

Mechanistic Exploration on Visible Light-Activated Carbon/TiO₂ Hybrid Dots Damaging Bacterial Cells

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Supplementary Materials

1. Materials

The colloidal TiO₂ sample (Degussa P25) was purchased from Aldrich. Ethanol (>99%) was obtained from VWR, oligomeric polyethylene glycol (PEG, average molecular weight ~1,500) from Fluka, and oligomeric polyethylenimine (PEI, average molecular weight ~600) from Polysciences. Silicon carbide powders (120 Grit) were supplied by Panadyne Abrasives, and dialysis membrane tubing (molecular weight cut-off ~25,000) by Spectrum Laboratories. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

2. Measurement

UV/vis absorption spectra were recorded on a Shimadzu UV2501-PC spectrophotometer. Fluorescence spectra were collected on a Horiba Jobin-Yvon emission spectrometer equipped with a 450 W xenon source, Gemini-180 excitation and Triax-550 emission monochromators, and a photon counting detector (Hamamatsu R928P PMT at 950 V). Powder X-ray diffraction measurements were performed on a Rigaku Ultima IV X-ray diffractometer with Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) at 25 °C.

3. C/TiO₂ Hybrid Dots

PEG-C/TiO₂-Dots.^{S1} The commercially supplied colloidal TiO₂ sample (Degussa P25) was dispersed in ethanol via vigorous sonication, followed by centrifuging at 1,000 *g* to collect the supernatant as an ethanol dispersion of the harvested TiO₂ nanoparticles. An aliquot of the

dispersion (50 mg of TiO_2) was mixed with PEG (2 g) in a glass vial via vigorous sonication. The resulting dispersion appeared homogeneous, and from the dispersion ethanol was removed by purging with nitrogen gas to obtain the desired precursor mixture of the TiO_2 nanoparticles dispersed in PEG for the microwave-assisted thermal carbonization processing.

The glass vial containing the precursor mixture was immersed in a silicon carbide bath (about 8 cm in diameter and 2.5 cm in height, containing about 50 g silicon carbide) in a conventional microwave oven. With the oven power set at 700 W, the sample was treated with microwave irradiation for 110 s, followed by taking the sample vial out of the oven for 60 s and then re-immersing the vial in the silicon carbide bath in the microwave over for the next heating-cooling treatment cycle. The same cycle was repeated for 7 more times. Then, to the reaction mixture was added more PEG (1 g) with ethanol for a dispersion via sonication, followed by the removal of ethanol via purging with nitrogen gas. The resulting mixture in the silicon carbide bath was treated with the above-described cycle of microwave heating at 700 W and then cooling for 11 repeats. Again, to the reaction mixture was added more PEG (1 g) with ethanol for the same dispersion and then solvent removal, was then added respectively, followed by 5 cycles of microwave heating at 900 W and then cooling. One more addition of PEG (1 g) and then the same processing cycles were performed to obtain the final reaction mixture. The mixture back at ambient temperature was dispersed in hot deionized water (15 mL) with sonication, and the resulting dispersion was dialyzed (molecular weight cut-off ~25,000) against fresh deionized water for 24 h to yield PEG-C/ $\text{TiO}_2^{(25\text{nm})}$ -Dots as apparently a homogenous aqueous dispersion.

PEI&PEG-C/ TiO_2 -Dots.^{S1} An aliquot of the same ethanol dispersion of TiO_2 (50 mg) was mixed with PEI (0.5 g) and PEG (1 g) in a glass vial with sonication to obtain a homogeneous dispersion, followed the removal of ethanol via purging with nitrogen gas. The

sample vial was immersed in the silicon carbide bath for the microwave heating at 900 W and for 110 s and then cooling in 5 repeating cycles. Then, in 3 repeats, each included the addition of more PEG (1 g) with ethanol to the reaction mixture for a homogeneous dispersion, followed by the removal of ethanol, and then for the resulting mixture to be treated with the same microwave heating and then cooling cycle for 4 times. The final reaction mixture back at ambient temperature was dispersed in hot deionized water (15 mL), followed by dialysis (molecular weight cut-off ~25,000) against fresh deionized water for 24 h to obtain the PEG-C/TiO₂-Dots in apparently a homogeneous aqueous dispersion.

4. Microscopy Results

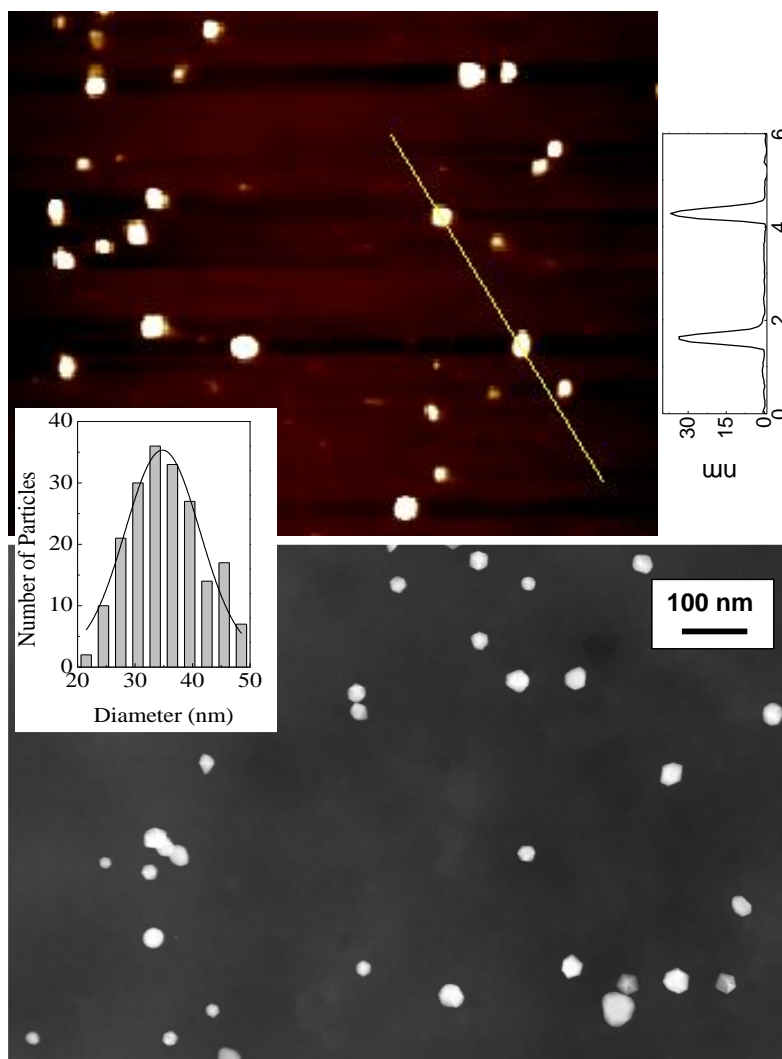


Figure S1 (the same as Figure 3 in ref. S1). Representative AFM (upper) and TEM (lower) images of PEI&PEG-C/TiO₂-Dots. Inset: Statistical analysis of dot sizes based on multiple AFM images.

5. Antibacterial Experiments

Bacillus subtilis culture was originally purchased from Carolina Biological Supply Co. (Burlington, NC). *B. subtilis* culture was grown in 10 mL nutrient broth (Fisher Scientific,

Pittsburgh, PA) by inoculating the broth with a single colony of a plated culture on a Luria–Bertani (LB) agar (Fisher Scientific, Fair Lawn, NJ) plate, and incubated overnight at 37 °C. Freshly grown *B. subtilis* cells were washed two times with phosphate buffered saline (PBS, 1X, pH7.4) (Fisher Scientific, Pittsburgh, PA) and then re-suspended in PBS for further experimental uses.

The treatment of bacterial cells with C/TiO₂ hybrid dots was performed in 96-well plates. Each well was added with 150 µL bacteria cell suspension and 50 µL of the dot sample with desired concentrations. The final bacterial cell concentration in each well was about $\sim 10^6$ – 10^7 CFU/mL, and the final dot concentration was varied in the range of 0.01 mg/ml to 0.1 mg/mL, depending on the needs of individual experiments. The actual dot concentrations used in given experiments were indicated in the figure captions. The plates were exposed to visible light (400–800 nm) from a commercially available 60 W-equivalent daylight LED placed 10 cm above the surface of the plate for 2 h or as stated otherwise.

The traditional surface plating method was used to determine the viable cell numbers in the controls and treated samples. Briefly, the bacterial samples were serially diluted (1:10) with PBS. Aliquots of 100 µL appropriate dilutions were surface-plated on LB agar plates. After incubation at 37 °C for 24 h, the numbers of colonies on the plates were counted, and the viable cell numbers were calculated in colony forming units per milliliter (CFU/mL) for all the treated samples and the controls. The reduction of the logarithmic value of the viable cell number in the treated samples in comparison to the controls was used to evaluate the antibacterial effectiveness of the C/TiO₂ hybrid dots. The greater the viable cell reduction, the higher the antibacterial effectiveness.

References

- S1. Pan, N.; Okonjo, P. A.; Wang, P.; Tang, Y.; Sun, Y.-P.; Yang, L. Optical and Photodynamic Properties of Carbon/TiO₂ Hybrid Dots in Different Nanoscale Configurations. *Chem. Phys. Lett.* **2020**, 763, 137208.