Supplementary Materials for

Synergistic NIR-Polydopamine Nanotherapy for Blood–Brain Barrier Crossing and Parkinson's Disease Intervention

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Supplementary Materials

Cell counting kit-8 (CCK-8) and ROS assay kit were obtained from Shanghai Beyotime Biotech., Inc., China. BAX, Bcl-2, IL-10, α-syn, β-actin, TH, NF, GFAP, IL-6, IL-1β, and TNF-α polyclonal antibodies were purchased from Proteintech Group, Inc. Ultrapure water (18.2 MΩ cm^{-1} at 25°C) was prepared using the Milli-Q system (Millipore, USA). All the other chemicals and reagents were of analytical grade or higher.

Supplementary Methods: Animal Procurement, Housing

C57BL/6J mice (SPF grade, 6-8 weeks, 18-22 g, male) were purchased from SPF Biotechnology Co. (Beijing, China), which were maintained at the Animal Research Center of the Chinese Academy of Sciences under constant temperature and humidity. After 7 d acclimatization period, PD models were established.

Supporting Information

Section S1.1 Physicochemical Characterization

The particle size and zeta potential of ZnO, ZnO@PDA, and PDA were tested under the following conditions: a mobility range of ±10 μcm/Vs, conductivity range of 0–200 mS/cm, and temperature range of 2–90°C. Optical absorption properties were measured using UV2600 UV-Vis spectroscopy (Shimadzu Corporation, Japan). Measurements were conducted at 35°C with humidity of 30-80%, scanning from 200 to 900 nm. The chemical composition and molecular structure were analyzed by FTIR (Thermo Fisher Niolet iN10, USA). The background spectrum was collected prior to sample measurement, with a resolution of 4 cm^{-1}, 32 scans, and a wavenumber range of 400/600-4000 cm^{-1}. The crystal structures of ZnO and ZnO@PDA were characterized by X-ray diffraction (XRD, Rigaku SmartLab SE). The morphology and elemental distribution were observed by XPS (Thermo Scientific K-Alpha, USA) and TEM, respectively.

Section S1.2 ROS Scavenging Activity of ZnO@PDA

ZnO@PDA solution (0-1 mg/mL, 0.2 mL) was mixed with 1.8 mL of 0.1 mM DPPH ethanol solution. After incubation at 37°C in the dark for 30 min, absorbance spectra (200-800 nm) were recorded using a Shimadzu UV2600 spectrophotometer under controlled conditions (15-35°C, 30-80% humidity). The radical-scavenging capacity was calculated based on the reduction in absorbance at 517 nm. For ·OH scavenging assay, the reaction system contained: 1.7 mL acetate buffer (pH 4.6), 0.1 mL ZnO@PDA dispersion (0-1 mg/mL), 0.1 mL 100 mM H_2O_2, and 0.1 mL of 100 mM TMB. After 3 h of incubation at room temperature, the blue reaction product was acidified with 0.2 mL 6 M HCl (yielding yellow color) and spectrophotometrically measured. For the $O_2{\cdot}^-$ scavenging assay, a WST-8-based superoxide dismutase assay kit was used: 160 μL

working solution, 20 μL sample solution (0-1 mg/mL), and 20 μL starter solution. After 30 min of incubation, absorbance was measured at 450 nm using a SPARK 10M microplate reader. The scavenging activity was determined from the kinetic curves.

Section S1.3 Assessing Cell Viability

PC12 cells were seeded into 96-well plates and incubated overnight (37°C, 5% CO_2). A 6-OHDA solution was prepared in 0.9% NaCl containing 0.2% ascorbic acid, at concentrations ranging from 0 to 500 μmol/L. After removal of the culture medium, the cells were treated with various concentrations of 6-OHDA for 24 h. Cell viability was assessed using the CCK-8 assay (10 μL/well, 1 h incubation), and absorbance was measured at 450 nm. The concentration of 6-OHDA that induced approximately 50% cell death was selected for subsequent experiments. To evaluate the protective effects of ZnO@PDA, cells were pretreated with ZnO@PDA (10–300 μg/mL) for 6 h prior to 24 h exposure to a predetermined concentration of 6-OHDA. Then, 10 μL/well of CCK-8 reagent was added and incubated for 1 h. Absorbance was measured at 450 nm. The concentration of ZnO@PDA that showed the greatest cytoprotective effect was selected for further studies.

Section S1.4 Cellular Uptake Assay

PC12 cells were seeded in 12-well plates and incubated overnight (37°C, 5% CO_2). The medium was replaced with fresh medium containing: 150 μg/mL Cy5-ZnO@PDA (control group) and 150 μg/mL Cy5-ZnO@PDA + NIR (808 nm laser, 0.3 W/cm² for 5 min, experimental group). Cells were incubated for 1 h. Subsequently, the cells were fixed with 4% paraformaldehyde for 15 min, followed by 5 μg/mL phalloidin solution for 15 min and 5 μg/mL DAPI solution for 15 min

(protected from light). Samples were visualized using a confocal fluorescence microscope. Fluorescence stability was evaluated (Figure S4-6).

Section S1.5 Flow Cytometry Assays

PC12 cells were seeded in a 6-well plate and divided into four groups: control, 6-OHDA, 6-OHDA + ZnO@PDA, and 6-OHDA + ZnO@PDA + NIR (808 nm laser, 0.3 W/cm², 5 min). After 12 h of drug treatment, cells were washed twice with ice-cold 1× PBS, digested with trypsin, and collected in sterile flow cytometry tubes. and centrifuged at 800 g for 6 min. Next, the supernatant was discarded, the cells were resuspended in 1X PBS, centrifuged again, resuspended in 1 mL PBS, and finally stained with the fluorescent probe (CM-H2 DCFDA) at 37°C for 30 min. The mean fluorescence intensity of cells in each group was determined using flow cytometry.

Section S1.6 Cell Apoptosis Detection

PC12 cells were allocated to four treatment groups as previously described. Following EDTA-free trypsinization and centrifugation, the cells were washed with PBS and resuspended in 100 μL of Annexin V Binding Buffer (1×). The cell suspension was stained with 5 μL Annexin V-AbFlourTM 488 and 2 μL propidium iodide (PI) for 15 min at room temperature in the dark. After adding 400 μL of binding buffer, the samples were analyzed by flow cytometry within 30 min of staining using standard FITC (excitation/emission: 491/517 nm) and PI (535/617 nm) detection channels within 30 min of staining.

Section S1.7 *In Vivo* Therapy

Fifteen PD model mice were randomly divided into five groups (n = 3 per group) and treated with intravenous ZnO@PDA (0.1 mL, 1 mg/mL) every three days for 30 days. The experimental groups were ZnO@PDA + NIR + US (NM + L + U), ZnO@PDA + US + NIR (NM + U + L), ZnO@PDA + NIR (NM + L), ZnO@PDA + US (NM + U), and ZnO@PDA only (NM). The treatment protocols were as follows: 1) NM + L + U group: ZnO@PDA was intravenously injected into the tail vein of mice. After 30 minutes, the treated area was irradiated with an 808 nm laser for 5 minutes (1.0 W/cm²), followed by ultrasound treatment for 10 minutes. 2) NM + U + L group: ZnO@PDA was intravenously injected into the tail vein of mice. After 30 minutes, ultrasound treatment was administered for 10 minutes, followed by irradiation with an 808 nm laser for 5 minutes (1.0 W/cm²). 3) NM + L group: ZnO@PDA was intravenously injected into the tail vein of mice. After 30 minutes, the treated area was irradiated with an 808 nm laser for 5 minutes (1.0 W/cm²). 4) NM + U group: ZnO@PDA was intravenously injected into the tail vein of mice. After 30 minutes, ultrasound treatment was administered for 10 minutes. 5) NM group: ZnO@PDA was intravenously injected into the tail vein of mice. This treatment regimen was repeated for all groups 4 hours post-administration. Three healthy mice were randomly selected as the controls.

Supporting Figure

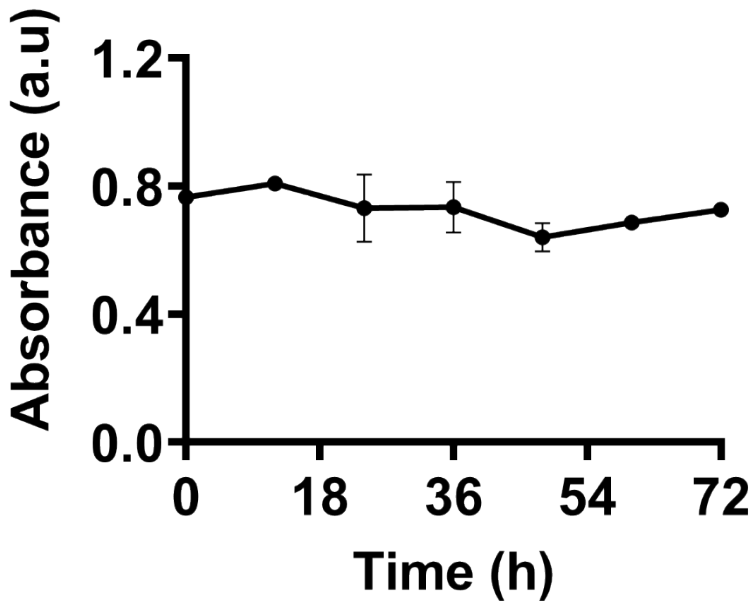

Figure S1. Stability assay of ZnO@PDA in serum over a period of 72 hours.

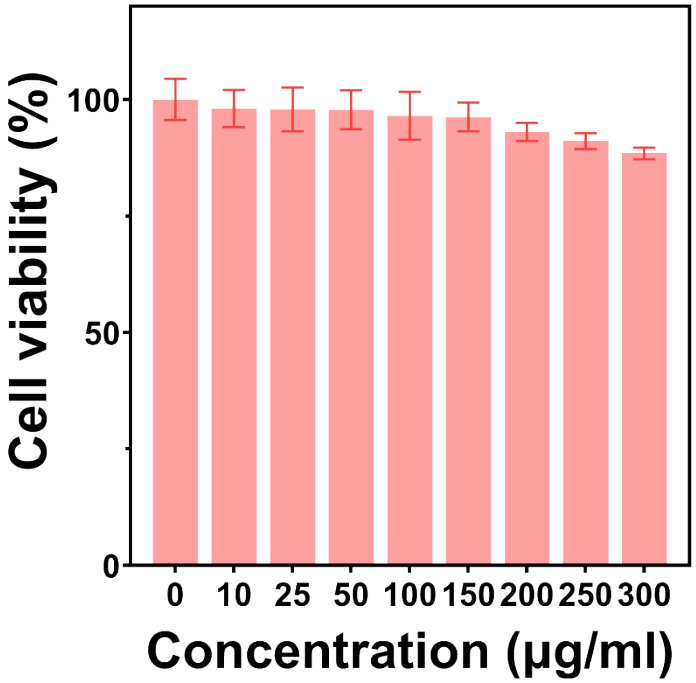

Figure S2. HUVECs after incubation with ZnO@PDA for 24 h by CCK8 assay.

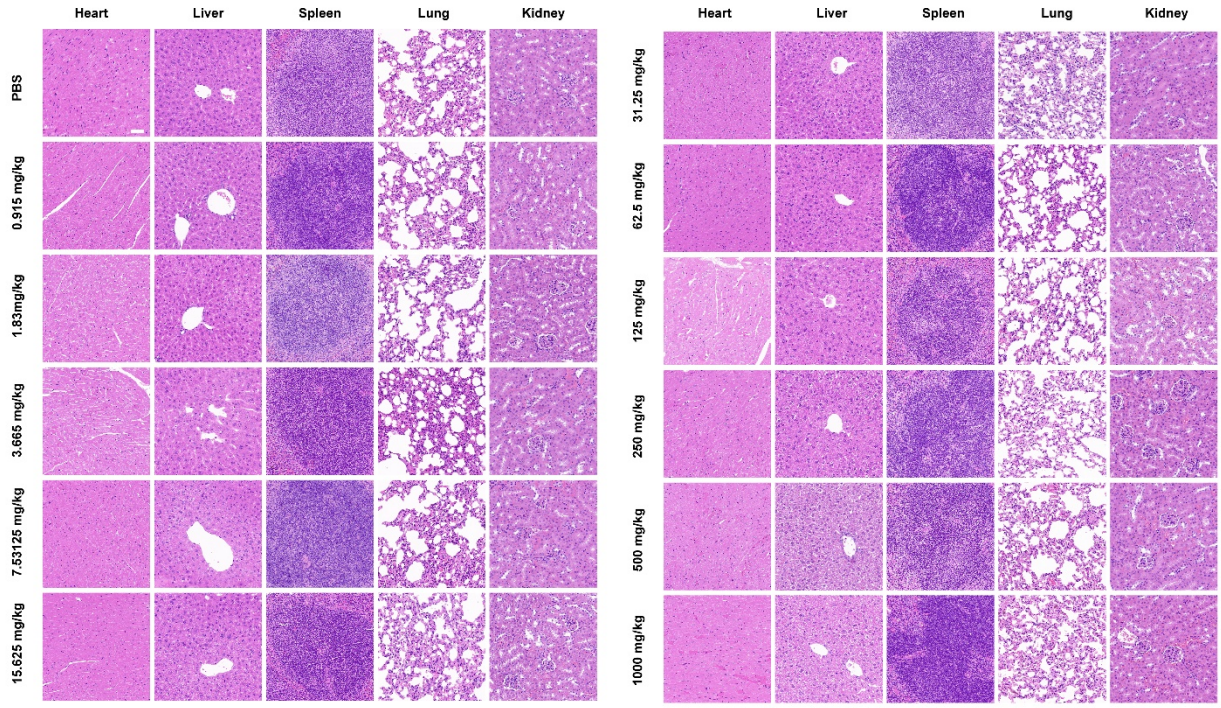

Figure S3. Systematic dose-response study of ZnO@PDA in healthy mice.

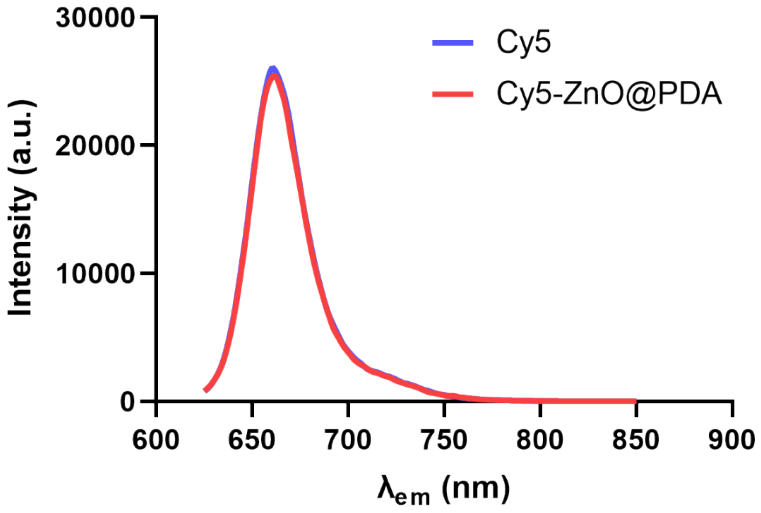

Figure S4. Fluorescence spectra of Cy5 and Cy5-ZnO@PDA at the same concentration at the initial stage.

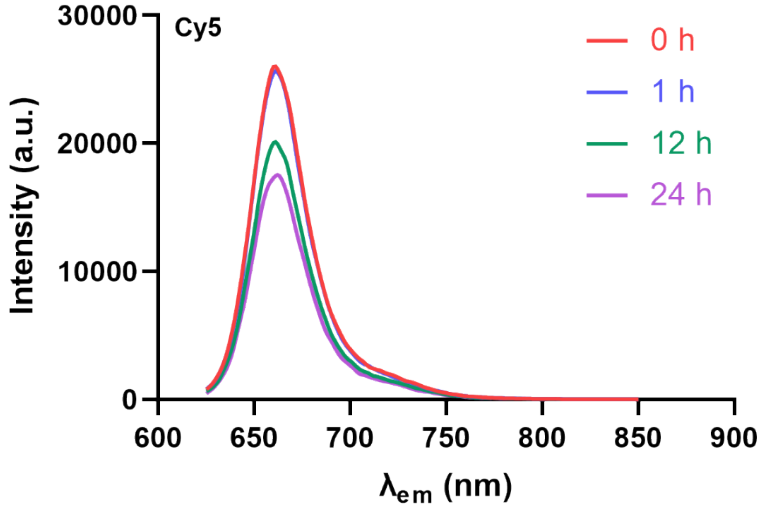

Figure S5. Fluorescence spectra of Cy5 at different time points (0h, 1h, 12h, and 24h).

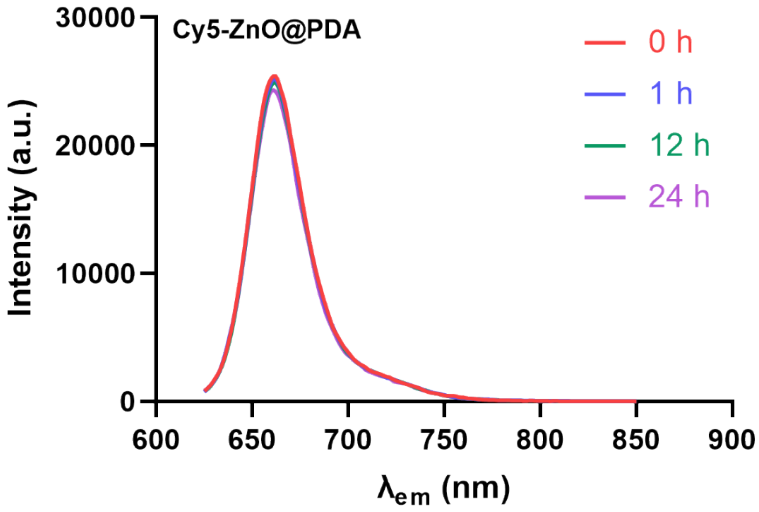

Figure S6. Fluorescence spectra of Cy5-ZnO@PDA at different time points (0h, 1h, 12h, and 24h).