Colloids and Surfaces B: Biointerfaces 170 (2018) 401–410

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Antimicrobial potency of differently coated 10 and 50 nm silver nanoparticles against clinically relevant bacteria *Escherichia coli* and *Staphylococcus aureus*

Anna-Liisa Kubo[a], Ivona Capjak[b], Ivana Vinković Vrček[c], Olesja M. Bondarenko[a], Imbi Kurvet[a], Heiki Vija[a], Angela Ivask[a], Kaja Kasemets[a], Anne Kahru[a,d,*]

[a] Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia
[b] Croatian Institute of Transfusion Medicine, Petrova 3, 10 000 Zagreb, Croatia
[c] Institute for Medical Research and Occupational Health, Ksaverska cesta 2, Zagreb, Croatia
[d] Estonian Academy of Sciences, Kohtu 6, Tallinn, Estonia

ARTICLE INFO

Keywords:
Library of silver nanoparticles
Biocides
Dissolution
Bioavailability
Recombinant Ag-sensor bacteria
Gram-negative and Gram-positive bacteria
Healthcare associated infections
Minimal bactericidal concentration
Flow cytometry
Nanoparticle-cell interactions

ABSTRACT

Silver nanoparticles (nanoAg) are effective antimicrobials and promising alternatives to traditional antibiotics. This study aimed at evaluating potency of different nanoAg against healthcare infections associated bacteria: Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. A library of differently coated nanoAg of two different sizes (10 and 50 nm) were prepared using coating agents poly-L-Lysine (PLL), cetyltrimethyl-ammonium bromide (CTAB), citrate (CIT), polyvinyl-pyrrolidone (PVP), polysorbate 80 (Tween 80), and dioctyl-sodium sulfosuccinate (AOT). Stability evaluation by means of agglomeration and dissolution behaviour was performed for all nanoAg under conditions relevant for this study.

Antibacterial properties of nanoAg were addressed by determining their minimal bactericidal concentrations (MBC) in deionised (DI) water to minimise the influence of silver speciation on its bioavailability. In parallel, $AgNO_3$ was analysed as an ionic control.

Studied nanoAg were efficient antimicrobials being remarkably more potent towards *E. coli* than to *S. aureus* (4 h MBC values for different nanoAg ranged from 0.08 to 5.0 mg Ag/L and 1.0–10 mg Ag/L, respectively). The toxicity of all nanoAg to *S. aureus* (but not to *E. coli*) increased with exposure time (4 h *vs* 24 h). 10 nm sized nanoAg released more Ag-ions and were more toxic than 50 nm nanoAg. Coating-dependent toxicity was more prominent for 50 nm nanoAg coated with Tween 80 or CTAB rendering the least toxic nanoAg. Obtained results showed that the antimicrobial effects of nanoAg were driven by shed Ag-ions, depended on target bacteria, exposure time and were the interplay of NP size, solubility and surface coating.

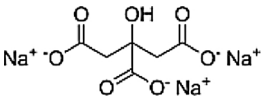
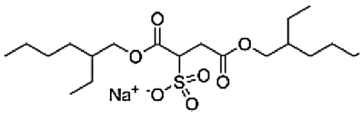
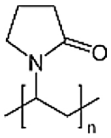
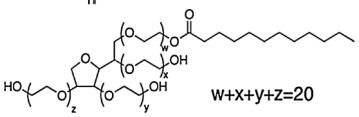
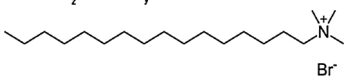
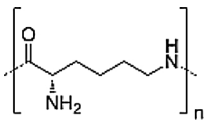
1. Introduction

The increased microbial resistance to antibiotics is a worldwide problem that significantly affects public health issue due to healthcare-associated infections (HAIs). O'Neill recently forecasted that the antimicrobial resistant bacteria (AMR) belonging to both, Gram-negative and Gram-positive bacteria [1] will kill more people than cancer by 2020 [2]. Unfortunately, prevention of HAIs is challenging due to rapid proliferation of bacteria and their profound ability to accommodate within unfavourable environment and develop resistance to nearly all existing antibiotics [3]. The use of silver nanoparticles (nanoAg) is one of the possibilities to combat the antibacterial resistance [1]. High antibacterial efficacy of nanoAg has often been demonstrated to originate from the effect of solubilised Ag-ions on the microbial membranes, specifically on the thiol-groups of proteins leading to enzyme inhibition [4] including enzymes of the respiratory chain [5]. Different nanoAg-based formulations have been recommended for high-touch surfaces in healthcare environment to avoid proliferation of pathogenic bacteria [6]. Also, as the risks of HAIs are often related with catheterization, silver-impregnated catheters are widely applied in acute-care hospitals [7]. In contrast to conventional antibiotics, bacterial resistance against silver has been reported just in a few hospital cases [8]. Additionally, synergic action of nanoAg with antibiotics commonly used against *E. coli* and *S. aureus* could be an effective antimicrobial strategy [9]. NanoAg are nowadays used as biocidal additives in many fields and in various products including dental resin composites, bone

* Corresponding author at: Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia.
E-mail address: anne.kahru@kbfi.ee (A. Kahru).

https://doi.org/10.1016/j.colsurfb.2018.06.027
Received 16 February 2018; Received in revised form 27 April 2018; Accepted 15 June 2018
Available online 18 June 2018
0927-7765/

Table 1
Coating/stabilising agents used in this study.

Name	Molecular structure	Molecular weight (g/mol)	Charge of the coating agent in deionised water	Conventional use
Trisodium citrate (CIT)		294.10 (dihydrate)	Negative	Buffering agent
Bis-2-ethylhexyl sulfosuccinate (AOT)		444.56 (Na-salt)	Negative	Anionic surfactant
Poly-vinylpyrrolidone (PVP)		40000	Neutral	Nonionic polymer
Polysorbate 80 (Tween 80)		1310	Neutral	Nonionic surfactant
Cetyltrimethyl-ammonium bromide (CTAB)		364.45	Positive	Cationic surfactant
Poly-L-lysine (PLL)		3000	Positive	Cationic polymer

cements, medical devices, water filters, textiles, detergents, soaps, toothpastes, wet wipes, washing machines, refrigerators, and many others [10,11]. According to the Nanotechnology Products Database (www.statnano.com), nanoAg are used in 75% nano enabled products for medical applications. The design of Ag-enabled biomedical nanomaterials is commonly performed by modulating the physico-chemical properties of nanoAg such as size, shape and surface properties [12,13]. Surface functionalisation is one of the important strategies to improve colloidal stability, controlled release of Ag-ions or targeted delivery of nanoAg [14]. Moreover, surface characteristics of nanoparticles (NPs) influence the interactions between NPs and microbes. A plethora of chemicals such as polymers, anionic, cationic or non-ionic surfactants, ionic liquids and reducing agents can be used to modulate NP surface properties providing protective, stabilising or functional surface coatings [14]. For metal-based NPs, such coatings control size and shape of NPs already during synthesis by interacting with metal ions and affecting the equilibrium of synthesis reaction, particle nucleation and growth rate [15]. According to Kvitek et al. [16], the aqueous dispersions of NPs can be stabilised (i) with the assistance of steric repulsion by using polymers (such as PVP) or non-ionic surfactants (Tween 80, Triton X-100); or (ii) by electrostatic repulsion using anionic (SDS) or cationic surfactants (CTAB).

A search in the Web of Science research platform (performed on Sep 15th 2017) on the use of coating/capping agents for stabilisation of nanoAg intended for use in biomedicine yielded altogether 4298 papers (Fig. S1). Literature search revealed that citrate (CIT) and polyvinylpyrrolidone (PVP) were the most frequently used coating materials (4.5 and 4.2% of the studies, respectively) followed by cetyltrimethyl-ammonium bromide (CTAB), dioctyl-sodium sulfosuccinate (AOT), poly-L-lysine (PLL) and polyoxyethylene sorbitan monolaurate (Tween 80). Other studies (~90%) reported the use of very diverse coating materials including different polymers (like PEG, chitosan, PVC, PEA, PAA, polypropylen), mineral-based materials (like HAP, silica, and iron oxide), surfactants, different biomolecules (like β-cyclodextrin, chitosan, cellulose, cysteine) or their combination. Chemicals applied as surface coating agents can protect NPs from direct interaction with the environment, oxidation [17], dissolution [18], or aggregation. However, stabilisation of NPs with functional coatings may significantly affect their biological activity. For example, Kvitek et al. [16] found a correlation between the stabilisation efficiency and increased antibacterial activity of SDS- and Tween 80-coated nanoAg concluding that non-aggregated NPs (but not spacious NPs aggregates) strongly interact with bacterial cell wall due to their high surface energy and mobility. Importantly, surface coating agent is not only attached to the NPs` surface, but exists also in free form in NPs suspensions [19]. Thus, the role (*e.g.*, potential toxicity) of coating agents should be addressed during evaluation of biological impact or toxicity of coated/stabilised NPs. Yet, the information on contribution of surface coatings to overall NPs biological effects is scarce [19].

This study aimed to evaluate antibacterial activity of differently coated nanoAg of two different sizes: 10 nm (10 nAg) and 50 nm (50 nAg). For this purpose, a library of 11 different nanoAg was prepared employing neutral (PVP and Tween 80), positively (PLL and CTAB) and negatively charged surface coatings (AOT and CIT). Biocidal activity of these nanoAg was evaluated against two clinically relevant pathogens: Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. Additionally, antibacterial effects of coatings themselves were investigated. The role of possible nanoAg dissolution on their biocidal effects was assessed by quantifying the released Ag-ions from nanoAg surface.

2. Materials and methods

2.1. Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) was used as a precursor of nanoAg. Capping/coating agents used for stabilisation of nanoAg are described in Table 1. CIT-coated 10 nAg were synthesised as described by Li et al. [20]. Other types of 10 nAg were synthesised by reducing $AgNO_3$ with $NaBH_4$ as described in [21]. 50 nAg coated with AOT, PVP, Tween 80 or CTAB were prepared *via* chemical reduction of the complex cation $[Ag(NH_3)_2]^+$ by *D*-glucose [22], while CIT-coated 50 nAg were prepared as