Highly bactericidal Ag nanoparticle films obtained by cluster beam deposition

Luca Gavioli, E. Cavaliere, S. De Cesari, G. Landini, E. Riccobono, G. M. Rossolini and L. Pallecchi

Interdisciplinary Laboratories for Advanced Material Physics (i-LAMP) & Dipartimento di Matematica e Fisica, Università Cattolica del Sacro Cuore di Brescia, Via dei Musei 41, 25121 Brescia, Italy

luca.gavioli@unicatt.it

Nanoparticles (NP) such as TiO_2, and Ag are promising alternatives to conventional materials [1,2,3] for the antimicrobial activity that has a wide range of important applications in medicine, water disinfection, and consumer products [3,4,5]. In this scenario, a big challenge is the synthesis and application of NP [6,7] to find effective control measures for reducing the incidence of healthcare-associated infections (HAI). HAI have become a global threat [8] due to the emergence and dissemination of microbial pathogens resistant to most antimicrobial agents available (extensively drug-resistant or totally drug-resistant phenotypes) [9,10]. Indeed, an estimated 20% to 40% of HAI have been attributed to cross infection via the hands of healthcare personnel, contaminated indirectly by touching contaminated environmental surfaces. [11] 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), is still a promising but not yet realized approach [10,11]. The challenge is directly related to the physical behavior of the NP (e.g. adhesion to surface determined by the NP-surface interactions) that would be the active material.

To date, the synthesis of Ag NP is largely based on wet chemical reduction, [4] posing several problems such as the solvents and synthesis process costs to avoid the NP aggregation in solution, the NP adhesion to metal surfaces requiring further functionalization. An alternative route to wet NP synthesis is the supersonic cluster beam deposition (SCBD), [12,13] based on the pulsed ablation of the material to be deposited and the subsequent formation of a NP beam. The method has also been shown to produce NP with mixed chemical composition, [13] allowing the combination of 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. The films (thickness can be tuned according to the experimental needs) are deposited at room temperature RT in medium vacuum (base pressure 1 x 10^{-6} mbar) conditions by SCBD [12,13] directly on microscope slides (SLG). Atomic force microscopy (AFM) of the as-deposited NP films is shown in Figure 1a. The NP are homogeneously distributed (rms roughness < 1 nm) with no coarsening over the entire covered area (~15 cm^2). XRD data (not shown) indicate that the NP are crystalline with a predominant (111) surface orientation and an average diameter of 7.4 x 0.1 nm. The NP density is 1.15±0.04 x 10^{11} NP/cm^2. Since the film density depends on the deposition time, it can be easily tuned from a few percent of the surface area up to a single layer and beyond.

The chemical state of the NP have been studied by Auger and X-Ray photoemission spectroscopy (XPS). A comparison of normalized MNN Auger spectra for the Ag NP film (curve b) and from a polycrystalline, metallic Ag reference (curve a), is shown in Figure 1b together with their difference (curve (b-a)). Spectrum b) presents a broader lineshape, reflected in the difference peak at 351±0.5 eV, while the feature at 357±0.5 eV is due to the appearance of a new structure: both consistent with the presence of Ag^+ ions. [14] The oxidation of the as-deposited NP film is confirmed by XPS data (not shown). Hence the NP are in a Ag_2O oxidation state. This is a required condition

for NP to release Ag^+ ions, which has been recently proposed as the more relevant mechanism with respect to the role of the NP size giving rise to the bactericidal activity of the NP.[6]

Ag NP films were found to exert a potent and broad-spectrum bactericidal activity, which was demonstrated both for reference strains and for a collection of clinical strains that exhibited extensively drug-resistant phenotypes and belonged in high-risk hyperepidemic clones(see Figure 2 and references [15,16,17,18] for strains characteristics). In particular, 24 hours of exposure to Ag NP films were able to reduce viable bacterial loads by more than 4 log with the majority of strains tested, including representatives of both Gram positive and Gram negative pathogenic species. The highest susceptibility was observed with members of the family Enterobacteriaceae (i.e. *E. coli and K. pneumoniae*), which are normal constituents of the intestinal microbiota and among the most common causes of HAI.[8] Interestingly, Ag NP films could almost completely sterilize a high inoculum (i.e. ~1 x 10^7 Colony Forming Units [CFU]) of extensively drug-resistant clinical strains producing two of the most worrisome resistance mechanisms recently emerged in enterobacteria and capable of pandemic dissemination, such as the NDM- and KPC-type carbapenemases. An overall strong bactericidal activity was also demonstrated against reference and clinical strains of *P. aeruginosa* and *A. baumannii*, including representative of extensively drug-resistant high-risk clones (Figure 2). Those microorganisms are major opportunistic pathogens in the hospital setting, with high propensity to evolve extensively drug-resistance and even totally drug-resistance phenotypes and to survive for long periods in the hospital environment (accounting for the occurrence of nosocomial epidemics through, contaminated taps, sinks or either antiseptic solutions).[11]

In conclusion we have demonstrated the realization of a Ag NP film with extremely controlled thickness and NP size directly on a substrate surface through a simple technique, SCBD. Such films present a high and broad-spectrum bactericidal activity that could be related to the oxidation state of the Ag NP. In perspective, the SCBD ability to modify the NP chemical composition will open large possibilities for tailoring the active material to optimize the precious metal amount, expanding the spectrum of antimicrobial activity, tailor the adhesion to metal surfaces to obtain a long lasting, low cost antimicrobial film.

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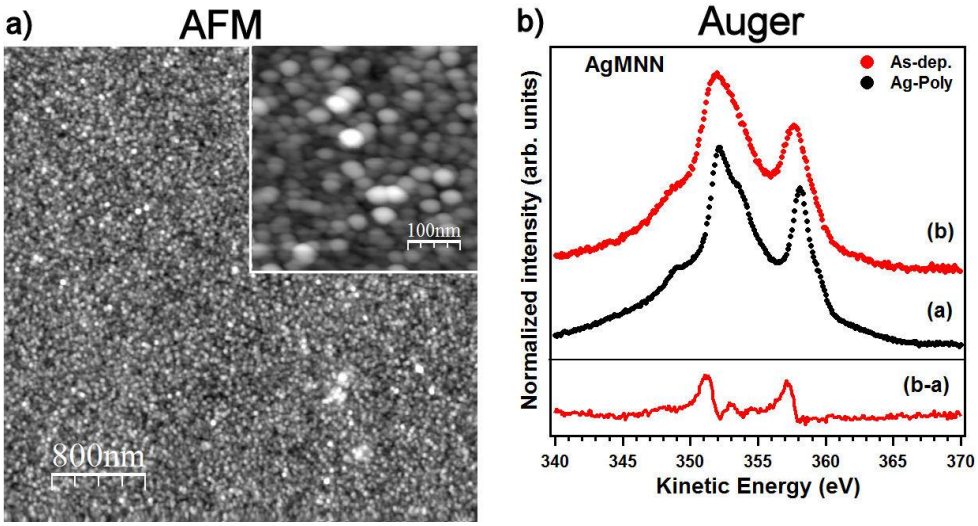

Figure 1. a) AFM image of the as-deposited Ag NP film, showing the uniformity at microscopic scale. In the inset, high resolution image where the NP size can be appreciated. b) Auger spectra of the as-deposited NP film (curve (b), red dots) and metallic polycrystalline Ag reference (curve (a), black dots), with the difference spectrum plotted in the bottom to highlight the oxidation state of the NP film.

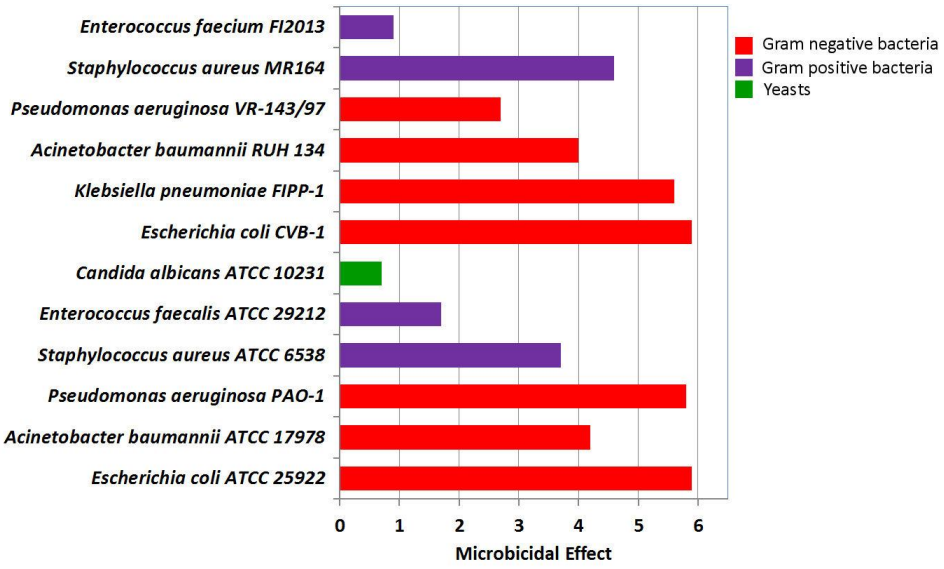

Figure 2. The microbicidal effect is calculated as log reduction of viable cells compared to control, after 24 hours of exposure.