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Nanoparticles in wound healing; from hope to promise, from promise to routine

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ABSTRACT

Chronic non-healing wounds represent a growing problem due to their high morbidity and cost. Despite recent advances in wound healing, several systemic and local factors can disrupt the weighed physiologic healing process. This paper critically reviews and discusses the role of nanotechnology in promoting the wound healing process. Nanotechnology-based materials have physicochemical, optical and biological properties unique from their bulk equivalent. These nanoparticles can be incorporated into scaffolds to create nanocomposite smart materials, which promote wound healing through their antimicrobial, as well as selective anti- and pro-inflammatory, and pro-angiogenic properties. Owed to their high surface area, nanoparticles have also been used for drug delivery as well as gene delivery vectors. In addition, nanoparticles affect wound healing by influencing collagen deposition and realignment and provide approaches for skin regeneration and wound healing.

1. INTRODUCTION

Wounds result from disruption of the normal anatomical epithelial lined tissue barriers and may be caused by trauma, tissue resection, or burns (6). Some wounds fail to heal in a timely fashion and become chronic as a result of co-existing conditions such as diabetes or peripheral vascular disease. Failure to heal might also result from post-operative wound infections which are estimated to affect up to 4% of patients who undergo surgery. Chronic non-healing wounds represent a growing health and economic burden and are associated with a high morbidity that adds significantly to the cost of medical care (7). The gold

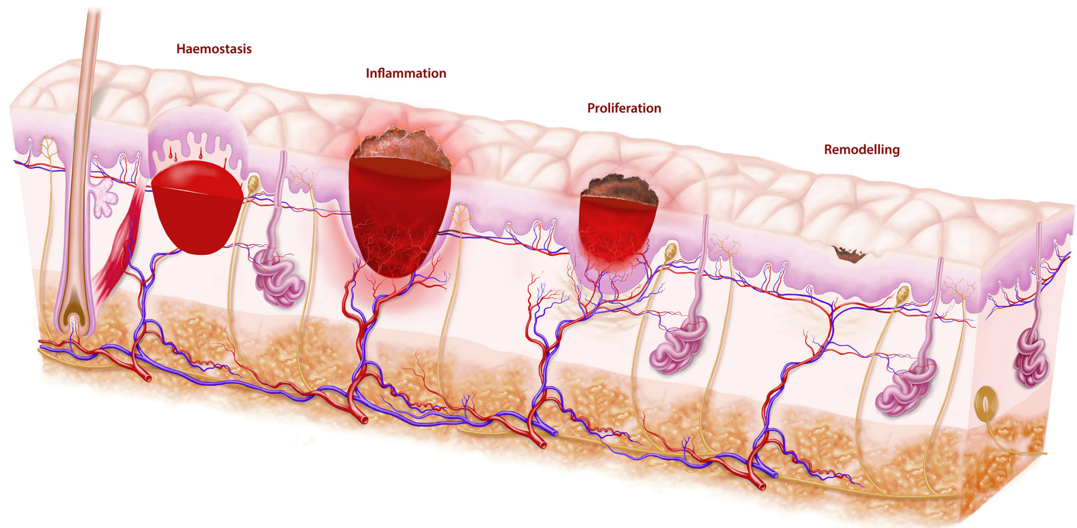

Figure 1. Schematic depiction of nanoparticles.

standard for the treatment of non-healing skin wounds is the transplantation of autologous skin. This strategy, however, might not be suitable in certain cases due to the lack of a donor site. In such cases, engineered skin substitutes are an alternative to autologous skin transplantation. Clearly, there is a need to develop strategies to promote wound healing and prevent scarring (8).

The use of cell therapy with or without of growth factors in experimental models has prevailed some positive results, but these therapies have not moved to clinical setting due to complications in scalable fabrication and storage, high costs, regulatory issues, and lack of standardisation. Moreover, their effectiveness and safety have not been demonstrated fully.

Nanotechnology is a rapidly expanding multidisciplinary scientific field, which combines the disciplines of material science and engineering. Nanoparticles (NPs), usually ranging in dimension from 1-100 nanometers (nm), have properties unique from their bulk equivalent. They possess unique physicochemical, optical and biological properties, which can be manipulated suitable for desired applications. Since ancient times, elements such as silver, gold, copper and titanium were used to treat a number of human conditions. More recently, researchers have developed insight in and awareness of nanoparticles and how these could be used for drug delivery, diagnostic and imaging, biosensor, and cosmetic purposes (9). Several nanomaterials for biological applications have been intensively investigated during the last several decades. These have included liposomes, dendrimers, quantum dots, fullerenes, carbon nanotubes, graphene, iron and titanium oxide, and gold and silver nanoparticles (Figure 1). Recently NP-based delivery of ions, such as calcium and oxygen has been used to promote angiogenesis (10). The application of nanomaterial-based scaffold with controlled delivery of calcium ions or oxygen would promote differentiation of ADSC to endothelial cells and angiogenesis (10).

Nanoparticles can be incorporated into biomaterials and scaffolds to create nanocomposite smart materials (Figure 1), which can aid wound healing through their antimicrobial (1), selective anti- and pro-inflammatory (2), and pro-angiogenic properties (3). They can be used as gene delivery vectors altering intracellular gene expression and protein synthesis related to the wound healing process (4). In addition, they can affect the wound healing process by influencing collagen deposition and realignment (5).

Wound healing either occurs by primary intention, where the wound edges are approximated and sutured, or by secondary intention, where the wound is left open to heal by a combination of granulation tissue formation, contraction, and re-epithelialisation. The wound healing process includes the subsequent and overlapping phases of haemostasis, inflammation, proliferation, and remodelling (Figure 2) (11). Haemostasis involves vasoconstriction, the formation of a platelet plug, and platelet degranulation. Inflammation occurs in the first two to three days after injury and involves the release of pro-inflammatory factors by platelets, which enhance inflammatory cell proliferation and migration. The proliferation phase overlaps the inflammatory phase and lasts up to 4 weeks. Here, inflammatory cells release chemo-attractants to fibroblasts, which migrate into the wound to deposit ground substance, type III collagen, and elastin. Angiogenesis occurs simultaneously. Finally, remodelling can last up to a year or longer and involves

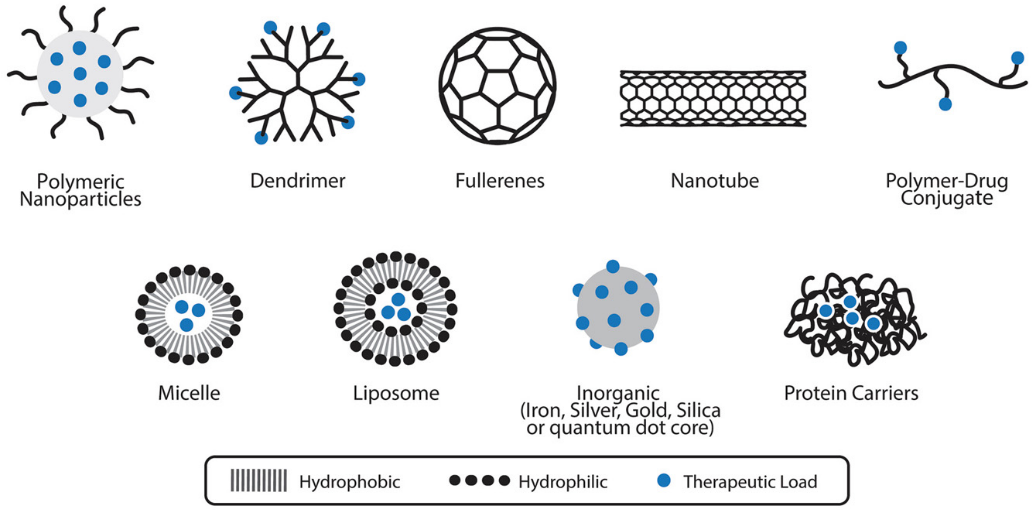

Figure 2. The four stages of wound healing; haemostasis, inflammation, proliferation, and remodelling.

re-arrangement and organisation of collagen fibres, as well as replacement of type III by type I collagen. It is a fine equilibrium between the inflammatory, proliferative, and remodelling phases that results in satisfactory wound healing.

Systemic and local factors that disrupt the weighed physiologic healing process can impede wound healing. Systemic factors are either congenital or acquired. Congenital factors include a range of genetic disorders associated with defective collagen synthesis, increased collagen degradation, defective elastin synthesis, prelamin an accumulation, and increased telomere decay. Acquired systemic factors include conditions such as diabetes mellitus, smoking, old age, vitamin deficiencies, and use of anti-inflammatory drugs. Examples of local factors are an infection, radiation, trauma, and poor tissue blood and neural supply.

Contrarily, excessive scarring following injury can result from a disruption in the equilibrium between the different wound healing phases. Keloids and hypertrophic scars differ from the healthy skin by a rich vasculature, high mesenchymal cell density, and thickened epidermal cell layer (12). In addition, they contain an abnormally high density of fibroblasts and unidirectional collagen fibrils (12). A prolonged or excessive inflammatory phase is believed to cause the onset of excessive scarring. Keloid scars can cause pain, pruritis, contractions, and are generally unaesthetic. Current treatment options include massage therapy, pressure garments, silicone gel sheeting, intralesional corticosteroid/5-fluorouracil injection, laser therapy, cryotherapy, radiotherapy, and surgery.

Both lack of appropriate healing and excessive scarring remain a common concern and an on-going challenge for clinicians. The incidence of refractory wounds is rising as a consequence of the ageing population, making the improvement of wound treatment a major healthcare issue (13). Current advances in wound healing aim to enhance regeneration and decrease scarring.

Nanotechnology has the potential to revolutionise the treatment of wounds through therapeutically active wound dressings using nanoparticles for the delivery of drugs, growth factors and pro-angiogenesis compounds such as calcium ions. Table 1 summarises recent studies investigating *in vivo* application of NPs in wound healing.

2. NANOPARTICLES IN WOUND HEALING

2.1. Nanoparticles with antimicrobial properties

Challenges facing the management of refractory wounds are often associated with microbial contamination and infection. The eradication of these is crucial for timely wound healing (33). The rise of multi-drug resistant pathogens has led to increasing use of nanoparticle-based anti-microbial remedies.

Currently, the metallic nanoparticles are thoroughly explored and extensively investigated as potential antimicrobials. The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms.

2.1.1. Anti-microbial activity of silver nanoparticles (AgNP)

The most vastly investigated nanoparticle with antimicrobial properties is silver. Several studies have confirmed the efficacy of AgNP and biomaterial

Table 1. Nanoparticles and wound healing preclinical studies.

Nanoparticle/ Nanoscaffold	Preclinical model	Wound	Procedure	Outcome
PLA nanosheets with AgSD	Mouse	Partial-thickness burns	Antimicrobial properties and cell viability assays	AgSD significantly reduced MRSA contamination both *in vivo* and *in vitro*
Pectin/copper exchanged faujasite	Rat and NIH3T3 fibroblast cell line	Burns	Assessment of membrane morphology, thermal stability, swelling and degradation	Cell viability of 89% was achieved, + improvement in wound healing & re-epithelialisation
NAC-SNO-NPs	Mouse	Burns	Histological examination of burn wounds for collagen deposition	Acceleration of the transition from inflammatory to proliferative wound healing
Quantum dots	Mouse	Laceration	*In vivo* optical system for assessment of wound healing.	Effective system for visualisation of wound healing.
Gold NPs (Au NPs)	Rat	Burns	Wound healing with Au NPs with microcurrent	Improved tissue repair due to enhanced mitochondrial function
Gelatin NPs	Rat	Full thickness laceration	Collagen and hyaluronic acid nanofibrous skin equivalent, with controllable release of angiogenetic factors	Acceleration of wound closure rate and elevated collagen deposition
AgNPs	Rat	Excision wound	Microwave irradiation of Naringi crenulata leaf extracts to synthesise bioactive AgNPs	Very effective wound repair and potential for tropical wounds
AgNPs coated with BC nanofibers	Rat	Partial thickness wound	Investigation of AgNP-BC for antibacterial properties and cytocompatibility	Reduction in inflammation and promotion of scald wound healing
Hypericin nanoparticles (HYNPs)	Rat	Infected excision wound	Antibacterial activity of hypericin	Improved epithelialisation, keratinisation, collagen deposition
Gelatin nanofibres	Rat	Excision wound	Development of gelatin nanofibrous mat loaded with epigallocatechin gallate / polyvinyl alcohol hydrogel	Significant increased in angiogenesis, re-epithilialisation and collagen synthesis
Pirfenidone NPs	Rat	Alkali burn in cornea	Assessment of corneal re-epithelialisation, haze and collagen deposition	Reduced collagen synthesis, prevented scarring/fibrosis, +improved corneal healing
Copper and zinc NPs	Rat	Soft tissue full layer excision wound	Wounds were either aseptic or infected	Regeneration attributed to antibacterial properties
Fibrin NPs coated with chitosan	Rat	Excision wound	Swelling, biodegradation, porosity, platelet activation and blood clotting	Faster wound healing and re-epithelialisation
AgNPs in alginate fibres	Mouse	Excision wound (2cm)	Investigation of AgNPs alone and AgNPs in alginate fibres with regards to wound healing	AgNPs in alginate fibres promoted fibroblast migration to the wound and increased epidermal
Elastin-like peptides & KGF (self-assembly)	Mouse	Excision wound	Assessment of efficacy of NPs in wounds of diabetic mice	These NPs offer a beneficial effect on chronic wounds
Fullerenes (carbon nanospheres)	Mouse & human skin, *ex vivo*	Skin irritation	Anti-inflammatory and anti-oxidant properties of fullerenes	Cell migration mediated human wound closure. Accelerated wound healing
AgNPs as a dressing	Dog	Severe burns (50% of TBSA)	AgNPs along with VAC dressing were assessed in wound healing	VAC and AgNPs successfully treated the dog
Mesoporous Silica NPs (MSN)	Rat	Achilles tendon injury	The effect of PDGF administration via MSN	Significant faster healing with PDGF incorporation
Lecithin NPs	Rat	Burns	Dihydroquecetin immobilised with lecithin NPs for healing of burns	Limitation of secondary necrotic zones in wounds and improvement of skin regeneration

Abbreviations: AgSD, silver sulfadiazine; PLA, poly (lactic acid) ; NPs, nanoparticles; NAC-SNO-NPs, N-acetylcysteine S-nitrosothiol nanoparticles; KGF, keratinocyte growth factor; VAC, vacuum assisted closure; TBSA, total body surface area; HA, hyaluronic acid.; BC, bacterial cellulose.

composites against bacterial contamination and infection (34-39). In terms of assessing the antibacterial efficacy of AgNPs of different sizes and surface conditions against Escherichia coli, Ag-resistant E. coli, Staphylococcus aureus, methicillin-resistant S. aureus (MRSA), and Salmonella sp, AgNP synthesized by base reduction with unmodified surfaces (sizes: 20, 50 and 80 nm) are toxic to all bacterial strains. AgNPs synthesised by base reduction followed by phosphate buffer washes (sizes: 20, 50 and 80 nm) and carbon-coated AgNPs (sizes: 25 and 35 nm) are toxic to all bacterial strains except Ag-resistant E. coli (40). Stable silver nanoparticles (AgNPs) generated via the active involvement of Bryonia laciniosa have shown antibacterial activity against both Gram negative and positive bacteria with no cytotoxicity observed *in vitro* (41). In addition, they lead to effective cytokine modulation. Preclinical wound healing showed AgNPs induced improved wound contracting ability in rats (41). Furthermore, AgNPs and carboxymethylcellulose gel formulation prepared by the reduction of silver nitrate *in situ* examined in simulated wound experiments showed that the gel was effective against the growth of both Gram-negative and positive strains including methicillin-resistant Staphylococcus aureus (MRSA) (42). Proliferation studies of human skin cells confirmed cytocompatibility of the composite (42).

In addition to anti-bacterial properties, AgNPs have also been shown to be effective against viral and fungal pathogens, including hepatitis B and HIV viruses (43-47). It is generally believed that various forms of Ag inactivate viruses by denaturing enzymes via reactions with carboxyl, amino, sulfhydryl, phosphate, and imidazole groups (48-52).

2.1.2. AgNPs combined with matrices as topical wound dressings

Silver-based dressings are currently in clinical use and have been evaluated thoroughly, including Ag-alginates, Ag-collagen preparations, Ag-hydrogels, Ag-hydrocolloids, Ag-fabrics, Ag-foams, and Ag creams and powders. However, the type of silver in many of these dressings is not specified. Several use ionic Ag whereas Acticoat uses nanocrystalline Ag. A retrospective analysis of burn wounds managed with Acticoat in humans showed a significant reduction in wound healing times for deep partial thickness burns compared to conventional paraffin gauze dressings (53). Generally, AgNP based dressings have shown superior results to more traditional Ag dressings such as Ag sulfadiazine cream (36, 54). Due to their beneficial antimicrobial activity and cytocompatibility, AgNPs often combined with hydrogels as silver nanocomposites have been widely investigated as antimicrobial wound dressings.

The use of nanocrystalline silver dressing (Acticoat) for the management of microbial contamination in cultured skin substitutes grafted to full-thickness wounds in athymic mice suggested that Acticoat may be suitable as a protective dressing to reduce contamination of cultured skin substitutes (55). AgNP polyvinyl alcohol (PVA) nanocomposite fibres for wound healing purposes showed significant inhibition of Gram-positive and negative bacteria (56). Preclinical studies combining AgNPs with a wide range of electrospun biomaterials as wound dressing materials have confirmed the antimicrobial properties of these constructs, as well as their positive effects on expediting the wound healing process. Electrospun poly (dopamine methacrylamide-co-methyl methacrylate) nanofibres functionalised with AgNPs through catechol redox chemistry showed effective AgNPs size and amount control with the minimum degree of aggregation. These dressings showed desirable antimicrobial activity against Gram-positive and negative bacteria. Following a rapid AgNP release in the first 24 hours, a sustained release was observed in the next 5 days. Preclinical study of the nanocomposite in full thickness skin wounds in rats showed expedited healing compared to controls (57). AgNP incorporated in the electrospun scaffold of a copolymer blend showed that a nanofibre membrane with good hydrophilicity and high porosity considerably facilitates *in vivo* wound healing especially at the early healing stage (58). Fibrous mats of electrospun poly (vinyl alcohol), chitosan oligosaccharides, and AgNP were also shown to accelerate *in vivo* wound healing over that of control gauze (37).

In addition to elecrospinning, scaffolds fabricated using other techniques combined with AgNPs have been evaluated preclinically. Silver and chitosan nanocomposite dressings, fabricated using a nanometre and self-assembly technology, tested on rats with deep partial thickness wounds showed significantly increased the rate of wound healing compared to Ag sulfadiazine with lower Ag levels in blood and tissues (59). Cellulose-chitosan-AgNP composite wound dressing showed faster wound healing in experimental wounds of rats compared to untreated control (60). Comparison between ionic and nanocrystalline silver and distilled water dressings showed increased wound contracture in rats treated with silver-based dressings (61). Similarly, guar gum alkylamine impregnated with AgNPs evaluated in rodents showed faster healing compared to a commercially available silver alginate cream (62). The nano biomaterial was observed to promote wound closure by inducing proliferation and migration of the keratinocytes at the wound site (62). Topical application of silver nanoparticles prepared from Naringi crenulata leaf extracts and evaluated preclinically in rats showed accelerated healing (20). AgNP-containing activated carbon fibres exhibited good biocompatibility *in vitro* and improved healing and collagen and granulation tissue deposition of infected wounds *in vivo* (38). AgNP incorporated into alginate fibres were shown to promote fibroblast migration, reduce inflammation, and improve wound healing both preclinical and *in vitro* (27). *In vivo* evaluation of AgNPs for wound healing showed these to exhibit antimicrobial properties in addition to the reduction in wound inflammation and modulation of fibrogenic cytokines (39). Biosynthesis of AgNPs using the Phytophthora infestans microorganisms showed stability and enhanced wound contraction ability in an *in vivo* excision wound model compared to Ag sulfadiazine (54). Synthesis of AgNPs using two glycosaminoglycans (chondroitin sulfate and acharan sulfate) as reducing agents supported the stability of these composites without any noticeable aggregation. A murine model of wound healing demonstrated that topical application of these nanocomposites stimulated wound closure and accelerated the deposition of granulation tissue and collagen at much lower Ag concentrations than commercial Ag sulfadiazine (36). Incorporation of silver-clay nanohybrid in poly (sulfobetaine) resulted in high, sustained, and diffusion-controlled antimicrobial activity of the silver-eluting polymer with antifouling properties resisting protein adsorption (63).

A matter that should be considered in the field of antimicrobial nanocomposite dressings is the selectivity towards microbes versus cytotoxicity to host cells and tissue. Silver preparations (55, 64), antimicrobial peptides (65), and antimicrobial photodynamic therapy (66) have been investigated for possible cytotoxicity towards human cells. AgNPs like other biocides are non-specific in action and cytotoxic to both microbial and human cells. They have been shown to be cytotoxic at high concentrations *in vitro*. The mechanisms underlying this cytotoxicity are believed to be agglomeration in cell nuclei and cytoplasm with induced intracellular oxidative stress (67), induced apoptosis via a mitochondrial pathway through ROS and JNK (68, 69), and DNA-repair gene-up regulation suggesting associated DNA damage (70, 71). Hence, dose regulation by entrapment in a matrix that utilises special drug carrier systems, as well as slow-release drug delivery systems may be useful in preventing their agglomeration. An *in vitro* study combining Ag sulfadiazine loaded lipid nanoparticles with chitosan found no cytotoxicity toward dermal fibroblasts and keratinocytes, suggesting that lipid encapsulation of Ag sulfadiazine prevents cytotoxicity (72). Similarly, polymeric micelles obtained by self-assembling of chitosan developed as carriers for Ag sulfadiazine showed a marked increase of Ag sulfadiazine concentration in micelle dispersion and reduced cytotoxicity (73). Nevertheless, it should not be neglected that several *in vitro* studies have attempted to approximate lethal and sublethal doses of silver solutions for keratinocytes, and values ranging from $7x10^{-4}$% to $55x10^{-4}$% in solution have been reported as toxic (74). A widely accepted mechanism of toxicity includes the release of Ag^{+} which readily interact with functional groups in proteins and can lead to enzymatic dysfunction and membrane damage, which manifest as nascent toxicity, symptomatically (75). Therefore, ensuring the dose is safe is equally important with administering a therapeutic dose, and the demonstrated cytotoxic potential of silver nanoparticles at higher doses should not be neglected.

Additionally, several preclinical studies have evaluated the toxicity of AgNPs. Toxicity studies of Acticoat in athymic mice with full-thickness wounds showed Acticoat to be toxic within 1 day, but *in vivo* exposure for a week did not injure the skin substitute or inhibit wound healing (55). A study looking at the safety of silver dressings including nanocrystalline silver in the treatment of MRSA-infected full thickness wounds in Sprague-Dawley and streptozotocin-induced diabetic rats showed that silver dressings induced slight liver damage in the diabetic rats (76). Although changes in serum chemistry caused by silver were observed, this did not indicate silver deposition in the organs and the hazards of silver-containing dressings were thought to be insignificant (76).

2.1.3. Recent Nanoparticles with antimicrobial properties

In addition to AgNPs, several other nanoparticles have been demonstrated to possess antimicrobial properties. Copper (Cu), graphene oxide, graphene, titanium oxide (TiO2), fibrin, polycationic NPs, and zinc oxide (ZnO) are amongst these nanoparticles (1, 77-83) often combined with biocompatible scaffolds as wound dressings (Figure 3). Photothermal treatment with the aid of NPs has also been described in the literature (22, 84), highlighting the efficacy of these constructs as antibiotic-free antimicrobial remedies promoting wound healing.

Graphene-based nanoparticles, in particular, have shown to promote wound healing through their antimicrobial properties (84-86). In addition to these properties, they can be combined with other materials to form nanocomposites, used for stem cell and/or growth factor delivery (87, 88), to enhance the bioactivity of materials (89-91), and to promote angiogenesis (92, 93). The intracellular formation of reactive oxygen and nitrogen species as well as activation of phospho-eNOS and phospho-Akt are believed to be the underlying mechanisms for graphene induced angiogenesis and antimicrobial properties (93). The results of these studies confirm the important role graphene NPs can play in wound healing.

2.2. Nanoparticles and angiogenesis

Angiogenesis, the formation of new blood vessels, plays a vital role in several physiological and pathological processes in the body. Angiogenesis is imperative for wound repair because new vessels provide nutrients and oxygen to support the actively proliferating cells.

Although several studies have suggested that gold nanoparticles (AuNPs) have anti-angiogenic properties (94-96), more recently, AuNPs have been

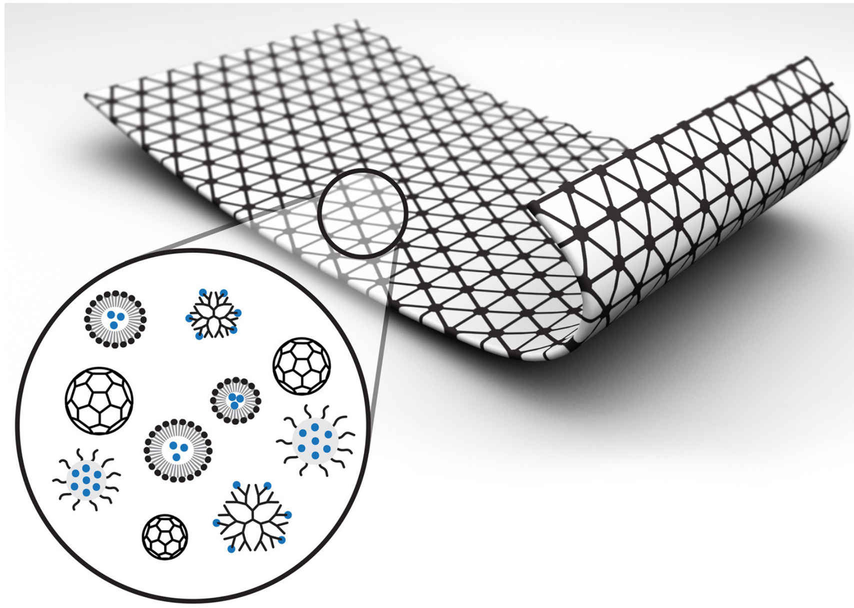

Figure 3. Nanomaterials incorporated into scaffolds for use in biomedical applications.

synthesised following a green chemistry approach, demonstrating their high stability and biocompatibility, as well as their excellent pro-angiogenic activity, through a series of *in vitro* and *in vivo* assays. Formation of reactive oxygen species and activation of p-Akt were believed to be the probable mechanism of angiogenesis (3). Similarly, the combination of AuNP, epigallocatechin gallate, and α-lipoic acid significantly accelerated diabetic cutaneous wound healing through angiogenesis regulation and anti-inflammatory effects (97). The combination led to an initial increase in vascular endothelial growth factor (VEGF) and angiopoietin-2 but not angiopoietin-1 expression (97).

It is highly likely that AuNPs modulate angiogenesis in healing wounds. Further preclinical studies are needed to draw conclusions about their angiogenic effects. Beside inherent angiogenic properties of NPs, these materials can also be used as drug delivery vectors for various factors, including some stimulating angiogenesis (98).

2.3. Nanoparticles and drug delivery

Nanoparticles have become potent drug delivery systems that have attracted much attention and interest as efficient carriers for various active compounds. The specific characteristics of nanoparticles make their use as drug delivery systems an interesting and suitable strategy. It is well established that growth factors (GFs) and other bioactive compounds play an important role in wound healing, inducing cell proliferation and migration, angiogenesis, and collagen deposition (99). There is a substantial potential for combining these factors and appropriate cells to treat wounds.

The majority of NP-based drug delivery systems aim to deliver growth factors to the wound site. Several studies have designed and described the effects of NP vectors that deliver VEGF, recombinant human epidermal growth factor (rhEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), granulocyte colony stimulating factor (GCSF), keratinocyte growth factor (KGF), and platelet-rich plasma (PRP), which contains an array of GFs.

Constructs incorporating VEGF, EGF, bFGF, and PDGF either directly embedded in collagen-hyaluronic acid (HA) nanofibrous matrices or encapsulated in gelatin NPs have also been made. Collagen-HA-gelatin NP constructs had similar mechanical properties to the human skin. In addition, the design of a particle-in-fibre structure allowed a slow controlled release of the GFs for up to 1 month. *In vitro*, these constructs stimulated the growth and maturation of endothelial cells. Application of these composite GF delivery systems on wounds of diabetic rats was associated with accelerated closure rate, together with elevated collagen deposition and enhanced maturation of vessels (19). Similarly, poly (ether) urethane-polydimethylsiloxane/fibrin scaffolds containing poly (lactic-co-glycolic acid) (PLGA) NPs loaded with VEGF and bFGF accelerated wound closure in genetically diabetic mice compared to scaffolds without growth factors or containing unloaded PLGA NPs. However, the closure rate was similar to that observed in mice treated with scaffolds containing free VEGF and bFGF. Both scaffolds containing growth factors induced complete re-epithelialization, with enhanced granulation tissue formation and maturity and collagen deposition compared to the other groups (100). Others designed a dual GF delivery system based on electrospun chitosan and poly (ethylene oxide) matrices loaded with VEGF and embedded with PDGF encapsulated poly (lactic-coglycolic acid) (PLGA) NPs. *In vitro* studies revealed that the nanofibrous composites delivered VEGF quickly and PDGF in a delayed manner. They supported fibroblast growth, exhibited anti-bacterial activities, and preclinical significantly accelerated wound healing by promoting angiogenesis, increasing re-epithelialization and controlling granulation tissue formation. For later stages of healing, evidence also supported quicker collagen deposition and earlier remodelling (101). Mohandas *et al.* developed VEGF-loaded fibrin NPs, incorporated into chitosan-HA sponges as dressings for diabetic wounds. The nanocomposites combined with human umbilical vein endothelial cells induced capillary-like tube formation *in vitro*, which was absent in control sponges, suggesting that the VEGF-fibrin NP-chitosan-HA constructs have the potential to induce angiogenesis (102).

PLGA-rhEGF NPs showed a controlled release of rhEGF encapsulated in the NPs and enhanced rhEGF effects on cell proliferation whilst shortening preclinical wound healing time in diabetic rats when compared to controls (free EGF, NPs, and phosphate-buffered saline (PBS). The PLGA-EGF NPs were uniform and dispersible and EGF release lasted for 24 hours (103). A similar topical delivery system composed of lipid NPs and rhEGF showed excellent bioactivity *in vitro* even higher than that of free rhEGF. *In vivo* examination in both diabetic mice and a porcine model showed improved healing evidenced by the number of arranged microvasculature, fibroblast migration and proliferation, collagen deposition and evolution of the inflammatory response, whilst rhEGF plasma levels were almost undetectable (104-106).

KGF, combined with elastin-like peptides fabricated using self-assembly to form a fusion protein and applied to wounds of diabetic mice, was associated with enhanced re-epithelialisation and granulation compared to controls (28). Another study utilised GCSF-loaded dextran NPs coated with PLGA and spray-painted on haemostatic gauze as a scaffold for application in post tumour resection wounds and demonstrated enhanced haemostasis and blood neutrophil counts *in vivo* (107). PRP contains many GFs and can also be used to enhance wound healing and GF delivery. PRP combined with heparin-PLGA NPs and fibrin gel was associated with a prolonged PDGF release compared to PRP and fibrin gel alone (108). Examination of the construct in a murine model resulted in much faster wound closure, as well as dermal and epidermal regeneration compared with PRP-fibrin gel and heparin-PLGA fibrin gel. Heparin-PLGA-fibrin gel PRP also accelerated angiogenesis (108).

In the same way, other bioactive molecules and compounds can be incorporated into NPs for sustained release at the wound site. These compounds may include antibiotics, analgesics, and peptides. A system based on HA and lipid NPs for the delivery of Astragaloside IV, the active compound of Astragali Radix (the root of Astragalus membranaceous plant) was used to accelerate wound healing and reduce scars (109). This construct enhanced the migration and proliferation of keratinocytes and increased drug uptake on fibroblasts *in vitro* ($p < 0.01$). It strengthened wound healing and inhibited scar formation *in vivo* by increasing wound closure rate ($p < 0.05$) and contributing to angiogenesis and collagen remodelling (110).

Delivery of topically applied opioids using NP can lead to efficient pain reduction. Opioids encapsulated in lipid and dendritic NPs yielded enhanced load delivery compared to unloaded NPs and free morphine (111). Interestingly, transforming growth factor beta1 (TGF-β1) was taken up by dendritic NPs. Opioid-lipid NPs enhanced keratinocyte migration, whereas opioid-dendritic NPs did not inhibit this. Another morphine-lipid NP delivery system tested in a human-based 3D-wound healing model showed accelerated re-epithelialisation and wound healing, suggesting that in addition to analgesic effects, opioids may improve wound healing (112).

LL37, an endogenous human host defence peptide that modulates wound healing and angiogenesis and has anti-microbial properties, encapsulated in PLGA NPs displayed antimicrobial activity against Escherichia coli (113). *In vivo* examination of the nanocomposite showed that treatment with PLGA-LL37 NPs significantly accelerated wound healing compared to PLGA or LL37 alone. PLGA-LL37 NP-treated wounds were characterised by advanced granulation tissue formation with higher collagen deposition, re-epithelialisation and neovascularisation (113). PLGA-LL37 NP improved angiogenesis, significantly up-regulated IL-6 and VEGF expression and modulated the inflammatory wound response (113).

Nanocarriers comprised of clarithromycin encapsulated in chitosan NPs are biocompatible *in vitro*, as well as able to increase the concentration of clarithromycin compared to a saturated water solution (114).

In conclusion, NPs can be effectively used for the targeted and sustainable delivery of several drugs, GFs, and other bioactive compounds, which can play an important role in the wound healing process.

2.4. Nanoparticles and immunomodulation

Cell populations and the complex signalling pathways that regulate the stages of wound healing depend on an intact and functional immune system.

Macrophages are pivotal cells in orchestrating the healing of wounds (115, 116). They debride unhealthy tissue and phagocytose invading bacteria. Macrophages are further activated within the wound to secrete a variety of cytokines that recruit other cells and enhance their proliferation, thereby promoting regeneration. Injection of activated macrophages into wounds of patients was reported to promote wound healing (117). Essential to wound healing, depletion of wound T lymphocytes decreases wound strength and collagen content, while selective depletion of the CD8+ suppressor subset of T lymphocytes enhances wound healing (118). Lymphocytes also exert a down-regulating effect on fibroblast collagen synthesis via several secreted lymphokines (interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α), and interleukin-1 (IL-1) and by cell-to-cell contact. Fine-tuning of the immune system response can aid the process of wound healing. Nanoparticles that modulate the immune response and inflammation phase of wound healing have been vastly studied in the literature.

AuNPs are among the NPs studied for their immunomodulatory properties. Silica–gold core–shell NPs (SiO2-Au) applied to wounds *in vivo* promoted wound healing, which was potentially related to the anti-inflammatory and anti-oxidation properties of AuNPs (119), although an exact mechanism was not detailed. *In vitro* evaluation did not show any cytotoxicity of the composites (119). A similar study examining the effect of AuNPs combined with epigallocatechin gallate, and α-lipoic acid on wound healing in diabetic mice showed that the nanocomposites significantly accelerated diabetic wound healing through angiogenesis regulation and anti-inflammatory effects (97). Immunoblotting showed a significant decrease of CD68 expression whilst VEGF significantly increased following treatment (97).

In addition to AuNPs, AgNPs also play an anti-inflammatory role in wound healing. Treatment of porcine wounds with nanocrystalline Ag significantly increased induction of apoptosis in the inflammatory cells present in the dermis (120). Additionally, decreased levels of pro-inflammatory cytokines TNF-α and IL-8, and increased levels of anti-inflammatory cytokine IL-4, EGF, KGF, and KGF-2 were observed (120). A similar study investigating dendrimers, as well as AgNPs, showed that both these NPs had anti-inflammatory properties. When combined in the form of dendrimer-AgNP composites the anti-inflammatory properties were further augmented, resulting in faster wound healing *in vivo* (2). Others (121) describe that nanocrystalline Ag dressing decreases adversely high levels of matrix metalloproteinases (MMP) -9, a proteolytic enzyme involved in wound healing. High MMP-9 levels promote TNF-α, IL-8, and TGF-β, all associated with exaggerated ongoing inflammation. Low levels impede keratinocyte migration (121). When used in a situation of minimal inflammation, these dressings may undesirably decrease the low levels of MMP-9 and adversely affect epithelialization.

Several other NPs have been investigated for their immunomodulatory effects. These include dendrimers (122), TiO2 NPs (80, 123), fullerenes (29), curcumin (124), tea tree oil NPs (125), and Cu NPs (83). As described previously, NPs can also be used as drug delivery tools loaded with immunomodulatory compounds such as α-gal (126) and indomethacin (127).

2.5. Nanoparticles and collagen deposition

AgNPs impregnated polyelectrolyte multilayer (PEM) have been shown to be non-cytotoxic yet bactericidal *in vitro*. A full-thickness, excisional murine wound healing model in both normal and spontaneously diabetic mice showed mildly increased collagen deposition in the silver dressing treated animals (128). Other studies have shed light to the excellent structural alignment of the collagen, as well as its increased deposition in improving tensile properties of tissues such as skin, after administration of silver nanoparticles post-wound healing (5). Silver is not the only nanoparticle able to induce collagen synthesis. Calcium-based nanoparticles also cause contracture of collagen lattices and stimulate fibroblast activity (129). This may show great potential and it will not be unlikely to see calcium nanoparticles used for wound healing in the future. Returning to the realm of the metals, with a more noble approach, one should not overwrite gold nanoparticles (AuNPs) from the scene. AuNPs have been used particularly for imaging of scars, as they are adept at accumulating at those sites, particularly large myocardial scars (130). In combination with other nanoparticles from this immense arsenal, these localisers of scars may prove very promising in the near future. Some managed to develop a platform of sustained release of nitric oxide, using nanoparticles, to achieve a reduction in inflammation, along with a marked increase in collagen deposition, accelerating wound healing (131). Other interesting designs have produced similar effects; carbon nanotube on polystyrene and polyaniline copolymer increased collagen gelation in a dose-dependent manner, whilst leaving D-periodicity and average fibril diameter of this immense protein, largely unchanged (132). An appreciation of the size of collagen molecules, along with the cross-linking required, results in more justice to the complexity of the process and the role of nanoparticles.

2.6. Gene delivery with nanoparticles for acceleration of wound healing

Nanoparticles have also been used in studies of genes for acceleration of wound healing. Several groups have tried different methods, with vascular endothelial growth factor (VEGF) transfection being the most common one. Viral vectors have been used to administer VEGF to diabetic patients and an effect in wound healing was observed as soon as 6 days post-transfection (133). Viral vectors, despite the amount of attenuation or other measures which reduce the likelihood of generating an immune response, should always be treated with caution. As a result, most groups focused on non-viral transfections, using nanoparticles; Peng *et al.* introduced VEGF into bone mesenchymal stem cells, using β-cyclodextrin linked polyethyleneimine. The results were more than promising, as wound closure and increased collagen formation were observed 72hours post transfection (134). They also looked at the effects of VEGF in a gelatin scaffold in terms of skin re-epithelialisation and collagen synthesis, both of which were increased (135). Another way to facilitate wound closure apart from inducing angiogenesis is to minimise local motility in the cellular level. Fidgeting-like 2 is a severin enzyme involved in this process, and inhibition with target delivery of siRNA using nanoparticles accelerates healing (4, 136). Intradermal delivery of key genes such as the sonic hedgehog has been performed, as well. The mode was biodegradable cationic poly (β-amino ester) nanoparticles, which resulted in greater transfection compared to the commonly used Lipofectamine 2000 (137). Other carriers, such as β-cyclodextrin and poly (amidoamine) dendron atoms have also been tested and proven successful in promoting wound healing in streptozocin-induced diabetic mice (138). Finally, hypoxia inducible factor (HIF-1α) has been entrapped in fibrin to improve the healing process in full thickness wounds, by being a potential inducer of VEGF (139). Although the evidence in this field is recent and limited, some fundamental questions regarding the safety and efficacy of this technique shall be elucidated in the years to come.

2.7. The role of nitric oxide nanoparticles in wound healing

The presence of Nitric Oxide (NO) plays a significant role in the wound healing process through modulation of angiogenesis, collagen deposition, and keratinocyte proliferation (131, 138-140). NO, and its reactive nitrogen species derivatives are effective in killing pathogens (141, 142) and potentially form a useful preventive and therapeutic strategy against skin infections (140).

NO synthetase (NOS) knockout mice have shown impairments in the wound healing processes that were only ameliorated after the addition of excess L-arginine substrate or re-introduction of the NOS gene through transfection (143). NO-releasing hydrogels and nanocomposites have shown significant antimicrobial activity with an acceleration of infected wound healing both *ex* and *in vivo* (144, 145). NO release decreases suppurative inflammation (131) and collagen degradation, minimises the bacterial burden (145), and inhibits fibroblasts to a lesser extent than clinically administered concentrations of antiseptics like povidone iodine (144). NO nanoparticles significantly accelerate wound healing (146) through modification of leukocyte migration and increasing tumour growth factor-β production with a subsequent promotion of angiogenesis (122, 139), leading to increased fibroblast migration and collagen deposition (131, 146). In infected wounds, stained NO nanoparticle-treated tissue depicts decreased neutrophil infiltrate and bacterial load, as well as rapid healing (145, 147-148).

Consequently, the fact that NO nanoparticles greatly expedite wound healing is not surprising. Together, these data suggest that NO nanoparticles have the potential to serve as a novel category of applied antimicrobials for the treatment of infected wounds and may also function as a novel wound healing strategy in the setting of immunocompromised states associated with defective wound healing.

2.8. Stem cell delivery with the aid of nanoparticles

This field is certainly one occupying high ambitions and hopes in the horizon. The domain is two-sided, as seen from the studies discussed below since nanoparticles can be used to guide stem cells, but so can the latter be utilised as a medium to deliver nanoparticle properties. Researchers have tried different combinations of various stem cell types with nanoparticles and managed to achieve localised drug delivery, inhibit neovascularisation where appropriate (e.g. diabetic retinopathy) and reconstruct trauma-distorted surfaces (e.g. ocular reconstruction) (149). Another group injected human MSCs with quantum dot NPs and seeded them onto a fibrin suture. When applied to the myocardium of rats, fibrosis was greater in non-hMSC seeded sutures, and the quantum dots provided a satisfactory media for imaging in both cases (150). Apart from soft tissue, success with stem cells and nanoparticles has also been recorded in bone. Ferucarbotran labelled MSCs guided with an extracorporeal device were successfully guided to the site of a rat bone fracture (151). Furthermore, PLGA has been used to deliver a plasmid for expression of bone morphogenetic protein into rabbit adipocytes (152). The technique offered significant healing advantage to the treated rats over the control group, in terms of chondrogenesis. Others modified adipocyte stem cells to express VEGF in a murine hindlimb model of ischaemia. The transfection was through nanoparticles, making them here the “therapeutic substance” delivered to the tissue of interest (153). This is possible due to the well described migratory properties of stem cells. However, it is noteworthy

Table 2. Clinical trials of nanoparticles in wound healing

Nanoparticle/ Nanoscaffold	Type of Wound	Procedure	Outcome	Year/ref
AgNPs and AgSD	Partial thickness burns	RCT of AgNPs compared to AgSD in 54 pts	AgNP was superior to topical AgSD for wound healing	2014 (158)
Aquacel Ag dressing	Venous leg ulcers	Multi-centre RCT comparing Aquacel Ag to Urgotul in 281 pts	Better wound healing progression with Aquacel Ag	2012 (159)
Ag nylon (Silverlon)	Colorectal surgical wound	Prospective RCT comparing Ag with gauze dressings in pts undergoing colorectal op	Silverlon was safe and three times more effective	2011 (160)
AgNPs (nanocrystaline silver)	Leg ulcers	RCT of AgNPs compared with cadexomer iodine in 281 pts	Similar antibacterial properties. Healing within 2 weeks AgNPs	2010 (161)
Ag hydrofibre	Pilonidal sinus	RCT of Ag hydrofibres compared to sponge dressings in 43 pts	Ag hydrofibre is more cost-effective & wound healing	2010 (162)
AgNP (Acticoat)	Freshly grafted burn	RCT of Acticoat compared to the standard management in 20 pts	Acticoat was cheaper & the effect on wound healing was similar	2007 (163)
AgNPs	Partial thickness burn	RCT of AgNPs compared to 1% AgSD in 191 pts	Similar bacterial colonisation. AgNPs significantly reduced healing time	2006 (164)
AgNPs	Superficial burn wounds	120 patients were randomised to receive: AgNPs/carbon fibre/hydrogel/ Vaseline gauze dressings	Water retention capacity was significantly higher in carbon fibre dressing	2007 (165)

Abbreviations: Pt, patient; AgNP, silver nanoparticles; AgSD, silver sulfadiazine; op, operations

to consider the limitations in this field; although application of nanoparticles offers great potential, the closer interaction with stem cells requires further investigation. Some nanoparticles, indeed, interfere with gene expression amongst other intracellular process in stem cells and thus, further research is required in this field to optimise the delivery. For instance, Au- and Ag- based nanoparticles affect the growth of embryonic neuronal stem cells (154). More specifically, whilst Au- based nanoparticles could be considered for delivering stem cells, they have been widely utilised to induce effects of interest in stem cells; an example being altered osteogenesis when culturing human mesenchymal stem cells with gold nanoparticles *in vitro* (155). In summary, different nanoparticles can have different effects on the proliferation and differentiation of stem cells and this has been exploited by scientists in previous experiments (156). However, it needs to be taken into consideration when attempting to deliver stem cells via nanoparticles and intend to avoid such interactions.

Perhaps an even better way to demonstrate the two-way relationship between stem cells and nanoparticles is through two eminent studies in the field. More specifically, one group have controlled the migration of mesenchymal stem cells (MSCs) magnetically, via filling them with Si-, Au- and Fe- nanoparticles. This technique allowed for very effective magnetic guiding to the site of atherosclerosis, and using photothermal therapy, the results were superior to conventional stenting (157). Finally, Peng *et al.* reveal the other side of the same spectrum, in a study of wound healing in the skin. By transfecting epidermal stem cells with β-cyclodextrin linked to polyethyleneimines, they managed demonstrated acceleration of hair follicle regeneration, skin re-epithelialisation, dermal collagen synthesis and VEGF synthesis, therefore concluding how nanoparticles can be integrated into stem cells for use as a gene reservoir in wound healing applications (137).

3. CLINICAL TRIALS OF NANOPARTICLES IN WOUND HEALING

A brief list of clinical trials in patients using AgNPs is summarised in Table 2, focusing on one major study for the different types of wounds.

Whilst the amount as well as the variety of the studies conducted in randomised patients (i.e. RCTs) is still not significant compared to preclinical studies, the results so far are promising. In particular, silver nanoparticles are undoubtedly at the core of human research due to their therapeutic properties. However, their potential for cytotoxicity shall be taken into consideration when tailoring treatment to a particular patient. Studies to come will shed more light on other candidates used in preclinical trials. The latter may serve a major role alongside AgNPs in the years to come, enriching the armamentarium for wound healing.

4. CONCLUSION AND FUTURE PERSPECTIVES

The field of nanotechnology as applied to wound healing is moving at a rapid pace. With further advances, it is likely that breakthroughs in nano-inspired treatments will significantly improve wound healing in the foreseeable future. Perhaps the most exciting aspects of nanotechnology, as applied to wounds, would be advances in NP-based growth factors delivery systems for angiogenesis, as well as NPs' inherent anti-microbial properties resulting in efficient skin regeneration. Therefore, it can be reasonably concluded that nanotechnology-based remedies will be the next frontier poised for breakthroughs in unmet clinical needs of skin regeneration and wound healing. The ideal wound dressing should have good flexibility, good mechanical strength, large porosity, and be non-adherent to the wound surface. They should also provide a cooling sensation and a moist environment, whilst acting as a barrier to microbes. This is a multi-billion pound industry with a large number of companies as well as academics working towards accelerating wound healing products based on nanotechnology.

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