Supplementary Information Available

Conjugated oligoelectrolyte-polyhedral oligomeric silsesquioxane loaded pH-responsive nanoparticles for targeted fluorescence imaging of cancer cell nucleus

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Experimental Section

1. Synthesis and Characterization

1.1 Materials

COE-POSS was synthesized according to the literature.[1] Diamine-poly(ethylene glycol) (PEG-$(NH_2)_2$, M_w: 5000) was purchased from Laysan Bio, Inc. Chitosan (CS, M_w: 50000-190000), ethylenediaminetetraacetic acid (EDTA), glutaraldehyde, *N, N'*-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), folic acid (FA), triethylamine (TEA), penicillin-streptomycin solution, trypsin-EDTA solution and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Fetal bovine serum (FBS) was purchased from Gibco (Lige Technologies, Ag, Switzerland). LysoTracker Green DND-26 was purchased from Invitrogen. Milli-Q water was supplied by a Milli-Q Plus System (Millipore Corporation, Breford, USA).

[1] K. Y. Pu, K. Li, X. H. Zhang and B. Liu, *Adv. Mater.*, 2010, **22**, 4186-4189.

MCF-7 breast cancer cells and NIH/3T3 fibroblast cells were provided by American Type Culture Collection.

1.2 Characterization Methods

The fluorescence spectra were determined using a fluorometer (LS-55, Perkin Elmer, USA). The UV-vis spectra were recorded on a Shimadzu UV-1700 spectrometer. The average NP size and size distribution were measured using laser light scattering (LLS) with a particle size analyzer (90 Plus, Brookhaven Instruments Co. USA) at a fixed angle of 90° at room temperature. The zeta potential of NPs was measured using a zeta potential analyzer (ZetaPlus, Brookhaven Instruments Corporation) at room temperature. The morphology of NPs was studied by field-emission scanning electron microscopy (FESEM, JSM-6700F, JEOL, Japan) at an accelerating voltage of 10 kV. The sample was fixed on a stub with a double-sided sticky tape and then coated with a platinum layer using an autofine coater (JEOL, Tokyo, Japan) for 60 s in vacuum at a current intensity of 10 mA. The morphology of NPs was also investigated by transmission electron microscopy (TEM, JEM-2010F, JEOL, Japan).

1.3 Preparation of COE-POSS CS/PEG NPs

15 mg of CS were first dissolved in 10 mL of acetic acid aqueous solution. 30 mg of PEG-$(NH_2)_2$, 0.1 mg of COE-POSS, 10 mg of EDTA, and 1mL of ethanol were added to the CS aqueous solution, respectively, to yield a solution with pH ~ 3. Subsequently, 0.2 N NaOH solution was added dropwise to the mixture under vigorous stirring, and the clear solution turned bluish when the pH was adjusted to 6. 7.5 μL of glutaraldehyde solution (50%) were then added to cross-link the obtained NPs at room temperature. The cross-linked NP suspension was dialyzed against PBS buffer (pH 7.4) for 24 h using a dialysis

membrane (12 kDa cutoff) to remove EDTA and residual small molecules.[2] The final product was purified by centrifugation at 12000 rpm for 10 min. The amount of COE-POSS loaded into the NPs was determined by comparing the absorption spectrum of NPs with reference to a standard curve of COE-POSS (Fig. S7). The COE-POSS encapsulation efficiency is defined as the ratio of the amount of COE-POSS encapsulated in NPs to the total amount of COE-POSS used in the fabrication of NPs.

1.4 Preparation of COE-POSS CS/PEG-FA NPs

The synthesis of folate-PEG-NH_2 was carried out according to the literature.[3] To prepare COE-POSS CS/PEG-FA NPs, 15 mg of folate-PEG-NH_2 and 15 mg of PEG-$(NH_2)_2$ were added to the CS aqueous solution, following the same procedure as that for the preparation of COE-POSS CS/PEG NPs. The amount of FA in NPs was evaluated by determining the fluorescence intensity at 440 nm upon excitation at 360 nm.[4] In brief, pure CS/PEG-FA NPs were first prepared using the same method as that used for COE-POSS CS/PEG-FA NPs, followed by freeze-drying for two days to get the fine powder of NPs. The COE-POSS was not loaded in order to eliminate the fluorescence interference to that of FA. 10 mg of freeze-dried CS/PEG-FA NP powder was then dissolved in 1 mL of DMSO and the fluorescence intensity at 440 nm was obtained upon excitation at 360 nm using a fluorometer. The concentration of FA in 10 mg of NPs was determined with reference to a standard curve of FA. To establish the standard curve, the fluorescence for a series of FA with known concentrations in 1 mL of DMSO was collected at 440 nm upon excitation at 360 nm, which was then plotted against FA concentration (Fig. S8).

1.5 *In vitro* COE-POSS Release from NPs

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[2] R. Guo, L. Y. Zhang, Z. S. Zhu and X. Q. Jiang, *Langmuir*, 2008, **24**, 3459-3464.

[3] J. Wu, Q. Liu and R. J. Lee, *Int. J. Pharm.*, 2006, **316**, 148-153.

[4] K. Li, J. Pan, S. S. Feng, A. W. Wu, K. Y. Pu, Y. T. Liu and B. Liu, *Adv. Funct. Mater.*, 2009, **19**, 3535-3542

Two release media including 1× PBS buffer with pH 7.4 and pH 5.0, respectively, were used to study the pH influence on COE-POSS release. Briefly, 1 mg of NPs were suspended in 1 mL of 1× PBS buffer (pH 7.4 and 5.0, respectively) in a centrifuge tube and shook at 37 °C in an orbital water bath shaker. At defined time intervals, the samples were centrifuged at 12000 rpm for 10 min. The amount of released COE-POSS from the NPs was determined by absorbance at 465 nm with reference to a standard curve of COE-POSS.

2. Cellular Imaging

2.1 Cell Culture

MCF-7 breast cancer cells and NIH/3T3 fibroblast cells were cultured in folate-free Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin streptomycin at 37 °C in a humidified environment containing 5% CO_2. Before experiments, the cells were precultured until confluence was reached.

2.2 Subcellular Localization of NPs

MCF-7 breast cancer cells were cultured in chamber (LAB-TEK, Chambered Coverglass System) at 37 °C. After 80% confluence, the medium was removed and the adherent cells were washed twice with 1× PBS buffer. The COE-POSS CS/PEG-FA NPs in FBS-free DMEM medium at 0.4 μM COE-POSS were then added to the chamber. After incubation for 1.5 h, the cells were washed three times with 1× PBS buffer and then incubated with 50 nM LysoTracker Green DND-26 for 5 min to visualize lysosomes. Thereafter, the cells were washed three times with 1× PBS buffer, fixed with 75% ethanol for 10 min and imaged by confocal laser scanning microscope (CLSM, Zeiss LSM 410, Jena, Germany) with imaging software (Olympus Fluoview Fv1000). The excitation/emission wavelengths were 488 nm/505-525 nm for LysoTracker and 488 nm/560 nm for NPs.

2.3 Targeted Imaging of Cancel Cells Nucleus

MCF-7 cells were cultured in chamber at 37 °C. After 80% confluence, the medium was removed and the adherent cells were washed twice with 1× PBS buffer. The COE-POSS CS/PEG NPs and COE-POSS CS/PEG-FA NPs in FBS-free DMEM medium at 0.4 μM COE-POSS were then added to the chamber, respectively. After incubation for 1.5 h, the cells were washed three times with 1× PBS buffer and then allowed to be incubated in culture medium for another 24 h for sufficient release of COE-POSS from the NPs. After that, the cells were fixed with 75% ethanol for 10 min and imaged by CLSM. The fluorescence signal from COE-POSS was collected upon 488 nm excitation with a 560 nm longpass barrier filter. NIH/3T3 fibroblast cells incubated with COE-POSS CS/PEG NPs and COE-POSS CS/PEG-FA NPs were also investigated following the same procedures. Two-photon fluorescence imaging based on COE-POSS CS/PEG NPs with and without FA functionalization was studied using laser scanning microscope (Olympus Fluoview FV300) equipped with two-photon Chameleon for both MCF-7 and NIH/3T3 cells as well. The detection of COE-POSS fluorescence was achieved by excitation at 800 nm with a 505 nm longpass barrier filter.

2.4 Cytotoxicity of COE-POSS CS/PEG-FA NPs

The cytotoxicity of COE-POSS CS/PEG-FA NPs against MCF-7 cancer cells was evaluated by MTT assay. Briefly, MCF-7 cells were seeded in 96-well plates (Costar, IL, USA) at an intensity of 4×10^4 cells/mL. After 24 h incubation, the cells were exposed to a series of doses of COE-POSS CS/PEG-FA NPs at 37 °C. After the designated time intervals, the wells were washed twice with 1×PBS buffer and 100 μL of freshly prepared MTT (0.5 mg/mL) solution in culture medium was added into each well. The MTT medium solution was carefully removed after 3 h incubation in the incubator. DMSO (100 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all the precipitates formed. The absorbance of MTT at 570 nm was monitored

by the microplate reader (Genios Tecan). Cell viability was expressed as the ratio of the absorbance of the cells incubated with sample suspension to that of the cells incubated with culture medium only.

Tables and Figures

Table S1. Characteristics of COE-POSS CS/PEG NPs in PBS solution at pH 7.4 and 5.0.

pH of PBS	Size (nm) [a]	Zeta potential (mV)	*In vitro* COE- POSS release at 1.5 h (%)	*In vitro* COE-POSS release at 24 h (%)
7.4	131.9 ± 2.7	2.3 ± 0.5	1.4	7.7
5.0	168.9 ±1.1	20.8 ± 4.2	18.3	46.7

[a] The size of NPs is measured by LLS.

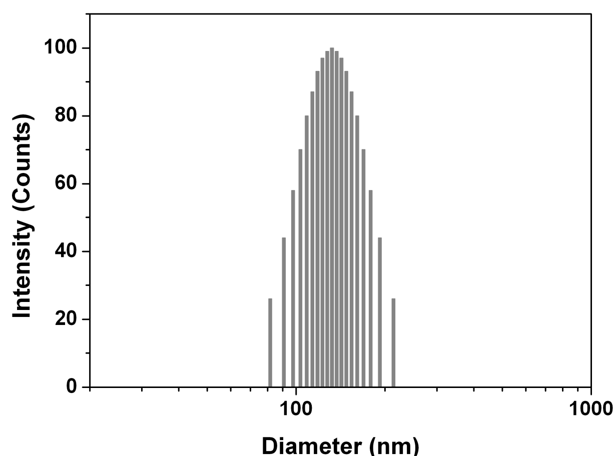

Figure S1. Particle lognormal size distribution of COE-POSS CS/PEG NPs in PBS solution at pH 7.4. The polydispersity index is 0.089 ± 0.020.

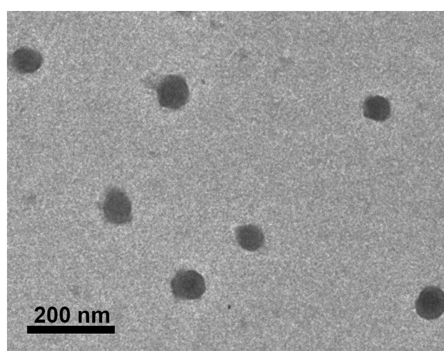

Figure S2. TEM image of COE-POSS CS/PEG NPs.

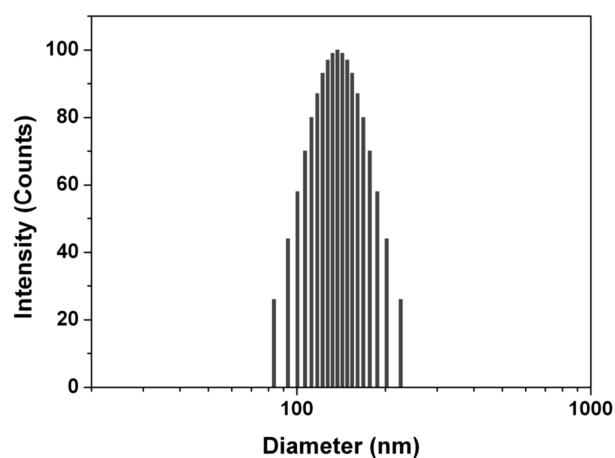

Figure S3. Particle lognormal size distribution of COE-POSS CS/PEG-FA NPs in PBS solution at pH 7.4. The polydispersity index is 0.104 ± 0.008.

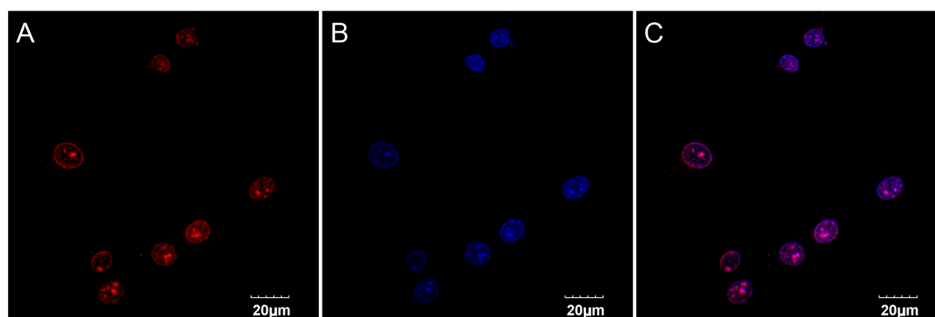

Figure S4. Confocal image of MCF-7 breast cancer cells after treatment with COE-POSS CS/PEG-FA NPs, followed by staining the cell nuclei with DAPI. The red fluorescence from COE-POSS (A) and the blue fluorescence from DAPI (B) in the cell nuclei are colocalized well (C).

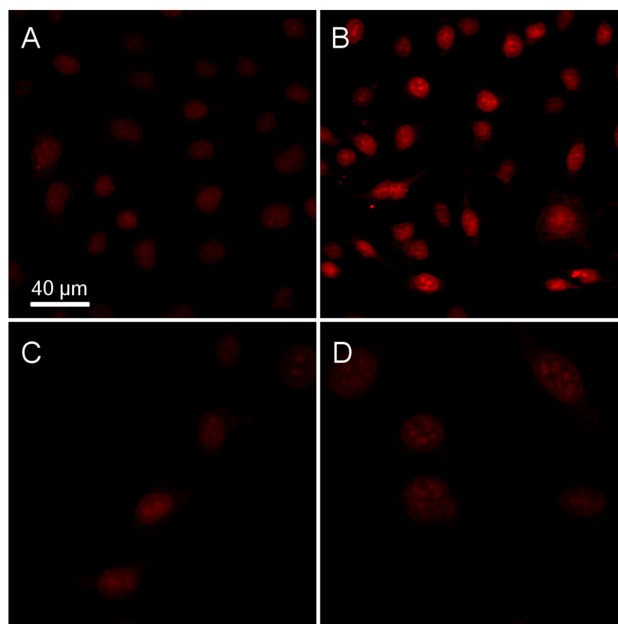

Figure S5. Two-photon excited fluorescence images of MCF-7 cancer cells treated with COE-POSS CS/PEG NPs (A) and COE-POSS CS/PEG-FA NPs (B) as well as the images of NIH/3T3 fibroblast cells treated with COE-POSS CS/PEG NPs (C) and COE-POSS CS/PEG-FA NPs (D). After incubation with the NPs containing 0.4 μM COE-POSS at 37 ^{o}C for 1.5 h, the cells were washed and then allowed to be incubated in culture medium at 37 ^{o}C for another 24 h. The images were recorded upon 800 nm excitation with a 505 nm longpass barrier filter. The bar is the same for all images.

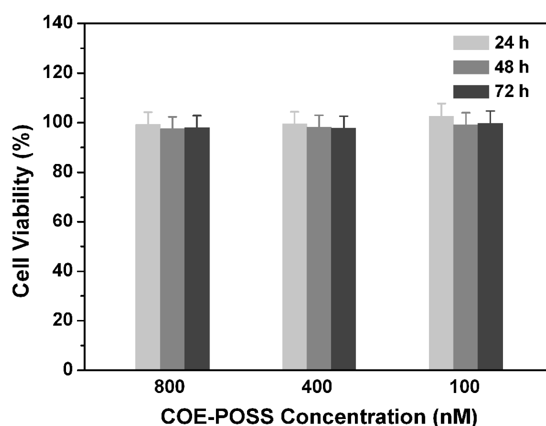

Figure S6. Metabolic viability of MCF-7 breast cancer cells after incubation with COE-POSS CS/PEG-FA NPs at various COE-POSS concentrations for 24 (light gray), 48 (gray), and 72 h (dark gray), respectively.

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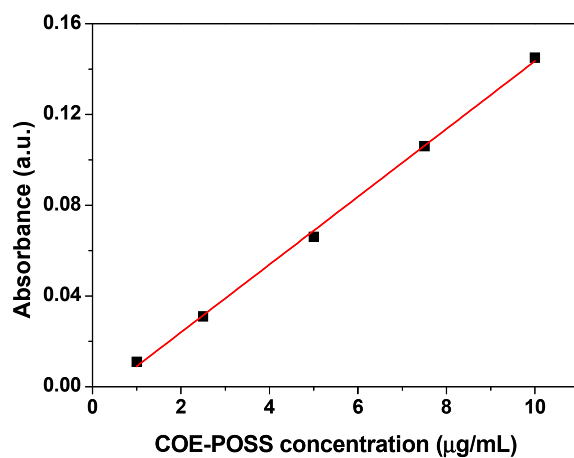

Figure S7. The standard curve of absorbance at 465 nm for COE-POSS in water versus concentration. Correlation coefficient $R^2 = 0.998$.

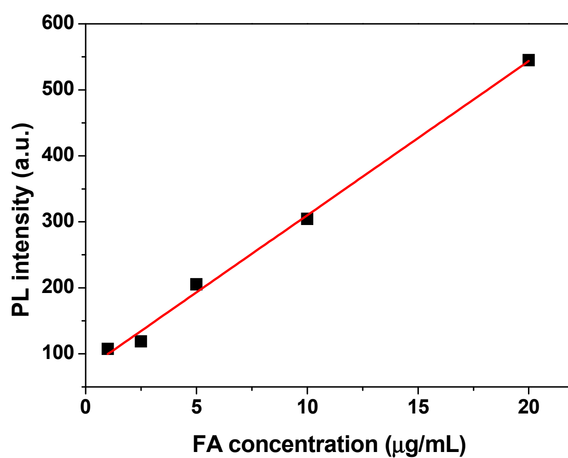

Figure S8. The standard curve of fluorescence intensity at 440 nm of FA in DMSO upon excitation at 360 nm versus concentration. Correlation coefficient $R^2 = 0.995$.