Supplementary materials for

Dual UV irradiation-based metal oxide nanoparticles for enhanced antimicrobial activity in *Escherichia coli* and M13 bacteriophage

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Running head: Dual UV irradiation-based metal oxide nanoparticles

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Measurement of dual UV spectra

UV spectra of lamp in CBD were measured using a UV spectrometer (JAZ system) with SpectraSuite software prior to UV treatment. Cylindrical UV lamp (ECOSET Co., Ltd) had two areas, so called coated and uncoated areas, divided by coating materials.

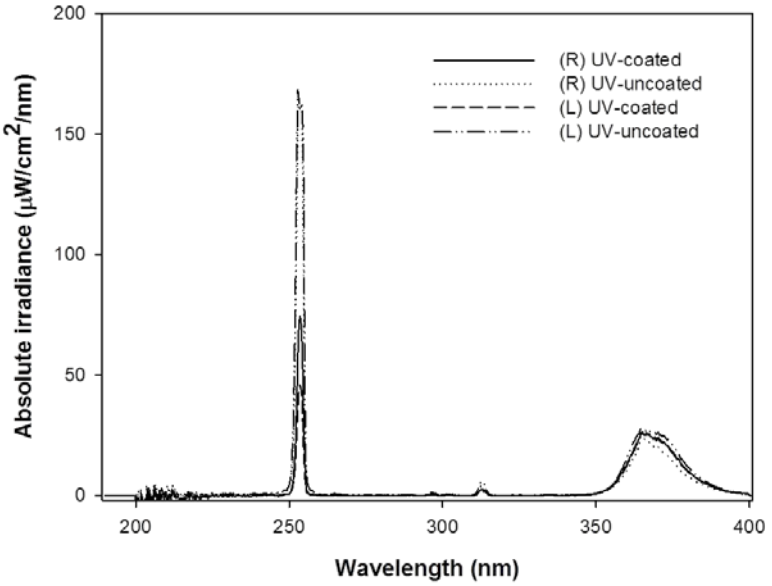

Figure S1 Dual UV spectra of UV lamp. UV-coated and uncoated areas had different UV intensities of UV-A and UV-C. In the spectrum, UV-C intensity from coated area was lower than that from uncoated area while UV-A intensity from coated area was similar to that from uncoated area.

Particle size measurement

Particle sizes of MO nanoparticles were measured using a light scattering (ELS-Z, OTSUKA Electronics, Japan) with a standard cell. MO nanoparticles were dispersed in 30% ethanol-distilled water. For the measurements, nanoparticles were diluted with absolute ethanol prior to analysis in order to obtain the appropriate intensity. The mean diameters (nm) of nanoparticles were determined at room temperature.

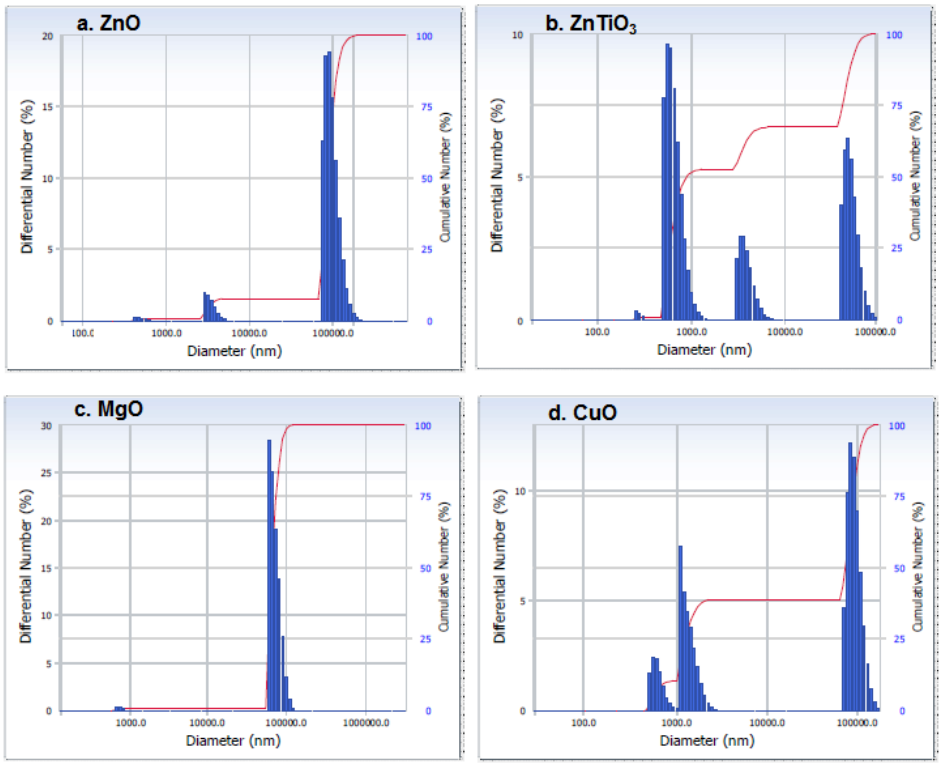

Figure S2 Size distributions of MO nanoparticles: (a) ZnO, (b) $ZnTiO_3$, (c) MgO, and (d) CuO. They were monitored in a number-weighted mode. MO nanoparticles were poorly dispersible in 30% ethanol-distilled water showing flocculates or aggregates of nanoparticles. Their particles were grown up to 100 μm-scale.

Scanning Kelvin probe microscopy (SKPM)

SKPM, enhanced EFM mode, in AFM (XE-100) was used to confirm electrostatic properties in nanoparticles of ZnO and $ZnTiO_3$. Samples were prepared as mentioned in Method section of EFM. SKPM was operated with SPM controller in a non-contact mode. San size was a 1 μm x 1 μm. In lock-in amplifier, measurement conditions of phase, frequency, and amplitude were 50°, 17 Hz, and 2 V, respectively.

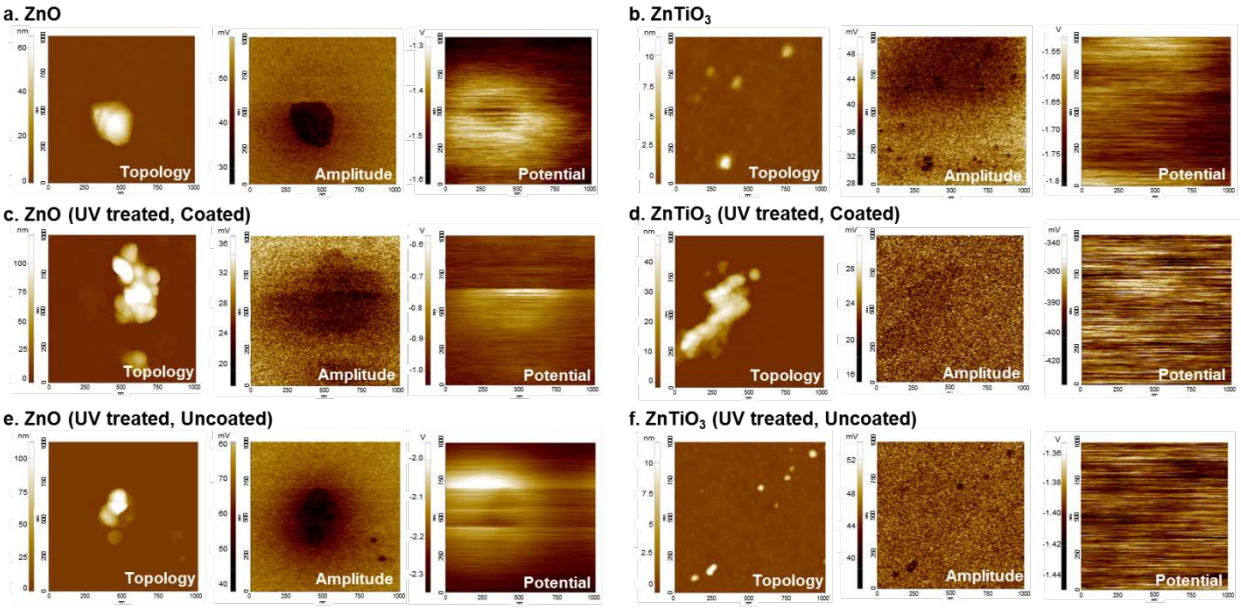

Figure S3 SKPM images of ZnO and $ZnTiO_3$ nanoparticles after dual UV irradiation. The electrostatic properties of dual UV-irradiated ZnO and $ZnTiO_3$ nanoparticles were monitored for topology, amplitude (mV), and surface potential (V) using SKPM and compared to those of ZnO and $ZnTiO_3$ nanoparticles. ZnO nanoparticle aggregates showed 28.2 ~ 48.8 mV and -1.5 ~ -1.4 V. In addition, $ZnTiO_3$ nanoparticles showed 31.8 ~ 43.8 mV and -1.7 ~ -1.6 V. However, their amplitude and potential values were changed after dual UV irradiation of a coated area (ZnO, 22.2 ~ 32.3 mV and -0.99 ~ -0.93 V; $ZnTiO_3$, 19.2 ~ 27.5 mV and -414.4 ~ -369.3 mV). Using an uncoated area for dual UV irradiation, ZnO nanoparticles showed 46.1 ~ 73.5 mV and -2.2 ~ -2.1 V, and $ZnTiO_3$ nanoparticles showed 39.7 ~ 48.2 mV and -1.40 ~ - 1.37 V.

Piezoelectric force microscopy (PFM)

PFM in AFM (XE-100) was determined to check the electromechanical changes in MO nanoparticles of ZnO after dual UV irradiation. Samples were prepared as mentioned in EFM of Method. PFM results were obtained using SPM controller operated in a non-contact mode. San size was a 1 μm x 1 μm. In lock-in amplifier, the measurement condition of amplitude was at a range of 0 to 5 V with a time interval of 1,000 ms.

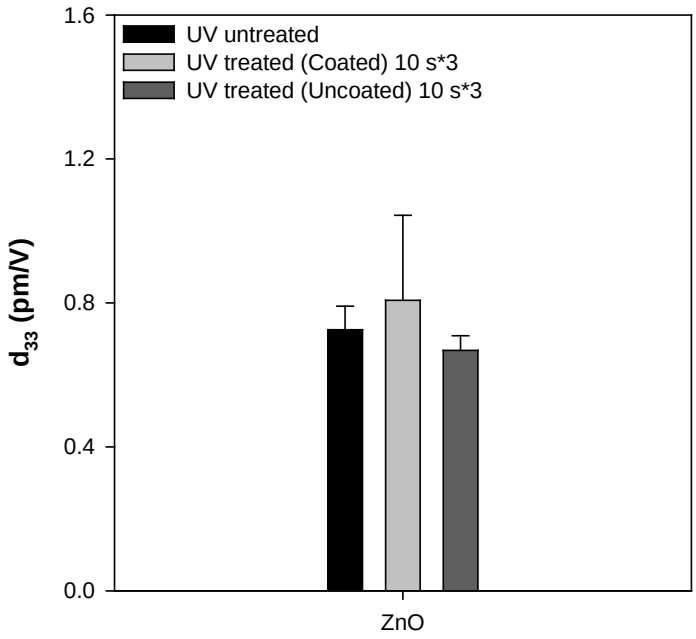

Figure S4 Piezoelectric constant (d33) levels of ZnO nanoparticles after dual UV irradiation. The d33 value (pm/V) represents a piezoelectricity, which means the linear interaction between mechanical and electrical properties. ZnO nanoparticles in water were maintained electromechenically after UV treatment of 10 s in 3 cycles for 30 min. The results are expressed as the means ± SD (n=3).

Inductively coupled plasma-mass spectroscopy (ICP-MS)

The concentrations of metal ions in water released from nanoparticles were monitored using an ICP-MS (NexION 300D, PerkinElmer, USA) with standard (Mg) and DRC modes (Zn, Ti, and Cu). MO nanoparticles were dispersed in water at the concentration of 0.02% (4 mg MO nanoparticles in 20 mL water, w/v). Dispersed samples were incubated at room temperature in the dark condition. Samples were collected at 1 and 7 days after incubation and directly injected into the equipment without further treatment at the flow rate of 0.5 mL/min. Operation conditions were 1,500 W, 18 L/min, 1.25 L/min, and 1.02 L/min of RF power, plasma gas flow, auxiliary gas flow, and nebulizer gas flow, respectively.

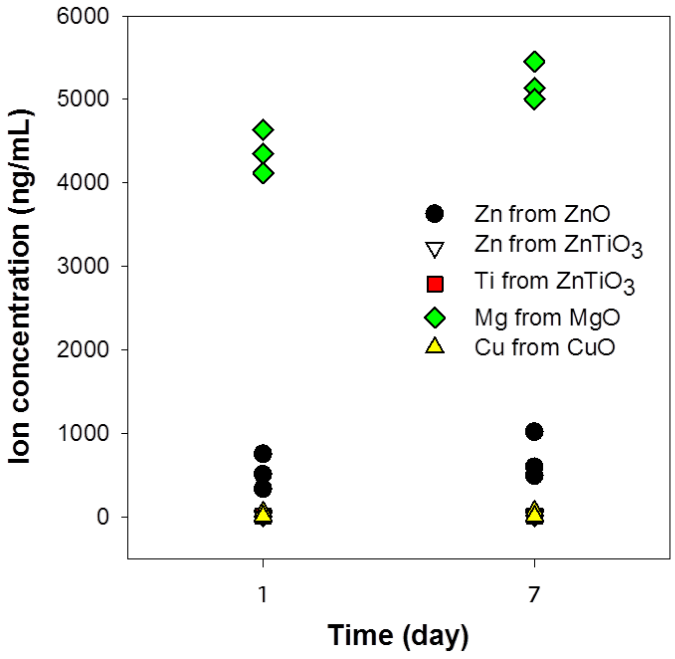

Figure S5 Cumulative release of metal ions from MO nanoparticles in water. Ion concentrations of Zn, Ti, Mg, and Cu were analyzed (n=3).

Antimicrobial activity of CuO nanoparticles

CuO nanoparticles were dispersed in water at 0.1 to 1.0 mg/mL. In the dark condition, *E. coli* (10^4 colony forming unit, CFU) was added to nanoparticles and was incubated for 30 min. Then, one-milliliter was collected and added to LB/agar medium. The resultants were poured into plates and incubated in the dark at 37°C overnight. On the other hand, phages (10^4 plaque forming unit, PFU) were mixed with CuO nanoparticles (0.1 to 1.0 mg/mL in water), and incubated for 30 min. Then, one-hundred microliters of samples were collected and incubated with overnight cultured bacteria at room temperature for 60 min. After the top agars were mixed with those and poured onto LB/IPTG/Xgal plates, the plates were incubated at 37°C overnight for phage growth. For UV irradiation, the same protocol was used as mentioned in the Experimental section. Briefly, UV was applied to CuO nanoparticles for 10 s once, 30 s once or for 10 s in three cycles (total 30 s) in *E. coli* or phages for 30 min. After UV irradiation, one-milliliter of samples for *E. coli* was collected, added to LB/agar medium, and poured into plates. Then, plates were incubated in the dark at 37°C overnight. For pages, one-hundred microliters of samples after UV irradiation were collected and incubated with overnight-cultured bacteria at room temperature for 60 min. Then, samples were mixed with the top agars and poured onto LB/IPTG/Xgal plates. The plates were incubated at 37°C overnight. Colonies and phage plaques were counted using Image J (NIH) after taking images.

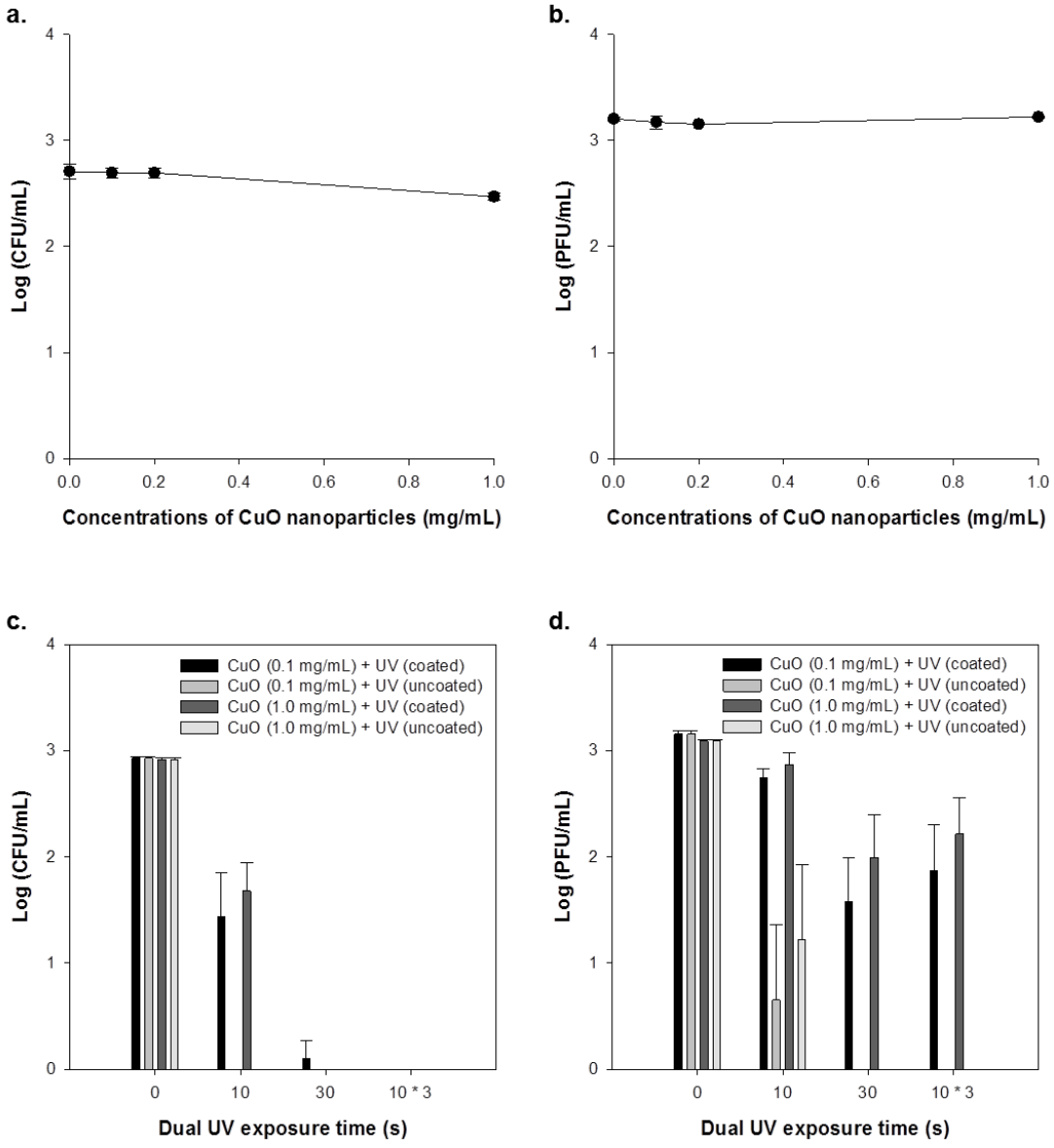

Figure S6 Antimicrobial effects of CuO nanoparticles: (ac) antibacterial effects on *E. coli*, (bd) inactivation effects on phages with and without dual UV irradiation.