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Smart silver nanoparticles: borrowing selectivity from conjugated polymers or antimicrobial peptides

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ABSTRACT

Silver nanoparticles (AgNPs) as novel antimicrobial agents are gaining tremendous exploration in various medical fields due to their broad spectrum activity, efficacy and low cost. The major problem associated with the AgNPs treatment is their narrow therapeutic window. To address this inherent shortcoming, significant efforts have been dedicated to reduce AgNPs cell toxicity and improve their therapeutic index. In this brief review, the emphasis would be placed on development of the combined mechanisms which can enhance the antimicrobial action of AgNPs, arising from investigating the biological differences between microbial and mammalian cells. Using one of our selected antimicrobial cell penetration peptide conjugated AgNPs as an example, we demonstrated that antimicrobial peptides (AMPs) anchored AgNPs produced enhanced antimicrobial activities, possibly through multimodal mechanisms including selective binding to microorganisms and producing the intracellularly controlled Ag^+ release, thus, improving the therapeutic index of AgNPs.

Keywords: *Silver nanoparticles, antimicrobial peptides, surface chemistry, therapeutic index.*

1. INTRODUCTION

Silver based antimicrobial agents have been used since Ancient Greece and Rome. Recently, they have attracted intensive attention in biomedical applications owing to the broad-spectrum biocidal effects against both Gram-positive and Gram-negative bacteria, fungi, yeast, viruses, parasites and even some drug-resistant super bugs[1-3]. For disinfection purposes, the frequently used Ag species are either in ionic or nanoparticulate form. For example, in order to prevent eye disease in infants born to mothers with gonococcal infectious diseases, instillation of 1% silver nitrate eye drop has been practiced worldwide [4]. Ag colloids or nanoparticles (AgNPs) between 1 and 100 nm in size are of particular interest in health-related fields. They are widely used in consumer products like apparel, footwear, paints, appliances, cosmetics, and plastics; in addition, contemporary advances in nano-scale silver synthesis have rendered the NPs rather effective in preventing bacterial colonization or biofilm formation on numerous medical devices, including but not limited to catheters, bone cements, and cardiac prostheses and valves. In the past decades, a number of comprehensive review papers have been published on the production and antimicrobialproperties of AgNPs [5, 6]. Meanwhile, the increasing application of AgNPs in consumer products and medical products has raised concerns of their toxic effect on environment and humans, as evidenced by the accumulation of publications on such topics [7-9].

The toxicity of AgNPs has been reported to be dependent on concentration, particle size, shape, surface charge and capping agents [10]. Although the therapeutical window for silver is often assumed to be narrow [11], from our viewpoint, there are still possibilities to improve the therapeutic index through surface modification of the AgNPs to achieve targeted Ag^+ release. Thus, this review is dedicated to analysing the recent efforts in improving the safety use of AgNPs for potential therapeutic applications.

2. SMART SILVER NANOPARTICLES

This brief review is focused on three main aspects: (1) the biological differences between microbial and mammalian cells that may provide targets for silver nanoparticles, (2) the mechanism of antimicrobial action mode of AgNPs, (3) recent methods to improve the selectivity of silver nanoparticles.

2.1. Opportunities provided by exploring the differences between eukaryotic and prokaryotic cells

At the first glance of Figure 1, eukaryotic (mammalian) cells and prokaryotic (bacterial) cells are very alike as both of them have a selectively permeable plasma membrane, a semifluid cytoplasm, and chromosomes. Fortunately, there are subtle differences existing between them, which may represent targets for antimicrobial AgNPs.

First of all, the overall surface of the bacterial envelope is more negatively charged than mammalian cells, although the magnitude of this charge varies from strain to strain. These negative charges are sourced from the anionic polymers like lipopolysaccharide (LPS) or teichoic acid (TA) that are widely dispersed in the envelope of Gram-negative or Gram-positive bacteria[12, 13]. In the plasma membrane of mammalian cells, there are five types of phospholipids. The outside leaflet consists mainly of neutral phosphatidylcholine and sphingomyelin, whereas

phosphatidylserine and phosphatidylinositol (both have negative charged head groups) together with phosphatidylethanolamine are located at the inner leaflet. The asymmetric distribution of phospholipids between the two halves of the membrane bilayer only results in a negative inner leaflet of cell membrane, which is quite different with the more negatively charged surface of bacterial cells. Moreover, this transmembrane potential of the mammalian cells is well documented to be less negative than that of bacterial cells; therefore the latter will be prone to be attacked by the positively charged antimicrobial agents [14].

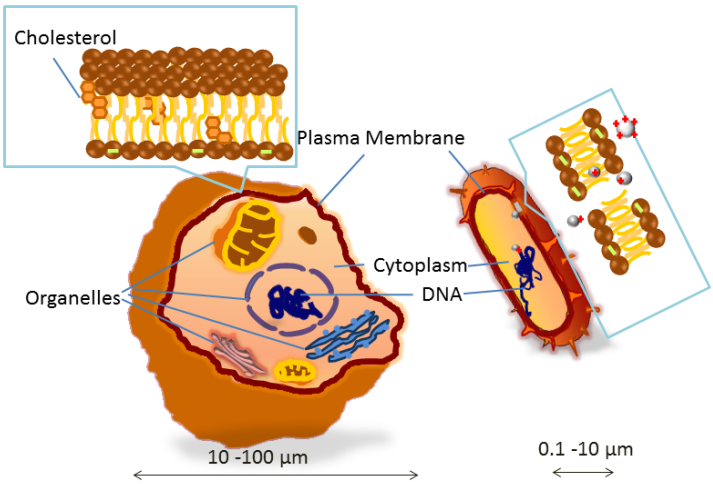

Figure 1. Depiction of cellular structures of mammalian (left) and bacterial cell (right). Targeted delivery of silver nanoparticles to bacterial cells may be realized by surface modification with antimicrobial peptide.

Cholesterol is another important component that is confined in the lipid raft of mammalian cells [15]. With its polar hydroxyl group facing the phospholipid head groups and rigid ring adjacent to fatty acid tails of phospholipids, the amphipathic molecule plays a distinct role in stabilizing the mammalian cell membranes. The vulnerability of bacterium to antimicrobial peptides has been associated with the absence of cholesterol in the membrane [16].

Moreover, mammalian cells contain membrane-encapsulated organelles in which metabolic activities take place or important DNA is packaged. In contrast, a lack of organelles in bacterial cells renders them more susceptible to external stress. For example, Cao et al. stained chromosomal DNA of *Staphylococcus aureus* and nucleus DNA of rat bone marrow mesenchymal stem cells (bMSCs) with Hoechst 33342 [17]. Under UV irradiation, violent fluorescence quenching of bacterial chromosomal DNA was clearly observed while the bMSC DNA quenching was very minimal. They concluded that mammalian and bacterial cells interact differently withthe AgNP decorated titanium oxide NPs, thus the biocompatibility and antibacterial activity of their materials are well balanced [17].

From these evidences, we could conclude that the characteristic structures of mammalian and prokaryotic cells may be explored to enable AgNPs to induce the selective toxicity to bacterial cells. More examples for peptide or polymer conjugated AgNPs with improved selectivity will be discussed in section 2.3. In the next section we will review the recent studies on the antimicrobial mechanism of AgNPs.

2.2. Opportunities provided by the antimicrobial mode of silver nanoparticles

Despite that the debate on the antimicrobial mechanisms of Ag species is still continuing, several recent studies have clearly revealed that the biocidal properties of silver are dependent on the presence of free silver ions [18, 19]. In general, the electrostatic interaction between positively charged Ag ions and negatively charged bacterial envelopes is supposedly the major mechanism to cause rapid and extensive membrane degradation with subsequent cell lysis [20, 21]. It is postulated that Ag^+, as a soft Lewis acid, has strong affinity for sulphur, nitrogen and phosphorous that are abundantly present in the DNA/RNA and numerous enzymes. Such silver ion binding may damage bacterial cells with multiple modes from interfering DNA replication to inactivating respiratory enzymes [22]. The silver ion binding to NADH dehydrogenase is also related to the generation of large quantities of reactive oxygen species, which has been found to be associated with the bactericidal activity of Ag^+ against *E. coli* [23, 24].

The reported mechanisms whereby AgNPs exert their killing power towards bacterial cells are still controversial. There are several inconsistent mechanisms proposed from various studies. Apparently, Ag^+ could be liberated from nanoparticles, thus they share the aforementioned mechanism in eradicating microorganisms [25]. Additionally, transmission electron microscopy (TEM) technique disclosed that AgNPs were accumulated on the cell membrane and caused cell envelope abnormalities [26]. Zhang and co-workers recently elaborated the influence of photo-generated reactive oxygen species (ROS) on uncoated silver nanoparticles and their antibacterial property. Their results showed that AgNPs generated both superoxide and hydroxyl radicals. ROS generation and the release of metal ion significantly enhanced the NPs' antibacterial activity under UV irradiation [27].

Evidence suggests that the biocidal activity of Ag^+ is non-specific; this leads to an inherent difficulty in balancing antimicrobial activity and cytotoxicity, which has limited biomedical applications of silver ions. In contrast to Ag^+, AgNPs are reported to be less cytotoxic in most studies, after being normalized by the mass of added silver [5]. The reduced toxicity of AgNPs may have been related to the controlled release of Ag^+, since an immediate and massive uptake of Ag^+ by eukaryotic cells may cause cell death. There are evidences available in the literature to support this hypothesis. For instance, the biocompatible AgNPs reviewed in the literature [28] were mainly immobilized on the medical devices. The reduced cytotoxicity is based on the fact that AgNPs cannot be uptaken and internalized by eukaryotic cells. A therapeutic window thus is obtained by restricting Ag^+ within the vicinity of the embedded NPs without damaging the surrounding organ cells [29-31]. Noteworthy is that almost all the incorporated AgNPs, either in burn wound dressing or in orthopaedic and dental implants, especially in vascular stents or prosthetic heart valves, have high chances to be released and reach vital organs in the human body. Needless to say, the toxicity

can be a crucial concern when such AgNPs are administered systemically. Surprisingly, few efforts have been directed toward creating mobile AgNPs with high selectivity as elaborated in the following section. Indeed, AgNPs have been considered as Ag^+ reservoir or modern Trojan horse in the literature [32]. It is highly viable to attach targeting molecules, such as antibody [33], antibiotic segments and peptide molecules to the surface of the AgNPs thereby the Trojan horse can be preferentially driven inside the bacterial cells and kill the microorganisms by controlled release of Ag^+intracellularly.

2.3. Strategies in improving therapeutic index (TI) of unrestricted AgNPs

Therapeutic index (TI) is defined as the ratio of the toxic dose to the therapeutic dose. In order to reduce the toxicity, coating AgNPs with "stealth" ligands is one of logical strategies. For example, Stevanović et al. pioneered in development of poly(l-glutamic acid)-capped silver nanoparticles (AgNP-PGA) and ascorbic acid (AscH) encapsulated within freeze-dried poly(lactide-co-glycolide) (PLGA) nanospheres. They obtained a nanomaterial with simultaneous osteoinductive, antioxidative, and prolonged antimicrobial properties [34]. Additionally, if the surface molecules possess antimicrobial property, the TI of Ag nanohybrid may potentially be increased dramatically due to the synergic effect.

Previously reported synthesis of AgNPs in the presence of coating agents, such as sodium dodecyl sulphate (SDS) [35] and hyperbranched poly(amidoamine) [36] has rendered the NPs better antimicrobial activity. Alarcon et al. [37] recently prepared spherical 3.5 nm diameter silver nanoparticles and stabilized them in type I collagen. Remarkable selectivity was observed when the biocomposite was examined for its bioactivity towards keratinocytes and fibroblasts and its anti-bacterial properties. The performance improvement observed in these studies may be attributed to the better dispersion of NPs provided by the coating agents.

Renowned for their good biocompatibility, remarkable antibacterial activities, and proved successful usage in delivering therapeutical drugs, chitosan has been employed to stabilize AgNPs reduced by ascorbic acid [36]. Antibacterial activities of Ag@chitosan were carried out against *S. aureus* and *E. coli*. The silver nanoparticles exhibited significant inhibition capacity towards these bacteria. Detailed studies on the biocompatibility of the silver@chitosannanocomposites were conducted with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and cell adhesion test. Results of the study confirmed that these nanocomposites were beneficial for the proliferation and adhesion of L-929 cells [38].

Cationic antimicrobial peptides (AMPs) have received increasing attention due to their broad-spectrum activities and ability to combat multi-drug-resistant microbes. An overview of their classification, biological functions, mechanism of action, and applicability as alternative therapeutic agents has been provided by Hancock [39] and Peters [40]. Similar to AgNPs, AMPs mainly target to bacterial membranes and the related biological functions. One of the hurdles that limit the initial AMPs' clinical application is their non-selective toxicity to mammalian and bacterial cells. In continuing the efforts to optimize the hydrophobicity, the net positive charge and other critical parameters, Juretić et al. successfully synthesized adepantin 1 peptide with TI > 200 [41]. Here TI = HC_{50}/MIC, where HC_{50} is the peptide concentration that causes 50% red blood cells lysis, and MIC is the minimal inhibitory concentration against bacterial growth. Another successful example of AMP is the use of an excellent target-specific antimicrobial peptide, G10KHc, which was synthesized by conjugatinga rationally designed Pseudomonas-specific targeting moiety (KH) to a generally killing peptide (novispirin G10). G10KHc displayed the capability for fast and selective elimination of *Pseudomonas* species [42].

Previously, we have prepared a novel class of core–shell nanoparticles formed by self-assembly of an amphiphilic peptide that have strong antimicrobial properties against a wide range of bacteria, yeasts and fungi. The nanoparticles showed a high therapeutic index (TI = 50) against *S. aureus* infection in mice and were more potent than their unassembled peptide counterparts [43]. Here TI = LD_{50}/ED_{50}, where LD_{50} is the lethal dose at which half of the mice were killed after intraperitoneal injection of S. aureus; ED_{50} is the effective dose at which half of the mice survived after intravenous injection of GGGRRRRRRYGRKKRRQRR (CG_3R_6TAT) peptide nanoparticles.

Inspired by these progresses, we employed the cell penetrating peptide G_3R_6TAT as the stabilizer and reductant to produce AgNPs. Our strategy for creating highly selective AgNPs is based on conjugation of the multifunctional (targeting and killing bacterial) peptide segment to an existing broad-spectrum AgNPs, thereby generating a bacterial specifically targeted Ag-pep [44]. The cell penetrating peptide catalyzed the formation of antimicrobial AgNPs in N,N-dimethylformamide. The novel Ag-pep demonstrated a distinctly enhanced biocidal effect towards bacteria (Gram-positive *B. subtilis*, Gram-negative *E. coli*) and pathogenic yeast (*C. albicans*), compared favourably to triangular and extremely small silver nanoparticles. For example, the Ag-pep MIC is even lower than MIC of $AgNO_3$ for *B. subtilis* (0.01 vs. 0.03 mM). A plausible reason for the effectiveness of Ag-pep in the destruction of Gram-positive bacteria may depend on three synergistic factors: (i) the G_3R_6TAT cell penetration effect [43], the antibacterial activity of the peptide and (ii) the intracellular Ag effect. In addition, a satisfactory biocompatibility was verified by a haemolysis test. At 0.1 mM (a concentration close to the MIC against E. coli), around 21% haemolysis was observed with the Ag-pep, while Ag^+ mediated 54% haemolysis at 0.025 mM, which was even lower than its MIC. These results provide a paradigm in developing strategies that can maximize the silver nanoparticle application potentials while minimizing the toxic effects.

To sum up, the differences between bacterial and mammalian cells together with the unique antimicrobial mode of AgNPs and their functionalizable surface with polymer or peptide, may offer a promising chemotherapy for topical or systemic infection treatment.

3. CONCLUSIONS

Novel antimicrobial agents with multimodal mechanisms of action are urgently required due to the outbreak of emerging infectious diseases associated with drug-resistant microbes. Silver nanoparticles have distinct size-related physicochemical properties such as large surface area to mass ratio, high affinity for sulfur and phosphorous elements that are abundant in bacterial cell membrane as well as in DNA. They can potentially be used as alternative antimicrobial therapy as demonstrated in numerous medical device studies. One major challenge of Ag nanomedicineis the selective delivery of nanoparticles to pathogenic microorganisms. From evidences presented in the literature, we believe the problem of the narrow therapeutic window of AgNPs is not an insurmountable obstacle. Results from our and other laboratories suggest the nanoparticle surface conjugation with an antimicrobial peptide or polymer that preferentially interacts with the bacterial cells may confer AgNPs with the desired selectivity, therefore, producing new therapeutic opportunities.

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