Divergence in Antiviral Activities of Carbon Dots *versus* Nano-Carbon/Organic Hybrids and Implications

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Supplementary Materials

Section S1: Synthesis of CS200, CS330, and CS_{MT} samples[1]

Chemicals

Citric acid was purchased from Alfa Aesar (Tewksbury, MA), oligomeric polyethylenimine (PEI, branched, average molecular weight ~600) from Polysciences, Inc. (Warrington, PA) and *N*,*N*-diethylaniline and 2,4-dinitrotoluene from Sigma-Aldrich. Dialysis membrane tubing (molecular weight cut-off ~500 or ~1000) was supplied by Spectrum Laboratories. Water was

deionized and purified by being passed through a Labconco WaterPros water purification system (Labconco, Kansas City, MO).

CS200 Sample

Citric acid (1 g) and PEI (0.5 g) were mixed in water (10 mL) and the resulting mixture was loaded into a stainless-steel tube reactor. The reactor was sealed for heating in a tube furnace at 200 °C for 3 h. Then, the reactor was cooled back to ambient and the reaction mixture in the reactor was collected. The mixture was placed in a membrane tubing (molecular weight cut-off ~1000) for dialysis against fresh water, followed by concentration. A light-brown aqueous solution was obtained as the sample from the carbonization synthesis at 200 °C, denoted as CS200.

CS330 Sample

Citric acid (1 g) was dissolved in water (2 mL) with brief sonication and the resulting solution was mixed well with PEI (0.5 g). Then, the mixture was loaded into a stainless-steel tube reactor. The reactor was sealed for heating in a tube furnace at 330 °C for 6 h. Upon the cooling of the reactor back to ambient temperature, the reaction mixture in the reactor was collected by washing with water. The aqueous mixture was placed in a membrane tubing (molecular weight cut-off ~1000) for dialysis against fresh water. Upon concentration and then centrifugation, the colored supernatant was collected as the sample from the carbonization synthesis at 330 °C, denoted as CS330.

CS_{MT} Sample

CA (1 g) in water (2 mL) was mixed well with PEI (3 g), followed by evaporation to remove water for a solid-like mixture. Separately, a bath of silicon carbide (150 g) in a silica crucible casting dish (about 8 cm in diameter and 2.5 cm in height) was prepared and pre-heated in a conventional microwave oven at 500 W for 3 min. The solid-like mixture of CA-PEI in a scintillation vial was immersed in the pre-heated silicon carbide bath for treatments with microwave irradiation, first at 200 W for ~4 min until no more bubbles in the sample and with the sample color turning dark orange and then 1000 W for 3 min, during which there were 5 brief pauses each of a few seconds. Post-treatment, the reaction mixture was cooled to ambient temperature and dispersed in deionized water with sonication. The resulting aqueous dispersion was centrifuged at 5000*xg* to keep the supernatant, followed by dialysis in a membrane tubing (molecular weight cut-off ~1000) against fresh water to obtain a colored aqueous solution of the sample denoted as CS_{MT}.

Section S2: Preparation of PEI-CDots[2,3]

Commercially acquired sample of carbon nanopowders (20-40 nm in size, 2 g) was refluxed in concentrated nitric acid (8 M, 200 mL) for 48 h. The reaction mixture was cooled back to ambient temperature, and centrifuged at 1,000x*g* to discard the supernatant. The residue was re-dispersed in deionized water, dialyzed in a membrane tubing (molecular weight cut-off ~500) against fresh water for 48 h, and then centrifuged at 1,000*xg* to retain the supernatant as an aqueous dispersion of small carbon nanoparticles. The nanoparticles could be recovered from the dispersion by removing water via evaporation.

The surface functionalization of the small carbon nanoparticles with oligomeric polyethylenimine (PEI, average molecular weight ~600, branched) for PEI-CDots was accomplished via microwave-assisted thermal processing. In a representative experimental protocol, the carbon nanoparticles (100 mg) as an aqueous slurry were mixed with PEI (2 g) and ethanol (2 mL) in a scintillation vial, and the resulting mixture was sonicated in an ultrasonic cleaner (VWR 250D) at 40 ℃ for 1 h, followed by a complete removal of solvent via evaporation for a solid mixture. Separately, a bath of silicon carbide (150 g) in a silica crucible casting dish (about 8 cm in diameter and 2.5 cm in height) was prepared and pre-heated in a conventional microwave oven at 500 W for 3 min. Several rounds of microwave heating treatments were as follows: (1) the vial containing the solid mixture was immersed in the preheated silicon carbide bath for microwave irradiation at 1,000 W for 3 min; (2) the sample vial was taken out of the bath for being cooled in the ambient, and more PEI (1 g) and ethanol (2 mL) were added and mixed well, followed by the removal of ethanol, and then microwave irradiation the same as in (1); (3) a repeat of (2) but with the microwave irradiation at 500 W for 8 min; and (4) a repeat of (3). After the microwave treatments, the reaction mixture was cooled to ambient temperature and dispersed in deionized water with vigorous sonication. The resulting aqueous dispersion was centrifuged at 5,000 *xg* to collect the supernatant, followed by dialysis (molecular weight cut-off ~1,000) against fresh water for up to 24 h to obtain the PEI-CDots in an aqueous solution.

Section S3: VSV Virus Propagation and Purification[4]

VSV, Indiana strain (VR-1238), and baby hamster kidney (BHK-21) cells (CCL-10) were purchased from ATCC (Manassas, VA). BHK-21 cells were cultured in high glucose Dulbecco's

modified Eagle medium (DMEM) (Hyclone), supplemented with 10% FBS (HyClone) and no antibiotics, at 37 °C under a 5% CO2 atmosphere. VSV was propagated in confluent monolayers of BHK-21 cells at a multiplicity of infection of 10. After the incubation at 37 °C for 1 h, DMEM supplemented with 2% FBS was added to the flask. Viruses were harvested approximately 18 h post inoculation by low-speed centrifugation at 5000 *xg* for 10 min to remove the cell debris.

To prepare the purified stock of VSV, the collected supernatant was centrifuged at 40,000 *xg* for 90 min at 4 °C using chilled Beckman rotor Type 70 Ti. The supernatant was discarded, and the pellet containing the VSV virus particles was resuspended in 3.5 mL of TNE buffer (Tris-EDTA (TE) buffer with NaCl) (100 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.4). Then, 27 mL of 10% sucrose solution (w/v in NTE) was added to a centrifuge tube, and 3.5 mL of virus suspension solution was layered on the top of 10% sucrose. The total volume was adjusted to 37 mL by adding additional 6.5 mL of NTE. The sample was centrifuged at 150,000 *xg* for 1 h at 4 °C. The supernatant was removed, and the purified VSV pellet was resuspended in 2 mL of sterile deionized (DI) H_2O. The protein concentration was determined by the spectrophotometric measurement, and the final protein concentration of the VSV sample was set at 1 μg/mL. The purified VSV samples were stored at refrigeration until the viral titer could be determined by plaque assay and for further experimental uses.

VSV Virus Titration by Plaque Assay. The VSV titer was quantified by plaque assay in BHK-21 cells. Briefly, BHK-21 cells were seeded into six-well plates at a density of ~1 x 10^6 cells/well and incubated overnight to form confluent cell monolayers. The VSV samples were 1:10 serially diluted with DMEM without FBS, and aliquots of 400 μL of the dilution series were added to inoculate the BHK-21 cell monolayers. The plates were incubated for 1 h at 37 °C with gentle

rocking every 10−15 min. After the infection, each well was overlaid with 1.2% agarose diluted 1:1 with DMEM supplemented with 2% FBS. After overnight incubation at 37 °C under a 5% CO2 atmosphere, the cells were fixed with 4% formaldehyde solution and stained with 0.05% crystal violet in 20% methanol for plaque visualization and counting, to calculate the plaque-forming units per mL (PFU/mL).

Figure S1: The relative antibacterial activities of PEI-CDots, CS200, CS330, and CS_{MT}.[1]

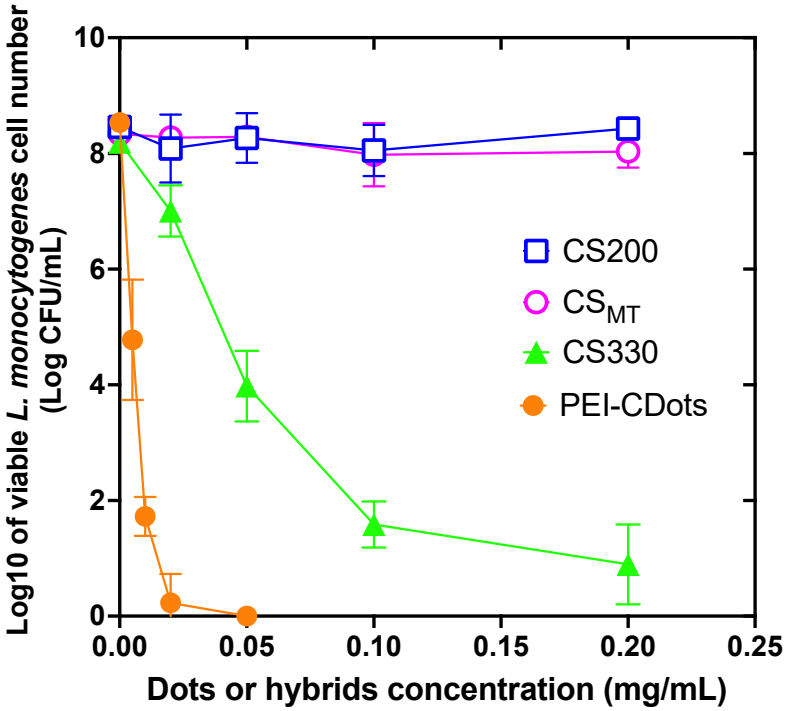

Figure S1: The visible light-activated antibacterial effects of PEI-CDots, CS200, CS300, and CS_{MT} against *L. monocytogenes* cells, expressed by the reduction in logarithmic viable cell number with

the increasing concentration of CDots or hybrids. Note: Viable light treatment 1 h; Concentrations of CDots or hybrids used in the treatments varied as shown in the figure.[1]

Figure S2: AFM images of CS330 and CS_{MT} Samples[1]

Atomic force microscopy (AFM) images were acquired using a Nanosurf CoreAFM instrument (Liestal, Switzerlan). Sample specimens for AFM were prepared on clean Si wafer pieces, with blank images obtained under the same conditions using the same Si wafers.

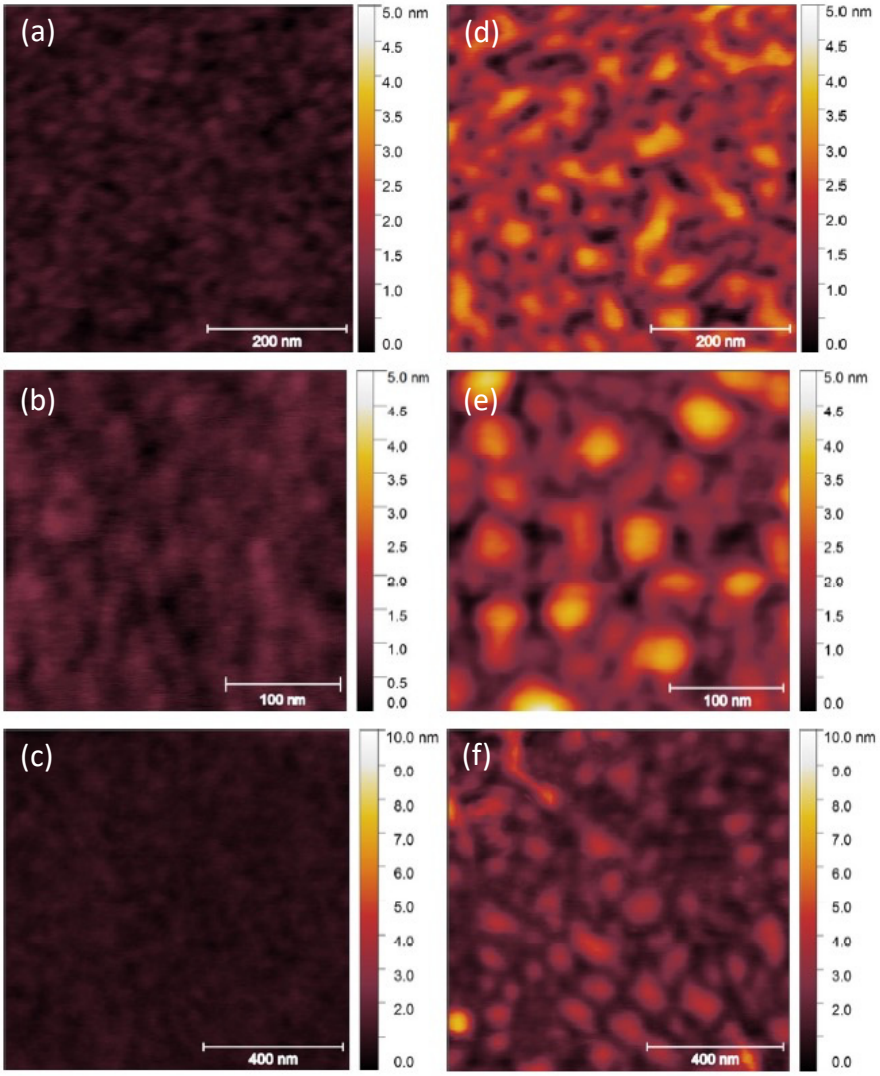

Figure S2: AFM images of the CS200 (d), CS330 (e), and CSMT (f) samples on Si wafers, with the corresponding images of clean Si wafers on the left (a–c).

References:

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