Supplementary Information

ROS-Responsive SERS Probes for Real-time Monitoring of Oxidative Stress in Traumatic Brain Injury

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1. Experimental Section

1.1 Instrumentation

UV-Vis absorption spectra were recorded by Nanodrop One microvolume spectrophotometer (Thermal Fisher, USA). Raman measurements were carried out by inVia Qontor Raman confocal microscope system (Renishaw, UK). Particle size distribution and zeta potential tests were performed by SZ-100 Z2 particle size analyzer (Horiba, Japan). Transmission electron microscopy (TEM) images were recorded using an HT 7800 (Hitachi, Japan) system at an accelerating voltage of 120 kV.

1.2 Synthesis of Au NPs

Gold nanoparticles (Au NPs) were obtained by the crystal seed growth method. Specifically, a 2.2 mM sodium citrate solution was prepared in a three-necked flask and heated to boiling under strong stirring. Then, 0.5 mL of 25 mM chloroauric acid was immediately added and kept boiling for 15 min. the solution was observed to change from colorless to light yellow and then to light red. The above solution was then cooled to 90 °C. 0.5 mL of sodium citrate solution (60 mM) and 0.5 mL of chloroauric acid solution (25 mM) were added sequentially at 2 min intervals for 12 cycles. Then, the temperature was maintained at 90 °C for 30 min and finally cooled to room temperature.

1.3 Synthesis of SERS probe

Firstly, 40 μL of $AgNO_3$ (10 mM) and 40 μL of ascorbic acid (AA, 10 mM) were added to 5 mL of Au NPs, which was stirred at room temperature for 2 h, and the color of the solution was changed from red to brownish red. Subsequently, 6 μL of 0.1 mM DTNB was added dropwise to the solution and stirred vigorously for 30 min. 10 μL, 2 mM of freshly prepared HS-PEG was then added to the above solution and stirred for another 30 min. Finally, the centrifugation (7000 rpm, 15 min) was performed and the solution was resuspended in 1 mL of ultrapure water to obtain the SERS probe storage solution. The performance of the SERS probe was tested by UV-vis, DLS, TEM and SERS.

1.4 Cell culture

Mouse brain microvascular endothelial cells (bEnd.3) were purchased from BFB

in Shanghai, China. cells were incubated in DMEM containing 10% FBS. All cells were cultured at 37 ℃, 95% humidity, and 5% CO_2.

1.5 Cytotoxicity of SERS Probe

The cytotoxicity of SERS probe was systematically evaluated using Cell Counting Kit-8 (CCK-8) assay. The bEnd.3 cells were seeded in 96-well plates at a density of 5 × 10^4 cells/well and allowed to adhere for 24 h under standard culture conditions (37 °C, 5% CO_2). Subsequently, cells were exposed to gradient concentrations of SERS probes (0-2.5 nM, 0.5 nM increments) for 24 h. Following double-wash with phosphate-buffered saline (PBS), 100 μL CCK-8 working solution was added to each well. After 2 h incubation protected from light, optical density (OD_{450}) was measured using a BioTek Synergy H1 microplate reader. Cell viability (%) was calculated as: $[(OD_{treatment} - OD_{blank}) / (OD_{control} - OD_{blank})] \times 100$. All experiments were performed in triplicate.

1.6 Haemolysis Assay

Healthy BALB/c mice were selected for transorbital venous plexus puncture for blood collection, and heparin anticoagulated blood collection tubes were used to collect whole blood. Blood samples were diluted in phosphate buffer solution (PBS, pH 7.4) and then centrifuged (4 °C, 2000 rpm, 10 min), repeated three times to achieve erythrocyte purification. The final precipitate was resuspended into 5% erythrocyte suspension using phosphate buffer (PBS, pH 7.4) at a ratio of 1:19 (v/v). The experiment was set up with a gradient concentration group (5-200 μg/mL SERS probe), a blank control (equal volume of PBS) and a complete haemolysis control (0.1 % Triton X-100 lysate). The mixture of all groups was continuously incubated in a constant temperature incubator for 120 min before centrifugation to obtain the supernatant. The collected supernatant drop was added to a 96-well plate and the OD value at 540 nm was determined by an enzyme marker (SYNERGY H1) and the haemolysis rate was calculated.

1.7 Intracellular SERS imaging in bEnd.3 cells

1.7.1 SERS imaging of ROS

Functionalized SERS nanoprobes (1 nM) were co-cultured with bEnd.3 cells (mouse brain microvascular endothelial cells) for 12 h under standard conditions. The probe-containing medium was aspirated followed by triple rinsing with ice-cold phosphate-buffered saline (PBS). Cells were pretreated with cytokine cocktail containing lipopolysaccharide (LPS, 5 μg/mL) across a time-course gradient (5, 10, 15, 30, 45, 60, 90, 120 min). Cellular SERS mapping was conducted on randomly selected triplicate samples using a 633 nm excitation laser with 1 s integration time, captured through a 50 × objective conditions.

1.7.2 SERS imaging for monitoring ROS fluctuations

The SERS probe (1 nM) was incubated with bEnd.3 cells for 12 hours. Petri dishes were washed three times with PBS to remove unbound probes. Cells were divided into three experimental groups: (a) SERS Probe: no treatment; (b) NAC + SERS Probe group: cells were pretreated with N-acetylcysteine (NAC) for 3 h; (c) SERS Probe + ROS group: cells were co-stimulated with LPS (5 μg/mL) for 4 h.

1.8 Establishment of TBI model

All mice procedures were performed with the approval of the animal care and use committee of Hainan Medical University (Pending NO. HYALL-2025-233). BALB/c mice (6~8 weeks) were purchased, weighing 20 ± 2 g. Mice were acclimatised and fed for one week prior to modelling, surgical instruments used were autoclaved before use, and surgical operations were performed in an SPF-grade environment. The specific modelling method was as follows: after adaptive feeding, the mice were anaesthetized by intraperitoneal injection, fixed in the prone position on the brain stereotactic apparatus, and the scalp was incised along the midline of the head, with the length of the incision being about 1.5 cm, the periosteum was separated with scissors, and the cranium was fully exposed. A circular bone window with a diameter of 5 mm was opened using a micro-grinding drill centred on 3.5 mm behind the fontanelle and 2.5 mm to the right of the midline paracentesis. The integrity of the dura mater was maintained during the surgical procedure, and strikes were initiated after the tail-clamping reflex appeared. The percussion method was the modified Feeney's free-fall method.

1.9 SERS imaging of TBI model

SERS imaging was performed at 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 90 min, and 120 min time points in vivo mice models and isolated brain trauma sites; mice were anaesthetised by using 3% sodium pentobarbital during the SERS imaging process, and the injection method was intraperitoneal injection. The mice were anaesthetised with 3% sodium pentobarbital and injected intraperitoneally. 10 μL~20 μL of SERS probe solution was sprayed at the brain injury site to detect the fluctuation of the glucose ROS level at the brain injury site. SERS imaging was performed on a Renishaw confocal micro-Raman spectrometer with the following parameters: laser excitation wavelength of 633 nm, laser power of 8.5 mW, acquisition time of 1 s, and step size of 120 μm. SERS data were processed using the WIRE 5.5 software, and the Raman intensity of the characteristic peak at 1337 cm^{-1} was rendered in pseudo-color.

2. Mechanism of DTNB Oxidation toward ROS

Upon exposure to reactive oxygen species (ROS), the disulfide bond (–S–S–) within DTNB undergoes a redox-mediated cleavage. The reaction proceeds in two steps: ROS species attack the disulfide bond as the primary oxidation, forming an unstable sulfenic acid intermediate (R–SOH). Then, the sulfenic acid is subsequently oxidized to a sulfonic acid derivative ($R–SO_3H$), with the concomitant release of one molecule of TNB (5-thio-2-nitrobenzoate), which serves as the SERS-active reporter. This mechanism illustration and the description have been added in the supporting information.

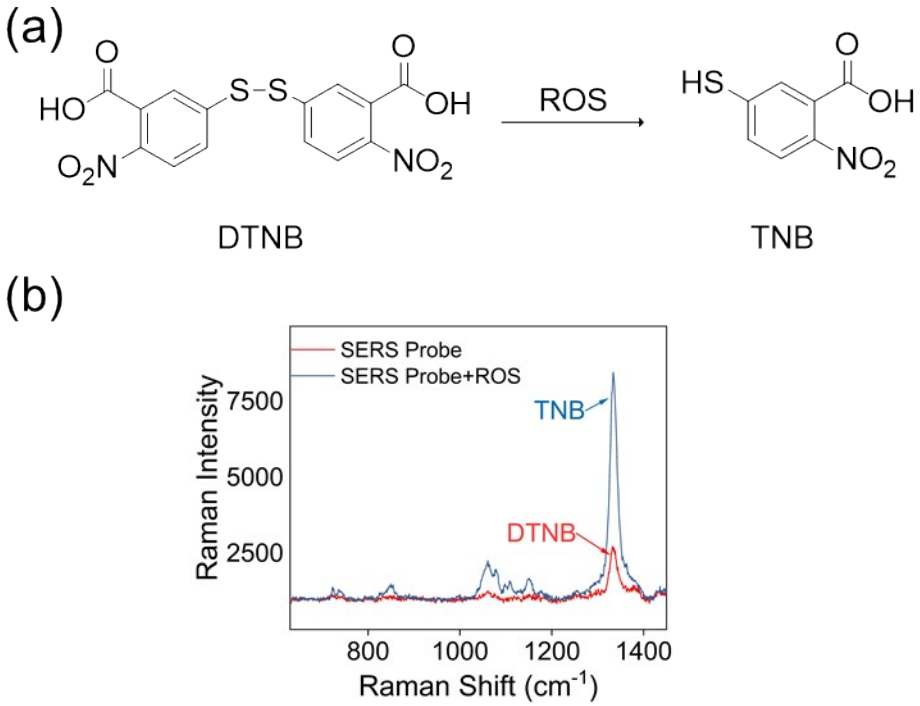

Fig. S1 (a) Illustration of the oxidation of DTNB on SERS probe by ROS to produce TNB. (b) Spectral changes of the SERS probe under ROS conditions.

Ref.

1. K. R. Williams, *J. Chem. Educ*. 2000, **77**, 626.

2. Y. Wang, S. Wu, Y. Yang, Y. Yang, H. Liu, Y. Chen and H. Ju, *Chem. Sci.*, 2024, **15**, 3901-3906.

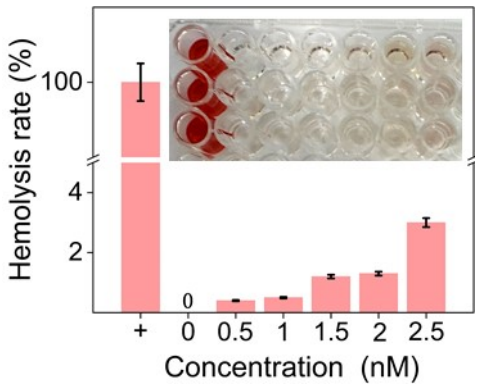

Fig. S2 In vitro haemolytic assessment of SERS probe.

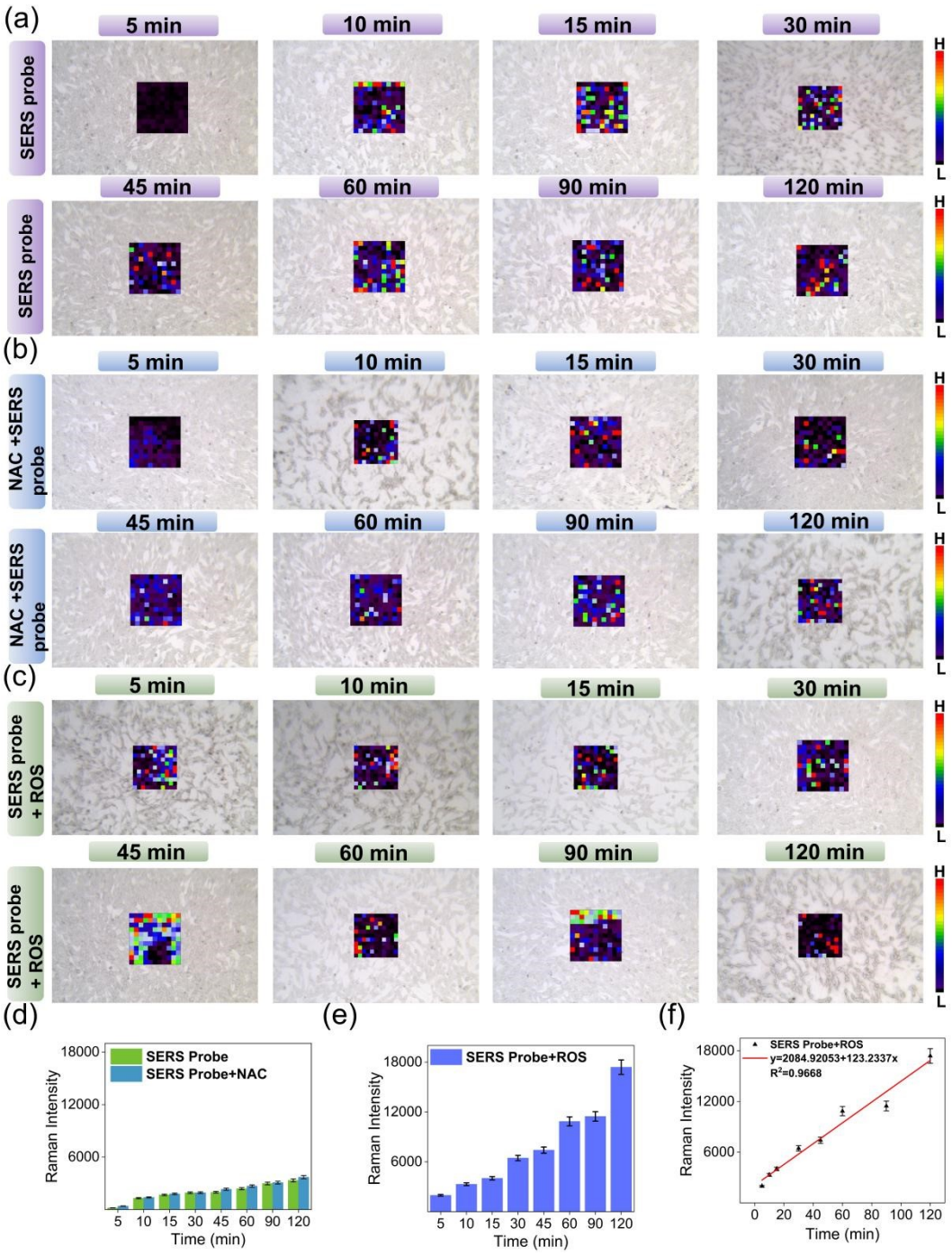

Fig. S3 (a) SERS mapping images of bEnd.3 cells after interaction with SERS probe at different incubation time points. (b) SERS mapping images of bEnd.3 cells co-treated with SERS probe at different time points after NAC pretreatment. (c) SERS mapping images of SERS probe co-treated with ROS for bEnd.3 cells

at different time points. (d) and (e) Quantitative analysis of Raman signal intensity for group a, b and c at different time points, respectively. (f) Calibration curve of SERS signal intensity at different time points in group c.