Electronic Supplementary Information

Silica/Porphyrin Hybrid Nanotubes for \textit{In Vivo} Cell Tracking by Near-Infrared Fluorescence Imaging

Koichiro Hayashi,* Michihiro Nakamura and Kazunori Ishimura

Department of Anatomy and Cell Biology
Institute of Health Biosciences
The University of Tokushima Graduate School
3-18-15 Kuramoto-cho, Tokushima, 770-8503, Japan
E-mail: khayashi@basic.med.tokushima-u.ac.jp
**EXPERIMENTAL SECTION**

**Synthesis of Silica/TCPP HNTs.** TMAPS (2 mM) and TCPP (1 mM) were dissolved in ethanol-water (29/1) mixed solvent. Aqueous sodium hydroxide (1.25 mM) was added to the solution with stirred. TEOS was added to the solution with stirring, and the reaction solution was stirred overnight at room temperature. The product was collected by centrifugation and then washed with three times with ethanol and then water.

**Synthesis of TCPP-Containing Silica NSs.** TCPP-containing silica NSs were synthesized under the same conditions as the silica/porphyrin HNTs except the reaction temperature was 80°C.

**Characterization.** SEM and TEM images of the silica/TCPP HNTs and the TCPP-containing silica NSs were obtained on a JSM-6700F electron microscope (JEOL, Tokyo, Japan) and a H-7650 electron microscope (Hitachi, Tokyo, Japan). FTIR spectra of TEOS, TMAPS, TCPP and the silica/TCPP HNTs were obtained using a FTIR-6200 instrument (JASCO, Tokyo, Japan). The hydrodynamic diameter in PBS of the silica/TCPP HNTs was measured by DLS (NICOMP 380 ZLS, Showa Denko, Tokyo, Japan). The amount of TCPP in the silica/TCPP HNTs was measured by differential thermal analysis-thermogravimetry (DTA-TG, Rigaku, TG 8120, Tokyo, Japan). The absorption spectra of TCPP, silica/TCPP HNTs and TCPP-containing silica NSs were measured using ultraviolet-visible (UV-vis) spectrophotometer (Hitachi, U-3000, Tokyo, Japan). The fluorescent spectra of TCPP, TCPP-containing silica NSs and the silica/TCPP HNTs were measured using a fluorescence spectrophotometer (F-2500, Hitachi, Tokyo, Japan). Fluorescence images of the silica/TCPP HNTs were obtained using the IVIS® Imaging System (Caliper Life Sciences, Hopkinton, MA).

**Animals.** C57BL/6 Alb Hr mice were used for the studies on toxicity and fluorescence imaging. All experiments were approved by the Committee on animals of the University of Tokushima.

**Cytotoxicity Assay.** Primary macrophages directly isolated from mice were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum using a humidified incubator in air containing 5% CO₂ at 37°C. The macrophages were plated in 96-well plates at a concentration of 2×10⁵ cells per well and treated with the silica/TCPP HNTs diluted in RPMI 1640 at 37°C. The macrophages were treated with the HNTs for 24 h. After 24 h, the fraction of macrophages surviving was determined by WST-1 assay.
**In Vivo Toxicity Assay (Biochemical Assay).** Phosphate buffered saline (PBS) and the silica/TCPP HNTs were intravenously injected into mice. A week after injection, the blood samples were collected and the serum was obtained by centrifugation of the whole blood at 3000 rpm for 15 min. Liver function was evaluated based on the serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Nephrotoxicity was determined by blood urea nitrogen (BUN). These biochemical parameters were determined by an automated biochemical analyzer (Hitachi 7180, Tokyo, Japan).

**Labeling of Macrophages with Silica/TCPP HNTs.** Primary macrophages (5×10^5 cells) directly isolated from live mice were plated a dish and were treated with 100 µg of the silica/TCPP HNTs for 1 h. The dish was washed with PBS several times to remove the HNTs outside the cells. Cellular uptake of the HNTs was observed using fluorescence microscopy (TE2000 fluorescent microscope Nikon, Kanagawa, Japan). The macrophages were stained with DAPI showed blue fluorescence under ultraviolet excitation. The fluorescence derived from the HNTs was observed using 635–675 nm excitation and 696–736 nm emission filters.

**Minimum Detectable Number of Cells In Vivo by Fluorescence Imaging.** The silica/TCPP HNTs-labeled macrophages were collected by detaching the cells from a dish using a cell dissociation solution (Sigma-Aldrich, St. Louis, MO) and centrifuging the cell suspension. The collected cells were diluted with PBS to suspensions containing 2×10^5 cells, 2×10^4 cells, 2×10^3 cells and 2×10^2 cells. These suspensions were subcutaneously administered to mice. The cells were detected by fluorescence imaging using the IVIS® 200 Imaging System (λ_ex=660–690 nm and λ_em=730–750 nm).

**In Vivo Cell Tracking.** The silica/TCPP HNTs-labeled macrophages were collected by detaching the cells from a dish using a cell dissociation solution and centrifuging the cell suspension. The collected cells (1×10^6 cells) were resuspended in PBS and intravenously injected into the cell donor mice. The distribution of the cells was observed by *in vivo* and *ex vivo* fluorescence imaging using IVIS Spectrum (λ_ex=660–690 nm and λ_em=730–750 nm).
Figure S1. Chemical structures of (a) TMAPS, (b) TCPP and (c) the TCPP anion. (d) Schematic representation of silica/TCPP HNTs.
Figure S2. FTIR spectra of (a) TEOS, (b) TMAPS, (c) TCPP and (d) silica/TCPP HNTs.
Figure S3. Transmission electron microscopy images of silica/TCPP HNTs.
Figure S4. Transmission electron microscopy image of TCPP-containing silica NSs: scale bar 2 \( \mu \)m. (Inset) Magnification: scale bar 100 nm.
Figure S5. DLS spectrum of silica/TCPP HNTs in PBS. (Inset) Photograph of silica/TCPP HNTs in PBS.
Figure S6. DTA and TG curve of silica/TCPP HNTs.
Figure S7. (a) Absorption spectra of TCPP (black) and silica/TCPP HNTs (red) in PBS. (b) Fluorescence spectra of TCPP (black) and silica/TCPP HNTs (red) in PBS. TCPP and the silica/TCPP HNTs were photoexcited at 635 nm and 670 nm, respectively. (c) Photograph and fluorescence images of silica/TCPP HNTs in PBS: (from the left) photograph, \( \lambda_{\text{em}} = 710–730 \) nm, 730–750 nm, 750–770 nm, 770–790 nm and 790–810 nm (\( \lambda_{\text{ex}} = 660–690 \) nm in all fluorescence images).
Figure S8. (a) Absorption spectrum of TCPP-containing silica NSs in PBS. (b) Fluorescence spectrum of TCPP-containing silica NSs in PBS: $\lambda_{ex} = 658$ nm. 
Figure S9. Cytotoxicity of silica/TCPP HNTs.
Figure S10. Biochemical assays in serum of mice injected with PBS and silica/TCPP HNTs (n=5). A week after injection, (a) the BUN, (b) AST, (c) ALT and (d) ALP values were measured.
Figure S11. Bright-field images of (a) intact macrophages and (e) silica/TCPP HNTs-labeled macrophages. Fluorescent images of (b, c) intact macrophages and (f, g) silica/TCPP HNTs-labeled macrophages: (b, f) $\lambda_{\text{ex}}=330–380$ nm and (f, g) $\lambda_{\text{ex}}=635–675$ nm. (d) Overlayed images of (b) and (c). (e) Overlayed images of (f) and (g).
Figure S12. Dependence of autofluorescence on wavelength. (a, b, e) Dorsal and (c, d, f) ventral fluorescence images of a mouse: (a, c, e, f) $\lambda_{\text{ex}}=625–655$ nm and $\lambda_{\text{em}}=670–690$ nm; (b, d) $\lambda_{\text{ex}}=660–690$ nm and $\lambda_{\text{em}}=730–750$ nm. The dependence of (g) total radiant efficiency (RE) and (h) average RE on wavelength: (red) dorsal side and (blue) ventral side.
Figure S13. *In vivo* image of mouse after subcutaneous injection of $2 \times 10^2$ cells, $2 \times 10^3$ cells, $2 \times 10^4$ cells and $2 \times 10^5$ cells of silica/TCPP HNTs-labeled macrophages to different areas.