Antibacterial and Wound Healing Activities of Silver Nanoparticles Embedded in Cellulose Compared to Other Polysaccharides and Protein Polymers

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Abstract

The aggregation of silver nanoparticles (AgNPs) in colloidal solution and the oxidative cytotoxicity towards human cells are two major hindrances for their thriving medicinal applications. Their incorporation in natural polymers such as cellulose, chitosan, alginate, collagen, gelatin, silk fibroin, carrageenan, hyaluronic acid, keratin and starch may be an alluring alternative strategy to sidestep these complications and attaining the advantageous wound dressings. Biocompatibility, bioavailability, biodegradability, and inherent therapeutic properties known for theses polymers, would accelerate the healing of infected chronic wounds. However, the low thermal stability, mechanical strength, rapid biodegradation, and weak washing resistance properties are some of the limitations for these polymers. Herein, recent advances, present challenges and future perspective for AgNPs incorporated nanocomposites (NCs) are discussed to realize ideal antibacterial activities by exploiting the abundant natural biopolymers.

Introduction

Hemostasis, inflammation, proliferation, and tissue remodeling are four main continuous phases of wound healing, which can be slowed by bacterial and fungal infections particularly in the case of diabetic foot ulcers (DFUs) (Ezhilarasu et al. 2020). Multidrug resistant (MDR) bacteria in infected wounds can complicate this situation by their adaptation mechanisms to a wide range of conventional antibiotics. Moreover, bacterial biofilms with complex and sessile community of bacteria and fungi embedded in extracellular matrix (ECM) composed mainly of exopolysaccharides (EPS) are more resistant to penetration of antibiotics (Pouget et al. 2020). Silver nanoparticles (AgNPs) have appropriate bacteriostatic and bactericidal traits but are cytotoxicity towards eukaryotic cells and hence their limited therapeutic applications. Micro- and nanoformulations based on natural polymers with appropriate biocompatibility, bioavailability, biodegradability, and therapeutic attributes can reduce the side effects of AgNPs under physiological conditions. Antibacterial mechanisms of AgNPs have been studied via the interaction of Ag^{+} with cell wall, membrane, and enzymes of the respiratory chain, proteins and nucleic acids of bacteria. Reactive oxygen species (ROS) such as superoxide ($^{\bullet}O_2^{-}$) and hydroxyl radical ($^{\bullet}OH$) can ensue from the reaction of Ag^{+} ions with molecular oxygen in outside and inside medium of bacteria (Alavi et al. 2019; Taran et al. 2016; Alavi and Karimi 2019, 2020).

Living organisms encompassing animals, plants, bacteria, fungi, and alga can synthesize natural polymers or biopolymers with higher biocompatibility, bioavailability, and biodegradability properties. These polymers may be classified based on their monomers, wherein polysaccharide, polypeptide, and polynucleotides are three major forms comprising glucose, amino acids, and nucleotides. Among the natural polymers, cellulose, chitosan, alginate, collagen, gelatin, silk fibroin, hyaluronic acid, keratin, carrageenan, and starch have garnered more attention owing to their unique physicochemical and biological properties (Kaczmarek et al. 2020).

Polysaccharide Polymers

Cellulose

Three major properties namely biocompatibility, biodegradability, and appropriate hydrophobicity are indicated for cellulose polymer. As forms of nano scale, cellulose nanocrystal (CNC) and cellulose nanofibril (CNF) can be prepared commonly by acid hydrolysis and high pressure homogenization (Alavi 2019). These nanomaterials (NMs) are employed to improve the mechanical strength of hydrogel, cryogels, and scaffold (Abdelgawad et al. 2020). Functionalization of cellulose can be completed by targeting its hydroxyl groups to improve mechanical strength, biochemical reactivity, and absorbency in physiochemical conditions. AgNPs are often synthesized via the reduction of Ag^+ ions and stabilizing of ensuing NPs by hydroxyl groups of this polymer without any additional stabilizers (Alavi and Nokhodchi 2020). Sustained release of Ag^+ ions is critical to hinder the growth of MDR bacteria and formation of biofilm at wound site. Ag nanowires (AgNWs) with higher aspect ratio (length to diameter ratio) can improve the release of Ag^+ ions and mechanical properties of wound dressings. Stretchable wound dressing has been made combining the bacterial cellulose (BC) with AgNWs in three volume ratios of 10 : 4.5, 10 : 3, and 10 : 1, wherein after 24 h, sustained release of Ag^+ ions was observed at the period 9 days for all volume ratios; highest tensile stress and Young's module were 1.56 MPa and 2.88 GPa, respectively for the volume ratio of 10 : 4.5. Additionally, the complete wound closure after 12 days as well as significant antibacterial effect (≈ 100% after 6 h) against *E. coli* and *S. aureus* bacteria were indicated for this volume ratio (Wan et al. 2020). Wound dressings based on polymeric hydrogels encompassing AgNPs are interesting alternative to enhance the antibacterial activity with wound healing properties *in vitro* and *in vivo*. AgNPs have been loaded on hydrogel composed of cellulose carbamate using Tween 80, cetyl trimethylammonium bromide (CTAB) and rarasaponin as values of 7.53, 9.94 and 10.15 mg/g, sequentially. Compared to other groups, higher antibacterial effect was indicated for AgNPs (99.4 μg) @hydrogel-CTAB as inhibition zone diameters (IZDs) of 18.98 and 19.84 mm toward *E. coli* and *S. aureus*, which may be caused by synergistic effect of AgNPs with cellulose carbamate and CTAB surfactant. It is worth noting that CTAB has positive charge with high affinity for the negative charge of cell membranes (Bundjaja et al. 2021). Stability and durability of AgNPs is critical factor for employing these NPs in a large-scale production. There are various derivatives for cellulose such as hydroxypropyl methylcellulose and ethylcellulose, which can contribute in synthesis and stabilization of AgNPs at 25 and 4.0 °C for long period (~ 3 months). Antibacterial activity of four types of AgNPs involving bare AgNPs, ethylcellulose (EC)-AgNPs, hydroxypropyl methylcellulose (HPMC)-AgNPs, methylcellulose (MC)-AgNPs and polyethylene glycol (PEG_{6000})-AgNPs toward *E. coli* and *S. aureus* were evaluated in a comparative way. Internalization of HPMC-AgNPs > EC-AgNPs > MC-AgNPs formulations into bacteria was higher compared to PEG-AgNPs and pure AgNPs for both bacteria significantly *E. coli* (Figure 1). Higher penetration ability of HPMC, EC, and MC into the bacterial cell membrane with phospholipid structure, more NPs production by these cellulose derivatives, and sustained release of silver ions may be reasons for this difference (Abdellatif et al. 2021).

Chitosan

The linear polymer polysaccharide containing D-glucosamine and *N*-acetyl-D-glucosamine by β-(1→4)-linkage, is obtained from the chitin shells of shrimp and other crustaceans such as crabs and lobsters by alkaline treatment (Alavi and Rai 2019). Suitably-designed scaffold with large pores size (≥250 μm) promote the adherence, proliferation and migration of skin cells which leads to rapid regeneration of dermal tissue. Further, chitosan ($C_{56}H_{103}N_9O_{39}$) with amine groups can interact with negative charge of cell envelope of bacteria and synergize the antibacterial activity of AgNPs. Wound dressing comprising chitosan-collagen-AgNPs (spherical by size in the range of 10-25 nm at concentration of 10 μg/mL) illustrated accelerated wound healing via promotion of the fibroblasts migration, more expression of α-smooth muscle actin (α-SMA), and macrophage performance (Figure 2). Dead cells at wound site can be eliminated by increasing the number of IL-10 levels and M2 macrophages in inflammatory phase (J. Wang and Xu 2020). In another study, a faster wound healing by abundant granulation tissue, higher collagen formation, lower levels of macrophage and vessel density were indicated for the electrospun chitosan-based NFs containing AgNPs at concentrations of 12 and 60 mg in comparison with control samples after 14 days of treatment. Moreover, this investigation demonstrated that the inorganic ions and proteins can slow down and block the release of Ag^+ from AgNPs (Shao et al. 2019).

Alginate

Brown alga such as *Turbinaria turbinata*, *Sargassum filipendula*, *Macrocystis pyrifera,Saccharina longicruris, Sargassum carpophyllum,* and *Sargassum siliquosum* as well as bacteria namely *P. aeruginosa* have the ability to produce hydrophilic polymer of alginate containing three types of block structure: G block (poly α-L-guluronic acid), M block (ß-D-mannuronic acid), and MG block (both polyuronic acids) with β-(1-4) glycosidic linkage (1-4) (García-Ríos et al. 2012; Valentine et al. 2020). AgNPs have been incorporated in the matrix of sodium alginate and collagen via interactions between the amine group of collagens and the carboxylate group of alginates with AgNPs. In this way, $NaBH_4$ and polyvinyl pyrrolidone (PVP) were employed, respectively as reducing and stabilizing agents to obtain spherical AgNPs with the mean particle size of 7 nm. AgNPs in concentration of 50 μM exhibited negligible cytotoxicity against NIH3T3 cell line with bacterial inhibition toward *E. coli* and *S. aureus* as inhibition zone diameters (IZDs) of 2.7 and 1.9 mm, respectively (Zhang et al. 2018). As an efficient strategy, polydopamine (PDA) spheres (a mean diameter of 0.430 μm) were decorated by AgNPs (size in the range of 50–70 nm) followed by immobilization on oxidized sodium alginate (OSA) wound dressing. Significant porosity value of 77.30 % (relative to hyaluronic acid/cationized dextran (73 %) and quercetin/duck's feet collagen/hydroxyapatite (76.36 %)) and blood compatibility at concentration of 200 ppm made this NC ideal as a safe wound dressing (Liang et al. 2020).

Gelatin

In addition to enzymatic extraction (Ahmad et al. 2017), partial acid and alkaline hydrolysis of type I collagen from the skin, bones, cartilage, and meat can produce gelatin types A and B, respectively (Yang

et al. 2019). Gelatin as an extracellular matrix (ECM) protein may be used to prepare drug and gene delivery systems, wound dressings, tissue engineering, and 3D cell culture (Han and Lv 2019; Afewerki et al. 2019). PVP-stabilized AgNPs have been aminated via 3-aminopropyltriethoxysilane (APTES) treatment at 70 ●C for 12 h and incorporated in the carboxylated CNF to achieve injectable nanoformulation for wound therapy in nursing infants. This formulation was employed to accelerate inflammation and proliferation phases of wound healing by increasing the number of white blood cells (WBCs) specifically neutrophils at wound site after 14 days of treatment compared to control group of CNF/gelatin (Gou et al. 2020). It is noteworthy that in the inflammation phase, increased capillary permeability and migration of cells particularly neutrophils to the wound tissue would help sterilize the wounds and release proteases to remove the denatured ECM followed by transformation of monocytes into macrophages in the wound site, regulated by cytokines such as monocyte chemotactic protein-1 (MCP-1) and transforming growth factor-β (TGF-β) (J. Wang and Xu 2020). Wound healing activities for combination of metal NPs (MNPs)/metal oxide NPs (MONPs) with important growth factors (GFs) involving fibroblast growth factor (FGF), epidermal growth factor (EGF), bone morphogenic proteins (BMPs), TGF-β, vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) in wounds' healing phases of hemostasis, inflammation, proliferation, and remodeling/maturation have been reviewed previously (Alavi and Rai 2020). Incorporation of AgNPs (0.004 M) in hydrogel formulated as sodium alginate/gelatin in the ratio of 80:20 displayed prowess for the topical application with MIC values toward *P. aeruginosa* (0.5 ppm) and *S. aureus* (53 ppm), higher consistency and a significant epithelialization after 14 days treatment compared to control and hydrogels without AgNPs (Figure 3) (Diniz et al. 2020).

Carrageenan (CAR)

Three types of CAR including kappa, iota, and lambda are commercial polymers, which are commonly deployed in food and pharmacy industries. The stabilization of MNPs/MONPs, gelling and thickening applications, specifically in food industry, have been reported for CAR as a sulfated polymer of galactose units linked alternatively by β-1,4 and α-1,3 (Pandey et al. 2020). CAR and carboxymethyl CNC were used to formulate cryogel loaded AgNPs with 100% reduction ability towards *E. coli* and *S. aureus*. This polymeric composition instigated desirable controlled and sustained release by 40 μg/mL of AgNPs within 250 min. rapid wound healing; suitable mechanical strength of this wound dressing originated from CAR and carboxymethyl CNC polymers, respectively (Abdelgawad et al. 2020). In addition to anti-planktonic and anti-biofilm activities towards *P. aeruginosa* and *S. aureus*, the stability of CAR-Ag NPs was indicated for 6 months in a colloidal medium which is due to the suitable interaction of CARs by their hydroxyl (-OH) groups with AgNPs (Goel et al. 2019).

Hyaluronic acid (HA)

HA, hyaluronate or hyaluronan, is anionic polymer of nonsulafted glycosaminoglycan (disaccharides of *N*-acetyl-D-glucosamine and D-glucuronic acid) with high molecular weight, and is present in prokaryotic and eukaryotic cells particularly in ECM section with the critical role in proliferation and migration of human cells in epithelial, neural, and connective tissues; inflammation response, angiogenesis and

granulation of wounds are influenced positively by HA (Alemzadeh et al. 2020). According to molecular weight (MW) property, 1–25 × 104, 25–10 × 104, > 1 ×106, > 6 × 106 Da were indicated for low MW, medium MW, high MW and very high MW of HA, respectively. All four stages of wound healing are influenced distinctly by low and high molecular weight of HA through interaction with the CD44 receptors related to granulocytes and monocytes as well as various interleukins such as IL-6, IL-1β, and IL-8. (Graça et al. 2020). Wrapping bacteria, reactive oxygen species (ROS) production, lipid peroxidation, membrane disruption, interaction with glutathione (GSH), adenine, and proteins leakage were indicated as main antibacterial mechanisms for graphene oxide (GO) (Nanda et al. 2016; Mohammed et al. 2020). HA-AgNPs NC was loaded on the GO sheet in solution of *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride and *N*-hydroxysuccinimide. Bactericidal activity against *S. aureus* could be synergized by heat conversion ability of HA-AgNPs-GO, resulting from the higher specific surface area of GO, under near infrared (NIR) irradiation (808nm for 2 min) from 25.5 ºC to 58.4 ºC. Wound healing was effected by controlled release of Ag^+ ions from HA-AgNPs under hyaluronidase enzyme (Ran et al. 2017). Inflammatory step of wound healing can be affected negatively by ROS species, which can be controlled by suitable formulations of Ag and HA. Polygalacturonic acid (PGA), an herbal polymer, was employed as a reducing and stabilizer agent to prepare AgNPs followed by incorporation in nanofibers (NFs) comprising HA and poly vinyl alcohol (PVA) by electrospinning technique. Antibacterial activities were not observed for (PGA/HA)-PVA, while incorporation of AgNPs imparted significant antibacterial effects on *B. subtilis*, *S. aureus*, and *E. coli*. In addition, the percentages of wound contraction for (PGA/HA)-PVA and (AgNPs-PGA/HA)-PVA after 14 days were 97.42 and 99.51% relative to 92.43% for Garamycin® cream as a control group (El-Aassar et al. 2020).

Pectin

Pectin, acidic hetropolysaccharides (linear chain of alpha (1-4) linked D-galacturonic acid; $C_6H_{10}O_7$) with high molecular weight (194.14 g/mol) are found in the cell walls of plant species. They are used as antioxidant, emulsifiers, food coloring agent, anti-infective agent, and stabilizers of NPs in the food and pharmaceutical industries (Mercado-Mercado et al. 2020; Jiang et al. 2020). Combination of pectin with other synthetic and natural polymers has been deployed as novel wound bandages to reduce the disadvantages of each individual polymer by itself. NFs comprising AgNPs/polyvinylalcohol (PVA)/polyvinylpyrrolydone (PVP)/pectin/mafenide acetate (MF) were electrospun in the weight ratio of 0.7 and 91.8 wt% for AgNPs and PVA as well as 2.5 wt% for PVP, pectin and MF, respectively. NPs were synthesized in PVA solution and mixed in solution of pectin, MF, and PVP followed by electrospinning on a grounded collecting drum. Higher antibacterial effect, although not adequate enough, was indicated against Gram-negative bacteria relative to Gram-positive bacteria owing to difference in thickness of bacterial cell wall. Moreover, the releases rate of AgNPs was influenced mainly by their interaction with the functional groups such as $-SO_2$ and $-NH_2$ of MF (Alipour et al. 2019). The size of AgNPs was affected by two parameters including weight percentage of pectin (extracted from citrus peel) (0.5, 1, and 2%) and temperature (20 and 60 ●C), wherein smaller diameter of NPs (8 nm) was obtained for 1% of pectin in 60 ●C. MIC values for pectin-AgNPs showed 31.25 and 500 µM towards *E. coli* and *S.*

epidermidis after 24 incubation compared to glutathione-AgNPs with MIC amounts of 140 and 1680 μM, respectively. In this regard, the ability of pectin to adhere to the bacterial membrane increases the antibacterial activity of AgNPs (Pallavicini et al. 2017).

Starch

Starch $(C_6H_{10}O_5)_n$, the linear polymer of glucose as amylose and amylopectin in the branched form, is a renewable and biodegradable polysaccharide and has been applied for various applications such as drug delivery, micro and nano formulations of therapeutic agents, production of pharmaceutical tablets (Athira et al. 2018; Chen et al. 2020). This polymer has reducing and stabilizing functions in MNPs/MONPs synthesis in one-pot operation. Synthesized Ag/Au bimetallic NPs have been shown to affect the morphology change of *E. coli* as lacking flagella in treatment samples relative to control. IC_{50} values for these bimetallic NPs compared to AuNPs were 4.92±0.81 and 6.95±1.70 μg/mL against multidrug resistant (MDR) *E. coli* and methicillin-resistant *S. aureus* (MRSA) (Lomelí-Marroquín et al. 2019). Isolated starch from babassu mesocarp, a by-product of babassu oil, was utilized to synthesize AgNPs with different diameter size of 124.2, 119.1, and 181.7 nm by microwave, autoclave, and water bath methods, respectively. In a comparative way, MBC value of >27 μg/mL was determined for all NPs as well as $AgNO_3$ salt against *S. aureus*. The prominent cell membrane damage, pore formation and the release of bacteria contents of *E. coli* was observed after treatment by AgNP prepared by water bath method (at sub value of MIC=6.75) (Bastos Araruna et al. 2020). Combination of AgNPs, starch, PVA, and GO as a scaffold showed synergic antibacterial effects with the Modulus and tensile strength of 145% and 26.81%, respectively (Usman et al. 2016). In this regard, GO can increase the antibacterial activities of AgNPs by lipid peroxidation resulting from carbon radicals (*C) and cellular membrane damage caused by sharp edge of GO (Alavi et al. 2020).

Cyclodextrin (CD)

Cyclodextrin (CD) is belongs to a cyclic oligosaccharides family with a macrocyclic ring of glucose subunits bonded by α-1,4 glycosidic links in three main types namely 6: α-CD, 7: β-CD, and 8: γ-CD, which can be used to encapsulate hydrophobic drugs in interior section due to improve its bioavailability, solubility, and stability. α-CD and γ-CDs types are more water soluble relative to β-CD (Tian et al. 2020). Carboxymethyl-β-cyclodextrin (CM-βCD) as a derivative of CD has solubility of 50 mg/mL, which can be used to load and stabilize AgNPs in various micro and nano formulations. Two concentrations of CM-βCD involving 50 and 25 mg were reacted with chitosan (50 mg), and glutaraldehyde (0.013 mM) to produce hydrogels 1 and 2 (H1 and H2). Incorporated constant amount of AgNPs in this formulation illustrated different antibacterial properties dependent on CM-βCD concentration, wherein 19, 15, and 41.8 mm were observed for H1 and H2, and gentamycin (30 μg) toward *E. coli* (Mohamadi Zahedi and Mansourpanah 2018). Gallic acid (GA) (3,4,5-trihydroxybenzoic acid), is a phenolic acid found in plants such as tea leaves, gallnuts, and oak bark and has antibacterial activities against *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *E. coli*, and *S. aureus* by irreversible changes in membrane and cell wall morphology (Li et al. 2019). Synergic effect of GA and its antibacterial activity in hydrogel

formulation was obtained by incorporation and loading of these materials in β-CD and GO, separately. Hydrogel film comprising PVA/GA@βCD/Ag-GO demonstrated improved tensile strength (>126.2 MPa), sustained and slower GA release over 2 h and higher antibacterial activity towards *E. coli* and *S. aureus* compared to PVA/GA@βCD and PVA/βCD (Pooresmaeil and Namazi 2019). Hydroxypropyl-βCD (HP-βCD) with solubility of 45% (w/v), as another derivative of β-CD, has been used as a delivery system to increase the solubility of therapeutic agents in aqueous media of biological conditions (Wei et al. 2017); 1 wt% AgNPs in NFs of AgNPs-HP-βCD showed 11.6 and 10.7 mm for *S. aureus* and *E. coli*, respectively. Higher antibacterial capacity and The larger fiber diameter were observed for 2 wt% AgNPs in these NFs (Celebioglu et al. 2019). Thiomers, thiolated polymers, are polymers employed in micro and nanoformulations to augment the absorption of therapeutic agents in vaginal, eyes, mouth, and nose routes. Thiomers with low molecular mass can impart higher permeability and mucoadhesive properties to polymeric formulations (Palazzo et al. 2017). For instance, β-CD-SH1200 and β-CD-SH600 illustrated 46.37-and 39.73- fold higher mucoadhesion relative to β-CDs, respectively (Moghadam et al. 2018).

Protein Polymers

Collagen

Collagen has been found as the frequent protein in terrestrial and marine animals with desirable tensile strength, biocompatibility, bioavailability, and biodegradability properties for various therapeutic applications particularly tissue engineering (Lim et al. 2019; Divakar et al. 2019). Scaffold of collagen can attract and guide the migration of fibroblast cells along a connective tissue matrix (Dill and Mörgelin 2020). By interaction with blood platelets, this polymer can lead to the wound closure and prompt the acceleration of hemostatic phase of wound healing (Ding et al. 2020). However, weak washing resistance, low thermal stability, mechanical strength, and rapid biodegradation properties are some limitations for this protein (Pietrucha 2005; El-Fiqi et al. 2013). Obtaining stable colloidal solution of AgNPs with uniform size distribution may be realized by the appropriate selection of suitable reducing/stabilizing agents. Dialdehyde xanthan gum (DXG) with favorable biocompatibility was employed to produce spherical AgNPs in the range of 12-35 nm followed by incorporation in collagen matrix by cross linkage of DXG (via Schiff's base reaction) to attain the wound dressing. In addition to antibacterial activities toward *E. coli*, *S. aureus*, and *P. aeruginosa*, this sponge illustrated the complete integrity after physical pressing in contrast to collagen sponge. Lack of collagen in wound tissue was indicated for the collagen sponge and the sterile gauze compared to collagen-AgNPs NCs after a period of 18 days treatment (Ge et al. 2018).

Keratin

The main component of the wool, feather, horns, hair, and hooves is keratin, which has been investigated widely owing to its intrinsic physicochemical, mechanical durability, biocompatibility, and biological properties. In contrast to other biopolymers such as chitosan, collagen, cellulose, and starch, cumbersome chemical conditions are required to dissolve this polymer in water and organic solvents for

appropriate extraction. The keratin extraction may be performed via the reduction, oxidation, microbial technique, microwave irradiation, and steam explosion pathways (Feroz et al. 2020). Based on mentioned advantages, a promising nanofibrous mat can be prepared from this type of polymer for apt wound bandages and tissue engineering (Esparza et al. 2017; Guo et al. 2019). For instance, a NC of keratin, polyurethane, and AgNPs can generate desirable antimicrobial impact. In this case, human hair was used as source of keratin followed by functionalization with iodoacetic acid to prepare *S*-(carboxymethyl) keratin. High concentration of AgNPs (5% of silver nitrate) revealed more antibacterial effects against *E. coli* and *S. aureus* relative to polyurethane and polyurethane -S-(carboxymethyl) keratin (Y. Wang et al. 2016).

Silk fibroin (SF) and sericin

Various species of silk worm such as *Bombyx mori* can be used to extract SF (70-80%) with cytocompatibility, high adhesiveness and suitable mechanical properties emanating from fibrillar structure with the β-sheet crystal of $(Gly\text{-}Ser\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ala)_n$, which can promote the adhesion of platelets and proliferation of stem cells (Patil et al. 2020; Anuduang et al. 2020). Anti-adhesion, anti-planktonic, and inhibition of biofilm was indicated for AgNPs/gentamycin-loaded SF-based film of titanium. The synergic antibacterial mechanism of AgNPs and gentamycin were adhering and accumulation on bacterial cell wall and membrane, disruption of cell wall and membrane, damaging of proteins/nucleic acids, and blocking of the transcription process by binding with 16S rRNA (Zhou et al. 2017). 20–30% of silk consist of sericin protein comprising 18 amino acids (glycine and aspartic acid as abundant amino acids) with glue-like structure, moisture absorbent, hydrophilic and mechanical stretching properties (Luo and Wang 2016). Tyrosine amino acid in this protein can contribute to AgNPs formation by redox reaction. Wound dressing composed of AgNPs/sericin/PVA illustrated the disruption of cell wall and membrane of *P. aeruginosa*, *S. aureus*, and *E. coli* upon treatment for 12 h of incubation (Figure 4) (Tao et al. 2019). Growth stages of bacteria displayed a different behavior toward antibacterial agents, wherein the lag phase for *S. aureus* (16 h) and *E. coli* (12 h) was delayed for AgNPs-sericin/PVA films prepared by UV-assisted method (He et al. 2017).

Conclusion

Toxicity of AgNPs towards eukaryotic cells is a main side effect for the applications of these MNPs in medicinal field. Many investigators have presented assorted materials in combination with AgNPs to increase the biocompatibility of MNPs under physiological conditions. Among these, polymers specifically biopolymers have garnered more attraction owing to their desirable biocompatibility, bioavailability, weak antigenicity, and biodegradability properties. Bacterial and fungal infections are one of the most critical complications that delay the wound healing specifically in chronic infectious wounds such as DFU. Inhibition of bacterial colonization, promising swelling and porosity properties, absorption ability of blood secretions, and moist environment can be delivered by the polymer-based wound

dressing. Compared to other wound dressings based on hyaluronic acid/cationized dextran and quercetin/duck's feet collagen/hydroxyapatite, NCs of OSA-PDA-AgNPs demonstrated more porosity of 77.30 %. In this review, antibacterial and wound healing activities of important applicable biopolymers including cellulose, chitosan, collagen, alginate, hyaluronic acid, silk fibroin, sericin, CAR, pectin, keratin, starch, and CD in combination with AgNPs are deliberated to acquire a clear viewpoint of these materials and their NMs forms in suitable micro and nanoformulations. Accordingly, each biopolymer can contribute to different stage of wound healing for example chitosan can increase antibacterial activities of AgNPs in infected chronic wounds as well as the application of collagen in wound bandages can attract fibroblast cells and promote deposition of new collagen fibers to the wound area. Cationic charge of chitosan is critical for interaction of this polymer with teichoic acids and lipoteichoic acids (containing phosphate groups) linked to respectively the peptidoglycan and the underlying plasma membrane in Gram-positive bacteria. This function can synergize the antibacterial mechanisms of AgNPs (oxidative stress, increased cell permeability, pore formation in cell envelope, blocking of electron transport chain, cell wall and membrane disruption, nucleic acid and enzymes damage) at low and safe concentrations compared to pure AgNPs. In the case of applicable wound dressing, the mechanical and chemo-physical properties of scaffold to achieve suitable water-retaining, tensile strength, and promotion cell growth properties can be efficiently attained by cellulose and its derivatives. Cellulose derivatives including HPMC and EC showed high physical stability and durability of AgNPs for 3 months period owing to contribution of C-O and OH groups in NPs synthesis and stabilization. Furthermore, a higher ability for internalization of cellulose functionalized AgNPs into bacteria cells was found for HPMC, EC, and MC derivatives.

As an example of protein polymer, collagen has a vital role in acceleration of hemostasis phase, fibroblasts migration along a connective tissue matrix, and nucleation process in wound area. Therefore, the formulation of several polymers is more efficient relative to the combination of one polymer with AgNPs, wherein, in some cases, synthetic polymers can be tremendously helpful to increase the mechanical strength of wound dressings.

Declarations

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Ethics approval

This study has not reported the results of studies involving humans and/or animals

Consent to participate

Not applicable.

Consent for publication

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Conflict of interest

The authors declare no competing financial interest.

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Figures

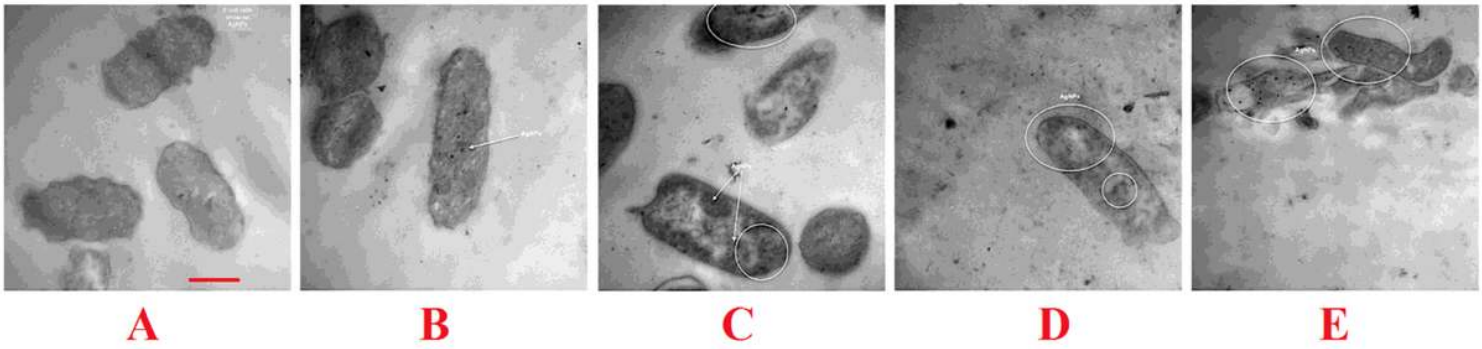

Figure 1

Different internalization of AgNPs into E. coli: A) Control, B) PEG-AgNPs, C) MC-AgNPs, D) EC-AgNPs, and HPMC-AgNPs; scale bar is 500 nm for all images (Abdellatif et al. 2021).

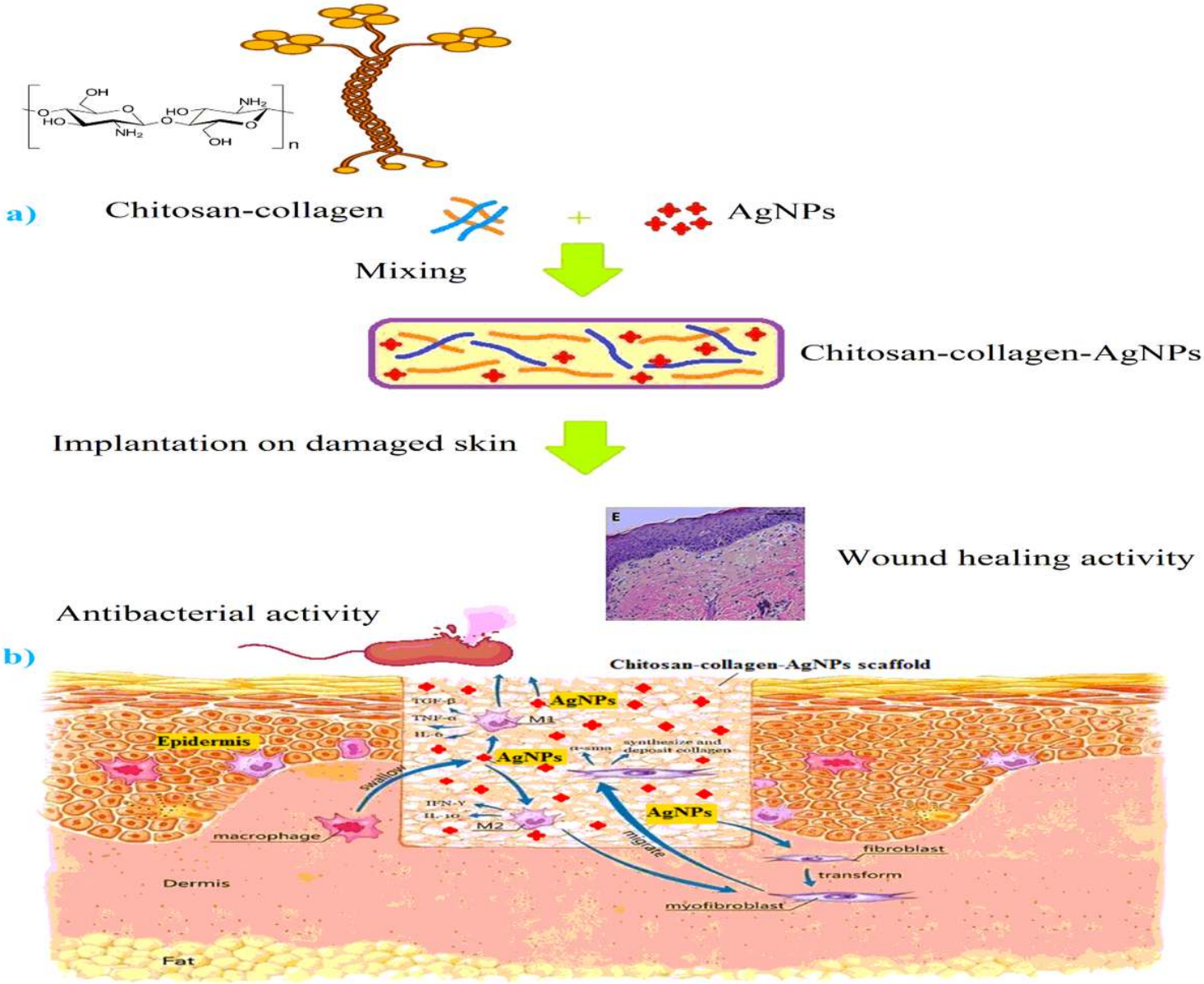

Figure 2

The possible mechanisms for accelerated cutaneous wound healing by scaffold of chitosan-collagen-AgNPs; tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interleukin 6 (IL-6), interleukin 10 (IL-10), interferon gamma (IFN-γ), α-smooth muscle actin (α-SMA), classical macrophage activation (M1) and alternative macrophage activation (M2) (Adapted and modified from (You et al. 2017) and BioRender.com).

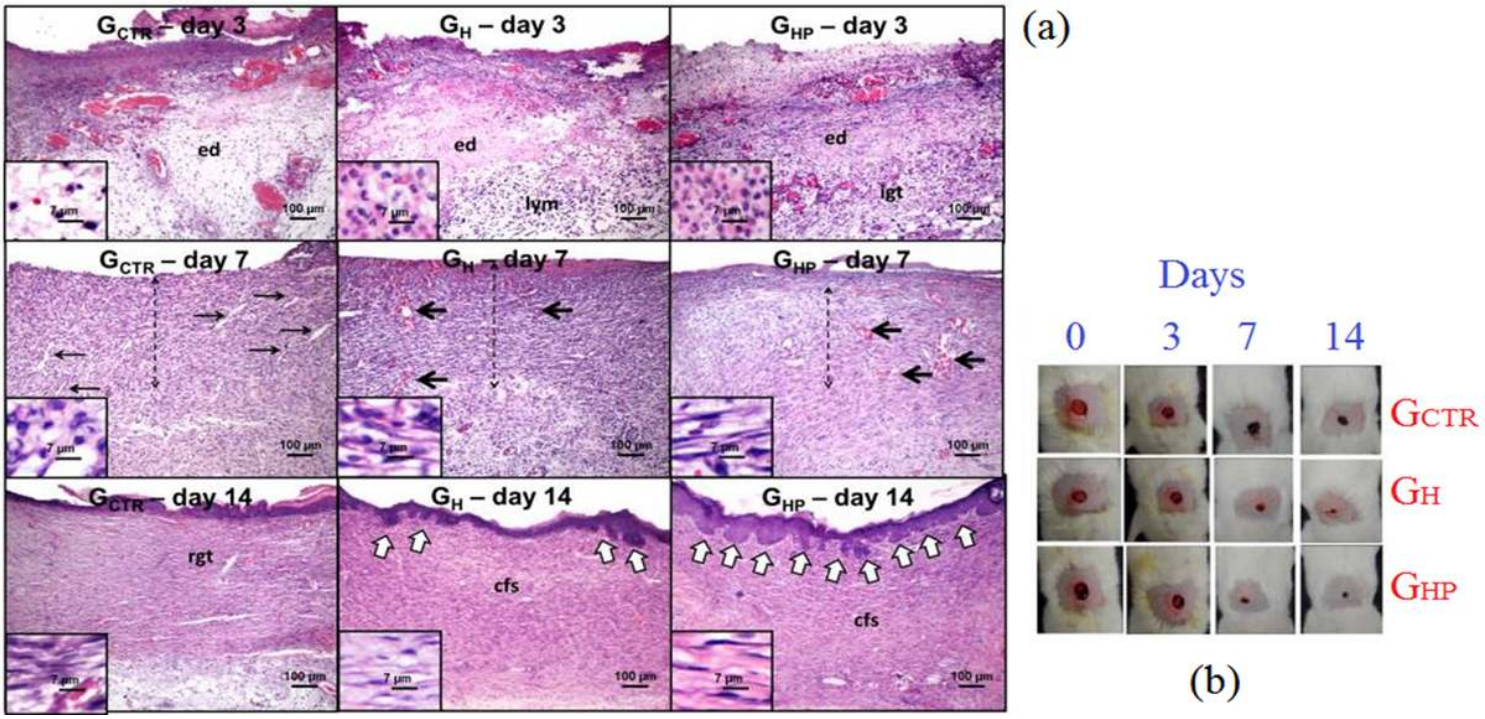

Figure 3

Histological sections (a) and images of wound closure (b) showing a striking wound healing in the period of 14 days of experiment. (Intense edema (ed), the lymphocyte-rich infiltrate (lym) and immature granulation tissue (igt), a cellular primary fibrous scar (cfs); groups of hydrogel with AgNPs 4 mM AgNO3 (GHP), hydrogel sodium alginate/gelatin (80:20) (GH), and control (GCTR)) (Adapted and modified from (Diniz et al. 2020)).

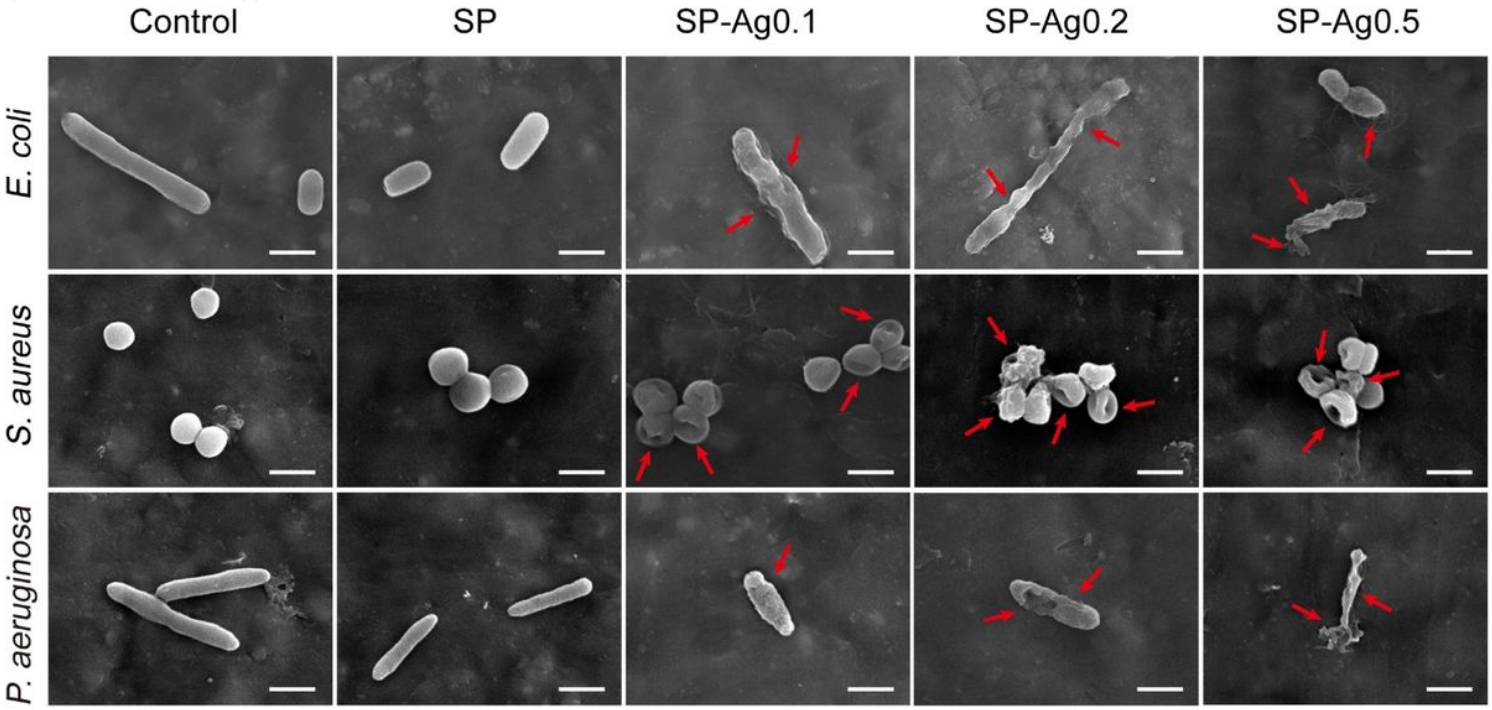

Figure 4

Morphology alteration of bacterial cell envelope under treatment of sericin/PVA (SP) and NCs of SP with different concentrations of AgNPs (0.1, 0.2, and 0.5 mM/L of $AgNO_3$). Complete cell wall deformation and disruption are mainly observable in the higher concentration of AgNPs (Tao et al. 2019).