Electronic Supplementary Information

Nanoelectrochemistry Monitoring of Intracellular Reactive Oxygen and Nitrogen Species Induced by Nanoplastic Exposure

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1 Experimental Sections

1.1 Materials and Instruments

SiC nanowires (SiC NWs) were purchased from Nanjing/Jiangsu XFNANO Materials Tech Co., Ltd (Nanjing, China). $H_2PtCl_6 \cdot 6H_2O$, peroxynitrite (sodium), $Ru(NH_3)_6Cl_3$, tetraacetoxymethylester (Calcein-AM), and propidium iodide (PI) were bought from Sigma-Aldrich (St. Louis, U.S.A.). 3-aminoprooyltriethoxysilane (APTES) was purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). Formic acid was purchased from Chinasun Specialty Products Co., Ltd (Changshu, China). 1400W dihydrochloride and mito-TEMPO were bought from MedChemExpress (Monmouth Junction, U.S.A.). Hoechst 33342 dye, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT), DAF-FM DA and DCFH-DA fluorescent probes were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). YF®555-Phalloidin was purchased from UElandy Biotechnology Co., Ltd (Suzhou, China). 30 nm amino functionalized polystyrene (PS-NH_2) and carboxy functionalized polystyrene (PS-COOH) nanoplastics were purchased from Huge Biotechnology Co., Ltd (Shanghai, China). Human alveoli cancer epithelial cells (A549 cells) and cell culture medium were obtained from Pricella Life Science & Technology Co., Ltd (Wuhan, China). Borosilicate capillary (1B100-4) was supplied by World Precision Instruments (U.S.A.). 80# Microcrystalline wax was purchased from Shuangfeng Wax Co., Ltd (Cangzhou, China). All other chemicals and solvents of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water (resistivity >18.2 MΩ·cm, Millipore Inc., U.S.A.) was used for rinsing and preparing all aqueous solutions during the whole experiment.

Scanning electron microscopy (SEM) images were taken by field-emission scanning electron microscopes (Zeiss Sigma and Zeiss Merlin Compact). Fluorescent images were taken by an inverted fluorescent microscope (AxioObserver Z1 and Axiovert 200M, Zeiss) or a confocal laser scanning microscope (LSM900, Zeiss). The DLS measurement of the NPs was characterized using a Zetasizer (nano ZS90, Malvern Instruments). A microforge (World Precision Instruments, ×40 objective) was used to

fabricate NWEs. Electrochemical measurements were conducted with a CHI 660e electrochemical workstation (CHI Instruments) in a two-electrode configuration with a homemade Ag/AgCl electrode to perform simultaneously as a reference and a counter electrode. Amperometric recordings were achieved with a patch clamp amplifier (EPC-10, HEKA Electronics) coupled with a micromanipulator (TransferMan 4r, Eppendorf).

1.2 Characterization of the Nanoparticles

A zetasizer was adopted to measure the hydrate particle size and zeta potential of PS NPs in ultrapure water.

1.3 Fabrication of SiC@Pt NWEs[1]

The SiC@Pt NWEs were fabricated as described before. Briefly, SiC NWs (~200 nm diameter) self-grown with dense Pt nanoparticles were obtained by reducing H_2PtCl_6 with HCOOH. Prepared SiC@Pt NWs dispersed in 20 mL ultrapure water were added dropwise to the center of a glass sheet and gently heated to evaporate water. Then the glass sheet was cut into two parts to allow for a partial protrusion of NWs over the glass slide edges.

A borosilicate capillary was pulled to form a micropipette with a tip diameter of about 3 μm under a laser micropuller, liquid metal was then injected into the glass micropipette and sealed with wax. The SiC@Pt NW was inserted into the glass micropipette with an exposed length of about 10 μm to form NWE.

1.4 Cell experiments

1.4.1 Cell culture

A549 cells were cultured in DMEM with 10% Ham's F-12K, 1% horse serum, and 1% penicillin-streptomycin at 37 °C with 5% CO_2 atmosphere. Before performing any of the experiments, A549 cells were seeded on small cell culture dishes (diameter 35 mm). After cell adhesion, the PS-COOH or PS-NH_2 NPs (30 μg/mL) were added to stimulate A549 cells.

1.4.2 Preincubation of A549 cells with the inhibitors

A549 cells were pretreated with 10 μM mito-TEMPO for 1 h or 10 μM 1400W for 24 h before PS-NH_2 NPs incubation.

1.4.3 Cell Staining Experiments

1.4.3.1 Fluorescence imaging of ROS/RNS in A549 cells

NP-incubated A549 cells were incubated with 20 μM DAF-FM DA or DCFH-DA solution for 20 min and washed 3 times with PBS. Bright-field and fluorescence images were then taken with an inverted fluorescent microscope as soon as possible.

1.4.3.2 Cell viability experiment

The A549 cells incubated with PS-NH_2 NPs for 6 h were treated with 1 mL fluorescence dye solution (Calcein-AM + PI) for 20 min, and residual dyes were washed off 3 times with PBS. Bright-field and fluorescence microphotographs were then taken as soon as possible.

1.4.3.3 Fluorescence imaging of actin and nucleus in A549 cells

The A549 cells (after 0, 0.5, 2, and 6 h of PS-NH_2 NPs incubation) were fixed with 4% paraformaldehyde for 5 min and then permeabilized with 0.1% Triton X-100 surfactant for 5 min. The A549 cells were then incubated with 555 phalloidin (dilution 1:40) and Hoechst 33342 dye (dilution 1:1000) for 30 min and washed three times with PBS. Confocal microphotographs were recorded by a confocal laser scanning microscope.

1.4.4 MTT experiment

Cell viability was assessed by the MTT assay. Briefly, NP-treated cells were incubated with a 5 mg/mL solution of MTT reagent at 37 °C for 4 h to allow MTT reduction to formazan blue by metabolically active cells. Then formazan crystals were solubilized at room temperature with 100 μL DMSO for 5 h. Optical density at 570 nm was measured in a microplate reader. Three replicate wells were used for each experimental condition in each experiment.

1.4.5 Amperometric data acquisition and analysis

All amperometric measurements involving living cells were performed on the stage of an inverted microscope placed in a well-grounded Faraday cage. A SiC@Pt NWE was firstly placed near the cell membranes with a micromanipulator and then the NWE was gently moved forward over 5 μm to ensure insertion inside the cell. The amperometric traces were recorded with a patch-clamp amplifier under a two-electrode

electrochemical system at a series of selected potentials (150, 550, 650, and 800 mV versus Ag/AgCl). Amperometric traces were sampled at 1 kHz, and we used bessel filtered at 2.9 kHz during the detecting process and performed smoothing of traces (Method: Savitzky-Golay, Points of Window: 10) to remove noise or irregularities.

1.5 Calculations of four primary ROS/RNS[2]

Each amount of the four primary ROS/RNS stored in A549 cells can be calculated shown in following equations, which represent quantitative current relationships corresponding to the four main ROS/RNS at different potentials.

$$Q_{150\,mV} = Q_{ONOO^-} + 0.1Q_{H_2O_2} \quad (1)$$

$$Q_{550\,mV} = Q_{ONOO^-} + Q_{H_2O_2} + 0.1Q_{NO} \quad (2)$$

$$Q_{650\,mV} = Q_{ONOO^-} + Q_{H_2O_2} + Q_{NO} \quad (3)$$

$$Q_{800\,mV} = Q_{ONOO^-} + Q_{H_2O_2} + Q_{NO} + Q_{ONOO^-} \quad (4)$$

where $Q_{potential}$ is the total charge measured at each selected potential, whereas $Q_{species}$ is the individual charge of the named species.

1.6 Production formulas of four primary ROS/RNS from precursors[3]

$$2O^{\bullet}{}_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (5)$$

$$O^{\bullet}{}_2^- + NO^{\bullet} \rightarrow ONOO^- \quad (6)$$

$$2ONOO^- \rightarrow 2NO_2^- + O_2 \quad (7)$$

2 Supporting Figures

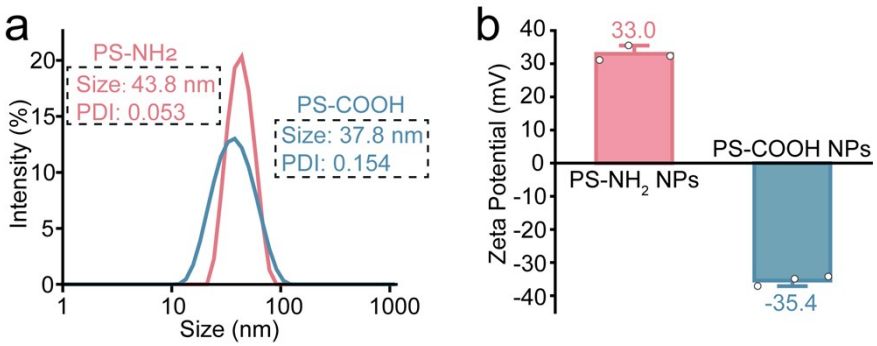

Fig. S1 (a) Size distribution and (b) zeta potentials of PS-NH_2 NPs (pink) and PS-COOH NPs (blue) dispersed in PBS (pH=7.4, n=3).

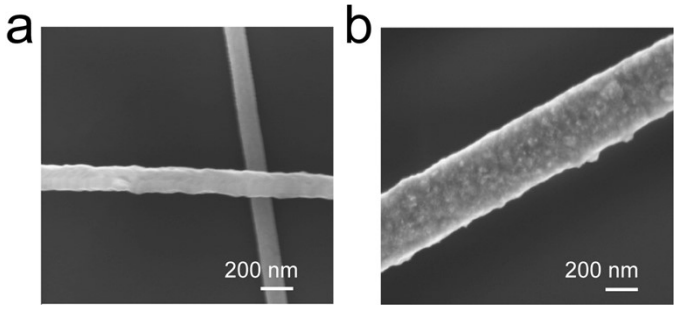

Fig. S2 SEM images of (a) SiC NWs and (b) SiC@Pt NW.

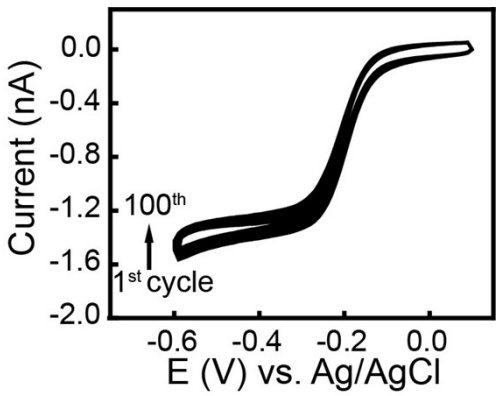

Fig. S3 Repetitive cyclic voltammograms (100 cycles) of 1 mM $Ru(NH_3)_6^{3+}$/1 M KCl of a SiC@Pt NWE.

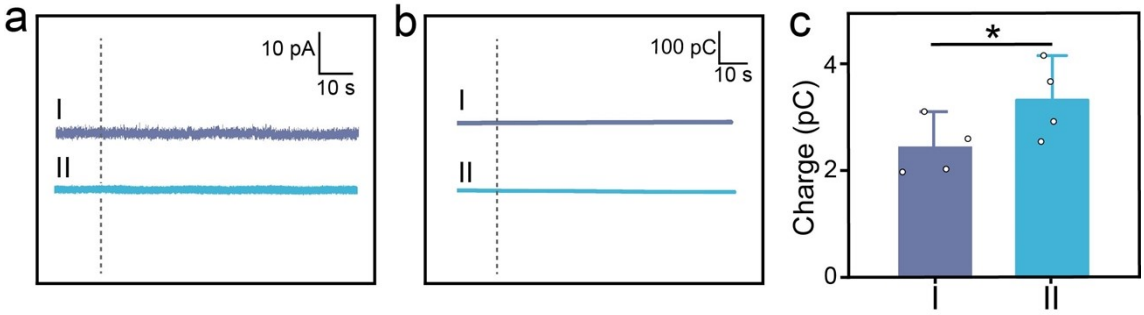

Fig. S4 (a) Amperometric traces and (b) corresponding charge traces recorded at +800 mV in PBS with PS-NH_2 NPs (I) or inside A549 cells without NP-incubation (II). The vertical grey dashed lines indicate the time of NWE insertion. (c) Statistical analysis of the charges (means and SEM, n = 4; *P < 0.05).

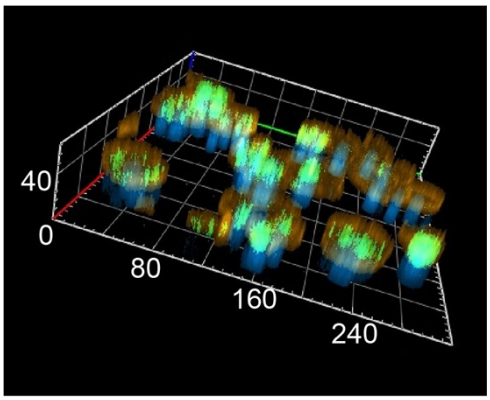

Fig. S5 Z-stacked confocal image of A549 cells after 6 h incubation of PS-NH_2 NPs. PS-NH_2 NPs (green), actin filaments (orange), and nucleus (blue).

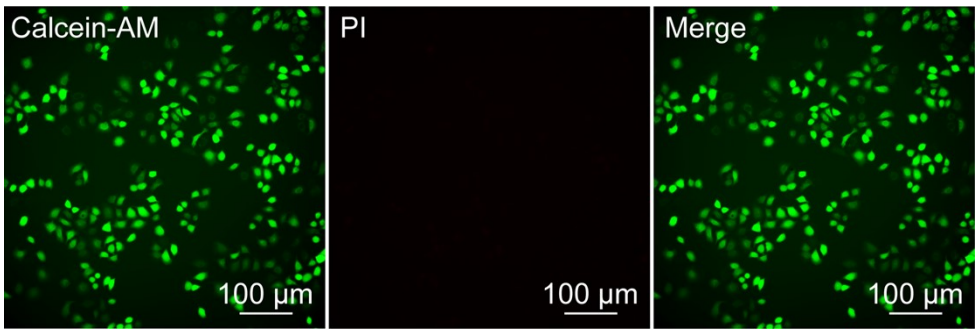

Fig. S6 Fluorescence microscopy images of A549 cells after staining with Calcein-AM (green) and propidium iodide (PI, red) as indicated (the "merge" view is obtained by the merging of Calcein-AM and PI fluorescence images).

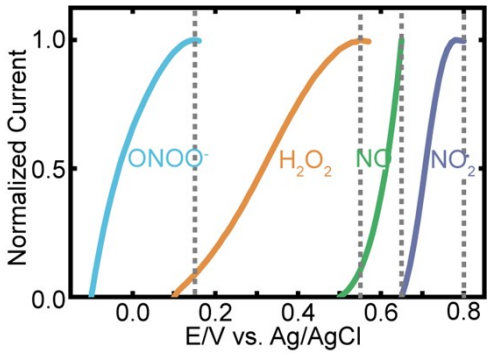

Fig. S7 Linear sweep voltammetric (LSV) curves of $ONOO^-$ (blue), H_2O_2 (orange), NO (green), and NO_2^- (purple) in PBS; currents were normalized to their maximum.

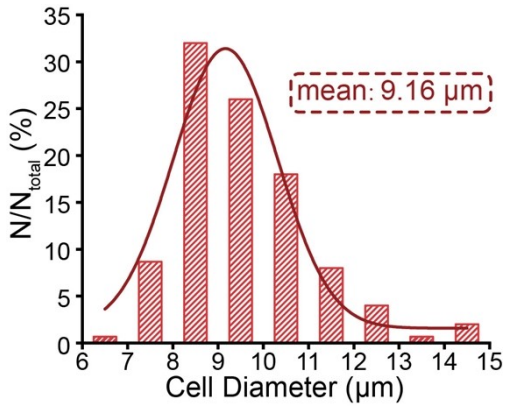

Fig. S8 The distribution and mean value of A549 cell diameters.

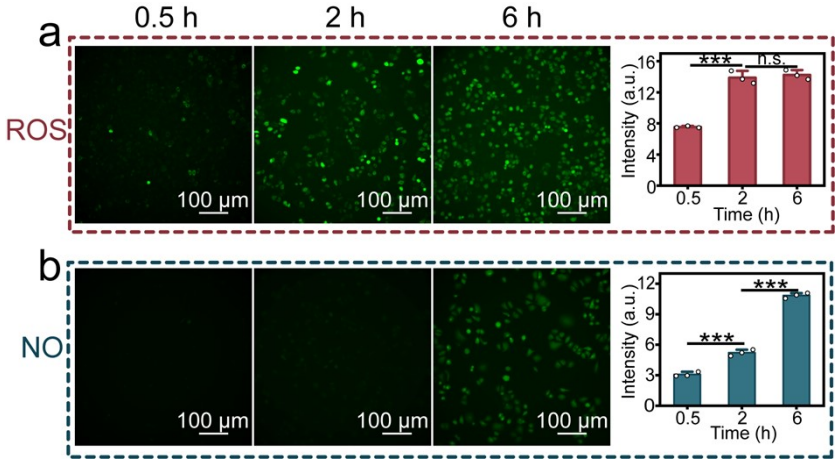

Fig. S9 Fluorescence images and statistical analysis of PS-NH_2 NPs induced ROS (a) or NO (b) production in A549 cells under different incubation times: 0.5 h, 2 h and 6 h (means and SEM, n=3; ***P < 0.001).

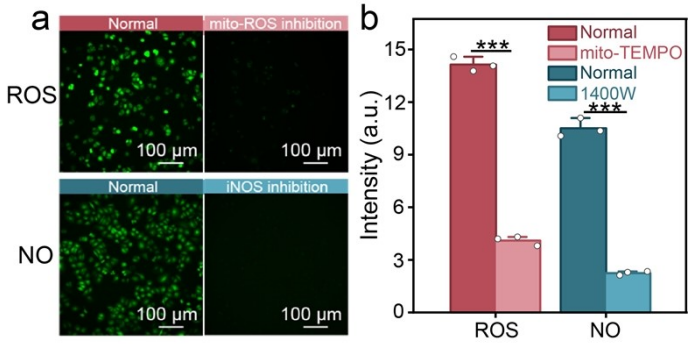

Fig. S10 (a) Fluorescence images and (b) quantitative results of PS-NH_2 NPs induced ROS (green) or NO (green) production in A549 cells with or without incubation of DPI/1400W (means and SEM, n=3; ***P < 0.001).

3. References

1 Y.-T. Qi, H. Jiang, W.-T. Wu, F.-L. Zhang, S.-Y. Tian, W.-T. Fan, Y.-L. Liu, C. Amatore and W.-H. Huang, *J. Am. Chem. Soc.*, 2022, **144**, 9723–9733.

2 Y.-T. Qi, F.-L. Zhang, S.-Y. Tian, H.-Q. Wu, Y. Zhao, X.-W. Zhang, Y.-L. Liu, P. Fu, C. Amatore and W.-H. Huang, *Nat. Nanotechnol.*, 2024, 1–10.

3 Amatore, S. Arbault, D. Bruce, P. de Oliveira, M. Erard and M. Vuillaume, *Chem. Eur. J.*, 2001, **7**, 4171–4179.