Supporting Information

High antiviral activity of mercaptoethane sulfonate functionalized Te/BSA nanostars against arterivirus and coronavirus

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Experimental Section

Synthesis of Te/BSA nanospheres

Te/BSA nanospheres were synthesized according to Zhou *et al.*.[1] Te/BSA nanostars were prepared as the previously reported with slight modification.[2, 3] Specifically, MES (0.1972 g) was added to 100 ml of ultrapure water and completely dissolved under stirring, followed by addition of sodium tellurite (0.066 g). When the color of solution turned from colorless to black, BSA (0.200 g) was added, followed by reaction at room temperature (RT) for 2 h. After reaction, the solution was dialyzed against ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity) using a dialysis membrane for 24 h to remove the unreacted low molecular weight by-products. The as-prepared Te/BSA nanostars were stored at 4 °C for further use.

The FITC was conjugated to Te/BSA nanospheres or Te/BSA nanostars as described by Ma *et al.* .[4] Briefly, 5.0 mL of FITC (177 μM) was mixed with 1.0 mL of Te/BSA nanospheres or Te/BSA nanostars in 0.10 M $NaHCO_3$ at RT. After 36 h, the unbound FITC was removed using a dialysis membrane with a molecular weight cut-off of 1000 Da against 0.10 M $NaHCO_3$ for 24 h and ultrapure water for additional 24 h.

Apparatus

The size and morphology of the synthesized nanomaterials were characterized by a JEM-2100F transmission electron microscope (JEOL, Japan) at an acceleration voltage of 200 kV. The UV-vis absorption spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan). Fourier-transform infrared (FT-IR) spectra

were detected by a Nicolet Avatar-330 spectrometer (Thermo, USA) using the KBr pellet technique. The Zeta potential and hydrodynamic diameters were obtained by using a Zetasizer Nano ZS90 dynamic light scattering (DLS) system (Malvern, England). The X-ray photoelectron spectroscopy (XPS) was measured with an Escalab 250Xi photoelectron spectrometer (Thermo, USA). X-ray diffraction (XRD) pattern was obtained using a Bruker D8 Advance X-ray diffractometer with Cu Ka radiation. Thermogravimetric analysis (TGA) was performed with a NETZSCH TG 209 analyzer.

Cells and viruses

MARC-145 cells (monkey kidney cell line) and Vero cells (African green monkey kidney cell line) were both obtained from the American Type Culture Collection (ATCC). These two cell lines were both maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS) in a humidified incubator at 37 °C under 5% CO2. Porcine reproductive and respiratory syndrome virus (PRRSV) strain WUH3, which was isolated in China at the end of 2006, was propagated in MARC-145 cells. Porcine epidemic diarrhea virus (PEDV) strain AJ1102, which was isolated in China in 2011, was propagated in Vero cells.

MTT assay

The cytotoxicity of Te/BSA nanostars on MARC-145 cells was detected by MTT assay as previously described.[5] Briefly, MARC-145 cells at a confluence of ~90% in 96-well culture plates were treated with 100 μL of Te/BSA nanostars at different

concentrations (30.0, 15.0, 7.50, 3.75 and 0 μg/mL) in DMEM (2% FBS) for 12, 24, 36 or 48 h, followed by the addition of 20 μL of MTT solution (3-[4,5-dimethylthiazol-2-thiazolyl]-2,5-diphenyl tetrazolium bromide, Sigma) to each well, and then incubation at 37 °C for 3 h. After removing the supernatant, the formazan crystals were dissolved in 150 μL/well dimethylsulfoxide. The absorption values were measured at 570 nm using an Enzyme Linked Immunosorbent Assay (ELISA) microplate reader and the cell viability percentage was calculated.

Likewise, the cytotoxicity of Te/BSA nanostars (30.0, 15.0, 7.50, 3.75 and 0 μg/mL) on Vero cells were evaluated after incubation for 24 h. The cell viability of MARC-145 cells treated with Te/BSA nanospheres (80.0, 60.0, 30.0, 15.0, 7.50 and 0 μg/mL) for 36 h was also tested. The cytotoxicity of MES (3.00, 1.50, 0.750 and 0 mmol/L) on MARC-145 cells was explored separately after treatment for 12, 24, 36 and 48 h.

Viral plaque assay

The 95% confluent MARC-145 cells cultured in 6-well culture plates were incubated with 10-fold serially diluted virus sample for 1 h. Then the cells were washed twice with serum-free DMEM, and overlaid with 1.8% (w/v) low melting point agarose (Promega) containing 2×DMEM supplemented with 3% FBS and 1% penicillin-streptomycin. After incubation at 37 °C for 2-3 day, the number of plaques was counted after staining with neutral red mixed with PBS at a 1:4 ratio for 2 h at 37 °C. The average plaque number and standard deviations were calculated from three

independent experiments. The titer of PRRSV was presented as plaque forming unit (PFU).

Western blot assay

MARC-145 cells were seeded in 6-well culture plates and infected or mock-infected with PRRSV (1.0 MOI) in the presence or absence of Te/BSA nanostars (15.0 μg/mL) for 12, 24, 36 and 48 h as described in *Antiviral assay*. After two washes with PBS, the cells were treated with 150 μL/well lysis buffer (2×LBA) (4% sodium dodecyl sulfate [SDS], 3% DL-dithiothreitol, 65 mM Tris-HCl [pH6.8], 40% glycerin). Next, the cell lysates were denatured in 5×SDS loading buffer by boiling for 10 min and then electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After transferring the separated proteins to 0.2 μm PVDF Membrane (Millipore), the membrane was blocked with 10% (w/v) nonfat dry milk for 4 h at RT, followed by incubation with mouse monoclonal antibody (mAb) against PRRSV nonstructural protein 2 (nsp2) at 37 °C for 4 h and then horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG for 1 h at RT. The β-actin was detected as a loading control. Protein bands were visualized using the Clarity Enhanced Chemiluminescence (ECL) reagent (Bio-Rad, Hercules, CA).

Indirect immunofluorescence assay

To evaluate the antiviral effect of Te/BSA nanostars, Te/BSA nanospheres and MES on PRRSV, MARC-145 cells were seeded in 24-well culture plates and incubated in DMEM (10% FBS) until ~90% confluence. Then, the cells were treated with Te/BSA

nanostars (15.0 μg/mL), Te/BSA nanospheres (15.0 μg/mL) or MES (1.50 mmol/L) and infected with PRRSV (1.0 MOI) as described in *Antiviral assay*. At 36 hpi (hours post infection), the cells were washed three times with PBS and fixed with precooling (4 °C) 4% paraformaldehyde for 15 min at RT. Next, the paraformaldehyde was discarded and precooling methanol (-20 °C) was added immediately to permeabilize the cells for 10 min at RT, followed by rinsing three times with PBS. After blocking with 5% BSA, the infected cells were detected with mouse mAb against PRRSV nucleocapsid (N) protein (primary antibody) and then Alexa Fluor 488-conjugated donkey anti-mouse IgG (secondary antibody). The cell nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen) for 15 min in the dark, followed by rinsing three times with PBS. All of the above steps were carried out on a shaker at the minimum speed. The fluorescence images were observed with an Olympus FV10 laser scanning confocal microscope (Olympus, Japan).

Similarly, the indirect immunofluorescence assay was also performed to explore the antiviral effect of Te/BSA nanostars (15.0 and 30.0 μg/mL) on PEDV (0.05 MOI) in Vero cells at 24 hpi, with PEDV-infected cells stained with mouse mAb against PEDV N protein (primary antibody) and then Alexa Fluor 594-conjugated donkey anti-mouse IgG (secondary antibody).

RNA extraction and quantitative real-time RT-PCR (RT-qPCR)

The total RNA of MARC-145 cells was extracted using TRIzol reagent (Invitrogen). Subsequently, 1.0 μg of each sample was reverse transcribed into cDNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche) with primers binding to

PRRSV negative-sense RNA: 5'UF (5'-GACGTATAGGTGTTGGCTC-3'), then the resulting cDNA was used in a SYBR green PCR assay (Applied Biosystems, USA) to test the levels of PRRSV negative-sense RNA. The PCR assay was performed using the following primers: q5'UTR (5'-GCATTTGTATTGTCAGGAGC-3' and 5'-AGCAGTGCAACTCCGGAAG-3'). The individual mRNA transcript in each sample was assayed five times.

Supplementary Figures

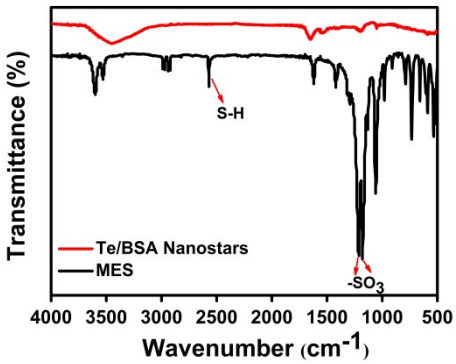

Figure S1. The FT-IR spectra of Te/BSA nanostars and MES.

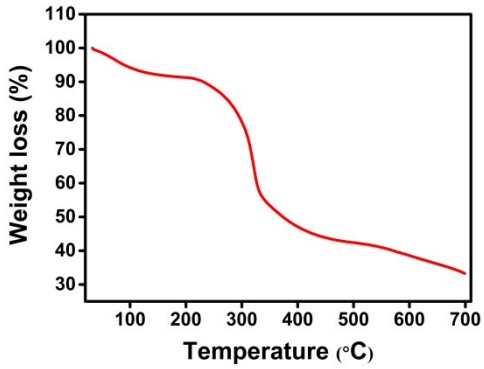

Figure S2. Thermogravimetric analysis (TGA) of Te/BSA nanostars showing the percentage weight loss of the sample as a function of temperature.

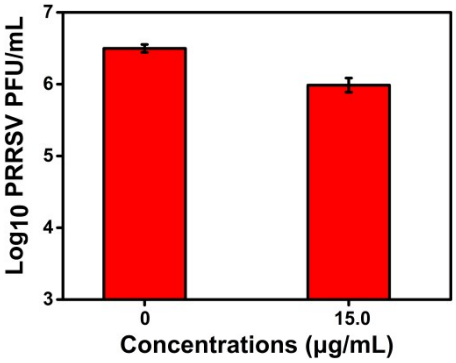

Figure S3. The direct effect of Te/BSA nanostars on cells as detected by viral plaque assay.

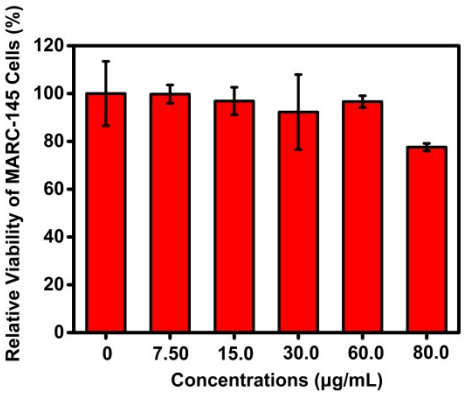

Figure S4. Cytotoxicity of Te/BSA nanospheres as detected by MTT assay after incubation for 48 h. All values were normalized to the control group (without Te/BSA nanospheres exposure). Error bars represent the standard deviation from three independent experiments.

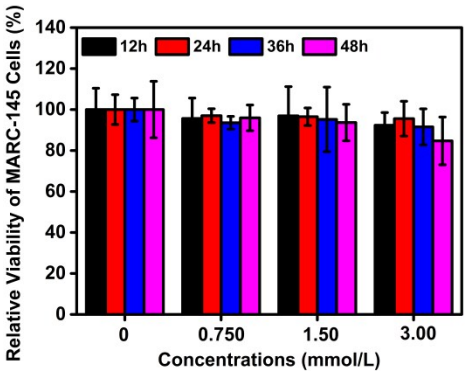

Figure S5. Cytotoxicity of MES on MARC-145 cells as detected by MTT assay.

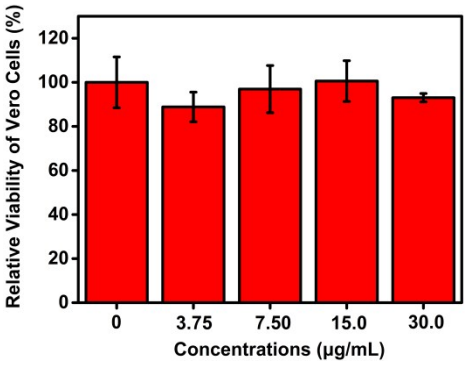

Figure S6. The cytotoxicity of Te/BSA nanostars on Vero cells as tested by MTT assay after treatment for 24 h.

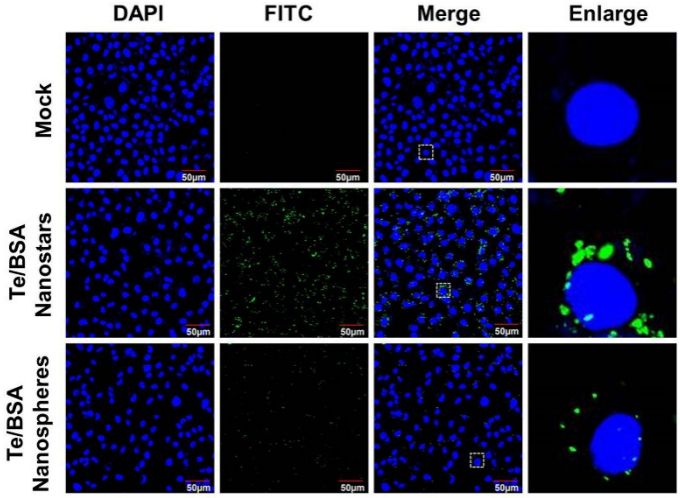

Figure S7. The cellular localization of Te/BSA nanostars and Te/BSA nanospheres in MARC-145 cells.

References

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