Supporting information for

Antibacterial Properties and Mechanisms of Toxicity of Sonochemically Grown ZnO Nanorods

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Method for the XTT reduction assay

In order to investigate the ROS production due to the production of superoxide anion ($O_2^{\bullet -}$), the XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay (Biotium) was used. The XTT reduction by superoxide anions results in the formation of XTT-formazan. The formazan produced has an absorption peak at 470 nm that can be used to quantify the relative amount of superoxide produced. For the XTT assay, manufacturer's instructions were followed. The assay consisted in mixing in the dark 5 mL of the XTT solution (1 mg mL^{-1}) with 25 µL of the activation reagent (5 mM phenazine methosulfate, PMS) to generate an activated XTT solution. An aliquot of 500 µL of the activated XTT solution was added to 1000 µL of ZnO nanorod solution. The final solution was mixed in a beaker at 400 rpm in the dark with a magnetic stirrer for 2 h. Aliquots of 250 µL were taken at 0, 1 and 2 h and the absorbance was read at 470 nm using a microplate reader. The changes in absorbance at 470 nm were monitored. In the assay, TiO_2 solution exposed to UV light was used as a positive control and the negative control was the XTT solution without ZnO. The results were shown below in **Fig. S1**.

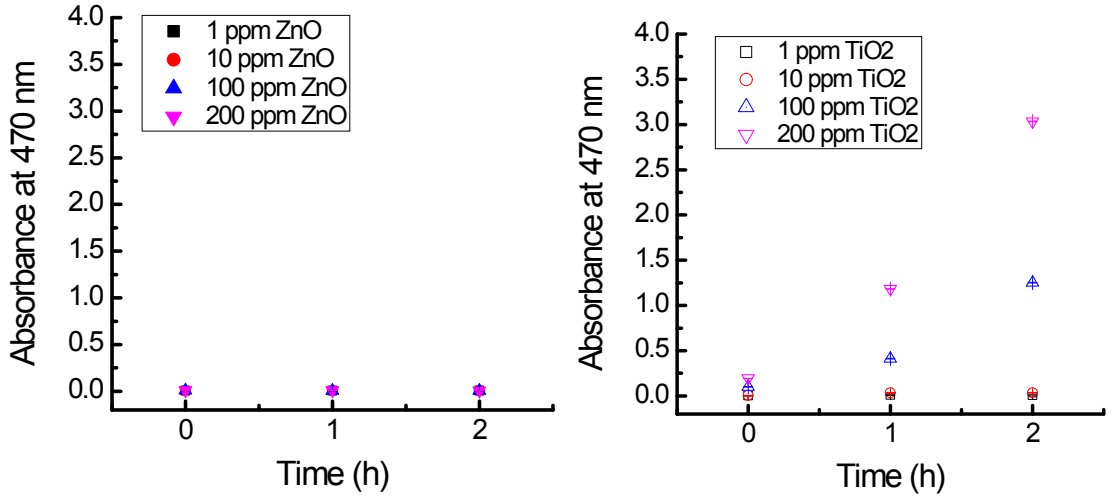

Fig. S1. The results of XTT reduction assay. In this experiment, TiO_2 was used as a positive control to determine the superoxide production. Even at higher concentrations, no superoxide production was seen with ZnO nanorods.

Determination of the Zn^{2+} ions

In order to investigate the toxicity mechanism of ZnO nanorods, zinc ions released from the surfaces of glass slides coated with ZnO nanorods and its possible effects to cells were determined.

To determine the released zinc ions from coated glass slides, glass slides were incubated as previously described in the "*Short term exposure*" section. At the end of the 5 h incubation, the solution was filtered through 0.2 μm syringe filters (VWR), and analyzed with an atomic absorption spectrometer (AAS) (Perkin Elmer). A standard curve was prepared prior to experiments using 1-15 ppm of $ZnCl_2$ solutions. The experimental results were tabulated in **Table-S1** below. In this table, all results were in ppm/cm^2. Four samples were the ZnO nanorods coated glass slides used to see the reproducibility of the experiment. Replicates 1, 2, and 3 are the reads in the AAS. Mean and standard deviation values were also given for each sample. Based on the experimental results, the overall mean of the released zinc ion concentration was calculated as 1.56±0.22 ppm/cm^2.

Table-S1. Zinc ion concentrations released from glass slides. In this table, all results were represented as ppm/cm^2.

Samples	Replicate 1	Replicate 2	Replicate 3	Mean	Standard deviation
Sample 1	1.218	1.218	1.258	1.231	0.023
Sample 2	1.718	1.768	1.768	1.751	0.029
Sample 3	1.728	1.748	1.738	1.738	0.010
Sample 4	1.518	1.508	1.498	1.508	0.010

To determine the released zinc ions from the nanorods, another set of experiments were done using the nanorods suspension described in "**Preparation and characterization of ZnO nanorods**" section, as used in XTT reduction assay above. ZnO nanorods suspensions at different concentrations were prepared, and sonicated for 15 min using a tip sonicator. Afterwards, the zinc ions were determined in these suspensions by AAS. The results were given below in **Fig. S2**.

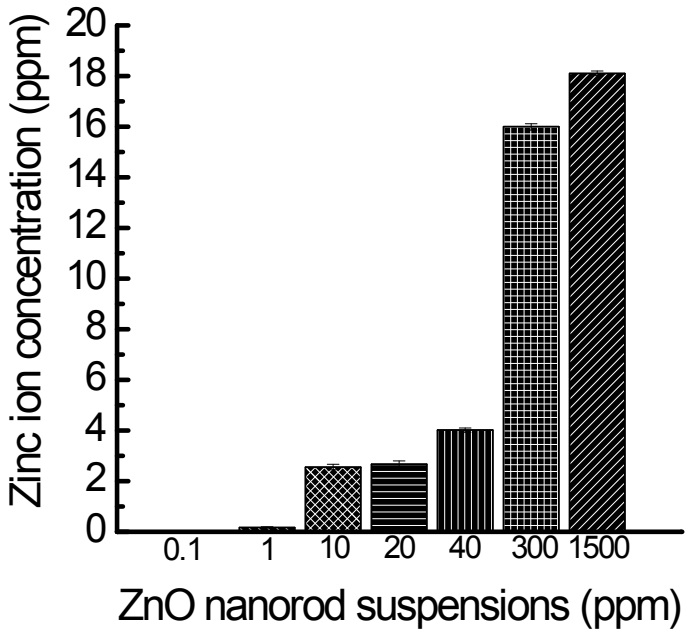

Fig. S2. Zinc ion concentrations in different ZnO nanorods suspensions.

Plate count method to test the toxicity of zinc ions

To investigate and confirm the toxicity mechanism of ZnO nanorods, a ZnO suspension of 10 ppm ZnO was prepared as described above. This concentration was selected since 10 ppm of ZnO leads to 2.5 ppm zinc ions and the zinc ion concentration released from the glass slides coated with ZnO nanorods was found to be 1.5 ppm. The cells were incubated in those suspensions for 2 h. Afterwards, the suspensions were used to count the cells by the plate count method as described in the manuscript. The control groups did not contain any ZnO nanorods. We also performed a minimum inhibitory concentration (MIC) for the ZnO (Fig. S3). We observed that after 100 ppm of ZnO the cells are almost 100% inactivated.

Table-S2. Plate count results.

E. coli + ZnO nanorods			*B. subtilis* + ZnO nanorods		
Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
4.6×10^6	4×10^6	7×10^6	8.6×10^6	3.4×10^6	3.5×10^6
E. coli control			*B. subtilis* control		
Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
6×10^6	7×10^6	9×10^6	5.7×10^6	2.8×10^6	3.2×10^6

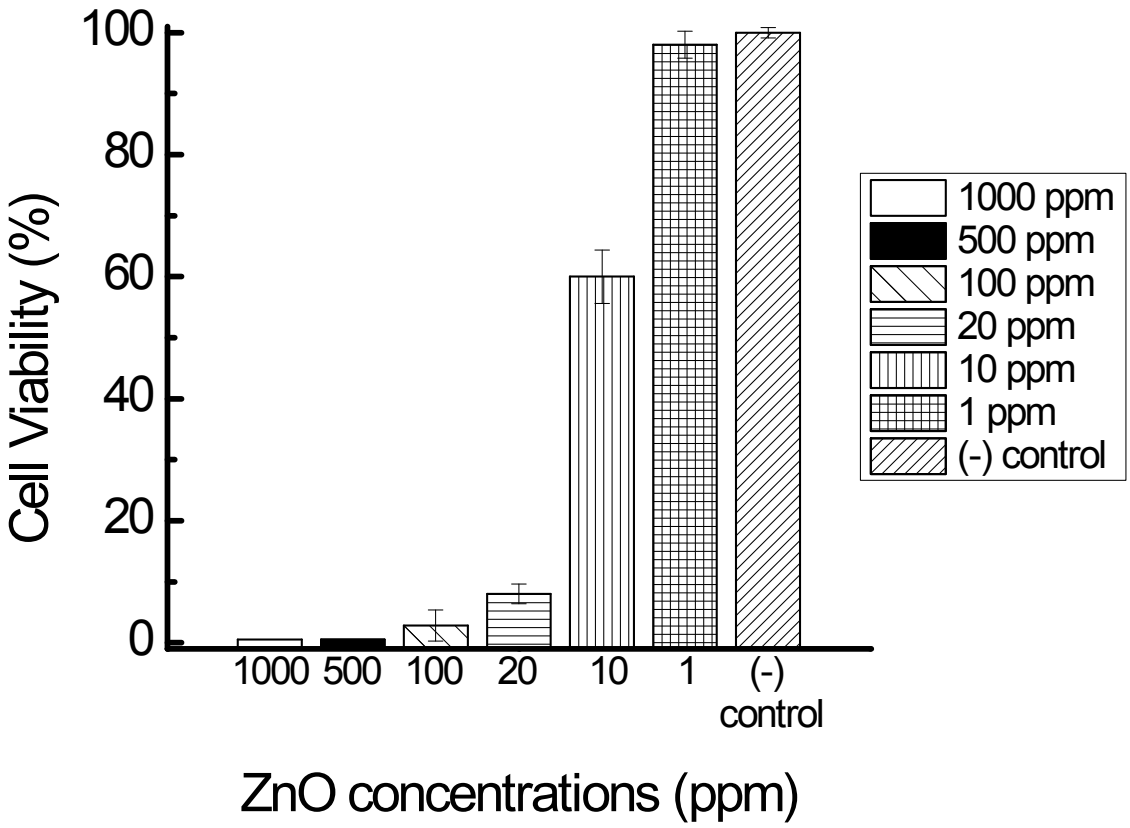

Fig. S3. Cell viability changes versus ZnO nanorods concentrations using E. coli cells.