SUPPORTING INFORMATION

Enzymatically Degradable Hybrid Organic-Inorganic Bridged Silsesquioxane Nanoparticles for In-Vitro Imaging

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I. GENERAL PROCEDURES

I.1- Materials. Absolute ethanol, 3-aminopropyltriethoxysilane (APTES), sodium hydroxide, fluorescein 5(6)-isothiocyanate (FITC), trimethylamine (TEA) and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich. Oxaly chloride was obtained from Alfa Aesar. Anhydrous dichloromethane (DCM) was purchased from Acros. All chemicals were used without further purification. Deionized water was used in all synthetic experiments. The human cervical tumor cell line (HeLa) was purchased from ATCC (USA). Eagle’s MEM medium (EMEM), phosphate buffered solutions (PBS, pH 7.4), fetal bovine serum (FBS), penicillin-streptomycin, paraformaldehyde, trypsin, 4,6-diamino-2-phenylindole (DAPI) were purchased from Invitrogen (USA).

I.2- Methods. $^1$H NMR and $^{13}$C NMR spectra were performed at 500 MHz with NB/CDCl$_3$ solutions at 5mg/ml with an Avance III Bruker Corporation instrument. Solid state NMR spectra were recorded using a Bruker WB Avance III HD spectrometer operating at magnetic field strength of 9.39 T, corresponding to Larmor frequency of 400 MHz for $^1$H nuclei, equipped with a Bruker 3.2 mm CP/MAS probehead spinning the sample at 15 kHz rate. $^{13}$C spectra were measured directly, while for $^{29}$Si cross-polarization technique was used with contact pulse durations of 2 ms. Chemical shifts were referenced to external TMS. IR spectra were recorded on a Thermo Scientific spectrometer (Nicolet iS10). Absorption spectra were recorded on a Varian Cary 5000 spectrophotometer and fluorescence data were collected on a Varian Cary Eclipse fluorimeter. Dynamic light scattering (DLS) and zeta-potential analyses were performed using a Malvern Nano ZS instrument. Transition electron microscopy (TEM) images were recorded with a Technai 12 T (FEI Co.) microscope operated at 120 kV. Scanning electron microscopy (SEM) images were recorded with a field emission Nova Nano 630 microscope (FEI Co.). Thermal gravimetric analyses were done on TG 209 F1 machine. The elemental mapping of particles was carried out with another TEM of model Titan G$^2$ 80-300CT from FEI Company (Hillsboro, OR) which was equipped with a post-column energy filter of model GIF tridium 863 from Gatan Inc. (Pleasanton, CA). The GIF was used in EELS mode in conjunction with HAADF-STEM. Moreover, Si-L$_{23}$ (99 eV), S-L$_{23}$ (165 eV), C-K (283 eV), N-K (401 eV) and O-K (532 eV)
energy-loss edges were utilized in making Si, S, C, N and O elemental maps, respectively. A
typical EELS spectrum showing the energy loss edges of these elements extracted from a
spectrum image dataset is shown in Figure S4. While the elemental maps of above listed elements
from a typical size NP is shown in Figure S5. Zeiss LSM 710 upright confocal microscope. N2
adsorption-desorption isotherm and corresponding pore size distribution were acquired to
characterize the mesoporous structure of BS NPs on a Micromeritics ASAP 2420 instrument.
II. SYNTHESIS AND CHARACTERIZATION OF THE BRIDGED ALKOXY SILANE PRECURSOR

II.1- Synthesis of OBA. First, a 50 mL round-bottom flask was dried under vacuum using a heat gun. Dry DCM (10 mL) was added to the flask via canula followed by the addition of TEA (0.42 mL, 3 mmol). Then, APTES (0.834 mL, 2.1 mmol) was added to this solution and flask cooled to 0°C in an ice bath. The DCM solution of oxalyl chloride was added dropwise (0.5 mL, 1 mmol, 2 M). After the complete addition, the reaction mixture was removed from the ice bath and stirred at room temperature for 1 h; the colorless solution gradually became yellowish. The solvent were evaporated under reduced pressure by a rotary evaporator. The obtained product was crystalline (475 mg, 95%) which is not characteristic for silanes and could be due to intermolecular hydrogen bonding reported previously for oxalic acid diamides.

![Figure S1. Synthetic pathway toward the bridged alkoxy silane precursor (OBA).](image)

II.2- Characterizations of OBA. $^1$H-NMR: (CDCl₃, 500 MHz) δ (ppm) 7.57 (s, J = Hz, 2H), 3.80 (q, J = 7 Hz, 12H), 3.29 (t, J = 6.7 Hz, 4H), 1.66 (m, J = 7.7 Hz, 4H), 1.20 (t, J = 7.1 Hz, 18H), 0.63 (t, J = 8.3 Hz, 4H). $^{13}$C-NMR: (CDCl₃, 125 MHz) δ (ppm) 159.9, 58.6, 42.1, 22.9, 18.3, 7.8. FTIR (cm⁻¹): 3317, 3017, 2973, 2936, 2895, 1668, 1522, 1449, 1371, 1276, 1203, 1110, 950, 783, 703, 572, 481. MS (ESI⁺) m/z (%): 496.3 (100) [MH⁺], 451.2 (55) [MH⁺ - one ethoxy fragment]. HRMS (ESI⁺): m/z calcd for C₂₀H₄₄N₂O₈Si₂: 496.2636, found 496.2636.

II.3- Synthesis of FITC-APTES.[Ⅲ] FITC (2.7 mg, 6.9 μmol) was dissolved in EtOH (1.5 mL) and APTES (6 μL, 25.6 μmol) was added. Reaction was conducted with magnetic stirring at room temperature during 2 h. The obtained product, FITC-APTES, was used directly without purification.
Figure S2. $^1$H (A) and $^{13}$C (B) NMR spectra of the OBA precursor.
Figure S3. FTIR spectrum of the OBA precursor.

III- SYNTHESIS AND CHARACTERIZATION OF BS AND BS-FITC NPS

III.1- Synthesis of BS NPs. The synthesis was performed according to a modified procedure of Croissant et al.\cite{2} A mixture of CTAB (6.4 mg, 17.5 µmol), distilled water (1.5 mL), ethanol (0.1 mL) and sodium hydroxide (NaOH, 6.68 µL, 1.28 M) was stirred at 75°C for 50 min at 1000 rpm in a 10 mL glass bottle. Then, the stirring speed was enhanced to 1400 rpm and ethanol solution of OBA precursor (0.3 mL, 48 µmol, 0.16 M) was added. After adding the bridged alkoxysilane the temperature was increased to 80°C. The condensation process was conducted for 2 h. Then, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and the NPs were collected by centrifugation during 10 min at 14000 rpm. The sample was washed twice with an alcoholic solution of ammonium nitrate (NH₄NO₃, 6 g/L in ethanol),\cite{3} and three times with ethanol, water, and ethanol. Each washing step involved a sonication of 10 min at 40°C; the collection was carried out in the same manner. The sample was finally dried under vacuum for few hours.
III.2- Synthesis of BS-FITC NPs. A mixture of CTAB (6.4 mg, 17.6 µmol), distilled water (1.5 mL), ethanol (0.1 mL) and sodium hydroxide (NaOH, 6.68 µL, 1.28 M) was stirred at 75°C for 50 min at 1000 rpm in a 10 mL glass bottle. Then, the stirring speed was enhanced to 1400 rpm and a mixture of alkoxy silanes was added (24 µmol of OBA in 150 µL of EtOH and 0.7 µmol of FITC-APTES in 150 µL of EtOH). After the addition the temperature was increased to 80°C, and the condensation process was conducted for 2 h. Then, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and the NPs were collected by centrifugation during 10 min at 14000 rpm. The sample was washed twice with an alcoholic solution of ammonium nitrate (NH₄NO₃, 6 g/L in ethanol),[3] and three times with ethanol, water, and ethanol. Each washing step involved a sonication of 10 min at 40°C; the collection was carried out in the same manner. The sample was finally dried under vacuum for few hours.

**Figure S4.** Typical background subtracted electron energy-loss spectrum of BS-FITC NPs.
Figure S5. STEM-EELS elemental mapping (silicon, oxygen, nitrogen, carbon, sulfur) of a representative BS NP spectrum image (top left). Scale bar of 50 nm.

Figure S6. FTIR spectra for the bridged alkoxy silane precursor, BS and BS-FITC NPs.
Figure S7. Solid state NMR of $^{29}$Si and $^{13}$C nuclei via CPMAS and HPDEC sequence in BS NPs. In the carbon spectrum, number indicate the followings: 1: Si-OCH$_2$CH$_3$, 2: HOCH$_2$CH$_3$, 3: CTAB residues.
Figure S8. Solid state NMR of $^{29}$Si and $^{13}$C nuclei via CPMAS and HPDEC sequence in BS-FITC NPs. In the carbon spectrum, asterisks indicate Si-OCH$_2$CH$_3$ peaks. FITC peaks are most likely in the noise of the spectrum because of the low content of the incorporated dye, of which the presence is unambiguously demonstrated by UV-Visible spectroscopy (Figure S15) and in-vitro imaging (Figure 5).
Figure S9. TGA analysis of BS NPs.

Figure S10. TGA analysis of BS-FITC NPs.
Figure S11. FTIR spectra for BS, BS-FITC NPs and CTAB.
Figure S12. TEM image of a representative BS NP at high magnification displaying the nanoscaled periodicity arising from the self-assembly of amide groups within the nanomaterial.
Figure S13. FTIR spectra for BS and enzymatically-degraded BS NPs, validating the cleavage of oxamide bonds as suggested by the decrease of its vibration modes.
Figure S14. TEM images of BS-FITC NPs in trypsin-PBS buffer after 24 h (A) and 48 h (B). DLS analyses of BS-FITC NPs before and after 24 and 48 h of enzymatic degradation (C).
**Figure S15.** UV-Visible spectra comparison of BS and BS-FITC NPs displaying the absorption of the dye at 505 nm in BS-FITC nanomaterial.

**Figure S16.** Zeta potential charge distributions of BA and BS-FITC NPs.
IV. BIOLOGICAL STUDIES OF BS NPs

Cells viability of HeLa cells treated with BS NPs. The cytotoxicity of silica incubated with HeLa cells were evaluated using the CCK-8 assay. Cells were seeded at a density of $1 \times 10^4$ cells per well in 96-well flat bottom plates and incubated with EMEM medium containing 10% FBS and 0.1% penicillin-streptomycin at 37°C in a humidified 5% CO$_2$ atmosphere for 12 h. After cell attachment, they were washed with DPBS and incubated with different concentrations (100, 10, 1, 0.1, 0.01, and $1 \times 10^{-3}$ μg/mL) of silica solutions in EMEM media for 24 h. Cell viability was evaluated by the CCK-8 colorimetric procedure.

![Figure S17. Cell cytotoxicity of HeLa cells incubated with BS NPs for 24 h.](image-url)
Figure S18. CLSM images (Z-stack gallery) of HeLa cells after incubation with BS NPs.
Figure S19. CLSM image (3D) of HeLa cells after incubation with BS NPs displaying the partial endocytosis and cell adhesion of BS NPs after 6 h of incubation.

V- REFERENCES