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Supporting Informations

Enhanced Tumor Targetability of PEGylated Mesoporous Silica Nanoparticles on In Vivo Optical Imaging According to Their Size

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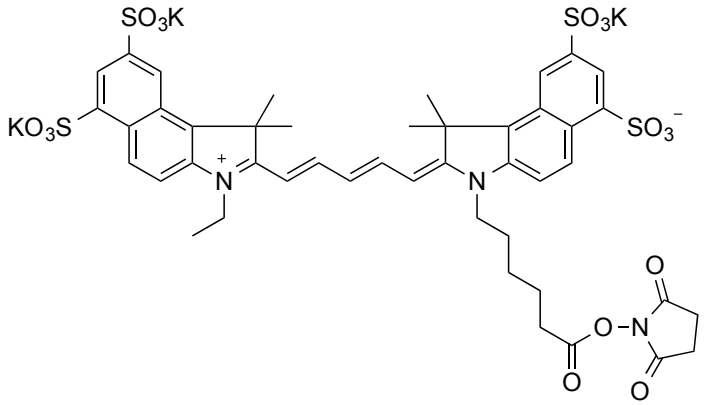
General. Unless otherwise noted all reagents and solvents were commercially available. Hexadecyltrimethylammoniumbromide (CTAB), Tetraethylorthosilicate (TEOS), Amino-propyltriethoxysilane(APTES) were purchased from Sigma Aldrich Chemical Co (St Louis, MO,USA). Methoxy-PEG_{12}-NHS ester was obtained from Thermo scientific (Rockford, USA). Phosphate Buffered Saline (PBS, pH7.2) was supplied from GIBCO (Grand Island, USA). Cyanine 5.5 monosuccinimidyl ester (Cy5.5-NHS ester) was purchased from GE healthcare (Uppsala, Sweden).

Preparation of Cy5.5-PEG-MSNs. NH_2-MSNs with three different particles sizes (≈ 30, 100-150 or > 300 nm in size) were synthesized according to the literature.[20, 21] MeO-PEG_{12}-COONHS (4.0 mg, 5.8 μmol) was added to the suspension of the NH_2-MSNs (2 mg) in phosphate buffer solution (PBS, pH 7.2, 3 mL) at 25 °C, and stirred for 6 h. The product, PEG-MSNs were purified by centrifugation (11000 rpm, 5 min) and washed five times with EtOH/water (3:1). For the labeling with Cy5.5, to the suspension of PEG-MSNs (1.0 mg) in PBS (pH 7.2, 2 mL) was added Cy5.5-NHS ester (0.3 mg, 0.26 μmol). The reaction solution was stirred at 25 °C for 6 h. Cy5.5-PEG-MSNs (≈ 30, 100-150 or > 300 nm in size) were collected by centrifugation (11000 rpm, 5 min), washed five times with EtOH/water (3:1), and dried overnight under vacuum. A sulfur elemental analysis of the Cy5.5-PEG-MSNs product showed that approximate 0.07 mmole of Cy5.5 moiety was tethered to per gram of Cy5.5-PEG-MSNs. Anal.: S 0.89 ± 0.012 (n = 5).

Cy5.5-NHS ester ≡



Characterization. The morphology of the MSNs were observed by high-resolution transmission electron microscopy (HR-TEM, JEM-2010, JEOL) operated at accelerating voltage of 200 kV. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin-Elmer spectrometer in the range between 4000 and 400 cm^{-1}. Zeta potentials were recorded on a zeta potential analyzer (ELS-600, Photal Inc.). Powder XRD patterns were recorded on a Philips X′Pert PRO SUPER X-ray diffractometer system with a CuKα radiation (λ= 1.542 Å, 40 kV, 30 mA) source. Nitrogen adsorption isotherms were measured on a surface area and pore size analyzer (Microtrac Inc.) at 77 K. All MSN samples were degassed at 523 K for 12 h for the analysis. The specific surface area was determined using the BET (Brunauer-Emmett-Teller)

method and the pore size distribution was computed using the BJH (Barret-Joyner-Halenda) model. UV-VIS Spectra were recorded on a HP8453 (Hewlett Packed) spectroscopy.

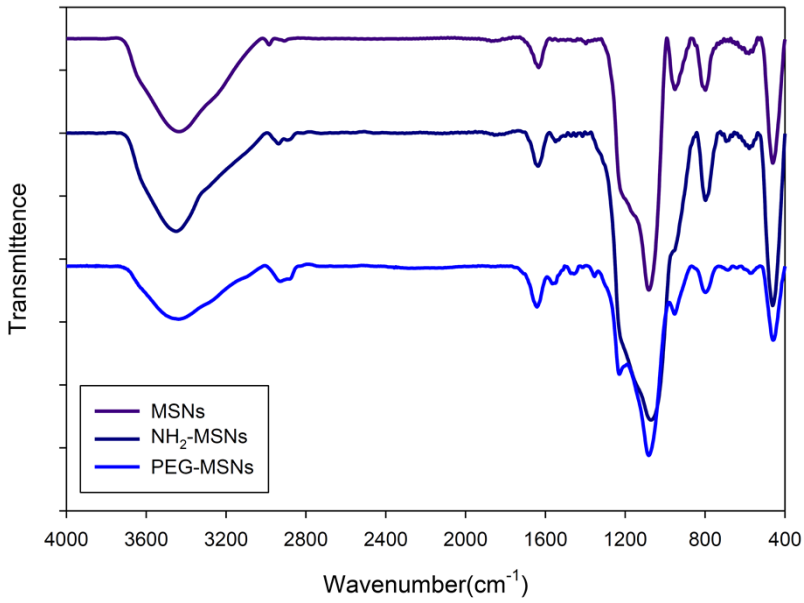


Figure S1. FT-IR spectra. MSNs, NH_2-MSNs and PEG-MSNs.

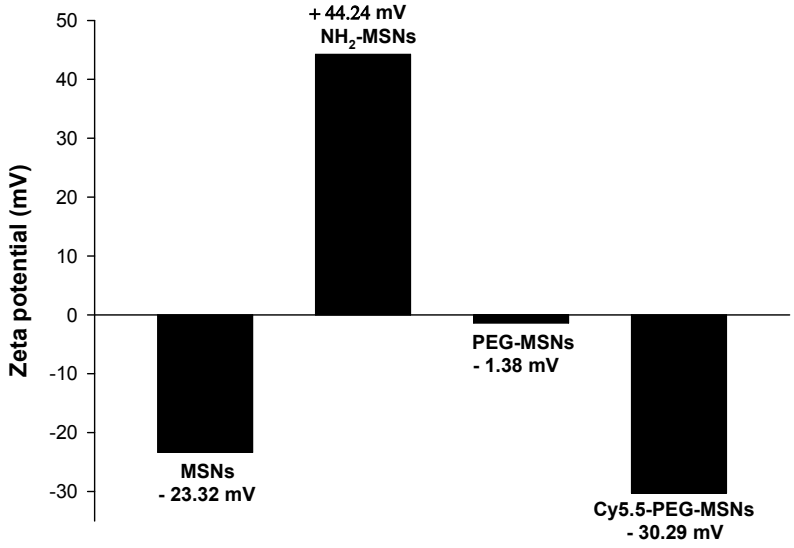


Figure S2. Zeta-potentials of MSNs, NH_2-MSNs, PEG-MSNs and Cy5.5-PEG-MSNs.

The introduction of functional groups, such as NH_2 and COOH, would result in the change of surface charges of MSNs after each reaction step. Therefore, zeta-potential assay was employed to further verify the covalently coupling of various functional groups to MSNs (-23.32 mV). In contrast to the zeta potential (ζ-potential) of +44.24 mV for NH_2-MSNs, the values of zeta-potential for PEG-MSNs and Cy5.5-PEG-MSNs in 1 mM PBS buffer (pH7.4) were correspondingly changed from -1.38 mV and -

30.29 mV, respectively. This result suggests that various items/groups were successfully coupled to MSNs.

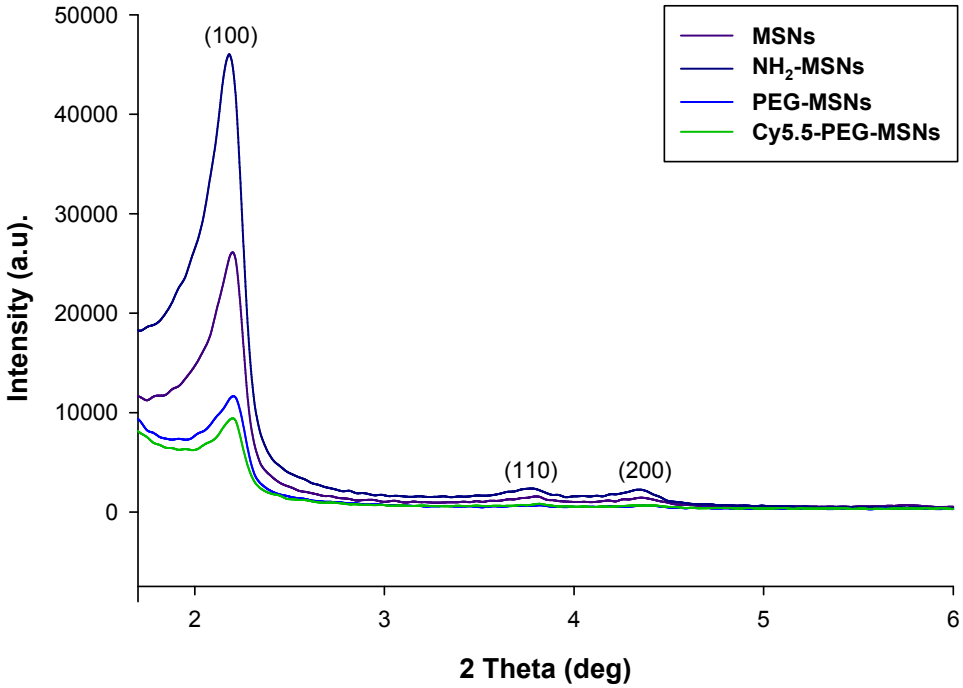


Figure S3. Low-angle XRD (1-6°) XRD patterns of the MSNs. Typical of hexagonal mesoporous silica nanoparticles are shown (100, 110, 200) in MSNs, NH_2-MSNs, PEG-MSNs and Cy5.5-PEG-MSNs.

a) b)

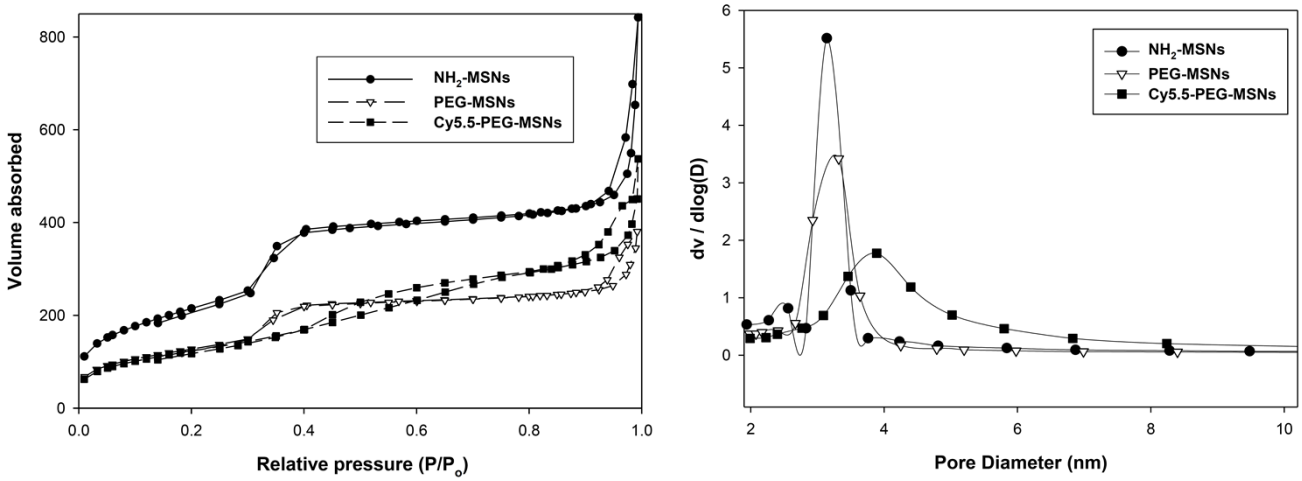


Figure S4. a) Nitrogen adsorption/desorption isotherms of NH_2-MSNs, PEG-MSNs, Cy5.5-PEG-MSNs. b) BJH narrow pore size distributions (based on adsorption branch).

Table S1. Specific surface area, pore volume calculated from N_2 adsorption-desorption isotherms and Zeta potential values for various MSNs.

Sample	BET Surface area S_{BET} [m^{-2}/g]	BET pore volume V_P (cm^3/g)	BJH Pore diameter W_{BJH}(Å)	Zeta Potential (mV)
NH_2-MSNs	798.26	1.368328	39.14	44.24
PEG-MSNs	467.43	0.631368	38.15	-1.38
Cy5.5-PEG-MSNs	455.41	0.862009	50.63	-30.29

Cell culture. U87MG human glioblastoma cell line obtained from the Korean Cell Line Bank (KCLB, Seoul). U87MG glioma cell were grown in RPMI-1640 modified medium (GIBCO, Carlsbad, CA) supplemented with 10 % (v/v) fetal bovine serum (FBS, GIBCO, Carlsbad, CA) and 1 % (v/v) penicillin streptomycin (Antibody, GIBCO, Carlsbad, CA) at 37 ^oC in humidified atmosphere containing 5 % CO_2.

Animal models**.** All animal experiments were performed in compliance with the policies and procedures of Institutional Animal Care and Use Committee for animal treatment. Female athymic nude mice (4-wk, *nu/nu*) were obtained from Orient-Bio (Seoul, Korea). Mice were anesthetized by subcutaneous injection with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg). After sedation, glioblastoma (U87MG) tumors were established by subcutaneous injection in the right flank of 5×10^6 cells mixed with matrigel (BD, Bioscience, San Jose, CA, USA). The mice underwent *in vivo* imaging and biodistribution studies when the tumor volume reached 150-250 mm^3 (eq. = length × W^2 × 0.5 4-5 wk after inoculation).

In vivo and ex vivo optical imaging study. In vivo optical images for each size of Cy5.5-PEG-MSNs (≈ 30, 100-150 or > 300 nm in size) were acquired using an IVIS system (Caliper Life science, Hopkinton, MA). After anesthetizing with isoflurane (2.0%), Cy5.5-PEG-MSNs (500 μg in 0.1 mL of PBS) were injected intravenously into the U87MG tumor-bearing mice. *In vivo* near infrared fluorescent optical imaging was performed at 1, 3, 6, 12 and 24 h after injection, using the Cy5.5 filter with the following settings: exposure time (1 s), f/stop (2), binning (8) and field of view (12.8). After acquisition of in vivo optical images, the mice were sacrificed and their major organs, such as heart, lung, liver, pancreas, stomach, spleen, kidney, intestine, muscle, bone and tumor, were imaged *ex vivo*.