

Review Article

Current Development of Silver Nanoparticle Preparation, Investigation, and Application in the Field of Medicine

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Received 15 September 2014; Accepted 14 October 2014

Academic Editor: Xiaoming Li

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The invited review covers different research areas of silver nanoparticles (AgNPs), including the synthesis strategies of AgNPs, antimicrobial and anti-inflammatory properties of AgNPs, osteoconductive and osteoinductive activities of AgNP-based materials, and potential toxicity of AgNPs. The potential mechanisms of AgNP's biological efficacy as well as its potential toxicity are discussed as well. In addition, the current development of AgNP applications, especially in the area of therapeutics, is also summarized.

1. Introduction

Silver has been used as an antimicrobial agent for centuries. For example, the Phoenicians used silver vessels to preserve water and wine during their long voyages [1]. In addition, ancient Egyptians believed that silver powder provided beneficial healing and antidisease properties; thus silver compounds were used for prohibiting wound infection prior to antibiotics [1]. In 1884, German obstetrician Crede introduced 1% silver nitrate (AgNO_3) as an eye solution for prevention of gonococcal ophthalmia neonatorum, which is most likely the first scientifically documented medical use of silver [1]. In modern society, topically used silver sulfadiazine cream is the standard antibacterial treatment for serious burn wounds and is still widely used in burn units today [2]. However, inadequate local retention and severe cytotoxic effects limited the clinical use of these ionic reservoir forms of silver materials [3–5].

Due to recent advances in nanotechnology, it is now possible to produce silver at the nanoscale [6, 7]. In addition to their potential electronic and transparent conductor applications, the emergence of nanosilver materials in antimicrobial consumer goods and medical products is driving

the growth of the nanosilver market, which is expected to grow in value from US\$290 million in 2011 to around US\$1.2 billion by 2016 (http://www.merid.org/Content/News_Services/Nanotechnology_and_Development_News/Articles/2011/Sep/14/nanosilver.aspx). Among the different nanosilver preparations, silver nanoparticles (AgNPs) are made up of 20–15,000 silver atoms, generally range from 1 to 100 nm in diameter and present in one dimension [8]. These extremely small dimensions lead to a high surface area to volume ratio and, subsequently, change the physical, chemical, and biological properties of AgNPs [9, 10]. This review critically outlines recent developments in the synthesis, physicochemical properties, toxicity, and biomedical applications of AgNPs.

2. Synthesis of AgNPs

AgNPs can be synthesized in a variety of different ways, each with its own advantages and disadvantages. Till now, the most common methods of AgNPs synthesis are through chemical reduction of silver salt (e.g., AgNO_3) using a reducing agent (e.g., sodium borohydride) [11]. During chemical reduction, silver ion (Ag^+) receives an electron from the reducing

agent and reverts to its metallic form (Ag^0) eventually clustering to form AgNPs. AgNO_3 is the most often used silver salt for these chemical methods of AgNP synthesis due to its low cost and high stability [10]. Generally, a capping agent [e.g., poly(*N*-vinyl-2-pyrrolidone) (PVP)] is used during chemical reduction to stabilize the nanoparticles and prevent them from aggregating [11]. However, reducing agents and organic solvents used in chemical reduction, such as *N,N*-dimethylformamide, hydration hydrazine, and sodium borohydride, are highly reactive and pose potential environmental and biological risks [12]. Moreover, the inability to control the size of the AgNPs obtained by chemical reduction could result in a wide distribution of sizes of AgNPs obtained.

Photoreduction of silver salt in the presence of PVP or citrate by ultraviolet (UV) light is another simple and effective strategy to produce AgNPs [11, 13, 14]. However, most photoreduction methods still use organic solvents, such as PVP, and are unable to control the size of obtained AgNPs [11, 13, 14].

Recent efforts have been focused on developing new *green chemistry* methods of AgNPs synthesis with the advantage of using natural products and avoiding toxic reducing agents, organic solvents, and wasteful purifications with high cytotoxic residuals. With these methods, molecules produced by living organisms such as bacteria, fungi, or plants replace the reducing and capping agents [15–18]. For example, AgNO_3 is added to chitosan obtained from various species of bacteria and dissolved in acetic acid. The silver ions are then reduced through gamma radiation and stabilized by chitosan [19, 20]. There have also been efforts to synthesize AgNPs in the lamellar space of montmorillonite/chitosan by utilizing the UV irradiation reduction method in the absence of any reducing agent or heat treatment [21]. Additionally, AgNPs have been synthesized via photoreduction of AgNO_3 in layered inorganic clay suspensions, laponite, which serve as stabilizing agents that prevent nanoparticles from aggregating instead of using PVP [22]. The disadvantage to using these types of *green* synthesis is the potential of contamination due to the pathogenic bacteria created during the purification [10]. Consequently, herbal extracts and essential oils are gaining in popularity for methods of *green* synthesis due to their antioxidant and antimicrobial properties while avoiding the use of pathogenic bacteria [23–25]. However, the disadvantage of using herbal extracts is once again the wide distribution of particle sizes obtained.

In addition, there have also been novel developments by using *green physical* methods for AgNP synthesis avoiding the use of chemicals completely. One such method, laser ablation, can synthesize pure colloidal AgNPs in solutions without the use of chemical reagents [27, 28]. However, the concentration and morphology of these AgNPs are inconsistent since they are dependent on many parameters such as number of laser shots, wavelength of the laser, laser fluence, liquid medium, and ablation time duration [27, 28]. Another chemical-free method for AgNP synthesis involves the application of an electrical current between two silver wires in deionized water causing surface silver atoms to evaporate and condense back into aqueous AgNPs [29]. Without the use of chemicals, there are no toxic residuals or contaminations. However, since

no capping agents are used, agglomeration of AgNPs may pose a problem [30]. To date, perhaps the most effective method for AgNP synthesis is the production through a novel, nonchemically based proprietary process (USPTO 7,282,167). In this process, silver granules with a purity of 99.99% are distilled into silver vapor by heating on a ceramic conductive element. By controlling the generation rate of silver vapor, gas flow, and reactor pressure, the nucleation rate of silver nanoclusters can be designed to produce nanosilver liquid droplets, which are then condensed in a stream of flowing helium gas into solid crystalline spherical AgNPs of desired size. The hot nanoparticles are then attracted to a cooled chamber wall by thermophoresis where they are mechanically collected and packaged for use. Furthermore, the active surface of the obtained highly purified (>99.9% pure) 20–40 nm AgNPs (15–25 m^2/g) is much greater compared to commercial nonnanoscale silver powder (1–2 m^2/g) and previously reported AgNPs (4 m^2/g) [31, 32].

3. Biological Properties of AgNPs

Currently, the unique antimicrobial properties of AgNPs have led to their application in areas such as clothing manufacturing, food preservation, and water purification [33–36]. More importantly, AgNPs are being increasingly utilized in the medical industry due to their antibacterial, antifungal, antiviral, anti-inflammatory, and osteoinductive effects as well as their ability to enhance wound healing [32, 37–42].

3.1. Antibacterial Properties of AgNPs. Antibiotics are standard antimicrobials used in medicine that bind to specific chemical targets of bacteria not present in humans [41]. However, this binding specificity narrows the number of bacterial species that are vulnerable to a specific antibiotic and contributes to the antibiotic resistance developed by bacteria, ultimately creating multidrug resistant bacteria [43]. With the increasing number of infections due to multidrug resistant bacteria, there has been a need for effective antimicrobial alternatives. Thus, silver is a great alternative because it is an antiseptic that targets a broad spectrum of Gram⁺ and Gram[−] bacteria as well as vancomycin-resistant strains [7, 31, 36, 40, 43]. Moreover, silver-resistant bacteria are rarely observed in hospital microbial germ flora because silver resistance requires one generation of bacteria to undergo three separate mutations in three different bacterial systems [31, 44].

Mechanistically, silver-based materials are thought to release silver ions (Ag^+) in aqueous solution, which account for their antibacterial properties by attaching to specific thiol (–SH) groups containing sulfur and hydrogen found in a variety of structural and functional bacterial proteins [45–47]. It should be noted that the reservoir form of the active silver ions might differ [43]. For instance, the reservoir form of AgNO_3 is a chemical combination of Ag(I) and nitrate, but AgNO_3 is not suitable for sustained local application due to its high solubility as an ionic salt. Nonnanoscale elemental silver [$\text{Ag}(0)$] used in silverware and jewelry is relatively insoluble in most fluids, resulting in minimal oxidative Ag^+ release [43]. Thus, neither the *in vitro* antibacterial action nor the clinical behavior of nonnanoscale silver-coated stainless

steel cortical screws significantly differed from that of the uncoated ones [48]. Due to the greater surface-to-mass ratio, AgNPs offer greater active surface, higher solubility, and chemical reactivity. As a result, although the reservoir form for AgNPs is also elemental silver [Ag(0)], AgNPs have higher release of oxidative Ag⁺ and/or partially oxidized AgNP with chemisorbed (surface-bound) Ag(I) and thus have higher antibacterial activity compared with conventional silver repartitions [40, 49, 50].

However, a comparative study of AgNPs, AgNO₃, and silver chloride (AgCl) found that AgNPs have higher antibacterial properties than free silver ions (Ag⁺) [51]. This implies that AgNPs have intrinsic antibacterial properties not dependent on the release of silver ions (Ag⁺). One possible mechanism of Ag⁺ releasing-independent bactericidal effects of AgNPs is partly due to the ability of AgNPs to attach to the bacterial cell wall and penetrate it, thereby increasing its permeability and, ultimately, leading to cell death [52]. Another possible mechanism attempts to explain the antibacterial effect of positively charged silver atoms (Ag⁺) on the surface of AgNPs through the formation of free radicals and the subsequent free radical-induced membrane damage [53]. Furthermore, a recent study suggests a novel mechanism for the antibacterial effect of AgNPs, namely, the induction of a bacterial apoptosis-like response [54]. This new proposed mechanism involves an accumulation of reactive oxygen species (ROS), increased intracellular calcium levels, and phosphatidylserine exposure in the outer membrane, all hallmarks of early apoptosis. It also includes disruption of the membrane potential and DNA degradation, indicators of late apoptosis, in bacterial cells treated with AgNPs [54]. Collectively, it is believed that AgNPs are thought to interact with the bacterial DNA, the peptidoglycan cell wall and plasma membrane, and the bacterial proteins involved with the electron transport chain to produce their bactericidal effects [30, 47, 55–57]. Although it is not fully understood which mechanism provides the main antibacterial effect, a combined effect of each mechanism provides broad-spectrum antibacterial resistance.

It is worth noting that the size and shape of nanosilver influence its antibacterial efficacy. Previous studies have demonstrated that 5–50 nm sized AgNPs are bactericidal [31]. Further investigation suggested that the greater the surface area of AgNPs is, the greater the antibacterial activity is [58–60]. In addition, Sadeghi et al. reported that nanoscale silver plates demonstrated the best antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* compared with nanoscale silver rods and particles [61]. Antibacterial activity of nanosilver was found to be dependent on the surface area of nanosilver shapes with nanoscale silver plates showing the greatest surface area [61]. Thus, nanosilver with the greatest surface area exhibits better antibacterial properties at lower concentrations [61].

As discussed, the evidence supporting AgNPs as an effective antimicrobial agent is abundant with AgNPs showing broad-spectrum resistance to bacterial strains such as *S. aureus* (including methicillin resistant strains, known as MRSA), *Staphylococcus epidermidis* (including methicillin resistant strains, known as MRSE), *Enterococcus faecalis*,

Enterococcus faecium, *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* and even vancomycin-resistant strains [7, 31, 32, 36, 40, 42, 43].

3.2. Antifungal Properties of AgNPs. Long-term, repetitive administration of standard antifungal drugs leads to increased fungal resistance, especially by the *Candida* species [39]. Therefore, new antifungal agents are constantly being investigated. AgNPs have displayed many antifungal properties against common fungi and thus offer potential as an effective antifungal agent. A recent study found that a AgNP-coated reverse osmosis membrane, which is used in water purification systems, exhibited good antifungal activities against fungal strains such as *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, and *Candida albicans* [36]. Additionally, Pulit et al. reported that AgNP suspensions created from aqueous raspberry extract acted as effective growth inhibition factors against two resistant fungal strains *Cladosporium cladosporioides* and *Aspergillus niger* [39]. Furthermore, a higher concentration of AgNPs induced stronger reduction of fungal growth in both cases. With the promising potential of AgNPs as a broad-spectrum antifungal agent, further investigation is warranted to elucidate the mechanisms concerning their antifungal properties.

3.3. Antiviral Properties of AgNPs. Various viruses, such as influenza, hepatitis, herpes simplex virus (HSV), and human immunodeficiency virus (HIV), can be life threatening [62]. Although many vaccines have been developed against various viruses, medicine has yet to develop a broad-spectrum antiviral vaccine. Furthermore, these viruses are developing antiviral resistance to current treatments and classical antiviral drugs, especially in immunocompromised patients [62]. In light of this, there is a pressing need for the development of new antiviral agents against a broad spectrum of viruses. AgNPs act as a broad-spectrum agent against a variety of viral strains and are not prone to developing resistance [10]. For example, studies have demonstrated antiviral activity of AgNPs against HSV-1, HSV-2, hepatitis B, and human immunodeficiency virus 1 (HIV-1) [63–66]. With regard to HSV-1, AgNPs are hypothesized to target the virus and to compete for its binding to cellular heparin sulfate through their sulfonate end groups, leading to the blockage of viral entry into the cell and the prevention of subsequent infection [63]. Additionally, a recent study demonstrated that 100 µg/mL of AgNPs could completely inhibit HSV-2 replication [66]. AgNPs at nontoxic concentrations were capable of inhibiting HSV-2 replication when administered prior to viral infection or soon after initial virus exposure. These findings suggest that the mode of action of AgNPs occurs during the early phases of viral replication [66]. Furthermore, there has been evidence of AgNP's antiviral activity against HIV-1 at noncytotoxic concentrations. AgNPs exerted anti-HIV activity at an early stage of viral replication, most likely as a virucidal agent or as an inhibitor of viral entry [65].

3.4. Anti-Inflammatory Properties of AgNPs. Despite the billions of dollars that have been spent on immunological

research, few effective anti-inflammatory drugs have emerged [72]. Thus, an urgent need for new drugs exists, as many inflammatory diseases are insufficiently responsive to current medications [72]. Recently, there has been increasing evidence that AgNPs are viable anti-inflammatory agents. Initially, anti-inflammatory properties of AgNPs were investigated by applying AgNP-coated, 0.5% silver nitrate (AgNO_3), or saline wound dressings to a porcine model of contact dermatitis [37]. AgNP-coated wound dressings outperformed other wound dressings as erythema, edema, and histological data showed that AgNP-treated pigs had near-normal skin after 72 hours, while other treatment groups remained inflamed [37]. Furthermore, AgNP-coated wound dressings decreased levels of proinflammatory cytokines transforming growth factor- (TGF-) β and tumor necrosis factor- (TNF-) α compared with other treatment groups. Similarly, another study evaluating the anti-inflammatory effects of AgNPs on an allergic rhinitis mouse model reported reduced nasal symptoms in sensitized mice and an inhibition of ovalbumin-specific immunoglobulin E, interleukin- (IL-) 4, and IL-10 [73]. Moreover, decreased inflammatory cell infiltration and goblet cell hyperplasia were also observed [73]. Further evidence of the anti-inflammatory effects of AgNPs has been demonstrated in a postoperative peritoneal adhesion model and through the use of AgNP-coated sutures [74, 75]. Wong et al. found that AgNPs are effective at decreasing inflammation in peritoneal adhesions without significant cytotoxic effects while Zhang et al. reported that using AgNP-coated sutures decreased inflammation and improved mechanical strength at intestinal anastomosis in mice [74, 75]. Our previous studies have also demonstrated that AgNP/poly(lactic-co-glycolic acid) (PLGA-) coated stainless steel alloy (SNPSA) materials significantly reduced bacterial infection and inflammatory cell infiltration in the intramedullary tissue in rat femoral canals (Figure 1) [76]. Mechanistically, the anti-inflammatory effects of AgNPs are not fully understood but are thought to be associated with the TNF- α pathway [74].

3.5. Osteoconductivity and Osteoinductivity of AgNP-Based Materials. Previously, by implanting AgNP/PLGA composite grafts for the healing of infected bone defects into grossly infected critical-sized bone segmental defects, we demonstrated that AgNP/PLGA composite grafts possess significant, antibacterial properties and osteoconductivity *in vivo* (Figure 2) [32]. AgNP/PLGA composite grafts displayed osteoconductive properties as they did not inhibit adherence, proliferation, alkaline phosphatase activity, or mineralization of ongrowth MC3T3-E1 preosteoblasts *in vivo* [32]. In a follow-up experiment, we unexpectedly found that SNPSA materials not only exhibited strong antibacterial activity *in vitro* and *ex vivo*, but also promoted MC3T3-E1 preosteoblast proliferation and maturation *in vitro* [42]. Furthermore, SNPSA implants induced osteogenesis while suppressing bacterial survival in contaminated rat femoral canals with no observed cytotoxicity (Figure 1) [42]. These findings require more research into the osteoconductive and osteoinductive properties of AgNP-based materials, but they offer promising therapeutic material for orthopedic surgery.

4. Toxicity of AgNPs

Applying silver as an antimicrobial agent has been hindered by its potential toxic effects, such as argyria, an irreversible pigmentation of the skin and eyes due to inappropriate silver deposition [3]. Thus, the toxicity of AgNPs must be investigated for commercial products and before widespread medical application. Also, environmental pollution must be considered to avoid ecological damage.

AgNPs enter the human body most often through the respiratory tract, gastrointestinal tract, skin, and female genital tract, as well as through systemic administration [77]. Recently, an *in vivo* oral toxicity study of AgNPs on Sprague-Dawley (SD) rats demonstrated the accumulation of AgNPs in the blood, liver, lungs, kidneys, stomach, testes, and brain, but showed no significant genotoxicity function after oral administration of AgNPs of 60 nm average size for 28 days at different doses [78]. Similarly, another *in vivo* 28-day inhalation toxicity study of 1.98–64.9 nm AgNPs on SD rats found no effects on respiratory function, hematology, or blood biochemical values [79]. In our previous studies, 3D porous graft and 43 μm thick PLGA coating composited by up to 2% 20–40 nm AgNPs and PLGA materials did not show significant cytotoxicity against preosteoblast MC3T3-C1 cells *in vitro* or toxicity during 8-week bone regeneration in SD rats *in vivo* [32, 42]. Other studies have shown that 20–25 nm AgNPs effectively inhibit microorganisms without causing significant cytotoxicity and that 10–20 nm AgNPs are nontoxic in mice and guinea pigs when administered by the oral, ocular, and dermal routes [32, 80]. In contrast, a 90-day whole body inhalation toxicity study of 18 nm AgNPs on SD rats reported that prolonged exposure to AgNPs elicited an inflammatory response within the alveoli and induced alterations in lung function at all particle concentrations [81]. Also, AgNPs have been reported to cross the blood-brain barrier in rats and induce neuronal degeneration and necrosis through accumulation in the brain over a long period of time [82]. Additionally, Vandebriel et al. and de Jong et al. found that intravenous administration of both 20 and 100 nm AgNPs in a 28-day repeated-dose toxicity study in SD rats induces suppression of the functional immune system [83, 84]. In the area of development, a study reported that AgNPs less than 12 nm in size affected early development of fish embryos, caused chromosomal aberrations and DNA damage, and induced proliferation arrest in cell lines of zebrafish indicating that AgNPs must be investigated for their potential teratogenic effects in humans [85]. *In vitro* studies have demonstrated that AgNPs are cytotoxic to various types of cells including neuroendocrine cells, mouse germ line cells, mouse fibroblasts, rat liver cells, human alveolar epithelial cell line, human peripheral blood mononuclear cells, normal human lung fibroblast cells, and human glioblastoma cells [86–91]. These conflicting results reveal the difficulty of pinpointing the overall toxicity of AgNPs to humans because the tremendous variation in particle size, particle aggregation, and concentration or coating thickness (in implants) of AgNPs has different profiles of silver release and bioactivity [43]. In short, much longer and detailed studies must be carried out to seriously consider AgNPs' potential toxicity in humans.

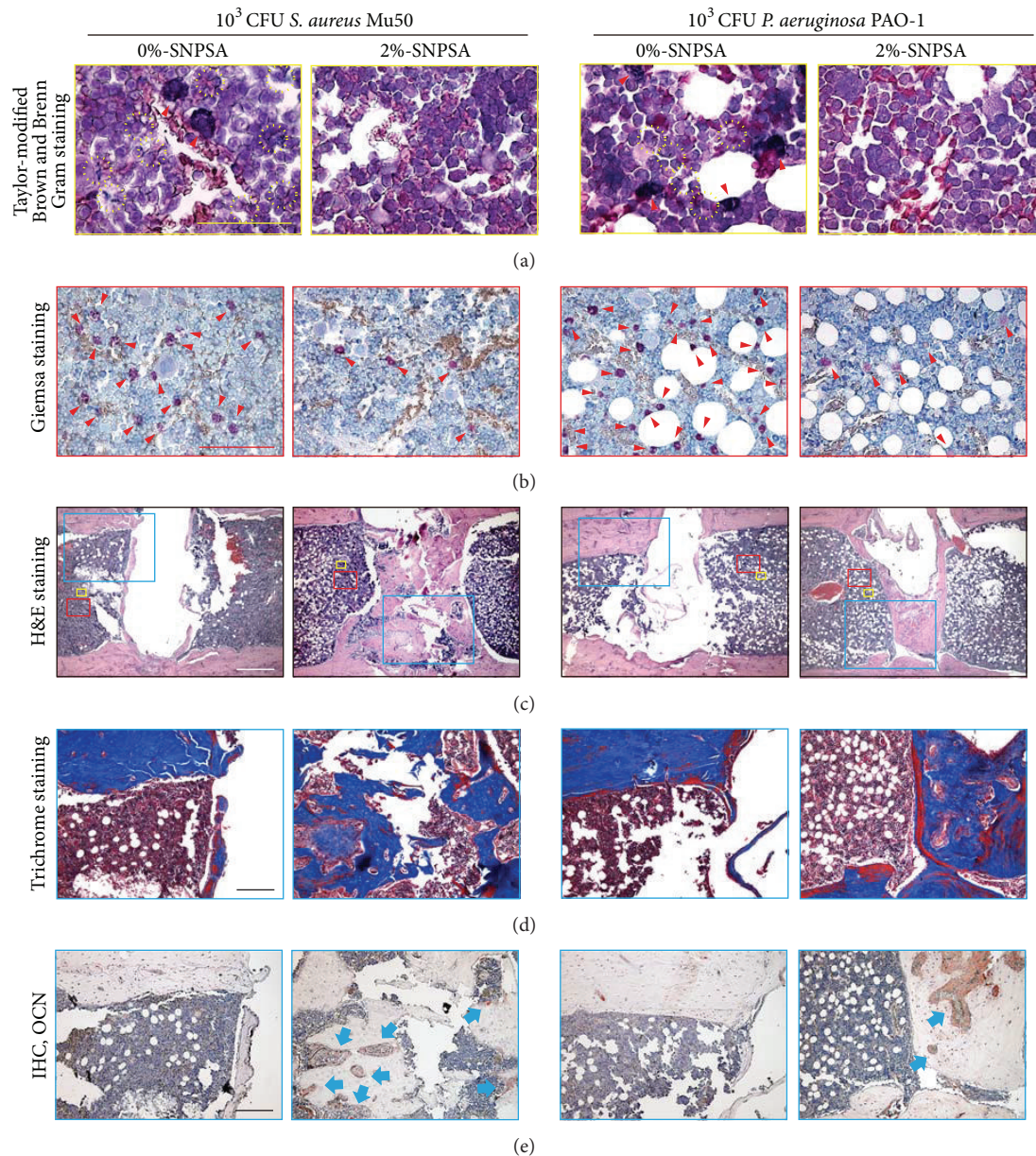


FIGURE 1: Histological and immunohistochemical staining of contaminated 0% and 2% AgNP/PLGA-coated 316L stainless steel alloy (SNPSA) implants in rat femoral canals (FCs) at 8 weeks after implantation. 10^3 colony forming units (CFU) *S. aureus* Mu50 or *P. aeruginosa* PAO-1 in $10\ \mu\text{L}$ phosphate buffered saline (PBS) (10^5 CFU/mL, the typical criteria for invasive tissue infection [26]) were pipetted into the canal before implantation for bacterial invasion. Taylor-modified Brown and Brenn Gram staining (a) and Giemsa staining (b) revealed bacterial persistence (yellow dotted circles) with massive inflammatory cell infiltration (red arrowheads) in the intramedullary tissue around 0% SNPSA implants in rat FCs. In contrast, no bacterial survival was evident around 2% SNPSA implants in the same situation, and inflammatory cell infiltration in the intramedullary tissues around the implants was minimal. Moreover, only minimal bone formation around the 0% SNPSA groups was observed, whereas significant bone formation (blue arrows) was detected around 2% SNPSA implants, as shown by hematoxylin and eosin (H&E) staining (c), Masson's trichrome staining (d), and immunohistochemical staining of high-intensity osteocalcin signals (e). Yellow scale bar = $50\ \mu\text{m}$; red scale bar = $100\ \mu\text{m}$; white scale bar = $500\ \mu\text{m}$; black scale bar = $200\ \mu\text{m}$. Reprinted from *Biomaterials*, Vol. 33, Y. Liu and Z. Zheng et al., "The antimicrobial and osteoinductive properties of silver nanoparticle/poly (DL-lactic-co-glycolic acid)-coated stainless steel," pp. 8745–8756, 2012, with permission from Elsevier.

A potential contributing factor to AgNPs cytotoxicity is the oxidative stress caused by the creation of reactive oxygen species after AgNPs enter the cell [89]. AgNPs also damage cellular components leading to DNA damage, activation of antioxidant enzymes, depletion of antioxidant molecules

(e.g., glutathione), binding and disabling of proteins, and damage to the cell membrane [92]. However, an emerging study suggests that cellular oxidative stress may not be the main cause of toxicity and that it might be a different mechanism all together. Two widely used *in vitro* human cell lines

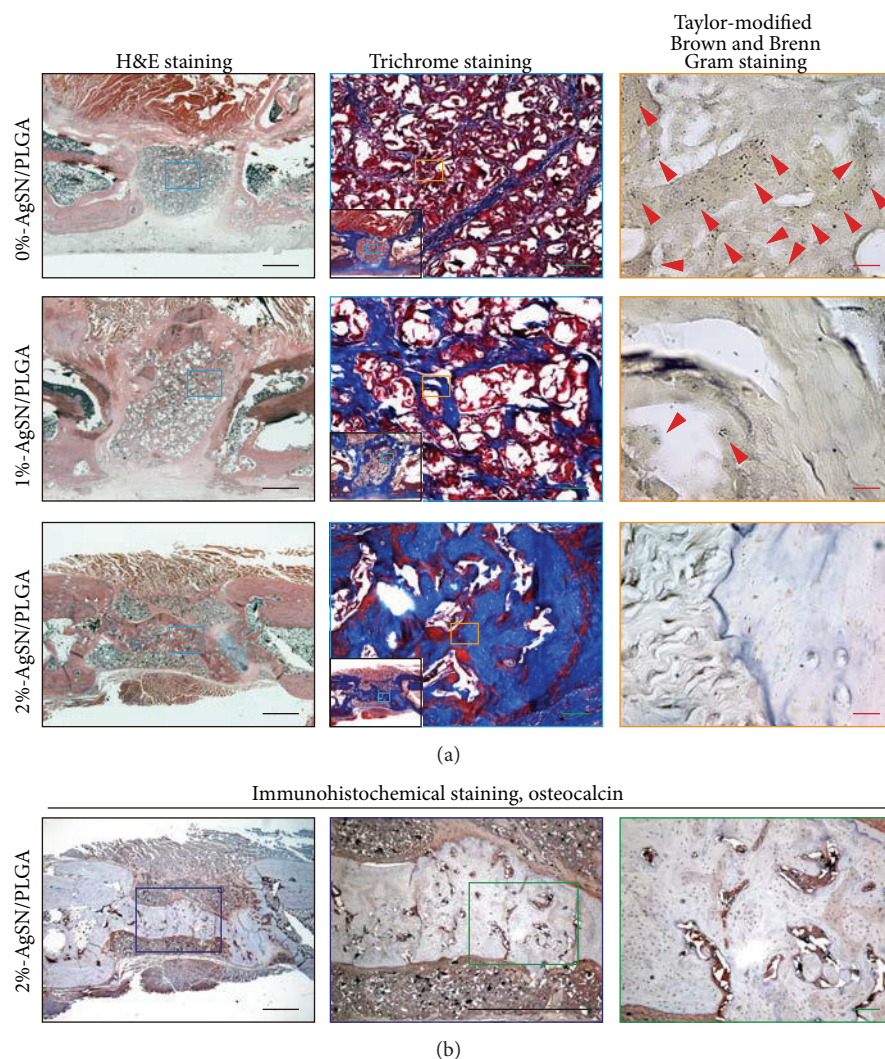


FIGURE 2: Histological and immunohistochemical staining of contaminated rat femoral segmental (FSD) defects implanted with 0.0%, 1.0%, and 2.0% AgNP/PLGA bone grafts coupled with 30 $\mu\text{g/mL}$ bone morphogenetic protein 2 (BMP-2) at 12 weeks after implantation. With a small oscillating saw blade, a 6 mm critical-sized middiaphyseal defect was created in 16–18-week-old male SD rat femur. The volume of the defect was approximately 75 μL . 10^8 CFU *S. aureus* Mu50 in 75 μL 20% gelatin gel was implanted into the defect before implantation. H&E staining and Masson's trichrome staining (a) revealed no bone regeneration in BMP-2/0.0% AgNP/PLGA (control BMP-2 coupled control PLGA) implanted groups with obvious continued bacterial contamination evidenced by Taylor modified Brown and Brenn Gram stain (red arrows). However, BMP-2/2.0% NS/PLGA grafts promoted significantly greater bone formation to form a mineralized bony bridge between the two defect ends by eliminating bacteria in the defect area (a). Immunohistochemical staining against osteocalcin showed active bone regeneration around the mineralized bridge and in the marrow-like cavities in the bridge (b). Black scale bar = 2 mm; green scale bar = 100 μm ; red scale bar = 10 μm . Reprinted from *Biomaterials*, Vol. 31, Z. Zheng et al. "The use of BMP-2 coupled—nanosilver-PLGA composite grafts to induce bone repair in grossly infected segmental defects," pp. 9293–9300, 2010, with permission from Elsevier.

for determining cytotoxicity in the liver and gastrointestinal tract, hepatoblastoma HepG2 and colon carcinoma Caco2, did not display any AgNP-induced oxidative stress when exposed to AgNPs at a concentration of 1–20 $\mu\text{g/mL}$ [93]. These results suggest that cellular oxidative stress did not play a major role in the observed cytotoxicity of AgNPs in HepG2 and Caco2 cells and that a different mechanism of AgNP-induced mitochondrial injury leads to the cytotoxicity. The HepG2 and Caco2 cells appear to be targets for AgNPs, suggesting that the differences in the mechanisms of toxicity induced by AgNP may be largely a consequence of the type

of cells used [93]. Another study reported a correlation between AgNP size and inhibitory effects on mitochondria, with smaller AgNPs of 15 nm significantly more toxic than larger AgNPs of 55 nm [94]. Thus, oxidative stress, cell type, and preparation of AgNPs all play a role in the toxicity. With regard to the environment, a recent study found that AgNPs have a significant impact on the microbial community and severely disrupt the natural seasonal progression of tundra assemblages [95]. This effect was evidenced by levels of differential respiration in 0.066% AgNP-treated soil that were only half that of control soils, a decrease in signature

TABLE 1: Commonly used burn topical antimicrobials.

Agent	Advantages	Disadvantages
0.5% silver nitrate solution Introduced in 1964	(i) Bactericidal against most gram positive, gram negatives, and yeast (ii) Avoids mucopurulent exudate formation (pseudoeschar) (iii) Minimal pain after application (iv) No hypersensitivity	(i) No eschar penetration (ii) Electrolyte imbalance (hypotonic solution depletes wounds cations) (iii) Needs frequent application (iv) Discolors wound bed (v) Possible methemoglobinemia (vi) Rare silver toxicity
Mafenide acetate—historically 11.1% in water-soluble cream, but newer formulation is 8.5% First introduced in 1964	(i) Bacteriostatic against most gram positives, gram negatives; may be more effective for clostridial and pseudomonal infections than silver nitrate or SSD (ii) Good eschar penetration	(i) Potential loss of bacteriostatic action at high ($>10^6$) bacterial loads (ii) Effective concentration in eschar drops below therapeutic levels after 10 hours—needing twice daily application (iii) Metabolic acidosis [drug and metabolite (p-carboxybenzenesulfonamide) inhibit carbonic anhydrase—can worsen ventilation] (iv) Pain after application (v) Potential hypersensitivity
1% silver sulfadiazine (SSD) water-soluble cream Developed in 1968	(i) Bactericidal against most gram positives and some gram negatives (ii) Minimal pain after application	(i) Less activity against certain gram negatives (<i>Enterobacter</i> and <i>Pseudomonas</i>) and yeast (ii) Poor eschar penetration (iii) Forms pseudoeschar that requires daily washings (iv) Neutropenia ^A (v) Potential hypersensitivity to sulfa component (vi) Rare silver toxicity
Mafenide acetate—5% solution First introduced in 1971	(i) Similar to cream (ii) Less pain after application versus cream	(i) Similar to cream (ii) Effective concentration in eschar drops below therapeutic levels after 6–8 hours—needing 3x–4x daily application
Acticoat Rayon/polyester core encased in dense polyethylene mesh coated with nanocrystalline silver	(i) Bactericidal against most gram positives, gram negatives, and fungus (ii) Sustained silver release for 3–7 days (iii) Minimizes pain from daily dressings	(i) Poor eschar penetration (ii) Requires maintenance of moist dressings for silver release (iii) Rare silver toxicity

Table information modified and compiled from the following references: [67–71].

^ANeutropenia thought to be due to transient depression of granulocyte-macrophage progenitor cells in the marrow [67].

bacterial fatty acids, and changes in both richness and evenness in bacterial and fungal DNA sequence assemblages [95]. Similarly, Völker et al. investigated the effects of AgNPs on *Potamopyrgus antipodarum*, a freshwater mudsnail, and found that AgNPs decreased their reproduction [96]. Thus, further assessment of the environmental pollution and risks due to AgNP exposure is required to prevent damage to the environment.

5. Applications of AgNPs

AgNPs have been widely used as an antibacterial coat in medical applications such as wound dressings, cardiovascular implants, catheters, orthopedic implants, and dental composites.

5.1. Wound Dressings. Silver wound dressings have been used for over a decade to clinically treat various wounds, such as burns (Table 1), chronic ulcers, toxic epidermal necrolysis, and pemphigus [30]. Surprisingly, compared to standard silver sulfadiazine and gauze dressings, AgNP wound

dressings significantly decreased wound healing time by an average of 3.35 days while simultaneously increasing bacterial clearance from infected wounds with no adverse effects [38]. Meanwhile, AgNP wound dressings could enhance healing in superficial burn wounds compared with conventional 1% silver sulfadiazine cream or plain petrolatum gauze [97]. However, AgNP wound dressings made no difference in the healing of deep burn wounds, compared with 1% silver sulfadiazine, suggesting that AgNPs accelerate reepithelialization but not new tissue formation, such as angiogenesis and proliferation [97].

As research in this area continues, new ideas for AgNP wound dressings with the intention of further increasing wound healing and antibacterial efficacy are being investigated. For example, a recent chitosan-AgNP wound dressing demonstrated significantly improved wound healing compared with 1% silver sulfadiazine along with the deposition of less silver, which may lower the occurrence of argyria or skin discoloration [98]. Additionally, chitin-AgNP wound dressings possessed a strong antibacterial potential for wound healing applications [99, 100].

5.2. Cardiovascular Implants. In order to reduce the occurrence of endocarditis, a prosthetic silicone heart valve was the first cardiovascular device coated with silver element [101]. The use of silver was intended to prevent bacterial infection on the silicone valve thereby reducing the inflammation response of the heart. However, clinical trials testing the silver heart valve found that silver causes hypersensitivity, inhibits normal fibroblast function, and leads to paravalvular leakage in patients [101]. Thus, efforts turned towards incorporating AgNPs into medical devices as a potential for providing a safe, nontoxic antibacterial coating. The development of a new nanocomposite with AgNPs and diamond-like carbon as a surface coating for heart valves and stents demonstrated antithrombogenic and antibacterial properties [102]. Additionally, studies have also found that incorporation of nanostructured materials into the backbone of polymers in polymeric heart valves improves biocompatibility, resistance to calcification, and durability [103].

5.3. Catheters. Catheters used in the hospital setting have a high propensity for infection, which can lead to unwanted complications. Thus, AgNPs have been investigated as a method of reducing biofilm growth on catheters. Recently, polyurethane catheters have been modified with a coat of AgNPs to create effective antibacterial catheters [104]. Multiple studies have reported that AgNP-coated catheters can effectively reduce bacteria for up to 72 hours in animal models [105–108]. Furthermore, a follow-up 10-day *in vivo* study in mice confirmed that the AgNP-coated catheter was nontoxic [104]. Similarly, a clinical pilot study investigating the prevention of catheter-associated ventriculitis (CAV) found no incidence of CAV, and all cerebrospinal fluid cultures were negative in the 19 patients that received a AgNP-coated catheter [109]. On the contrary, in the control group of 20 patients, 5 were positive for CAV [109].

5.4. Dentistry. AgNPs have also been applied to dental instruments and bandages. Akhavan et al. demonstrated that incorporating AgNPs into orthodontic adhesives could increase or maintain the shear bond strength of an orthodontic adhesive while simultaneously increasing its resistance to bacteria [110]. A recent study by Chladek et al. found that incorporating AgNPs into dental composites could reduce the microbial colonization of lining materials, enhancing the antifungal efficiency [111]. Similarly, AgNPs incorporated into endodontic fillings displayed an increased antibacterial effect against *Streptococcus milleri*, *S. aureus*, and *E. faecalis* [112].

5.5. Orthopedic and Orthodontic Implants and Fixations. Implant associated and joint replacement bacterial infections are high at 1.0–4.0% and are one of the most serious complications in orthopedic surgery because they are extremely difficult to treat and result in increased morbidity and significantly worse outcomes [31, 32, 41, 42]. Thus, AgNPs have been incorporated into plain poly(methyl methacrylate) bone cement, used for the secure attachment of joint prostheses hip and knee replacement surgery, as a way to reduce bacterial resistance [31, 41]. Similarly, our lab investigated

incorporating AgNPs into PLGA grafts as a potential for implant and fixation material in orthopedic and orthodontic surgery [32, 42]. All studies reported markedly increased antibacterial activity without significant toxicity [31, 32, 41, 42]. Additionally, our results indicated that AgNP/PLGA coating is a practical process that is nontoxic, easy to operate, and free of AgNP aggregation (Figures 1 and 2) [32, 76], suggesting that AgNP/PLGA-coated implants have excellent clinical potential for use in orthopedics and orthodontics.

5.6. Other Applications. AgNPs are also available in many commercial products such as water filters and purification systems, deodorants, soaps, socks, food preservation, and room sprays [34–36, 57, 77], which contributes to the increasing market of AgNPs.

6. Future Outlook

The use of AgNPs is already established for many commercial applications and certain medical applications, such as wound dressings, while many new potential applications are being heavily investigated. AgNPs possess great potential due to their antibacterial, antifungal, antiviral, and anti-inflammatory properties while our recent research has revealed novel osteoinductive properties as well. However, the mechanisms and biological interactions behind these properties are not fully understood. For example, the relationship between the size and shape of AgNPs and their biological properties and toxicity is not clearly revealed and thus requires further investigation as AgNP use continues to increase. Therefore, there is a pressing need to fully elucidate the mechanisms concerning the efficacy and toxicity of AgNPs before widespread medical application can occur.

Conflict of Interests

Kang Ting, Chia Soo, and Zhong Zheng are inventors of silver nanoparticle-related patents filed from UCLA.

Acknowledgments

The authors would like to thank Nadia Barakat from the University of California, Berkeley, for assistance in the preparation of this paper. This research is supported by the National Center for Advancing Translational Sciences UCLA CTSI Grant UL1TR000124.

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