Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Antibacterial Properties of Silver Dendrite Decorated Silicon Nanowires

*Hashim Alhmoud, Bahman Delalat, Xavier Ceto, Roey Elnathan, Alex Cavallaro, Krasimir Vasilev, Nicolas H. Voelcker**

Future Industries Institute, University of South Australia, X Building, University Boulevard, Mawson Lakes SA 5095, Australia.

Keywords: Antibacterial substrates, silicon nanowires, Ag nanoparticles, bacterial capture.

1. Experimental Section:

1.1 Dissolution of Ag dendrites

In cases mentioned in the text where the Ag coating on the SiNWs was dissolved deliberately, the sample was immersed in a concentrated solution of HNO_3 (70%) for up to 5 min with gentle swirling, until all the Ag visible on the surface gradually dissolved in solution.

1.2 SEM analysis

All SEM analysis was carried out using a field emission SEM (Merlin®, Zeiss, USA), fitted with a GEMENI II® column (Zeiss, USA) and a secondary electron detector, operating at 1 kV in high vacuum mode. Measurements of Ag agglomerate dimensions were performed using the open source software ImageJ.

1.3 Preparation of BacLight™ Live/Dead assay solution

BacLight™ Live/Dead assay kit was purchased from Life Technologies™ (Australia). Each of the kit components, namely A (SYTO 9) and B (propidium iodide), was diluted in saline solution (0.14 M NaCl, pH 7.2) by adding 25 µL of each component to 8 mL of saline. The solution was then used without alteration in all bacterial staining procedures.

1.4 LB-agar plating

Bacterial cultures (10^8 CFU/mL) were prepared from overnight cultures in TSB and 60 µL of the cultures were grown for 1 h in a 96 well plate as well as on Ag-free SiNWs, and Ag-coated SiNWs (5x5 mm^2) in a self-humidifying incubator at 37 ^oC. The solutions were recovered and diluted in fresh TSB to make up 100 µL. The diluted solutions were then plated on pre-prepared LB-agar plates and

incubated at 37 ^{o}C for 16 h. Following the incubation period, optical photographs were taken of each plate.

1.5 Turbidity measurements

Bacterial cultures (10^8 CFU/mL) were prepared from overnight cultures in TSB and 60 µL were grown for 1 h in a 96 well plate, Ag-free SiNWs, and Ag-coated SiNWs (25 mm^2) at 37 ^{o}C. The solutions were recovered and diluted in fresh TSB to make up 200 µL. the diluted solutions were placed in separate wells in a 96 well plates and incubated for 24 h at 37 ^{o}C. During the incubation period, the turbidity of the solutions was monitored every 2 h using an ELx800 plate reader (BioTek), at 600 nm.

1.6 Live/dead viability assay

Diluted bacterial solutions (108 CFU/mL, 60 µL) were incubated over SiNW samples and flat Si (5x5 mm^2) for 1 h. Subsequently, the bacterial solution was recovered and deposited on microscope glass slides. The bacterial solutions were allowed to incubate on the glass slides for 30 min to allow the bacterial to attach to the glass substrate. BacLight™ Live/Dead assay staining solution (20 µL, prepared previously) was then added to the bacterial solutions for 15 min in the dark. The majority of the staining solution was then removed and 50 µL of NaCl buffer was

added. Cover glass slides were then applied, and the samples were investigated using fluorescence microscopy on a Nikon Eclipse Ci-L upright microscope. Manual cell counts were performed in order to determine cell viability. Bacterial cell viability was calculated by subtracting the number of dead bacterial cells (red stained cells) from the total number of cells (green stained cells), and the result was divided by the total number of cells.

$$\text{Viability (\%)} = \left(\frac{Total\ No.\ of\ cells - No.\ of\ dead\ cells}{Total\ No.\ of\ cells}\right) \times 100$$

1.7 Mammalian cell culture

Human foreskin fibroblast cells (HFF), were grown and maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C with 5% CO_2.

The substrates were placed in a sterile 48-well plate. The substrates were then sterilized with 400 U/mL of penicillin, 400 µg/mL of streptomycin and 1000 ng/mL of amphotericin B (Invitrogen) in sterile PBS for 1 h and washed three times in sterile PBS. HFF cells were seeded at a density of 5×10^4 cells/mL in complete DMEM onto the substrates. As controls, cells were also plated onto sterile flat silicon and 48 well plate (n = 3) at the same density. Loosely attached cells were removed by rinsing with PBS approximately 2 h after seeding. Some samples were stained for cell attachment analysis. Following rinsing, all HFF cells

were cultured in complete DMEM at 37 °C in a humidified atmosphere with 5% CO_2 for 48 h to determine the effect of the substrates on cell viability by means of a lactate dehydrogenase (LDH) assay.

1.8 Mammalian cell viability assay

The viability of HFF cells grown on SiNWs was determined by measuring the percentage of LDH released in culture supernatants using an established assay (Abcam LDH-Cytotoxicity Assay Kit II) according to the manufacturer's instructions. After 48 h of cell incubation on SiNWs in DMEM, 100 µl of the DMEM was collected and centrifuged at 600 x g for 10 min, and the supernatant was transferred into a 96-well plate. 100 µl of the manufacturer supplied LDH reaction mix was added. After 30 min of incubation at room temperature, the absorbance was measured at 450 nm. All experiments were carried out in triplicates.

1.9 Mammalian cell attachment

Following 2 h of HFF cell incubation on the surfaces, the cultured cells were gently rinsed with PBS to remove culture media and serum proteins. The cell samples were fixed in a solution containing 4% formaldehyde EM grade (Electron Microscopy Sciences) for 10 min and subsequently permeablized in PBS-0.25%

Triton X-100 (Sigma-Aldrich) for 5 min at room temperature, followed by washing with PBS 3 times for 5 min each time. In order to visualize the cells, their nuclei were stained with 2 μg/mL Hoechst 33342 (Life Technologies) for 10 min at room temperature. The actin filaments in the cytoplasm were also stained with 100 μM TRITC-labelled phalloidin (Sigma-Aldrich) for 60 min. Finally, the samples were washed in PBS and imaged using an inverted fluorescence microscope (Nikon Eclipse Ti-S, Japan) equipped with appropriate filters. Images were obtained using identical sensitivity parameters and analysis was repeated in triplicates.

Results:

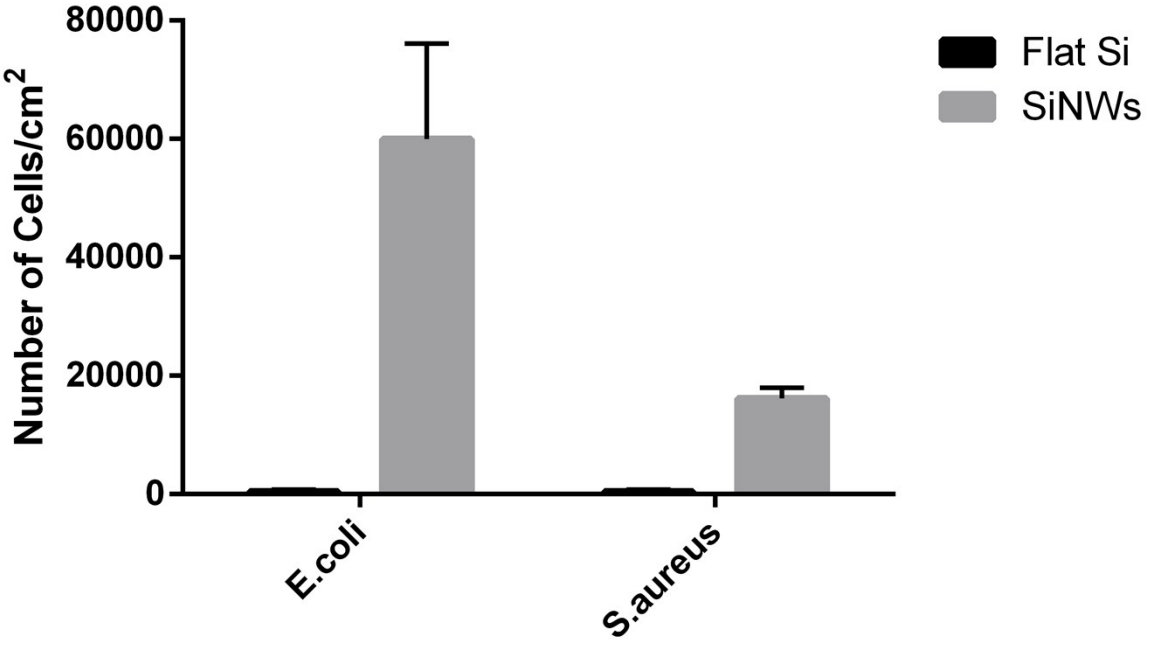

Figure S1. Number of bacterial cells of *E.coli* and *S.aureus*, counted after growth on flat Si surfaces and on SiNWs. The numbers were normalized per unit area as the number of cells/cm^2 of sample. The measurements were carried out in triplicates for each sample. The error bars represent standard deviation from cell counts performed in triplicates.

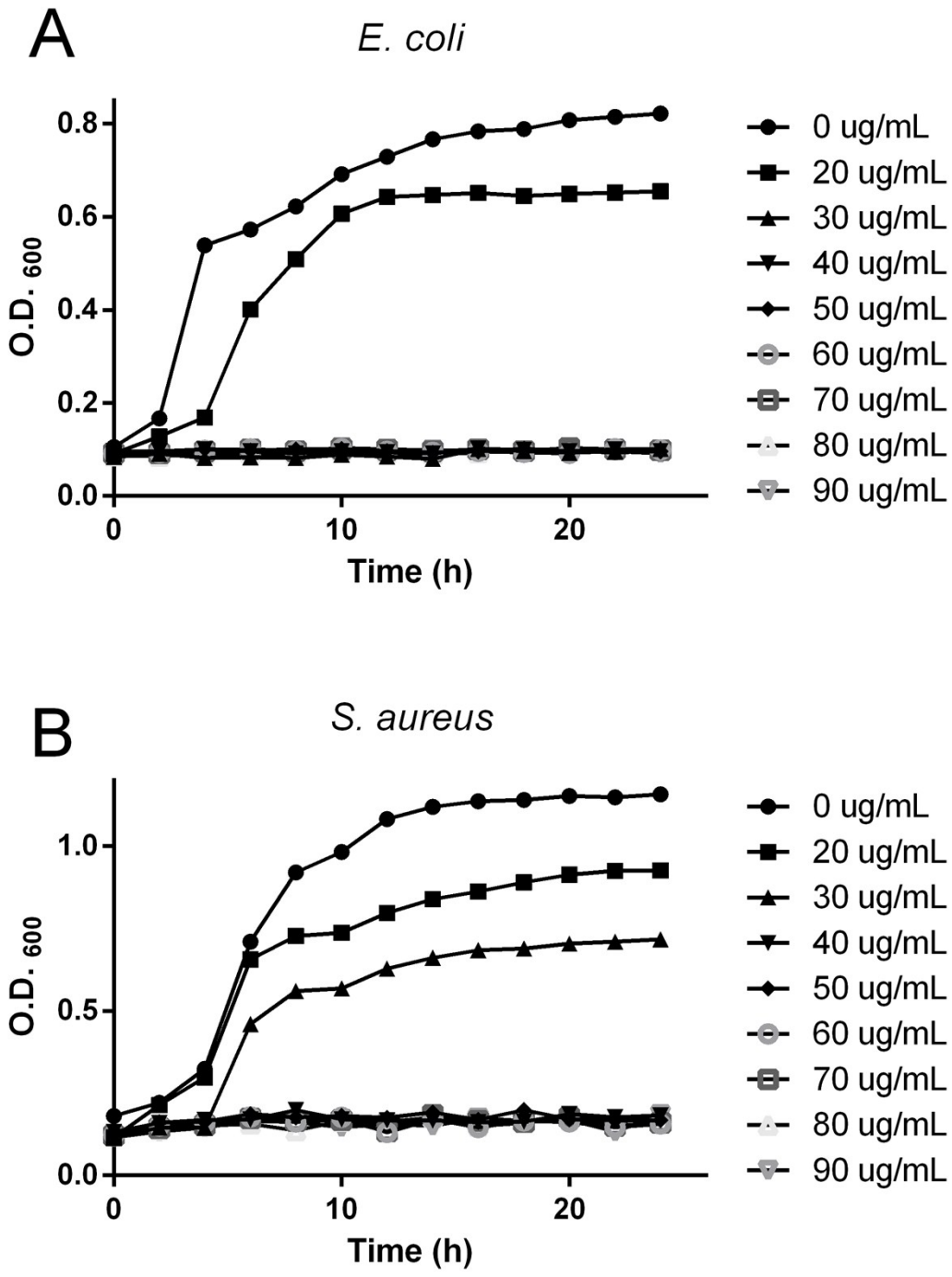

Figure S2. Optical density of bacterial solution measured at 600 nm as a function of time where measurements were taken every 2 h for up to 24 h and for different $AgNO_3$ concentrations. The optical density is a measure of solution turbidity and corresponds to the number of bacterial cells in solution, namely, *E. coli* (graph A) and *S. aureus* (graph B). Both strains were grown in TSB containing a series of $AgNO_3$ dilutions from 20 µg/mL to 90 µg/mL including a negative control using 0 µg/mL.