Supporting Information

One-Pot Microwave Synthesis of Water-Dispersible, High Fluorescence Silicon Nanoparticles and Their Imaging Applications *in Vitro* and *in Vivo*

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References

Experimental Section

Chemicals and Reagents. (3-Aminopropyl) triethoxysilane (APTES, 98%), trisodium citrate dehydrate (SC, ≥ 99.0%), glycerol (GEL), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), hydrochloric acid (HCl), sodium hydroxide (NaOH), fluorescein isothiocyanate (FITC), were purchased from J&K Scientific Ltd. (Beijing, China). Human cervical carcinoma cell lines (HeLa), and wild-type zebrafish were supplied by Nankai University School of Medicine. A Cell Counting Kit-8 (CCK-8, Dojindo, Japan) was used to measure the cytotoxicity of the as-prepared SiNPs. 4',6-diamidino-2-phenylindole (DAPI, Sigma) was used to stained the nuclei of HeLa cells. All chemicals were analytical grade and employed without additional purification. Milli-Q water (Millipore, 18.2 MΩ.cm) was utilized to dilute the solution throughout.

Instrumentations. Transmission electron microscopy (TEM), high resolution transmission electron microscopy (HRTEM) and selected-area electron diffraction (SAED) were performed on a Tecnai G^2 F20 transmission electron microscope at an accelerating voltage of 200 kV (FEI, The Netherlands). Dynamic light scatter (DLS) data were measured by a nanoparticle analyzer Nano-ZS instrument (Malvern, Britain). Fourier transform infrared spectroscopy (FTIR) spectrum (4000-400 cm^{-1}) was recorded by a Vector 22 FTIR spectrophotometer (Bruker, Germany). X-ray photoelectron spectrometry (XPS) data were measured by an Axis Ultra DLD spectrometer equipped with a mono chromatic Al Ka X-ray source ($h\nu$=1486.6 eV), hybrid (magnetic/electrostatic) optics and a multichannel plate and delay line detector (Kratos Analytical Co., UK). Inductive coupled plasma emission spectrometer (ICP) (Thermo, USA) was used to measure the content of the Silicon element in the as-prepared SiNPs. A UV-2450 spectrophotometer (Shimadzu, Japan) and a fluorescence spectrophotometer (F-4500, Hitachi, Japan) were employed for recording UV-visible (UV-vis) absorption spectrum and photoluminescence (PL) spectra, respectively. The value of absolute photoluminescence quantum yield (APLQY) was measured and calculated by an FLS-920 spectrophotometer (Edinburgh, UK) equipped with an integration sphere attachment under setting 440 nm as the excitation wavelength. Likewise, photoluminescence lifetime decay curves were obtained and fitted by the FLS-920 instrument using 405 nm lasers as the

excitation light. Two-photon excited fluorescence spectra were measured using a Chameleon Ultra II: Ti: sapphire femtosecond laser (Coherent, USA) as exciting light source (800 nm) with a pulse width of 140 fs and a repetition rate of 80 MHz. USB2000 (Ocean Optics Inc., USA) was employed as the recorder. Microwave reactor was purchased from Xi'an Yu Hui instrument co., Ltd (Shaanxi, China). All optical measurements were utilized at room temperature. The bright-field photographs and the fluorescence images of the HeLa cells were captured with an FV 1000 confocal microscope (Olympus, Japan) through setting 458 nm as the excitation wavelength with the emission window of 500-600 nm. The morphological photographs of HeLa cells were monitored under inverted phase contrast microscope (IX71, Olympus, Japan). The images of embryos or larvae were captured with a DP72 digital camera mounted on an SZX16 dissecting microscope (Olympus, Japan). The fluorescence photographs of embryos and larvae were captured with a DP71 digital camera mounted on a BX51 fluorescence microscope (Olympus, Japan).

The pH Stability of the As-prepared SiNPs. 10 mM phosphate buffer with different pH values from 5 to 9 were utilized to estimate the stability of the as-prepared SiNPs. HCl and NaOH were adopted for adjusting the wide pH values from 2 to 11. A fluorescence spectrophotometer was employed to record the PL intensity of the as-prepared SiNPs with the various pH values.

Cell Culture. HeLa cells were incubated in complete Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) and 1% penicillin-streptomycin.

Cytotoxicity Assay. The cytotoxicity against HeLa cells was evaluated by CCK8 assay according to the manufacturer's protocols. Briefly, the cells were seeded onto 96-well plates at a density of 5×10^3 cells per well and treated with 100 μL DMEM or SiNPs (50 and 100 μg/mL) for 4, 12, 24 and 48 h, respectively. Five parallel replicates of each sample were measured simultaneously. The morphology of HeLa cells were investigated after being treated with the as-prepared SiNPs. The detailed steps were as follows. The cells were seeded into 6-well plates at 5×10^4 cells per well including 2 mL complete DMEM and allowed to attach at 37 ℃ for 24 h in CO_2 incubator. Then, HeLa cells were incubated with 100 μg/ml SiNPs for

0, 4, 12, 24 and 48 h, respectively. The free-SiNPs groups, marked as the control, were incubated at the same conditions.

***In vitro* Bioimaging of the As-prepared SiNPs.** HeLa cells were seeded onto 24-well plates with a density of 5×10^4 cells per well. The cells were incubated with different concentrations of SiNPs-complete DMEM (.i.e. 100, 50, 25, and 12.5 μg/mL) at 37 ℃ for 4 h. Free-SiNPs cells were used as the control groups. The cells were washed three times with phosphate-buffered saline (PBS, pH 7.2) for 10 minutes each time, fixed in 4% paraformaldehyde (PFA) for 15 minutes at room temperature, stained the nuclei with 4',6-diamidino-2-phenylindole (DAPI, diluted at 1:1000; Sigma) for 5 minutes, and finally sealed with mounting media on glass coverslips. Images of the cell fluorescence were captured with an FV 1000 confocal microscope (Olympus, Japan). The excitation wavelength of DAPI and SiNPs were 405nm and 458 nm ,and the emission window were 425-475nm and 500-600 nm, respectively.

Zebrafish Maintenance. Wild-type zebrafish (AB strain) was used as the animal model in this study. According to Westerfield's method,[2] the zebrafish were raised at 28.5 ^{o}C for a 10/14-hour dark/light cycle. After removing feces and unfertilized eggs, the embryos were incubated in E3 medium (5 mmol/L NaCl, 0.17 mmol/L KCl, 0.33 mmol/L $CaCl_2$, 0.33 mmol/L $MgSO_4$, pH 7.2) at 28.5 ^{o}C, and developmentally staged by hours post fertilization (hpf). All of the procedures involving animals were approved by the Institutional Animal Care Committee of Nankai University.

Toxicity Test and Bioimaging on Zebrafish. Aqueous exposure to SiNPs on zebrafish was performed according to Organization for Economic Co-operation and Development guidelines.[1] At one- to four-cell stage, embryos were placed into 24-well plates with 50 embryos in each well. Three exposed groups were treated continuously with SiNPs-E3 medium at concentrations of 15, 7.5 and 3.75 μg/mL in dark. The same number of embryos was incubated only in E3 medium as the unexposed group. After 3 hours' incubation, the embryos were washed three times with E3 medium for five minutes each time and returned to E3 medium. The survival rates and hatching rates of the embryos were counted at 0, 12, 24, 36 hpf and 48, 60, 72 hpf, respectively. For *in vivo* bioimaging, one- to four-cell stage

embryos were exposed continuously to SiNPs-E3 medium in dark with the as-prepared SiNPs concentration of 7.5 μg/mL for 4 hpf. Starting from 12 hpf, embryos were grown in SiNPs-E3 medium containing 0.003% 1-phenyl-2-thiourea in dark to block pigmentation, and mediated visualization till 72 hpf. Each experiment was repeated 3 times as previously described.

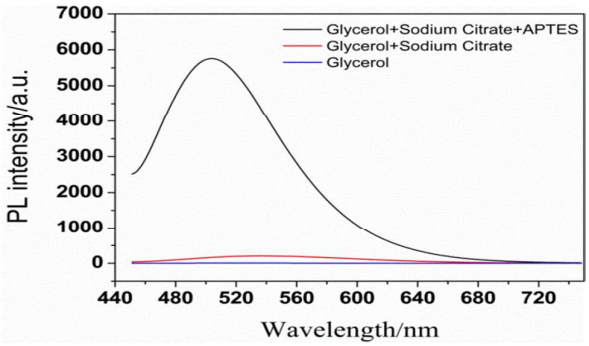

Figure S1. The control experiments.

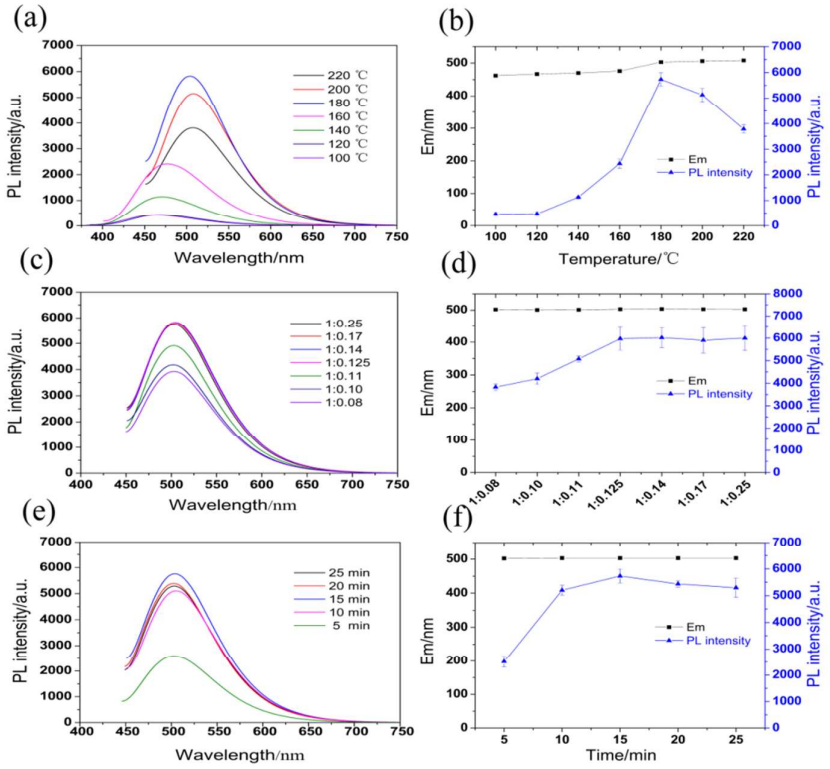

Figure S2. The optimization of reaction conditions. (a, b) Reaction temperature; (c, d) The molar ratio of APTES and citrate; (e, f) Reaction time.

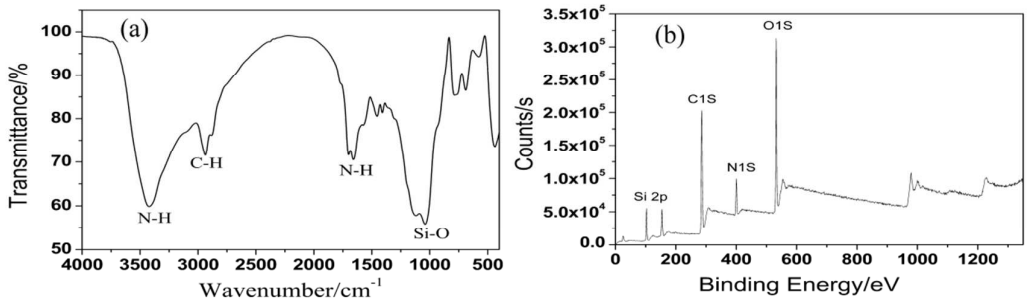

Figure S3. (a) FTIR spectrum and (b) the survey XPS spectrum of the as-prepared SiNPs.

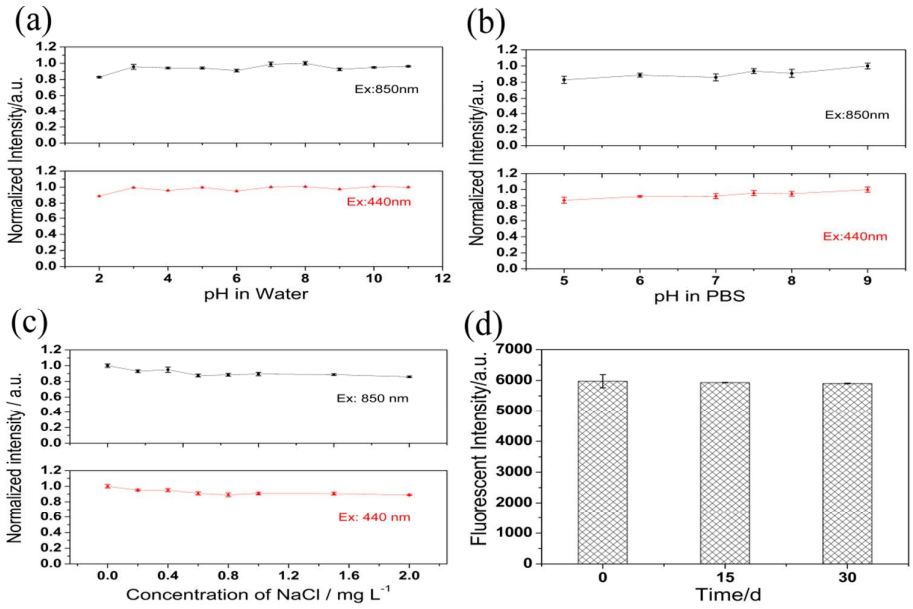

Figure S4. The stability of the as-prepared SiNPs. (a) pH stability in water; (b) pH stability in PBS; (c) Salt effect; (d) Storage stability.

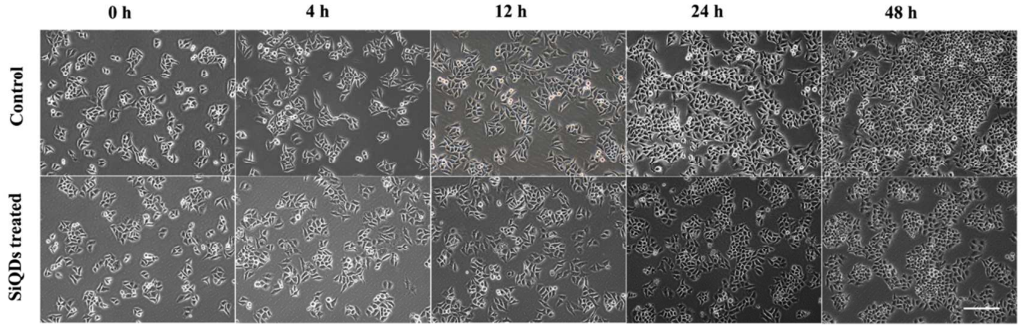

Figure S5. The morphology images of HeLa cells treated with the as-prepared SiNPs (100 μg/mL) by phase contrast microscope at different time points (0, 4, 12, 24, 48 h). Scale bar: 200 μm.

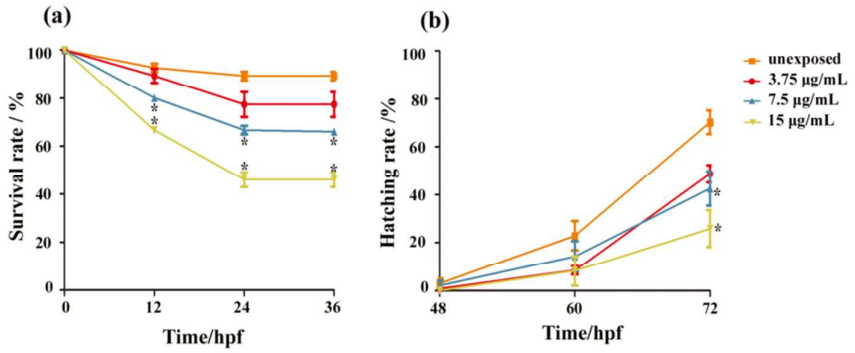

Figure S6. Statistical analysis of (a) the average survival rates and (b) hatching rates of the zebrafish embryos following aqueous exposure to SiNPs.

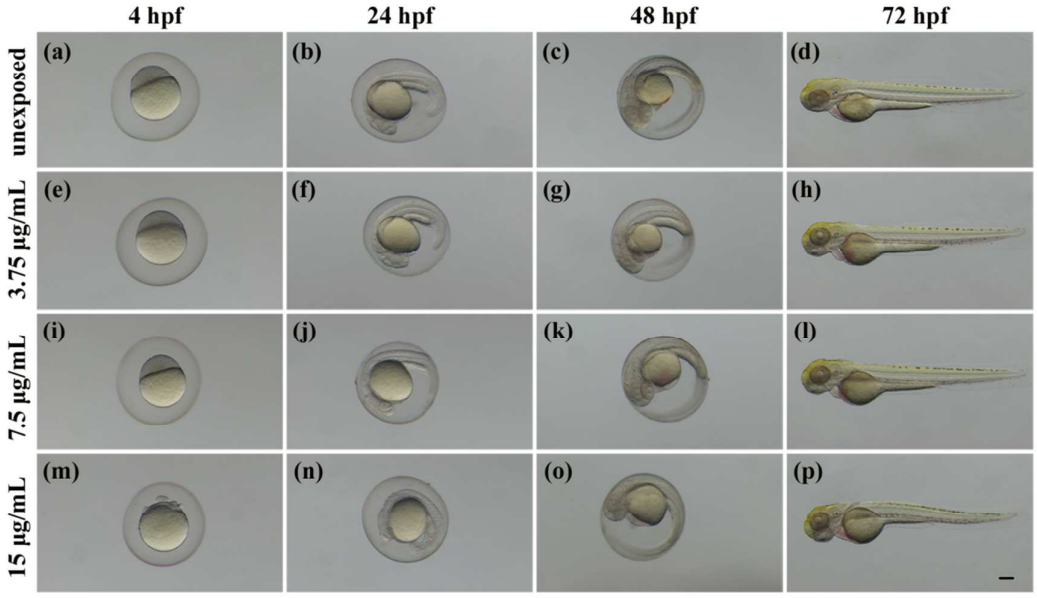

Figure S7. Phenotypes of the zebrafish embryos following aqueous exposure to the as-prepared SiNPs. Notes: (a-p) Phenotypes of embryos from 4 to 72 hpf in (a-d) the unexposed group and SiNPs-exposed groups at concentrations of (e-h) 3.75 μg/mL, (i-l) 7.5 μg/mL, and (m-p) 15 μg/mL. Dorsal is up and rostral is left in (d, h, l and p). Scale bar: 200 μm.

References

(1) Buschmann. *Methods Mol. Biol.* **2013,** 947, 37-56.

(2) Westerfield, M. *Univ. of Oregon Press, 4th ed.*; Eugene: **2000**.