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Highly bactericidal Ag nanoparticle films obtained by cluster beam deposition

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Abstract

The recent emergence of bacterial pathogens resistant to most or all available antibiotics is among the major global public health problems. As indirect transmission through contaminated surfaces is a main route of dissemination for most of such pathogens, the implementation of effective antimicrobial surfaces has been advocated as a promising approach for their containment, especially in the hospital settings. However, traditional wet synthesis methods of nanoparticle-based antimicrobial materials leave a number of key points open for metal surfaces: such as adhesion to the surface and nanoparticle coalescence. Here we demonstrate an alternative route, i.e. supersonic cluster beam deposition, to obtain antimicrobial Ag nanoparticle films deposited directly on surfaces. The synthesized films are simple to produce with controlled density and thickness, are stable over time, and are shown to be highly bactericidal against major Gram positive and Gram negative bacterial pathogens, including extensively drug-resistant strains.

Key words: Ag nanoparticle-based antimicrobial films; Supersonic cluster beam deposition; Atomic force microscopy; Electron spectroscopies; Bactericidal activity; Extensively drug-resistant bacteria

Backgrounds

Nanoparticles (NPs) are promising alternatives to conventional materials in many branches of science and technology[1-3] since the nanoscale allows to access physical properties and functionalities that often differ significantly from their bulk counterparts. As an example, the antimicrobial activity of nanomaterials such as fullerenes, TiO_2, and Ag NPs has a wide range of important applications in medicine, water disinfection, and consumer products (e.g. cosmetics, laundry detergents, toys, accessories).[3-6] In this scenario, a current challenge is the synthesis and application of NPs[7-9] to implement effective control measures to reduce the incidence of healthcare-associated infections (HAIs). HAIs have become a global threat due to the emergence and dissemination of microbial pathogens that are resistant to most or even all antimicrobial agents available for their treatment (extensively drug-resistant or totally drug-resistant phenotypes).[10,11] HAIs are in fact a major cause of patient morbidity and mortality.[12] Most of the principal nosocomial pathogens can asymptomatically colonize the human host, with colonization representing either a risk factor for infection or a key step for their dissemination. Indeed, an estimated 20% to 40% of HAIs have been attributed to cross infection via the hands of healthcare personnel, who may become contaminated indirectly by touching contaminated environmental surfaces.[13] Besides the strict adhesion to hand-hygiene practices and classical environmental cleaning procedures, the development of antimicrobial surfaces/coatings characterized by a long-lasting microbicidal effect to be applied in high-touch hospital devices (e.g. buttons or handles), has

Abbreviations: NP, nanoparticle; HAI, healthcare-associated infection; SCBD, supersonic cluster beam deposition; AFM, atomic force microscopy; XPS, X-ray photoemission spectroscopy; XRD, X-ray diffraction; CFU, colony forming units; ME, microbicidal effect.

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been advocated as a promising approach.[11,13] Some of the challenging aspects concerning fabrication of antimicrobial films are the adhesion to metal surfaces, determined by the NP–surface interactions, the ability to maintain the microbicidal effect over time and the lack of non-wet synthesis methods.

Among the large number of NPs investigated so far for antimicrobial features, Ag NPs have been considered the most promising ones for potential medical applications.[5] Ag NPs exert an antibacterial activity through a multifactorial process which has not been clearly elucidated, and involving either damaging of bacterial cell wall and plasma membrane, or inhibition of DNA replication and protein synthesis.[14] NP morphological characteristics (i.e. shape, size) have been proven to affect antibacterial efficacy of Ag NPs, with a recent work demonstrating that this could be related to a differential release of Ag^+ ions (i.e. the actual effectors of bactericidal activity).[8]

To date, the synthesis of Ag NPs is largely based on wet chemical reduction starting from a molecular precursor containing Ag in an oxidized state.[15] Such methods allow a great control over size and shape of the Ag NPs, but pose several problems such as the use of colloidal stabilizers, the presence of impurities, the solvents and synthesis process costs to reduce or avoid the problem of NP aggregation in solution.[16,17] Moreover, the mere synthesis of the Ag NPs does not imply a good adhesion of the obtained NPs to the substrate of need. The layer-by-layer approach[16] has been proposed to obtain surfaces on which thin films/layers of Ag NPs are deposited or formed as a molecular self-assembled monolayer, while pre-functionalization of glass has been used to stabilize the Ag NPs on the surface, further complicating the synthesis and deposition process.[17]

A so far unexplored alternative route to Ag NP wet synthesis is provided by the supersonic cluster beam deposition (SCBD).[18,19] The source is based on the pulsed plasma ablation of the material to be deposited and the subsequent formation of an NP beam. The method, which is intrinsically environmentally-friendly since it does not employ solvents, has also been shown to produce NPs with mixed chemical composition,[19] hence allowing the possibility of combining different elements to engineer the material properties. To our knowledge, this method has not been applied yet to synthesize Ag NP films with antimicrobial properties. In the present work, we obtain for the first time Ag NP films via SCBD directly on the surface of microscope slides, with an easy control over film thickness and density. The films are extremely uniform and composed of pure Ag NPs with an average diameter of 8 nm. Data show that the NP oxidation state is Ag_2O and the films present a high bactericidal activity against a wide range of clinically relevant pathogens (including extensively drug-resistant Gram positive and Gram negative strains).

Methods

NP film synthesis and characterization

Nanostructured Ag films were deposited at room temperature (RT) in medium vacuum (base pressure 1×10^{-6} mbar) conditions by SCBD based on the pulsed microplasma cluster source (see Figure S1 of Supplementary Materials)[18-20] directly on the surface of soda lime glass (SLG) microscope slides (electron microscopy sciences). The nominal film thickness and deposition rate were measured during deposition by a quartz microbalance, while film physical properties where obtained *ex situ* by atomic force microscopy (AFM) (Solver-pro NT-MDT), X-Ray photoemission spectroscopy (XPS), Auger Spectroscopy, X-Ray diffraction (XRD) and optical absorption spectroscopy. Once deposited in the vacuum chamber, the films are extracted to air and either transferred to the measurement apparatus or left exposed to the environment. More details on the experimental characterization procedures can be found in Supplementary Materials.

Antimicrobial activity of Ag NP films

Antimicrobial activity of Ag NP films was investigated against a panel of clinically relevant microorganisms, representative of both Gram negative and Gram positive bacteria, and yeasts. They included six reference strains (i.e. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* PAO-1, *Acinetobacter baumannii* ATCC 17978, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231) and ten clinical strains exhibiting antimicrobial resistance phenotypes of concern and/or being recognized as members of high-risk epidemic clones (see Table 1 and references therein[21-27] for clinical strains characteristics).

Antimicrobial activity testing was performed using the procedure proposed by Pallavicini et al,[28] with some minor modifications. Briefly, 1 ml of an overnight culture was washed twice with Phosphate Buffered Saline (PBS) pH 7.4, and 10 μl of microorganism suspension (i.e. range 5.4-7.3 log Colony Forming Units [CFU], see Table T1 of Supplementary Materials for details) was deposited both in a glass slide containing the Ag NP film (challenge slide) and in an unmodified glass slide (control slide), and a glass coverslip was applied (20 × 20 mm). After 24 h of incubation at 25 °C in damp environment, microorganisms were suspended in 10 ml of PBS, appropriately diluted, and 240 μl of each dilution was plated for viable cell count (i.e. enumeration of CFU). The log reduction rate was expressed as Microbicidal Effect (ME), following the formula: $ME = \log N_C - \log N_E$ (where N_C and N_E represented the number of CFU obtained with control slides and challenge slides, respectively). The detection limit of viable cell count was 4.2×10^1 CFU. All microorganisms were tested in three independent experiments and results were averaged. In order to calculate standard deviations (SDs), when no viable cells were counted, the result was arbitrary assumed as 4.2×10^1 CFU, representing the detection limit value. All microorganisms were grown aerobically at 37 °C in a shaker incubator (200 rpm) in Mueller–Hinton II broth (BD, Becton, Dickinson and Company, Sparks, MD, USA), except for *Enterococcus* spp. and *C. albicans* for which Brain-Heart-Infusion broth (BD) and Yeast Extract-Peptone-Dextrose broth (BD) were used, respectively. Viable cell count was performed in Sabouraud-Dextrose agar (Oxoid, Milan, Italy) for *C. albicans*, and in Luria-Bertani Agar (LBA) (Oxoid) for all other microorganisms.

Results

A representative atomic force microscopy (AFM) image of the as-deposited NP films synthesized in the present work is