

Supplementary Information

Immobilized Silver Nanoparticles Enhance Contact Killing and Show Highest Efficacy: Elucidation of the Mechanism of Bactericidal Action of Silver

Shekhar Agnihotri^a, Soumyo Mukherji^{a, b, c}, and Suparna Mukherji^{a, d, *}

^a Centre for Research in NanoTechnology and Science, ^b WRCBB, Department of Biosciences and Bioengineering, ^c Centre of Excellence in Nanoelectronics, ^d Centre for Environmental Science and Engineering, Indian Institute of Technology-Bombay, Powai, Mumbai 400076, India

*corresponding author: mitras@iitb.ac.in, 91-22-2576-7854 (Ph), 91-22-2576-4650 (Fax)

S1. ICP-AES analysis

Inductively coupled plasma-atomic emission spectroscopy (ARCOS ICP-AES spectro, Germany) was performed to determine the total amount of silver nanoparticles immobilized on the surface. The AgNP-glass substrate was immersed in a 5% HNO₃ solution (3ml), which facilitates retrieval of all AgNPs from the glass surface and converts them into ionic form. After required dilution, the silver content was detected based on its secondary wavelength of 338.28 nm and final concentration was automatically determined based on the calibration curve generated using silver standards. Finally, the amount of silver immobilized on AgNP-glass (g/cm²) was calculated by normalizing the amount of silver extracted from the substrate, with the total surface area of AgNP-glass substrate. The samples were analyzed over an entire spectrophotometric range of 130-770 nm to cover UV and Visible range using an Argon plasma with a resolution of 8.5 pm. All analyses were performed in duplicate.

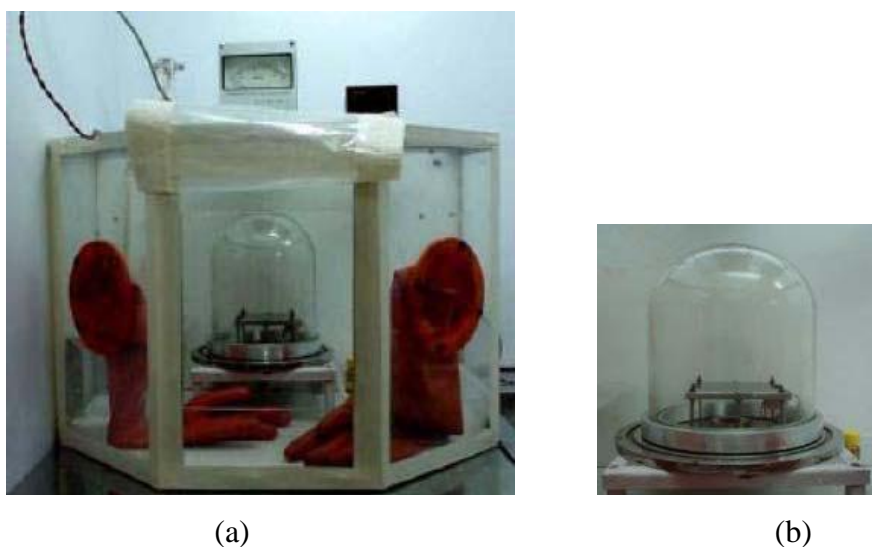


Figure S1. Photographs of the (a) silanization set-up (b) Substrate heater with Bell jar for vacuum drying

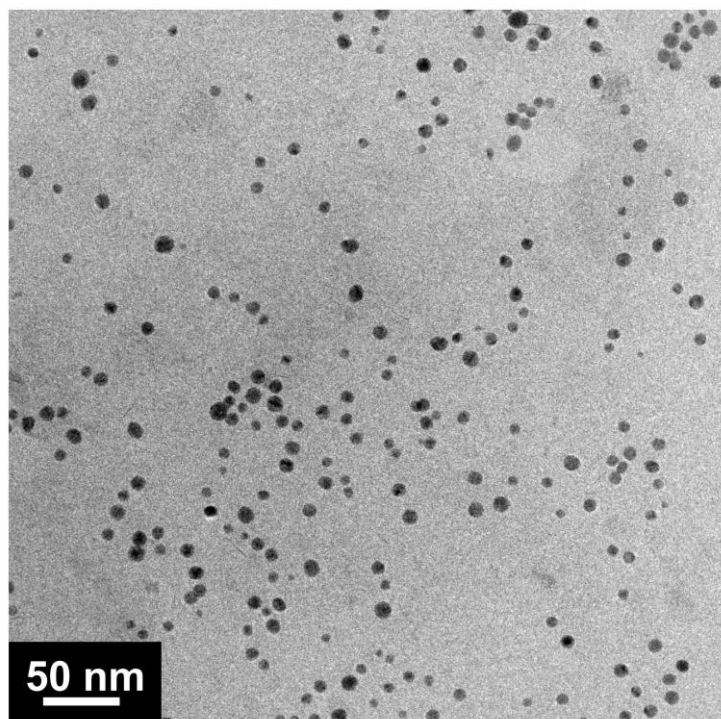


Figure S2. Representative FEG-TEM image to show particle distribution of as synthesized silver nanoparticles (total particle count : 476).

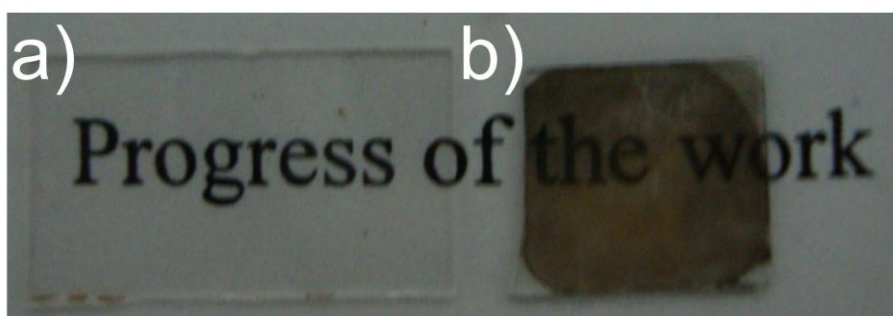


Figure S3. Photographic image of (a) pristine amine-silanized glass (b) AgNP immobilized glass

S4. Reactor fabrication with AgNP immobilized on glass substrate

The reactor was prepared using 100 ml beaker and the top part was sealed with a circular acrylic sheet in which a single large hole of about 3.5 mm diameter was drilled to serve as the sampling port. This port was covered with parafilm later during the experiments. The antibacterial substrate i.e., AgNP immobilized on glass (1x1 cm² dimension) was suspended into the beaker with the help of a wire having a clip. Position of the suspended substrate was adjusted in such a way that it was centrally placed within the reactor.

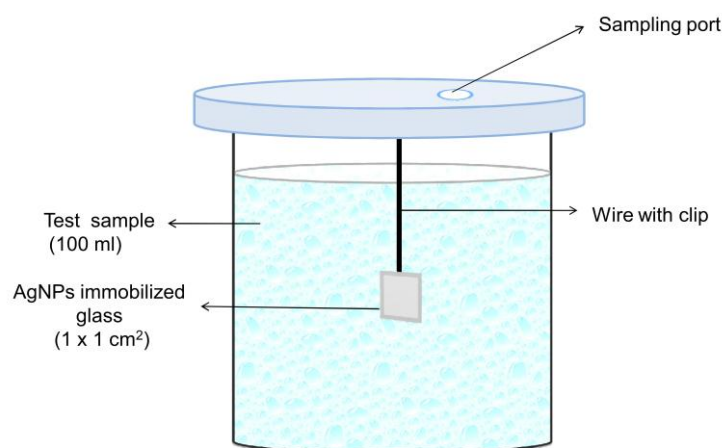


Figure S4. Representative image of the batch reactor (100 ml) containing AgNP immobilized glass substrate (1 x 1 cm²) used for disinfection and silver release studies.

S5 Surface coverage of Silver nanoparticle immobilized glass substrate: AFM

The surface coverage area of silver nanoparticles was demonstrated from the AFM image using the following equation:

$$Q (\%) = \frac{N \pi D^2}{4A} \times 100 \quad (\text{Equation})$$

where, Q is the percentage coverage, D is the diameter of the nanoparticles and, N is the number of nanoparticles detected per unit area A.

For the given AFM micrographs, the numbers of AgNPs immobilized on these two surfaces were counted manually (Image J, 1.42q, National Institute of Health, USA).

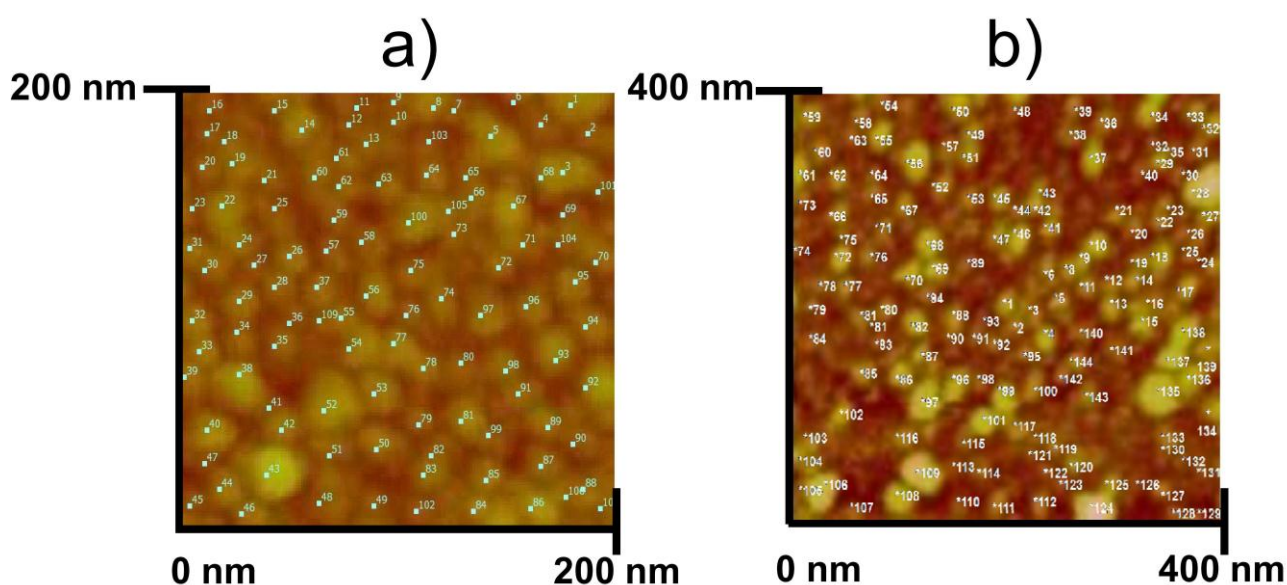


Figure S5: AFM micrograph with particle count for (a) freshly prepared silver nanoparticles immobilized glass substrate, (**no. of particles, 109**). **Scale bar: 200 nm** (b) 11 times used silver nanoparticle immobilized glass substrate, (**no. of particles, 144**). **Scale bar: 400 nm.**

S6. EDX analysis of the treated bacterial (*E. coli*) cells

Comparative EDX analysis of untreated, AgNP-glass substrate treated, and colloidal AgNPs treated bacterial cell at three different locations in bacteria i.e., outside cell membrane (p^*), just inside cell membrane (q^*) and interiors of the cell (r^*).

(1) Untreated E coli	Outside cell membrane (p^*)	Just inside cell membrane (q^*)	Interiors of the cell (r^*)
Element	Atomic%	Atomic%	Atomic%
C K	63.74	69.80	72.59
N K	7.56	5.05	1.03
O K	26.21	23.32	25.13
P K	1.39	0.87	0.00
S K	0.20	0.24	0.19
K K	0.90	0.72	1.06
Ag L	0.00	0.00	0.00
Total	100.0		

(2) AgNPs (immobilized) treated E. coli	Outside cell membrane (p^*)	Just inside cell membrane (q^*)	Interiors of the cell (r^*)
Element	Atomic%	Atomic%	Atomic%
C K	58.15	54.90	64.71
N K	10.02	16.34	10.20
O K	30.35	28.32	25.09
P K	1.41	0.44	0.00
S K	0.00	0.00	0.00
K K	0.90	0.72	1.06
Ag L	0.07	0.00	0.00

(3) AgNPs(colloidal) treated E. coli	Outside cell membrane (p^*)	Just inside cell membrane (q^*)	Interiors of the cell (r^*)
Element	Atomic%	Atomic%	Atomic%
C K	64.29	63.38	62.67
N K	6.77	5.05	10.04
O K	21.46	24.30	24.54
P K	0.00	0.00	0.00
S K	0.00	0.00	0.00
K K	0.90	0.72	1.06
Ag L	7.47	7.27	2.75
Total	100.0		