CHAPTER

4

Nanoparticulate systems for wound healing

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1. Introduction

Wounds attributed to injuries, traumas, and diseases are increasing in incidence with time, creating a financial burden for patients and the health care system [1]. The process of wound healing involves four stages: hemostatic phase, inflammatory phase, proliferative phase, and remodeling phase (Fig. 4.1). As shown in the figure, the wound healing process involves an inflammatory stage, which would benefit from the delivery of antiinflammatory drugs to overcome excessive tissue destruction and necrosis, as well as a proliferative phase that involves the formation of new blood vessels, hence would benefit from the delivery of angiogenesis and collagen-promoting therapeutic molecules. Topical delivery of pharmaceuticals is currently the mainstay of therapy in wound treatment, however, topical delivery is hampered by the barrier nature of the stratum corneum. Nanoparticle-based delivery has recently emerged as a very promising approach for treatment of dermatological diseases [2–4], among which is wounds attributed to different causes, in order to accelerate wound healing to overcome the increased risk of infection accompanying the delayed healing process [5]. Nanoparticles were reported to penetrate the stratum corneum and reach the deep skin epidermal and dermal layers owing to one or several combined possible mechanisms: their small size, their deformable properties, their lipidic nature, or their lipid-fluidizing nature [6–9]. One of the commonly researched areas is the use of nanoparticles for wound healing, in which nanoparticles were loaded with several therapeutic molecules, or themselves exhibited wound healing properties without drug loading. Therefore, the aim of this chapter is to highlight the most commonly used nanoparticles with emphasis on lipid-based systems (vesicular, emulsions, and solid matrix based) as representatives of organic nanoparticles and gold nanoparticles, silver nanoparticles, and metal oxide nanoparticles as representatives of inorganic nanoparticles.

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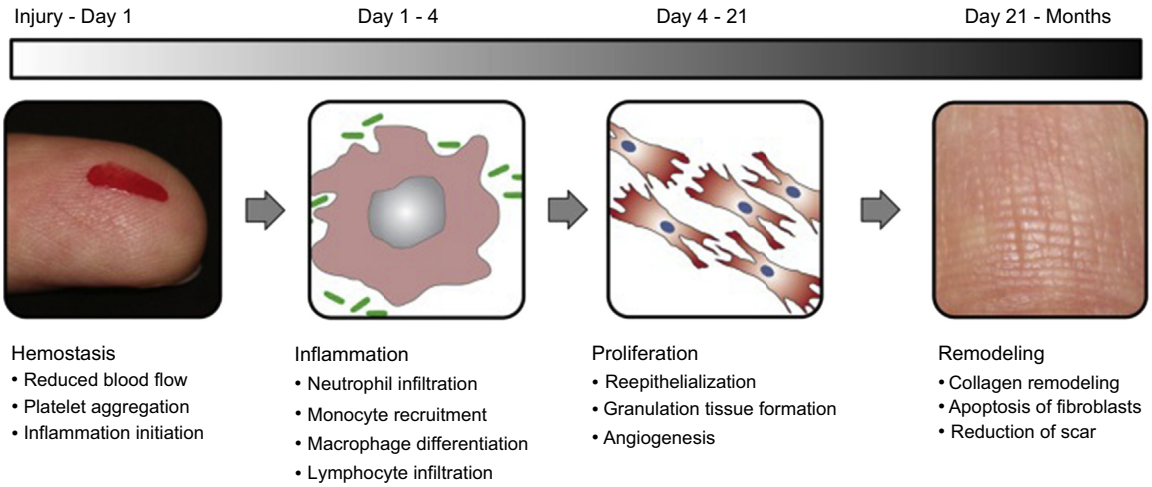

FIGURE 4.1 The four stages implicated in wound healing. *Abstracted with permission from Das S, Baker AB. Biomaterials and nanotherapeutics for enhancing skin wound healing. Front Bioeng Biotechnol 2016;4:82.*

2. Lipid-based nanoparticles

Lipidic nanoparticles are those comprised of one or more lipidic/oily substance in their composition. Nanoparticles can penetrate the skin through several mechanisms, as demonstrated in Fig. 4.2. Lipidic nanoparticles were reported to increase skin hydration, and allow rearrangement of skin cells, which may either induce fusion with the nanoparticles or enhance their permeation across skin layers [5]. The most commonly reported categories of lipid-based particles are either vesicular in nature such as liposomes, penetration enhancer vesicles, ethosomes and transfersomes, emulsion-based in nature (nanoemulsion and microemulsions), or contain a solid lipid matrix such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs).

2.1 Liposomes

Liposomes are the most commonly reported vesicular systems, and the term "liposomes" implies that they are only formulated using phospholipids, with no other additives such as surfactants or penetration enhancers, and hence they could be referred to as first-generation vesicles. Liposomes owe their popularity to their phospholipid content, which is biocompatible with skin lipids [8]. Liposomes act as reservoirs allowing controlled release of drugs, but they are mainly confined to the skin's upper layers. Despite their inflexible nature, many papers reported their effectiveness as topical delivery systems. Incorporation of liposomes within hydrogels was reported to aid the process of wound healing by the semiocclusive nature of the latter, which promotes angiogenesis and allows the growth of granulation tissue [10]. Moreover, functionalization of gauzes with liposomes was reported to offer high biocompatibility with human skin fibroblasts, and was reported to be the best vehicle for incorporation within gauzes [11]. Table 4.1 summarizes some of the promising uses of liposomes for wound healing, in which the described vesicles are devoid of any penetration enhancer/surfactant content.

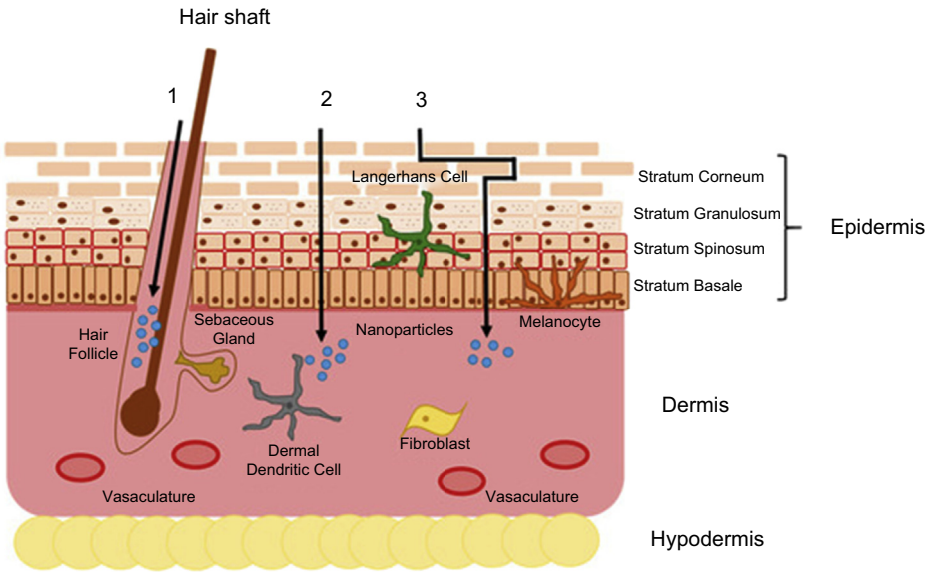

FIGURE 4.2 Mechanisms of nanoparticles penetration across the skin: (1) Transfollicular route, (2) Intracellular route, (3) Intercellular route. *Abstracted with permission from Palmer BC, DeLouise LA. Nanoparticle-enabled transdermal drug delivery systems for enhanced dose control and tissue targeting. Molecules 2016; 21(12):E1719.*

TABLE 4.1 Liposomal formulations used for wound healing.

Formulation and composition	Status of investigation	Active ingredient	Observations/Advantages	References
Liposomes prepared from phosphatidylcholine and cholesterol	In vitro/ In vivo	Curcumin	Sustainment of drug release for 24 h, and better wound diameter reduction on the 14th day postinduction compared to the unencapsulated form, verified for better skin penetration using fluorescence imaging	[12]
Liposomes prepared from phosphatidylcholine and cholesterol	In vitro/ In vivo	Danggui Buxue extract	The extract-loaded liposomes placed in a thermosensitive gel significantly improved collagen synthesis and angiogenesis, hence improving wound healing compared to blank vehicle—treated and model control groups	[13]
Liposomes prepared from lecithin and cholesterol, and coated with chitosan	In vitro	Substance P neuropeptide	The chitosan-coated liposomes provided significant reduction in cellular gap closure of HaCaT cells, delineating the system as a promising topical carrier for further in vivo wound healing experiments	[14]
Liposomes prepared from (1,2-dioleoyl-sn-glycero-3-phosphocholine), 1,2-dioleoyl-sn-glycero-3-phospho-(1′-rac-glycero1) sodium salt] and cholesterol	In vitro/ In vivo	Glycyl-l-histidyl-L-lysine	The liposomal encapsulated tripeptide displayed better angiogenic effect and shortened wound healing time compared to the free peptide	[15]

(*Continued*)

TABLE 4.1 Liposomal formulations used for wound healing.—cont'd

Formulation and composition	Status of investigation	Active ingredient	Observations/Advantages	References
Liposomes prepared from phosphatidylcholine and cholesterol	In vitro	Quercetin	Sustainment of drug release for 24 h	[16]
Liposomes prepared from egg lecithin on a sucrose-based powder	In vitro	recombinant human epidermal growth factor (rhEGF)	Enhanced skin permeation of the human epithelial growth factor when encapsulated in liposomes compared to its free form	[17]
Liposomes prepared from soybean lecithin and cholesterol	In vitro/ In vivo	Basic fibroblast growth factor bFGF	The wound healing effect of bFGF liposomes was dose dependent, demonstrating faster collagen generation with prolonged effect caused by encapsulation	[18]
Liposomes prepared from dipalmitoyl-phosphatidylcholine and cholesterol	In vitro/ In vivo	Epidermal growth factor (EGF)	Liposomal encapsulated EGF demonstrated superior wound healing effect compared to mere EGF administration and the silverdine ointment, with enhanced epidermal thickness	[19]
Undisclosed phospholipid type	In vivo	Polyvinyl-pyrrolidone iodine	Enhanced tissue repair property of liposomes led to faster wound healing for the liposomal hydrogel group loaded with polyvinyl-pyrrolidone iodine	[20]
Liposomes prepared from lecithin and phosphatidylserine	In vitro/ In vivo	Buflomedil	Accelerated wound closure around days 9 and 13 in normal and ischemic wounds, respectively, compared to untreated control	[21]

2.2 Penetration enhancer vesicles

Penetration enhancer vesicles (PEVs) are the same as liposomes, with the exception that they contain additional penetration enhancers in their composition such as polyethylene glycol, labrasol, and transcutol. Compared to conventional vesicles, the penetration enhancers content causes additional disordering of the skin [22], hence they can be categorized as flexible vesicles that can facilitate dermal penetration and allow better fibroblast cell uptake. Table 4.2 summarizes some of the reported successful attempts for delivering active ingredients with PEVs for wound healing purposes.

2.3 Ethosomes

Ethosomes are identical in their structure to liposomes, in the sense that they contain phospholipids as the bilayer forming agent, in addition to ethanol, which acts as a skin penetration enhancer [26], hence they are categorized as flexible vesicles. Only a few papers reported the use of ethanol as topical vesicular treatment of

TABLE 4.2 Penetration enhancer containing vesicles used for wound healing.

Formulation and composition	Penetration enhancer used	Status of investigation	Active ingredient	Observations/Advantages	References
PEVs prepared from soybean lecithin and tween 80 (a penetration enhancer −modified formulation version of transfersomes)	Sorbitol	In vitro	Baicalin	The modified sorbitol PEVs formulation displayed better skin accumulation in all layers, provided higher proliferative stimulation of fibroblasts, and provided better skin protection compared to transfersomes, and were proven more superior in promoting cellular-based wound closure	[23]
PEVs prepared from egg yolk lecithin and cholesterol	Polyethylene glycol (PEG 1500)	In vitro/ In vivo	Madecassoside	Vesicles exhibited higher deposited amount of the drug in the skin compared to its solution form, and enhanced wound healing rate accompanied with reduced scar formation by day 12 in wound healing animal model	[5]
PEVs prepared from soybean phospholipids	Ethylene glycol	In vitro/ In vivo	Polyphenolic phytocomplex from *Fraxinus angustifolia*	PEVs encapsulating the extracted polyphenols resulted in significant reduction in edema with concomitant decrease in myeloperoxidase activity compared to unencapsulated extracts, with normal skin appearance in animal inflammatory model	[24]
PEVs prepared from phospholipids mixture	Polyethylene glycol (PEG 400)	In vitro/ In vivo	Quercetin	PEVs-encapsulated quercetin exhibited significant reduction in tissue damage with significant tissue regeneration manifested by increase in collagen fibers compared to unencapsulated drug	[25]
PEVs prepared from soybean phospholipids	Polyethylene glycol (PEG 400)	In vitro/ In vivo	Quercetin and curcumin	PEVs loaded with quercetin and curcumin exhibited better antiinflammatory activity and more significant dermal deposition of drugs, with superior effect encountered with curcumin compared to quercetin vesicles in animal inflammatory model	[22]

wounds, probably owing to their ethanolic content, which might be an irritant to open wounds. A study conducted by Godin et al. [27] reported that the topical application of hydroalcoholic solution loaded with erythromycin was not as effective as a wound healing modality compared to topically applied ethosomal erythromycin, hence delineating the importance of the vesicular structure in promotion of skin penetration.

In a study reported by Partoazar et al. [28], ethosomal curcumin was found to significantly inhibit gram-positive and gram-negative

bacteria isolated from wounds compared to the unencapsulated curcumin, and to significantly reduce the wound area in a second-degree burn wound model in rats owing to its strong reepithelization, angiogenesis, collagen synthesis, and granulation tissue promotion potential. Moreover, an ethosomal gel loaded with *Sesamum indicum* seed extract was proven to exhibit significantly higher wound contraction percentage by the 16th day in rat excision model compared to povidone iodine ointment, correlating with high collagen synthesis inferred from the significantly higher hydroxyproline levels achieved with the ethosomal form [29]. Finally, an ethosomal formulation loaded with silver sulfadiazine displayed faster wound healing process cascade compared to the silver sulfadiazine gel or a marketed product, and was found to exhibit higher wound contraction percentage [30].

2.4 Transfersomes

Transfersomes are another example of modified vesicular generation, in which a surfactant (edge activator) is incorporated within the phospholipid bilayer, conferring flexibility to the vesicles, and consequently enhancing their skin delivery potential [31]. Like PEVs and ethosomes, transfersomes can be considered as flexible vesicles.

Regarding wound healing Chhibber, Kaur, and Kaur [32] conducted an interesting study in which they encapsulated bacteriophages rather than drugs (*Staphylococcus aureus* lytic phages MR-5 and MR-10 in transfersomes composed of phosphatidylcholine, cholesterol, tween 80, and stearylamine to overcome the decreased persistence of the phages at the place of the wound. Results showed that the transfersomal-encapsulated phages exhibited significant enhancement of their wound persistence, accompanied with better wound closure and faster healing rate in a diabetic wound animal model compared to the nonencapsulated form. In addition, when transfersomes prepared from phosphatidylcholine, cholesterol-gellan, and tween 80 were loaded with baicalin, they were shown to exhibit complete skin healing in inflammatory animal model compared to betamethasone cream [33]. Transfersomes composed of phosphatidylcholine and one of either tween 20, 40, 60, or 80 and loaded with tocopherol were prepared by Caddeo et al. [34], in which the authors proved the superiority of tween 80 transfersomes in providing skin accumulation of tocopherol, with faster HaCaT cell layer regeneration and complete wound closure in 3T3 cells compared to other transfersomes. Moreover Lei et al. [35], proved that transfersomal gel prepared from phosphatidylcholine and tween 80/span 80 as edge activator mixture in an atopic dermatitis-induced animal model managed to deposit tacrolimus into the deep skin layers with significant suppression of inflammation, and the associated wounds managed to grow a scab after 7 days compared to ointment and liposomal gel groups, confirming the superiority of transfersomes.

2.5 Nanoemulsions

Nanoemulsions are nano-lipid-based soft delivery systems, composed of water, oil, and surfactant/cosurfactant [36]. Owing to their biocompatible and safe components, several studies were reported in the literature for their use as wound healing therapies. Microemulsions are also often referred to as nanoemulsions, but they contain more surfactant/cosurfactant concentration and are prepared using a delicate ratio of oil/water/surfactant components, mostly using the water dilution method [9]. Since microemulsions exhibit nanometer-size range, they will be referred to as nanoemulsions in this work.

The oily phase of the nanoemulsion was reported to influence its wound healing

potential, since it affects the release of the ingredients from the oily core [37], and the inclusion of actives within nano/microemulsion formulations was shown to exhibit enhanced wound healing potential. Cinnamon oil nanoemulsion was found to be more effective than the oil alone in accelerating wound healing in animals [38]. Moreover, nanoemulsions can contain more than one active ingredient, in which Shanmugapriya et al. [39] loaded astaxanthin and alpha tocopherol in nanoemulsion formulation that managed to improve closure of cells in scratch wound healing cellular assay compared to control, delineating this nanoemulsion system as a potentially promising topical carrier. Similarly, phenytoin nanoemulsions improved cellular closure in scratch assay compared to the free phenytoin [40]. Nanoemulsion hydrogels were also loaded with growth factor combinations, displaying better skin permeability compared to the unencapsulated form, and were delineated as promising systems worthy of experimentation as wound healing systems [41]. In addition Cao et al. [42], prepared benzalkonium chloride nanoemulsion, and proved their antiinflammatory activity and therapeutic efficacy in skin abrasion wound model compared to control. Moreover, fusidic acid loaded in nanoemulsion gel accelerated wound healing in animals compared to the marketed cream of the drug [43].

2.6 Solid lipid nanoparticles/ Nanostructured lipid carriers

Among the popular lipidic delivery systems are solid lipid nanoparticles and nanostructured lipid carriers. Both systems are composed of solid lipid matrix, with NLCs additionally containing oil in their matrices [44]. Both systems can be used on their own or can be incorporated within additional dressings [45].

Quercetin-loaded SLNs accelerated the healing of wounds compared to quercetin alone [46]. SLNs also managed to create a combination therapy loaded with a peptide LL37 and an elastase inhibitor Serpin A1 that accelerated wound healing in cellular experiments [47], and another combination therapy of curcumin and ampicillin, which also accelerated wound healing, but in animal model [48]. When tetrahydrocurcumin was encapsulated in SLNs gel, it displayed faster wound healing compared to the unencapsulated form [49], and similarly, the topically applied SLN-loaded astragaloside IV displayed significantly higher wound closure percent compared to the unencapsulated form [50].

Regarding NLCs, those prepared using olive oil and loaded with eucalyptus oil caused significant reduction in wound areas of rats [51]. A comprehensive research reported by Gainza et al. [52] studied several wound healing parameters in porcine full-thickness excision wound model, in which they compared the effectiveness of NLCs-encapsulated recombinant human epidermal growth factor with the free form, and reported that even when smaller dose of the growth factor was administered in NLCs form, it exhibited better wound healing in terms of healing wounds percentage, epithelization, collagen formation, lower inflammation than the higher dose administered of the free form, attributed to its protection of the latter against enzymatic degradation. Another interesting study by Alalaiwe [53] reported that loading of oxacillin antibiotic within cationic NLCs enhanced the bactericidal activity of the antibiotic against methicillin-resistant *S. aureus* MRSA, and resulted in almost complete treatment of the abscesses, with significant reduction of water loss from infected wound compared to mere antibiotic administration, accompanied with much less inflammation of the wound.

3. Inorganic nanoparticles

Among the various types of inorganic nanoparticles, metallic nanoparticles such as silver (Ag), gold (Au), selenium (Se), and copper (Cu) nanoparticles, as well as zinc oxide (ZnO), iron oxide (Fe_2O_3), and titanium dioxide (TiO_2) nanoparticles have attracted significant attention lately in the area of wound healing, due to the proven biomedical applications of their metallic content, in addition to the delivery ability and unique properties of nanoparticles that reduce the cytotoxicity of the metals and increase their stability and therapeutic efficacy [54,55]. Therefore, the next sections of this chapter summarize studies conducted on the different inorganic (especially metallic) nanoparticles for wound healing and regeneration.

3.1 Silver (Ag) nanoparticles

Different forms of silver (both metallic and ionic) have long been used for wound healing applications due to their broad-spectrum antimicrobial effect, antiinflammatory traits, and wound healing promoting properties. The importance of silver was displayed thousands of years ago when the therapeutic applications of silver powder started to be discovered. The importance of silver nitrate was identified around the 17th and 18th centuries, in which it was applied clinically for treatment of skin ulcers and wounds, and was used as a disinfecting agent for wounds during the World War I era. The use of silver salts declined consequent to the introduction of antibiotics in 1940 due to the cost and doubtful toxicity of the metal. The interest in the use of silver for wounds was regained following the emergence of bacterial antibiotic resistance [54]. This resulted in the development of silver dressings and their commercial use for burns and wounds. Silver was applied to burns, either in the form of impregnated dressing or as the benchmark silver sulfadiazine cream. Nowadays, several forms of silver-based dressings are currently available commercially, either as fibers or polymeric scaffolds impregnated or coated with Ag salt or metallic Ag in nanoparticulate form. Recently, it has been proposed that silver performs its antibacterial activity through interacting with the bacterial cell wall, altering the bacterial DNA, and blocking its respiratory pathways, resulting in its death. Clinically, some studies have confirmed their safety for patients while others have raised concerns about their cytotoxicity on fibroblasts and keratinocytes [56].

Silver nanoparticles (NPs) (AgNPs) have been shown to possess unusual physical, chemical, and biological properties. Silver nanoparticles have been reported to possess antibacterial, antifungal, antiviral, antiinflammatory, antiangiogenesis, and antiplatelet activity. Nanoparticles have many advantages, such as possessing a large surface-to-volume ratio resulting in high reactivity, in addition to the ability of NPs to sustain the release of silver, hence prolonging its effect and minimizing its toxicity [57]. AgNPs have been used extensively as antimicrobial agents against different pathogens, especially against dermal pathogens, including *S. aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pyrogens*, as well as the methicillin- and oxacillin-resistant *S. aureus* (MRSA and ORSA) [58]. The mechanism of action of AgNPs doesn't differ greatly from that of Ag ion or metal. The presence of Ag in a nanoparticulate form results in better contact with the cell membrane of microorganisms. Moreover, it provides better interaction with sulfur present in cell membrane proteins as well as cellular DNA that contains phosphorus. This is followed by the interaction of NPs with the respiratory chain and cell division. Moreover, AgNPs were also reported to improve tensile properties of repaired skin by influencing collagen alignment [59].

Tian et al. [60] compared the antibacterial property of silver nanoparticles (at a lower amount) to 1% silver sulfadiazine cream both

grafted on a dressing, and compared their efficacy in healing of wounds. Two types of wounds were induced, one formed by thermal injury as an acute wound model and another diabetic wound as a chronic wound model in mice. The animals treated with silver nanoparticles showed higher healing rates in both acute and diabetic wound models with almost 11 days difference in the case of acute wounds. Moreover, healed skin after treatment with AgNPs showed the most resemblance to normal skin in comparison to that treated with sulfadiazine cream and control. For confirmation, the antibacterial and antiinflammatory activity of both formulations was studied throughout the wounds' healing process and compared. Silver nanoparticles inhibited bacterial growth for 7 days post injury while silver sulfadiazine group showed bacterial growth after 3 days. By monitoring the level of inflammatory mediators throughout the healing process, they found that the level of some mediators as IL-6 mRNA was significantly lower in the wound areas treated with AgNPs. Several studies showed that the therapeutic effects of AgNPs (in suspension form) depended on important nanoparticulate features, including particle size (surface area and energy), particle shape (catalytic activity), particle concentration (therapeutic index), and particle charge (oligodynamic quality) [55,58].

Biosynthetic methods of AgNPs have been recently investigated as an alternative to chemical and physical ones. Sundaramoorthi et al. [61] investigated the potential usefulness of *Aspergillus niger* in the production of AgNPs extracellularly, and evaluated their wound healing activity using both excision and thermal wound models. Results confirmed the superior wound healing activity of AgNPs synthesized from *A. niger* compared to silver nitrate. Synthesis of AgNPs from plant origin has been also reported by Dhapte et al. [62] where they synthesized AgNPs from *Bryonialaciniosa* leaf extract and tested their wound healing efficacy in comparison to sulfadiazine cream. The NPs exhibited better cytocompatibility, faster and more complete healing of the wounds.

Regarding toxicity of AgNPs, recent studies demonstrated their possible toxic effects on human fibroblasts and keratinocytes through decreasing mitochondrial function, shrinking of cells, as well as production of reactive oxygen species (ROS), which was found to be both particle size and concentration dependent [56]. Another group monitored mitochondrial functionality in human fibroblasts, and they found signs of reduced activity with lack of apoptosis or cell death signs. This reduction occurred temporarily as a cell protective mechanism against AgNPs and didn't affect the cell viability. Reproliferation of mitochondria would occur once the silver was removed, resulting in the renewal of the dermal tissue in vivo [55,56].

The concept of surface modification or coating of metallic nanoparticles, especially AgNPs, has been recently introduced with the aim of improving the biocompatibility and decreasing the toxicity of Ag ions through delaying their release. Table 4.3 displays some examples for coated AgNPs. Keletemur et al. [63] coated AgNPs with oligonucleotide [5′−HS− (CH2)6-TAATGCTGAAGG-3] and compared their activity to uncoated AgNPs on an in vivo mouse wound model. Surface functionalization of AgNPs resulted in acceleration of the proliferative phase of wound healing through faster deposition of fibroblasts and collagen in the wound, hence achieving more rapid wound healing. Similar results were obtained by Im et al. [64] upon modifying the AgNPs with chondroitin sulfate and acharan sulfate. Several other studies coated AgNPs with different polymers and proved the efficacy of the modified system in enhancing their antimicrobial effect, healing and regeneration of wounds besides decreasing the toxicity of Ag. The utilized polymers were chitosan/poly(vinyl alcohol) nanofibers [65], hyaluronan [66], and pectin [67]. Pallavicini et al. [67] utilized pectin for dual purposes, as a reducing agent and as a coating material for

TABLE 4.3 Surface-coated silver nanoparticles for wound healing.

Coating material	Model	Wound healing	Source
Oligonucleotide ([5′−HS− (CH2)6-TAATGCTGAAGG-3])	BALB/C mice excision wound model	Faster fibroblasts and collagen deposition associated with faster wound healing	[63]
Chondroitin sulfate and acharan sulfate	Male ICR mice incisional wound model	Enhanced wound healing activity	[64]
Hyaluronan	Nondiabetic and diabetic rat models	Enhanced antibacterial activity and wound healing activity	[66]
Chitosan/poly(vinyl alcohol)	Male sprague−Dawley rats incisional wound model	Enhanced cytokines and collagen production, and accelerated wound healing	[65]
Pectin	In vitro wound healing model	Enhanced antibacterial activity, fibroblasts proliferation, and wound healing activity	[67]

AgNPs. The association of AgNPs with pectin greatly enhanced their antibacterial activity against both *Escherichia coli* (gram negative) and *Staphylococcus epidermidis* (gram positive) bacteria. Moreover, it enhanced the wound healing activity through accelerating fibroblasts proliferation.

Regarding antimicrobial dressings, they were found to decrease the risk of multiple infections and provide a favorable environment for promoting the normal healing process. Nanosilver dressings possess unique features for wound management due to their antimicrobial effects in controlling infection and inflammation, ability to balance moisture content in the wound and manage epithelial regeneration. Nowadays, natural biomaterials (collagen, gelatin, chitosan, fibroin, and keratin) and their derivatives are used as constructing materials for biodegradable wound dressing [54]. One of the most advantageous biodegradable polymers used in the fabrication of wound dressings is chitosan. AgNPs in chitosan dressing was synthesized by Lu et al. [68] and its wound healing activity was compared to sulfadiazine dressing and plain unloaded chitosan dressing. AgNPs-chitosan dressing showed 21% and 15% acceleration in wound healing rates compared to sulfadiazine and mere chitosan dressing, respectively, and hence, the incorporation of NPs in the mentioned wound dressing enhanced both its antibacterial and wound healing abilities. Singh and Singh [57] prepared AgNPs embedded in chitin membranes and tested their antimicrobial wound healing ability. They found that 100 ppm AgNPs in chitin membranes showed promising antimicrobial activity against common wound pathogens.

3.2 Gold (Au) nanoparticles

Besides the various known therapeutic applications of gold nanoparticles in tumor therapy and diagnosis, the use of gold nanoparticles (AuNPs) in wound healing, alone or with other antioxidants, has been investigated owing to its antioxidant properties and its skin penetration capabilities through interaction with skin lipids and opening of the stratum corneum [59,69].

The combination of AuNPs with two antioxidants, epigallocatechin gallate (EGCG) and α-lipoic acid (ALA), by Leu et al. [69] showed accelerated wound healing on diabetic mouse wound through promoting the proliferation and migration of dermal fibroblasts and keratinocytes. Complete wound healing in the group treated with the AuNPs/antioxidants combination was exhibited by the seventh day after injury. By further investigation to elucidate the

mechanism of wound healing of the system, it was found that AuNPs/antioxidants combination accelerated wound healing through exhibiting antiinflammatory and antioxidant effects. Significant increase of vascular endothelial cell growth factor and angiopoietin-1 protein expression was shown after 7 days, whereas CD68 protein expression decreased and Cu/Zn superoxide dismutase increased significantly in the wound area in the group treated with the combination compared to other groups, thus explaining the significant antiinflammatory and antioxidant effects of the combination of AuNPs with other antioxidants. Similarly, Huang et al. [70] reported high acceleration in wound healing in diabetic mice wound model upon combining AuNPs with EGCG and applying the mixture through topical gas injection using the GNT GoldMed Liquid Drug Delivery. As similarly encountered with the other authors, a significant increase of the vascular endothelial cell growth factor on day 7 and the Cu/Zn superoxide dismutase expression from day 3 to day 7 was reported. Moreover, a significant increase in collagen I, III and hyaluronic acid protein expression was detected in the wound area after 7 days.

Recently, AuNPs alone were used as antioxidant to overcome the oxidative stress generated during wound healing process delineated by the increased ROS production in the wound area during photobiomodulation therapy (PBMT), which is a technique that depends on light application to stimulate cellular function, cellular migration, angiogenesis, and enhance quality of wound. Typically, ROS is generated as an active by-product during the healing process, especially with PBMT, which if not restricted would damage DNA, RNA, protein and inhibit growth. The application of AuNPs with powerful antioxidant effects in this case resulted in significant increase in wound healing rate owing to enhanced epithelialization, collagen deposition, and fast vascularization [71].

3.3 Metal oxide nanoparticles

Some studies investigated the use of metal oxides as zinc oxide (ZnO), titanium dioxide (TiO_2), and iron oxide for wound healing purposes. Therefore, examples of the therapeutic potential of these nanoparticles will be provided in the following section.

3.3.1 *Zinc oxide nanoparticles*

Zinc oxide NPs (ZnONPs) are incorporated into a variety of wound healing skin coatings due to their proven antimicrobial and/or antifungal properties, which becomes even more pronounced in the NPs rather than microparticles forming due to the higher surface-to-volume ratio of the former.

For control of postoperative wounds, ZnONPs were fabricated and impregnated in cefazolin nanofiber and compared with individual components. The combination achieved high entrapment efficiency and sustained release behavior for ZnO. Higher antimicrobial activity was achieved with ZnONPs and cefazolin combination of 1:1 w/w. A conducted in vivo study on Wistar rats showed higher wound healing rate in the group receiving the ZnONPs/cefazolin combination compared to plain cefazolin and ZnONPs-loaded dressings, which was related to their enhanced cell adhesion, epithelial migration, hence faster and more efficient collagen synthesis [72]. Raguvaran et al. [73] synthesized ZnONPs and embedded them in a biodegradable matrix with a known reported wound healing activity (sodium alginate-gum acacia hydrogel matrix) with the aim of decreasing toxicity and increasing efficacy of ZnONPs. The embedded NPs showed significant antibacterial effect on *P. aeruginosa* and *Bacillus cereus* at lower concentration than ZnONPs alone.

3.3.2 *Iron oxide nanoparticles*

The application of different forms of iron NPs in wound healing has also been recently reported. Moradi et al. [74] attempted a triple

combination approach toward better management of acute wounds, aiming for faster healing and better skin characteristics after healing. They combined the PBMT laser therapy with the application of curcumin-bound iron oxide (Fe_3O_4) superparamagnetic NPs, and compared the antibacterial and in vivo wound healing activity to other groups (control group, curcumin suspension group, laser only group). Results showed significantly higher antibacterial and wound closure rate in addition to better skin strength after healing with the curcumin-bound iron oxide NPs.

3.3.3 *Cerium oxide nanoparticles*

Cerium oxide nanoparticles were reported to possess autoregenerative and radical-scavenging properties, which eliminate oxidative stress in the wound area by scavenging the excess of ROS [75]. Moreover, cerium oxide nanoparticles were reported to induce hydroxylproline and collagen production, resulting in increased wound tensile strength and reduced wound closure time activity [76]. Recently, Rather et al. [77] fabricated cerium oxide NPs functionalized with polycaprolactone (PCL)-gelatin nanofiber (PGNPNF) by electrospinning and evaluated their antioxidative and proliferative potentials. The PGNPNF exhibited strong antioxidant property, which was evidenced by the strong ROS scavenging potential measured by fluorescence microscopy. Consequently, the viability and proliferation of cells increased by three-fold. Moreover, it has been recently reported that diabetic wound healing is usually impaired due to increased inflammation and decreased expression of the regulatory microRNA (miR-146a), which is a key regulator of inflammatory response. Therefore, a new study by Zgheib et al. [75] conjugated miR-146a to cerium oxide nanoparticles and tested its antiinflammatory, antioxidant, and wound healing efficacy in diabetic wound model. The group treated with 100 ng of the nanoparticles in conjunction with miR146a showed faster healing rate, where wounds were fully closed at day 14 post injury compared to more than 18 days in other groups treated with miR-146a or cerium oxide nanoparticles alone. In addition the healed skin in the cerium oxide nanoparticles-miR146a-treated group was more tensile and elastic with improved biomechanical properties (increased maximum load and modulus), suggesting their promising nature.

3.3.4 *Titanium dioxide nanoparticles*

Titanium dioxide (TiO_2) has several applications in drug, cosmetics, and pharmaceutical fields owing to its therapeutic effects, safety, and corrosion resistance. It is applied pharmaceutically in the form of nanotube films to support bone and stem cells, prevent bacterial adhesion, and stop hemorrhage. TiO_2NPs have been applied in the area of wound healing either alone or in conjugation with AgNPs due to their germicidal and antimicrobial activities. Moreover, as previously mentioned, the NPs provide slow release of the metal, achieving better control of the microorganisms and better wound healing results). Archana et al. [78] combined TiO_2NPs of previously reported antimicrobial, antiinflammatory, and wound healing capabilities with the biocompatible polymers chitosan and PVP. The in vivo wound closure rates of open excision wounds in albino rat model were significantly higher in case of the TiO_2/chitosan/PVP combination compared to conventional gauze, soframycin skin ointment, and chitosan-treated groups with 100% complete wound closure after 16 days whereas other groups required significantly longer periods. Similar results were obtained by Javanmardi et al. [79] who synthesized TiO_2/gelatin composite and compared its wound healing efficacy on burn models in male albino rats to different groups (control group, group receiving silver sulfadiazine, and group receiving gelatin-based ointment). The best results were achieved with the TiO_2/gelatin combination, hence delineating the composite as promising wound healing modality.

3.4 Copper (Cu) nanoparticles

Copper was reported to exhibit a powerful wound healing activity, owing to its antimicrobial, antiinflammatory, immune boosting, angiogenesis enhancing, and antioxidant properties. The copper's antioxidant activity is exhibited by acting as cofactor for enzymes such as superoxide dismutase and cytochrome oxidase. It augments immunity by stimulating the production of interleukin-2, and stimulates angiogenesis through induction of vascular endothelial growth factor (VEGF) expression. For better activity in wound healing, its presentation in nanoform as copper nanoparticles (CNPs) would provide more catalytic activity and better bioavailability. Newly emerging approaches have been tried to minimize the toxicity of CNPs and increase their efficacy [80]. One of them was to find safer methods of production of these nanoparticles such as biosynthesis from microorganisms and green synthesis from plants. Moreover, coating of the nanoparticles with biocompatible and biodegradable polymers has been reported as an alternative approach. CNPs were biosynthesized from *P. aeruginosa* and their wound healing activity was tested in vivo on rat excision wound model. The pace of wound healing was faster in case of CNPs than control and native copper group with 92% healing rate achieved in only 10 days in CNPs treated group [80]. Coating and stabilization of CNPs was tried by other authors using different biodegradable polymers. Xiao et al. [81] stabilized CNPs by folic acid and assessed its cytotoxicity on cells and wound healing on splinted excisional dermal wound model in diabetic mice. Lower cytotoxicity and enhanced cell migration was recorded in folic acid−stabilized CNPs group, which was attributed to the slower release of copper ions from the composite. Moreover, folic acid−stabilized CNPs composite enhanced angiogenesis, collagen deposition, and reepithelialization, consequently resulting in faster wound closure rates.

Owing to the several advantages of the biodegradable polymer chitosan, it has been extensively investigated as a dressing material on wounds and burns with different nanosystems. Besides its biocompatibility and biodegradability, chitosan possesses an inherent antimicrobial property that made it an excellent polymer for wound dressings, especially when combined with drugs or NPs for wound healing. Recently, Jayaramudu et al. [82] synthesized two types of chitosan-capped copper nanocomposites, which showed good antibacterial activity elicited by large inhibition zones, with better activity against gram-negative *E.coli.* compared to gram-positive *Bacillus* microorganism.

3.5 Silicon nanoparticles

Silicon is an abundant trace element in the human body, which was reported to exhibit a controversial effect on skin, bone, and blood vessels. Silicon-based formulations, such as gels, dressings, bioactive glass ointment, and silica gel fiber fleeces, have been reported to be effective in wound healing. It was proposed that they act as both excellent dressing medium by providing favorable environment for healing and also perform a crucial action in healing through being released from the dressing, and reaching the dermal and epidermal layers. It was assumed that they work by increasing epidermal and dermal fibroblasts proliferation through enhancing the expression of β-FGF fibroblasts growth factor through the release of orthosilicic acid Si(OH)4 molecule. Therefore, the formulation of silicon in the form of silicon NPs would provide a slow release of the active molecule in addition to being more easily internalized, thus providing more efficient control of the wound [83].

Furthermore, the loading of silicon nanoparticles with drugs or bioactive molecules with the aim of increasing the efficacy of wound healing has been tried. A recent study loaded

Flightless I (Flii) siRNA into porous silicon nanoparticles, which is an actin remodeling protein that increases in wounds and is responsible for wound progression. The siRNA of this protein is responsible for silencing the protein, hence interfering with wound progression and promoting wound healing. The loading of siRNA in NPs is expected to overcome the drawbacks of applying this molecule alone, such as its inability to cross cell membranes, its sensitivity, and degradation by the endogenous nuclease enzyme. NPs would also provide sustained release of the siRNA, providing an additional advantage for its nanoencapsulation. For better control of the release and augmented escape from endogenous siRNA degrading enzymes, the system was coated with a chitosan layer, and the in vivo wound healing potential of the prepared system on acute excisional wounds was tested in comparison to siRNA alone and siRNA-unloaded NPs. Significant reduction in wound area (20% after 6 or 7 days) was observed with the siRNA NPs system compared to other groups and control group. Therefore it was concluded that the proposed chitosan-coated siRNA-silicon NPs can effectively deliver a sufficient dose of siRNA to the wound to induce wound closure and healing [84].

3.6 Selenium nanoparticles

Selenium is an essential trace element, with several reported medical applications such as prevention of cardiovascular diseases, cancer, hypercholesterolemia, and diabetes. Its recently proposed use in wound healing is attributed to its inherent antioxidant property, in addition to being a central constituent of many antioxidant enzymes and vitamins, which makes it an excellent candidate for use in wound healing preparations when formulated as NPs [85]. Selenium NPs were synthesized from *Streptomyces minutiscleroticus* M10A62 bacteria ,which was isolated from magnesite mine and proved the antioxidant, antiproliferative, and antibiofilm properties of selenium NPs. Rostami et al. [86] studied the in vivo wound healing activity of selenium NPs prepared using chitosan as modifier and stabilizer on rat wound excision model, in which the animal group treated with selenium/chitosan NPs showed significantly higher rate of new blood vessels formation and fibroblasts proliferation, hence higher wound healing rate.

4. Conclusions

Nanoparticles proved to be a versatile platform for wound healing purposes. Whether they were organic or inorganic in nature, their unique properties cause them to overcome the problems of conventional treatment modalities and induce improved treatment outcome. With the advancements in the discovery of functional materials, futuristic studies on composite nanoparticles customized for wound healing are expected to increase, and to eventually replace the conventional therapies.

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