Arginine assisted immobilization of silver nanoparticles on ZnO nanorods: An enhanced and reusable antibacterial substrate without human cell cytotoxicity

Shekhar Agnihotri,a Geetika Bajaj,a Suparna Mukherji,a,b and Soumyo Mukherji a,c,d*

*E. mail: mukherji@iitb.ac.in; Tel: 091-22-2576-7767; Fax: 091-22-2572 3480

S1. Synthesis of ZnO nanorods

A typical sol-gel method ¹ was used for the formation of ZnO seeds. Ethanolic solution of zinc acetate dihydrate (0.25 mM, 50 ml) was refluxed in a condenser at 80°C for 2 hours with stirring and was subsequently cooled down to 0°C. The obtained zinc precursor (sol) was hydrolyzed using ethanolic solution of lithium hydroxide monohydrate (0.35 mM, 50 ml), added drop wise under vigorous stirring in an ice bath at 0°C for 30 min. This caused precipitation of the zinc precursor in the form of gel and the entire solution became turbid. After the reaction was complete, ZnO seeds were collected by centrifugation and washed thrice with ethanol in order to remove the excess ions. ZnO seeds (average size, 5 nm) were finally redispersed in deionized water for use in the subsequent growth process.

Prior to the growth process, the ZnO seeds were first drop coated on cleaned glass substrates and annealed at 55°C. These seeds acted as nucleation sites for nanorod formation. ZnO deposited glass substrates were then immersed in an aqueous solution containing 0.2 mM zinc nitrate [Zn(NO₃)₂.6H₂O] and 0.4 mM hexamethylenetetramine followed by heating at 90°C for one hour. The resulting composite contributes for the fabrication of randomly aligned ZnO nanorods. The samples were removed from solution, rinsed with deionized water, and dried at 50°C before storage.
S2. Instrumentation

The vibrational modes of various functional groups present on the samples were identified using Fourier transform infrared spectroscopy (FTIR, Vertex 80 FTIR System, Bruker, Germany). Spectra were obtained in transmission mode (4000–500 cm\(^{-1}\) range) at a resolution of 0.2 cm\(^{-1}\) at room temperature. The extinction spectra of various ZnO samples were recorded over 200-800 nm range using a UV-Vis spectrophotometer (Perkin-Elmer Lambda 35, USA). Raman studies were done using Ramnor HG-2S Spectrometer (Horiba Jobin Yvon, France) coupled with a 4W Ar laser. The samples deposited on glass substrates were tested in triplicate using data acquisition time of 120 s at 20X magnification. X-ray diffraction (XRD) patterns were recorded on a diffractometer (PANalytical X’pert PRO, The Netherlands) with a slow scan speed (0.017° per 25.18 s) over a 2θ range of 20–80° using CuK\(\alpha\) radiation. To determine the size and morphology of silver nanoparticles over ZnO nanorods using transmission electron microscopy (FEG-TEM, JEOL JEM 2100F, Japan), each specimen was prepared as follows: AgNP/ZnO nanorods were physically scratched from the glass substrate, resuspended in ethanol and instantly dropped (6 µl) over a carbon-coated Cu grid. The sample loaded grid was then allowed to dry at room temperature for 15 minutes and was immediately analyzed. The lattice fringes of silver and zinc with their corresponding d-spacings were determined in the high resolution (HRTEM) mode. The surface morphology and topography of ZnO nanorods after immobilizing AgNPs were also observed using scanning electron microscopy (FEG-SEM, JEOL JSM-7600F, Japan). For this, substrates mounted on sample stub were coated with a platinum sputter (10 mA, 15 s) and then analyzed. X-ray photoelectron spectroscopy (XPS, Thermo VG Scientific MultiLab, USA) was performed to analyze the chemical state of the AgNP loaded ZnO surface. High resolution spectra of individual elements were obtained using a microfocused monochromatic X-ray source (Al K\(\alpha\) = 1486.6 eV) operated at 250 W with a step size of 0.1 eV. Deconvolution, background subtraction and multiple peak fitting was done using XPS peak 4.1 software. The mass loading of silver and zinc on substrates (2 x 2.3 cm\(^2\)) were quantitatively estimated after acid digestion using nitric acid (0.1 M) and analyzed by inductively coupled plasma-atomic emission spectroscopy (ARCOS ICP-AES spectro, Germany). Acid digestion was used for conversion of AgNPs on the Ag/ZnO hybrid nanorods to their ionic form (Ag\(^+\)). The silver content was determined using ICP-AES after calibrating the instrument using silver standards. Silver was measured at its secondary wavelength of 338.28 nm. Specific surface area
of Ag/ZnO-20 deposited on a glass substrate was determined based on nitrogen sorption measurements conducted using a BET (Brunauer-Emmett-Teller) surface area analyzer (ASAP2020, Micromeritics, USA). After scapping off from the glass substrate, Ag/ZnO-20 sample was stored under vacuum before conducting the analysis. Subsequently, the sample was degassed at 150°C for 6h while maintaining relative pressure in the range 0.05 to 0.3.

**S3. FTIR and Raman spectroscopy: Detailed analysis**

The FTIR spectrum of ZnO nanorods loaded with different amount of silver was compared to its pristine form (Fig. S1a). The comparative absorption spectrum demonstrated that incorporation of AgNPs did not affect the structural morphology and integrity of ZnO surface. Rather, a few characteristics peaks appeared at 1300-1700 cm\(^{-1}\) region, which indicates successful immobilization of AgNP using arginine molecule on the ZnO template. Peaks at 1356, 1633, and 1687 cm\(^{-1}\) confirm the existence of stable silver-arginine complexes. The absorption bands in 500-1000 cm\(^{-1}\) region are attributed to ZnO. For pure ZnO, a peak near 3398 cm\(^{-1}\) indicated the presence of a sufficient amount of hydroxyl groups at the surface of the ‘as-prepared’ ZnO under ambient conditions. In case of Ag/ZnO HNRs, sharp peaks near 1556 cm\(^{-1}\) can be ascribed to N-H stretching due to arginine assisted immobilization of AgNPs on ZnO NRs. The role of arginine as a linker was also evidenced by the shift in the absorption spectrum of C=O and N-H indicating formation of stable bonds between Ag with oxygen and nitrogen atoms present on the arginine molecule. Peaks near 3000-3400 cm\(^{-1}\) can also be assigned to the combination of O-H and N-H vibrations of arginine coated ZnO NRs in the IR spectra, which cannot be discriminated due to several associations of silver and zinc with different functional moieties of arginine. A sharp peak at 2929 cm\(^{-1}\), ascribed to the symmetric stretching of NH\(_2\) group, was absent in case of pure ZnO sample. It indicates the presence of arginine over ZnO surface treated with arginine-silver solution. Analyzing the various FTIR spectra, increase in absorption intensity of characteristic peaks (1356, 1556, 1633, 1687, and 2929 cm\(^{-1}\)) indicated that arginine could possibly enhance the stability of silver nanoparticles in case of Ag/ZnO-20 HNRs and contributed for the best immobilization strategy. For higher concentration of silver loading on ZnO (i.e., Ag/ZnO-25 and Ag/ZnO-75 (not shown), corresponding peaks were not observed. It was observed that increasing concentration of silver in arginine-silver mixture leads
to more intense/sharp Raman signals for hybrid Ag-ZnO substrates (Fig. S1b). Results obtained through Raman studies were in good agreement with FTIR analyses where increase in intensity of peaks corresponding to N-H vibrations (1358 and 1560 cm\(^{-1}\)), C-N vibrations (1533 cm\(^{-1}\)) was observed with increase in arginine-silver concentration. The highest Raman intensity for various peaks was obtained in case of Ag/ZnO-20 HNRs, where even a small increase in the amount of

**Fig. S1.** FTIR (a) and Raman (b) spectroscopy of various Ag/ZnO HNR samples
silver (i.e., 25 mM in Ag/ZnO-25 HNRs) caused complete absence of several peaks. This enhancement in Raman intensity can be extrapolated for qualitative determination of the extent of AgNP immobilization on ZnO substrate, which is primarily governed by arginine. Based on a few recent studies 7, 8, it can be inferred that a stable arginine-silver interaction is favorable for immobilizing AgNPs on ZnO template. Therefore, arginine-silver mixture having 20 mM AgNO₃ concentration is expected to contribute for the most efficient immobilization of AgNPs on ZnO nanorods.

Fig. S2 Antibacterial effect of Ag/ZnO HNRs substrate (2 x 2.3 cm²) when placed in contact with agar plate with bacterial lawn (E. coli, 10⁵-10⁶ CFU ml⁻¹) for 1 hour and then removed, followed by incubating for 24 hours at 37°C. The inhibition zone (ZoI) is shown in the centre of the plate.
Fig. S3 (a) FEG-TEM micrograph of untreated (bare) *E. coli* cells. (b) EDX analysis of *E. coli* cells treated with Ag/ZnO HNRs showing the presence of silver (Ag).

**Table S1:** EDX analysis of Ag/ZnO hybrid nanorods

| Element | Weight (%) | Atomic (%) |
|---------|------------|------------|
| C       | 4.23       | 9.26       |
| O       | 17.11      | 47.58      |
| Zn      | 49.05      | 31.52      |
| Ag      | 29.61      | 11.64      |
| Total   | 100.00     |            |
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