

Supporting Information

NIR Light Active ZnO Based Nanohybrids for Bacterial Biofilm Treatment

Damayanti Bagchi¹, V.S. Sharan Rathnam², Peter Lemmens³, Indranil Banerjee² and Samir Kumar Pal*¹

¹*Department of Chemical, Biological and Macromolecular Sciences, S. N. Bose National Centre for Basic Sciences, Block JD, Sector III, Salt Lake, Kolkata 700 106, India*

²*Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, Odisha 769008, India*

³*Institute for Condensed Matter Physics, and Laboratory for Emerging Nanometrology, TU Braunschweig, Mendelsohnstrasse 3, 38106 Braunschweig, Germany*

*Corresponding Author

E-mail: skpal@bose.res.in

Telephone: +91 033 2335 5706-08

Fax: +91 033 2335 3477

Determining concentration of the nanohybrids:

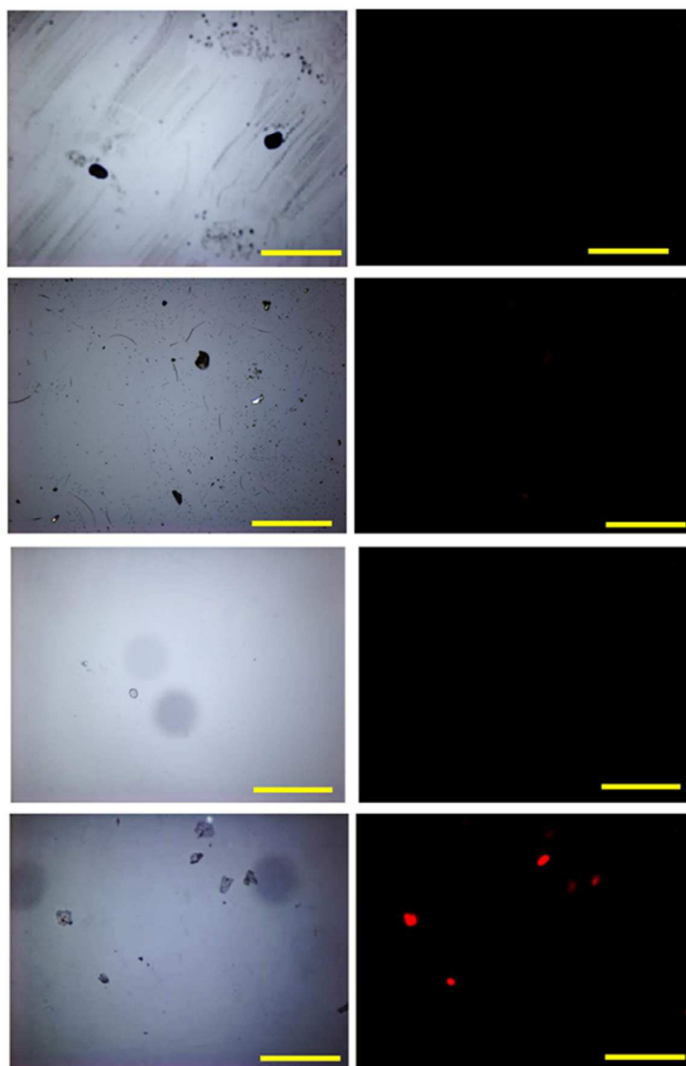
To determine the concentration of the individual counterparts, we have used absorption spectra of the nanohybrids. From the absorption peak value at 650 nm, we have calculated the SQ concentration ($\epsilon_{SQ} = 3.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$). Similarly, using subtraction, we have calculated the ZnO concentration using absorption peak value at 370 nm. The concentration of nanohybrids refers to the corresponding SQ concentration e.g. 140 nM of nanohybrids correspond to the presence of 140 nM SQ in the nanohybrids.

Details of bacteriological assay:

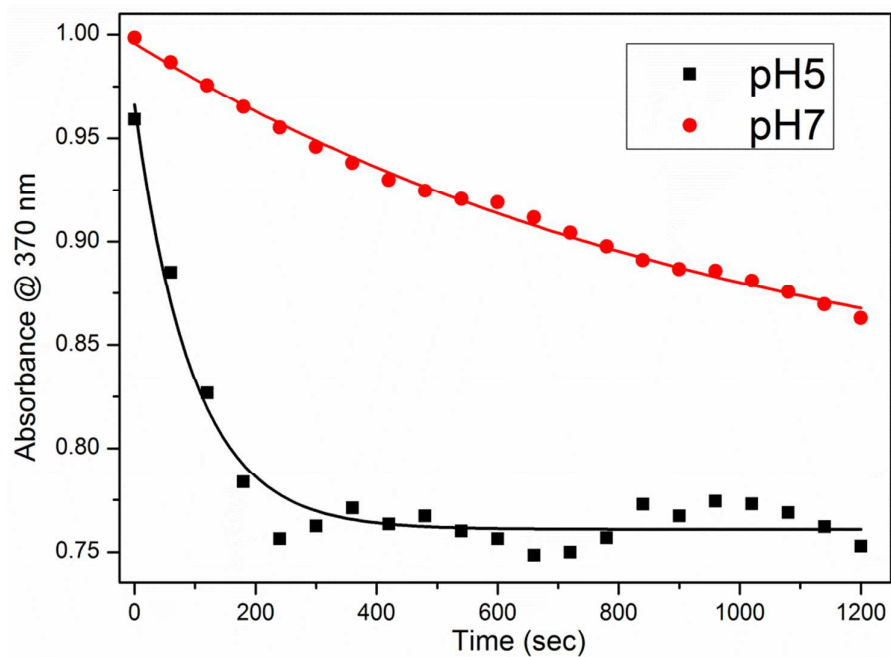
The bacteriological assays were performed using the strain *Staphylococcus aureus* (ATCC 25923). The *S.aureus* cells were cultured at 37 °C in liquid Luria–Bertani (LB) medium. When the optical density reached ~0.6, the culture was serially diluted 1000 times with LB medium and treated with variable concentration of drugs. The cells were incubated with 140 nM of nanohybrids (concentration is calculated wrt SQ loading,) followed by red light illumination ($\lambda_{\text{max}} = 640 \text{ nm}$) for 30 min. The photodynamic action was measured by using LB agar based colony formation assay following overnight incubation at 37°C.

Morphological analysis of bacterial biofilms using SEM:

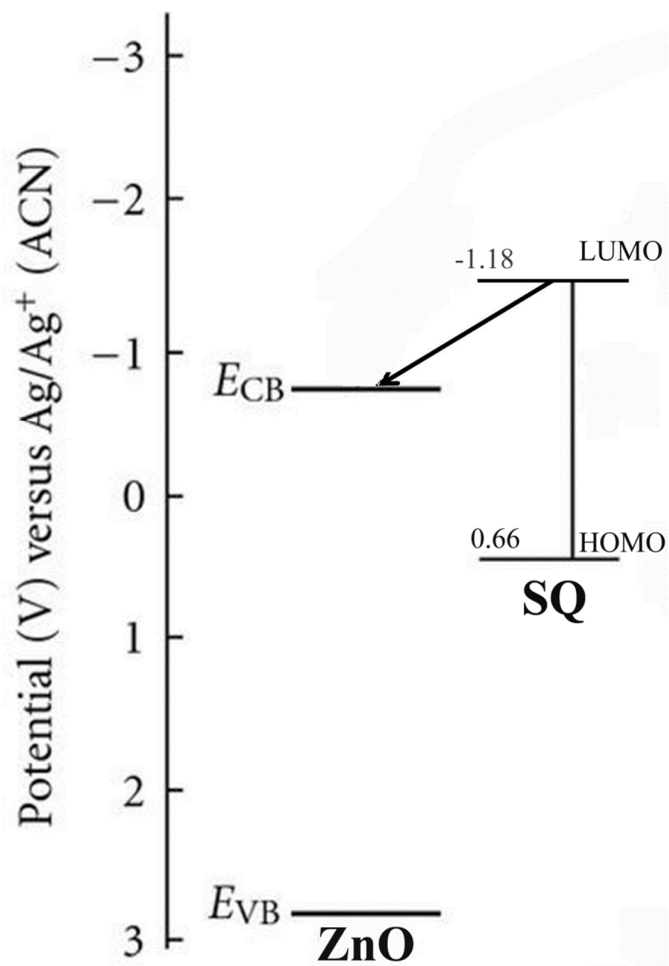
The bacterial biofilms have also been cultured over implant mimic titanium foils. The morphological changes of the biofilms were determined using SEM analysis upon different treatment conditions. 100 μL of the respective bacterial broth was kept over coverslips for 48 h at 37 °C, followed by washing in water. The samples were fixed with 2.5% glutaraldehyde followed by successive dehydration in alcohol and air. A qualitative assessment of the appearance of the biofilms was performed by scanning electron microscopy. The coverslips were coated with gold and scanned in a field emission scanning electron microscope (Quanta FEG 250: source of electrons, FEG source; operational accelerating voltage, 200 V to 30 kV; resolution, 30 kV under low vacuum conditions: 3.0 nm; detectors, large field secondary electron detector for low vacuum operation).



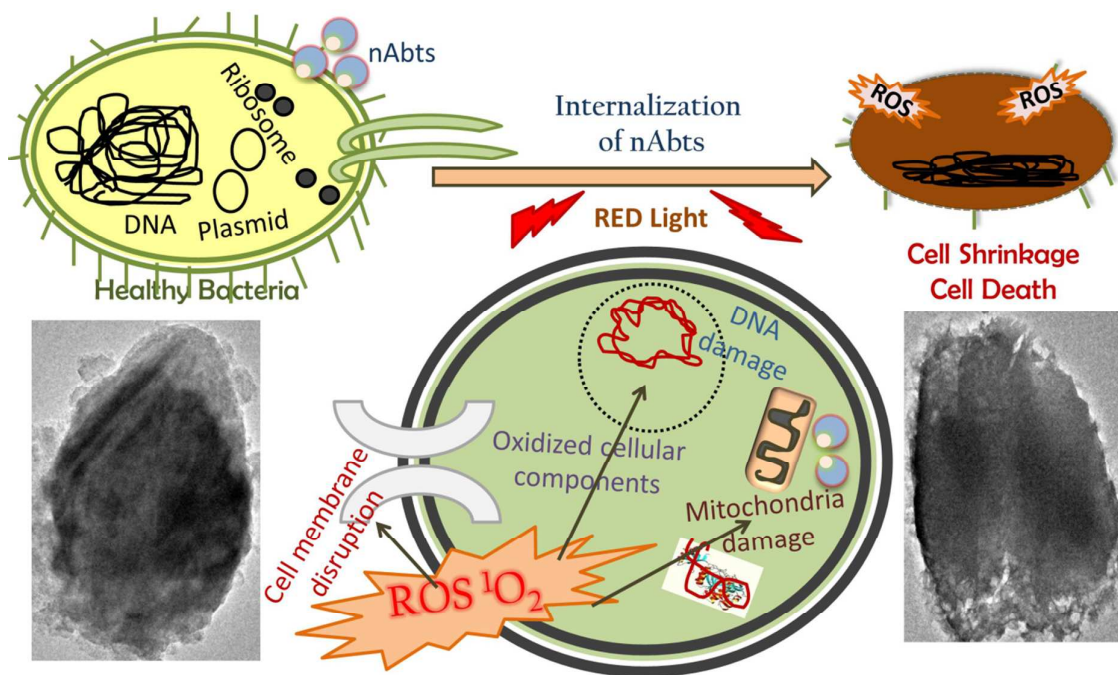
FigureS1: Propidium iodide staining assays of *S.aureus* under different treatment conditions. Microscopic images of (left panel-bright field and right panel-fluorescence) of control under (1st row) dark, (2nd row) red light illumination, ZnO-SQ treated under (3rd row) dark, (4th row) red light illumination. Scale bar is 100 μ m.



FigureS2: pH dependent dissolution kinetics of ZnO-SQ nanohybrids. The experimental values are represented by symbols. The solid lines correspond to single exponential fitting curves.



Scheme S1: Schematic energy level diagram and charge separation path at the ZnO-SQ interface.



SchemeS2: Pictorial representation of disruption in different cellular mechanism by nanohybrid internalization followed by photoinduced ROS generation.

TableS1: Fitting parameters of dissolution kinetics of ZnO-SQ nanohybrids at different pH

pH value	Rate constant (sec)
pH5	96
pH7	1031

Experimental curves are fitted with single exponential decay