

## Silver Nanostar-based Biocide Surfaces

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### Introduction

**Hospital-acquired infections** are a major concern given the current multiple resistance to antibiotics developed by the involved bacteria. Bacterial pathogens can grow in surfaces and eventually form **biofilms**, that adds an extra **layer of complexity to fight their proliferation**.<sup>[1]</sup> Inhibiting the proliferation of such microorganisms on surfaces is the first step for material's safety.

The effect of **silver ions (Ag<sup>+</sup>) as a bactericidal agent** is well known<sup>[2]</sup>, so in this work we used **silver nanostars as a coating agent** that can work as a "reservoir" of Ag<sup>+</sup>.

### Methods

We covered 0.13-0.16 mm thick, 9 mm diameter **glass disks with silver nanostars (AgNSs)** using a **deposition by centrifugation** method<sup>[3]</sup> of the synthesised AgNSs<sup>[4]</sup>, resulting on the pieces visible in Figure 1. The proliferation of two bacterial species – *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 – was assessed by several methods, including **LIVE/DEAD cell viability staining assays** and **atomic force microscopy (AFM)**.

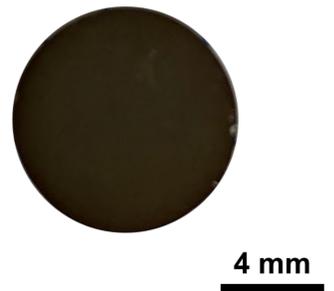


Figure 1 – Photograph of the AgNSs-coated surfaces.

### Results

Figure 2A shows that, after 24 h, *P. aeruginosa* cells **were fragmented/damaged**. For the non-coated surface (Figure 2B), some of the cells present an irregular morphology **but others are still intact**.

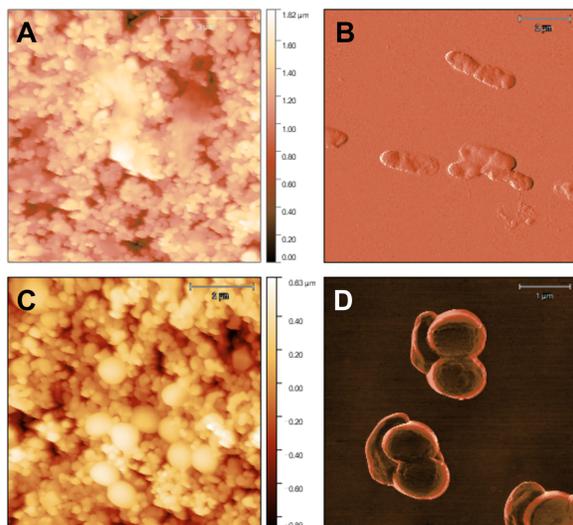


Figure 2 – AFM images of *P. aeruginosa* ATCC 27953 (A and B) and of *S. aureus* ATCC 25923 (C and D) deposited on AgNSs-coated (A and C) and non-coated surfaces (B and D), after 24 h.

This distinction was **not observed for *S. aureus* cells** (Figure 2C–D). However, this does not indicate that the cells kept their viability in both surfaces. As Gram-positive bacteria, they have **thicker peptidoglycan layer** of the cell wall, providing **structural strength**, making any **morphological changes harder to be observed**.

In Figure 3, bacteria showing as green are viable, while red bacteria are dead. *P. aeruginosa* stained majorly/totally red in all conditions. However, **in non-coated surfaces the number of cells was higher**, meaning that the **initial inoculum** deposited on the surface **was able to proliferate**, in **opposition to AgNSs-coated surfaces**. For *S. aureus*, there are more viable cells on non-coated surfaces, but they are **totally dead after 24 h for the AgNSs-coated surfaces**.

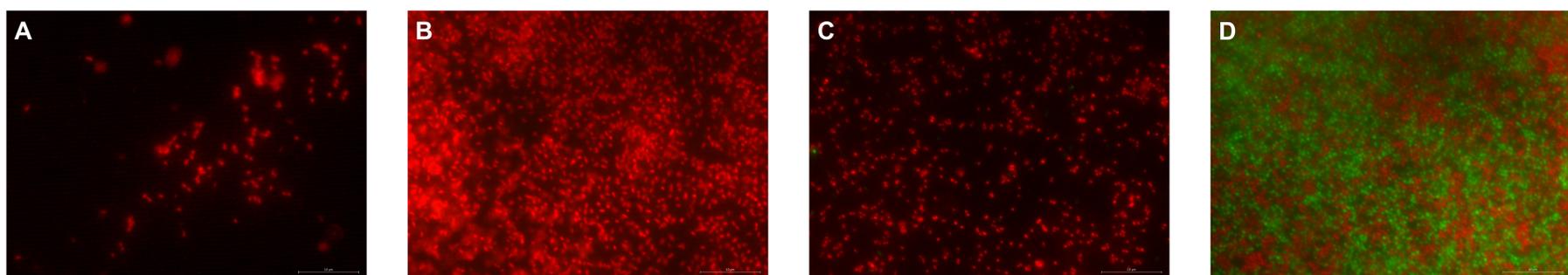


Figure 3 – LIVE/DEAD staining fluorescence microscopy micrographs of *P. aeruginosa* ATCC 27853 (A and B) and of *S. aureus* ATCC 25923 (C and D) deposited on AgNSs-coated (A and C) and non-coated surfaces (B and D) for 24 h.

### Conclusions

- It was possible to **easily produce** AgNSs-coated surfaces, uniformly coated to the naked-eye;
- The bacteria deposited on top of these surfaces **did not proliferate** and were found **totally dead after 24 h**;
- The AgNSs described here showed **great potential in being used to coat surfaces**, which become **strongly biocidal**.

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