γ has been demonstrated to be a potent antagonist of fibrogenesis through its ability to inhibit fibroblast proliferation and matrix production, its control of TGF- β production may play a role.⁹⁴ Vascular endothelial growth factor (VEGF) has been shown to promote healing.⁹⁵ Much higher levels of VEGF messenger RNA (mRNA) are detected in keratinocytes at the wound edge and in keratinocytes that migrate to cover the wound surface. Besides a few mononuclear cells, VEGF expression is not found in other cell types in the wound.⁹⁶ Tian et al.⁹³ suggest that keratinocytes in the wound are a major source of VEGF. As VEGF is highly specific for endothelial cells, it is likely to act in a paracrine manner on the sprouting capillaries of the wound edge and granulation tissue.⁹³ Several studies have indicated that TGF- β is able to induce keratinocytes to produce VEGF gene expression.^{59,97} Tian et al.⁹³ found that TGF- β increased and reached a peak on day 3 in the silver nanoparticle-treated animals and may explain why significantly higher VEGF mRNA levels were maintained in the early stage of wound healing.⁹³ Tian et al.⁹³ concluded that silver nanoparticles can modulate local and systemic inflammatory response following burn injury by cytokine modulation (Table 3). Since cytokines play an important role in wound healing, the authors investigated the expression patterns of IL-6, TGF- β 1, IL-10, VEGF, and IFN- γ with quantitative real-time polymerase chain reaction (PCR). Levels of IL-6 mRNA in the

wound areas treated with silver nanoparticles were maintained at statistically significantly lower levels throughout the healing process, while mRNA levels of TGF- β 1 were higher during the initial period of healing in the site treated with silver nanoparticles. The same trend was observed for IL-10, VEGF, and IFN- γ mRNA. Moreover, in this study, better cosmetic results were observed in animals treated with silver nanoparticles.⁹³ In terms of wound healing, enhanced expression of TGF- β 1 mRNA was found in both keloids and hypertrophic scars. Cumulative evidence has suggested that TGF- β 1 plays an important role in tissue fibrosis and postinjury scarring. The authors demonstrated that lower levels of TGF- β coincided temporally with increased levels of IFN- γ before wound closure in the silver nanoparticle-treated group. Since IFN- γ has been demonstrated to be a potent antagonist of fibrogenesis through its ability to inhibit fibroblast proliferation and matrix production, its control of TGF- β production may play a role in the positive effects of silver on wound healing. Regarding angiogenesis, it is well known that VEGF promotes healing⁹⁸ (Table 4).

Wong et al.⁹⁹ investigated the anti-inflammatory effect of silver nanoparticles in a postoperative peritoneal adhesion model. In vitro and in vivo experimental findings show that silver nanoparticles are effective at decreasing inflammation in peritoneal adhesions without significant toxic effects.⁹⁹ Nadworny et al.¹⁰⁰ found that nanocrystalline silver-derived solutions appear to have anti-inflammatory and prohealing activity, predominantly with a starting pH of 9. Solutions have been generated differently having various silver species with varying concentrations, only some of which are anti-inflammatory.¹⁰⁰ These

Table 4 Compilation of Findings on Effect of Silver Nanoparticles on Different Mediators During Impaired Wound Healing

Mediators	Findings	Reference
TGF- β , TNF- α	Immunohistochemical staining revealed higher levels of the proinflammatory cytokines TGF- β and TNF- α in the saline and silver nitrate groups. The negative control and nano crystalline silver groups showed similar levels of staining, which might be mediated by reducing cytokine release, decreasing lymphocyte and mast cell infiltration, and inducing apoptosis in inflammatory cells.	100,55
IL-6, TGF- β 1	Lower levels of IL-6 mRNA were detected in the wound areas treated with nano silver throughout the healing process. The mRNA levels of TGF- β 1 were higher in the initial period of healing in the ND group; however, this decreased and maintained a lower level during the latter phase of healing.	93
VEGF	Much higher levels of VEGF mRNA are detected in keratinocytes at the wound edge and in keratinocytes that migrate to cover the wound surface. As VEGF is highly specific for endothelial cells, it is likely to act in a paracrine manner on the sprouting capillaries of the wound edge and granulation tissue.	93
IL-10	Increased expression of IL-10, which can be produced by keratinocytes as well as inflammatory cells involved in the healing process, including T lymphocytes, macrophages, and B lymphocytes. One of the unique actions of IL-10 is its ability to inhibit the synthesis of proinflammatory cytokines, which also include IL-6. IL-10 also inhibits leukocyte migration toward the site of inflammation, in part by inhibiting the synthesis of several chemokines, including monocyte chemoattractant protein 1 and macrophage inflammatory protein 1a.	93
IFN- γ	The mRNA levels of IFN- γ stayed much higher in the nano silver-treated group relative to those of the standard group. The authors demonstrated that lower levels of TGF- β coincided temporally with increased levels of IFN- γ before wound closure in the group treated with silver nanoparticles. Since IFN- γ has been demonstrated to be a potent antagonist of fibrogenesis through its ability to inhibit fibroblast proliferation and matrix production, its control of TGF- β production may play a role in the positive effects of silver on wound healing.	93
MMP-9	Nanocrystalline silver dressings significantly reduced MMP-9 levels in a porcine model and improved wound healing, although no mechanism was proposed.	109

IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; mRNA, messenger RNA; ND, nano drug; TGF, tumor growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

solutions show promise for a range of anti-inflammatory treatment applications.

Role of Silver Nanoparticles in Impaired Wound Healing

Impaired wound healing is a common complication of diabetes mellitus.¹⁰¹ Healing in patients with diabetes mellitus is characterized by reduced tensile strength of wounds when compared with controls, suggesting either defective matrix production or deposition. In the human mammal, diminished perfusion resulting from the presence of peripheral arterial disease as well as decreased sensory nerve function caused by peripheral neuropathy may contribute to impair healing.^{102,103} It is presumed that diabetic complications result from periods of poor glycemic control. However, aberrant growth factor expression or factors secondary to diabetes, such as advanced glycation and cross-linking of matrix protein, may also be involved.¹⁰⁴ Growth factor involvement has been implicated not only in diabetic wounds but also in other diabetic complications, such as diabetic

retinopathy and nephropathy.¹⁰⁵ VEGF is one of the most potent known angiogenic cytokines and promotes all steps in the cascade process of angiogenesis. In particular, it induces degeneration of the extracellular matrix of existing vessels by proteases, causes migration and proliferation of capillary endothelial cells, and determines the tube proliferation of endothelial cells.¹⁰⁶ VEGF action is associated with a variety of physiological and pathologic neovascular events, such as embryonic development, tumor growth, and wound repair in particular.¹⁰⁷ VEGF is related to platelet-derived growth factor and has 4 different isoforms, VEGF121, VEGF165, VEGF189, and VEGF206, which are generated by alternative splicing of mRNA.¹⁰⁸ VEGF is produced by keratinocytes that, together with macrophages, represent the most important source of this growth factor during normal wound healing.

Tian et al.⁹³ investigated wound healing in diabetic mice. In this model, excised wounds treated with silver nanoparticles completely healed in 16 ± 0.41 days after injury, whereas mice in the control group required 18.5 ± 0.65 days ($P < .05$). In the nondiabetic littermates, silver nanoparticles still accelerated wound healing relative

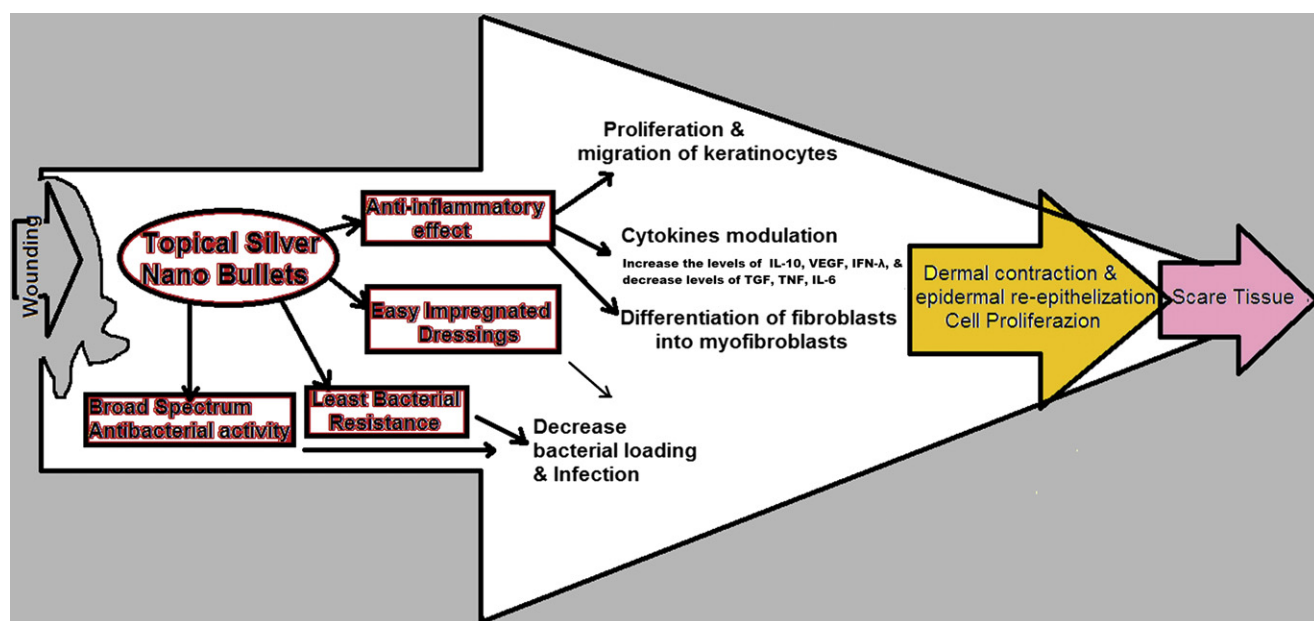


Figure 6 The Remarkable Properties of Silver Nanoparticles Bring the Wound Closer to the Normal Skin. Silver nanoparticles play a distinct role in preventing infection, decreasing bacterial load in the wound by their broad-spectrum antimicrobial properties, and their surface modification properties provide easy incorporation of nano silver into cotton fabrics and drugs to improve the wound-healing treatment. Along with the above properties, the potent anti-inflammatory properties of nano silver mediated through cytokine modulation lead to better therapeutic direction in wound treatment. IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

to the control group. Tian et al.⁹³ investigated VEGF expression patterns by using quantitative real-time PCR and found that TGF- β increased and reached a peak on day 3 in the silver nanoparticle-treated group, which may explain why significantly higher VEGF mRNA levels were maintained in the early stage of wound healing. Tian et al.⁹³ detected much higher levels of VEGF mRNA in keratinocytes present at the wound edge and in those that migrated to cover the wound surface.⁹³ Besides a scarce expression in mononuclear cells, VEGF was not expressed in other cell types in the wound, indicating that keratinocytes are the major source of VEGF in the wound. As this factor is highly specific for endothelial cells, it is likely to have a paracrine function in the sprouting of capillaries on the wound edge and in granulating tissue. It appears from these findings that silver treatment not only acts as an antibacterial, but also directly acts on dampening the process of inflammation, thus promoting scarless wound healing and the effect of silver nanoparticles on different mediators during impaired wound healing shown in Table 4.⁹³

Mechanism of Action of Silver Nanoparticles on Wound Healing

The recent emergence of nanotechnology has provided a new therapeutic modality in silver nanoparticles for use in various wounds. Nonetheless, the beneficial effects of silver nanoparticles on wound healing remain unknown. Tian

et al.⁹³ investigated the wound-healing properties of silver nanoparticles in an animal model and found that rapid healing and improved cosmetic appearance occur in a dose-dependent manner. Furthermore, through quantitative PCR, immunohistochemistry, and proteomic studies, they showed that silver nanoparticles exert positive effects through their antimicrobial properties, reduction in wound inflammation, and modulation of fibrogenic cytokines.⁹³ First they investigated that the wound-healing property of silver nanoparticles is due solely to their antimicrobial property, confirmed that silver nanoparticles are a more effective antibacterial agent, and compared silver nanoparticles with amoxicillin and metronidazole, both commonly used antibiotics. Wounds treated with silver nanoparticles completely healed in 25.2 ± 0.72 days after injury, whereas those treated with antibiotics required 28.6 ± 1.02 days ($P < .01$). This finding suggests that other factors are also involved in the mechanism of action of silver nanoparticles.⁸⁷ Then they investigated the expression patterns of IL-6, TGF- β 1, IL-10, VEGF, and IFN- λ by using quantitative real-time PCR. Here, levels of IL-6 mRNA in the wound areas treated with silver nanoparticles were maintained at statistically significantly lower levels throughout the healing process ($P < .01$). Also, mRNA levels of TGF- β 1 were higher during the initial period of healing in the silver nanoparticles group; however, this decreased and maintained a lower level during the latter phase of healing ($P < .01$). For IL-10, VEGF, and IFN- λ , mRNA levels stayed higher in the silver nanoparticle group relative to

those of the silver sulfadiazine group at all times monitored during healing ($P < .01$). The differences found in mRNA levels of various cytokines confirm that silver can modulate cytokine expression (Table 4). Similarly, Lee et al.¹¹⁰ investigated the effect of silver nanoparticles in dermal contraction and epidermal reepithelialization during wound healing and suggested that silver nanoparticles could increase the rate of wound closure. This was achieved, on one hand, through the promotion of proliferation and migration of keratinocytes.¹¹⁰ On the other hand, silver nanoparticles could drive the differentiation of fibroblasts into myofibroblasts, thereby promoting wound contraction. Finally, silver nanoparticles play a distinct role in preventing infection and decreasing bacterial load in the wound by their broad-spectrum antimicrobial properties, and their surface-modification properties provide easy incorporation of nano silver into cotton fabrics and drugs to improve the wound-healing treatment. Along with the above properties, the potent anti-inflammatory properties of nano silver mediated through cytokine modulation lead to better therapeutic direction in wound treatment (Figure 6).

Conclusion and Future Perspectives

An effective and complete process of wound healing is critical for the general well-being of any patients. In recent times, tremendous progress has been made in discovering the cellular and molecular mechanisms underlying the wound healing process. In current clinical treatments of wounds and ulcers, medications such as topical antimicrobial agents are still relevant. Moreover, applying nanotechnology and incorporating knowledge of cellular, subcellular events occurring during the typical healing process, could obviously get better future therapeutic interventions. Nanotechnology offers great opportunities for improving wound treatments. The nanometer scale opens the way for the development of novel materials for use in highly advanced medical technology. Silver nanoparticles exhibit remarkable biological properties, such as anti-inflammatory, antiviral activities and antibacterial properties with less bacterial resistance. Silver nanoparticle dressings are now the new gold standard in the conservative treatment of wounds and burns. The full potential of this technology has yet to be discovered. The mechanisms underlying the impressive wound-healing properties of silver nanoparticles are still not understood, and understanding them is a priority for future research in vivo.

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