Electronic Supplementary Information (ESI)

Stability of small mesoporous silica nanoparticles in biological media

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Experimental details

1. Materials: All chemicals were used as received. n-Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), dimethyl sulfoxide (DMSO), ammonium molybdate tetrahydrate, 4-methylaminophenol sulfate, sodium sulfite, oxalic acid, silicon standard (1000 mg/L) and 10X phosphate buffered saline (PBS) were purchased from Sigma Aldrich. 2-[Methoxy(polyethyleneoxy)propyl] trimethoxysilane, (PEG-silane, MW 596-725 g/mol, 9-12 EO) was obtained from Gelest. Ammonium nitrate (NH_4NO_3), hydrofluoric acid (HF), nitric acid (HNO_3) and ammonium hydroxide (NH_4OH, 28-30 wt% as NH_3) were obtained from Mallinckrodt. Hydrochloric acid (HCl) and acetic acid were obtained from BDH. 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl-tetrazolium bromide (MTT) was purchased from Invitrogen. Absolute anhydrous 99.5% ethanol and 95% ethanol were purchased from Pharmco-Aaper. The de-ionized (D.I.) water was generated using a Millipore Milli-Q system. Heat inactivated fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) with 4.5 g/L glucose, 110 μg/mL sodium pyruvate, 4.00 mM L-glutamine, and phenol red were purchased from Hyclone. Trypsin-EDTA and penicillin streptomycin (PS) were obtained from Gibco. Powder DMEM without phenol red and sodium pyruvate was purchased from SAFC Biosciences.

2. Characterization: Transmission electron microscopy (TEM) micrographs were taken on a JEOL 1200 EXII with a 100 kV voltage. Powder X-ray diffraction (XRD) patterns were measured on a Siemens Bruker-AXS D-5005 X-ray diffractometer using filtered Cu Kα radiation (λ= 1.5406 Å) at 45 kV and 20 mA. Data were recorded by step scan with a step size of 0.040° and a step time of 1.0 second. N_2 adsorption-desorption isotherms were taken on a Quantachrom Autosorb-1 analyzer at 77 K. Samples were degassed at 120 °C for at least 12 hours prior to measurements.

The total surface area was calculated using the Brunauer-Emmett-Teller (BET) equation at P/P_0 <0.3. The pore size distribution was calculated from the branch of the adsorption isotherm using a Barrett-Joyner-Halenda (BJH) method. UV-vis measurements were performed on a Perkin Elmer Lambda 12 spectrometer. Hydrodynamic diameter data were measured at particle concentration of 1 mg/mL using dynamic light scattering (DLS) with a Brookhaven 90Plus/BIMAS particle analyzer equipped a 655 nm laser. Three runs and one minute run duration were set for each measurement. The DLS size distribution was plotted using a lognormal analysis method. Cell viability and hemolysis percentage were measured at 570 nm using a Bio-Rad iMark microplate reader.

3. Preparation of mesoporous silica (MS) nanoparticles with 42 nm diameter (MS42)

(a) bare MS42 nanoparticles: The bare MS nanoparticles were prepared using our recent published procedure.[1] First, 0.29 g of CTAB was dissolved in 150 mL of 0.256 M NH_4OH solution at 50 ^oC. After one hour, 2.5 mL of 0.88 M ethanolic TEOS was added under vigorous stirring. After one hour, the stirring was stopped and the colloidal solution was aged for 20 hours at 50 ^oC. After aging, the as-synthesized colloidal solution was passed through a 0.45 μm GH propylene (GHP) filter and diluted to 40 mL with D.I. water. Herein, two methods were used to remove surfactant. One was a centrifugation method, and the other was a dialysis method. With centrifugation, the filtered as-synthesized MS42 colloids were transferred to 50 mL of 6 g/L ethanolic ammonium nitrate by centrifugation (30,000 rpm=66226g, 30 minutes) and heated to 60 ^oC for one hour with stirring. The MS42 nanoparticles were then transferred to 50 mL of acidic ethanol solution (1 mL of HCl/1L of ethanol) via centrifugation and heated to 60 ^oC for two hours under stirring. The extracted MS-42 nanoparticles were further washed with 95% ethanol and then 99.5% ethanol once. Finally, the surfactant-free MS42 nanoparticles were suspended in 99.5% ethanol and stored at 4 ^oC. The MS42 nanoparticle extracted by the centrifugation method is designated as MS42-c. For dialysis purification, the surfactant was removed from the as-synthesized MS42 nanoparticles using a dialysis process described by Urata et al.[2] The as-synthesized sample was transferred to regenerated cellulose dialysis tubing (with a molecular weight cut off, MWCO, of 12,000-14,000, Fisherbrand) and placed into a 250 mL acid solution composed of 95% ethanol and 2M acetic acid. The acid solution was replaced every 24 hours and repeated two times. The particles were then dialyzed against 500 mL of D.I. water three more times. Finally, the dialyzed MS42 nanoparticles were filtered through a 0.45 μm GHP filter and stored at 4 ^oC until use. The MS42 nanoparticles purified by the dialysis method are designated as MS42-d.

(b) Pegylated MS42 nanoparticles with hydrothermal treatment: Typically, 0.29 g of CTAB was added to 150 mL of 0.256 M NH_4OH solution at 50 ^oC. Then, 2.5 mL of 0.88M ethanolic TEOS was added to solution under continuously stirring. After one hour, 450 μL of PEG-silane was added to the as-synthesized colloidal solution. The mixture solution was stirred for 30 minutes and then aged at 50 ^oC for 20 hours. The as-synthesized pegylated MS42 colloidal solution was filtered with a 0.45 μm GHP filter and diluted to 50 mL with D.I. water. The filtered colloidal solution was then heated at 90 ^oC for 24 hours in a sealed vessel. The surfactant removal steps followed the centrifugation method described above. Finally, the surfactant-free pegylated MS42 nanoparticles were filtered using a 0.2 μm polytetrafluoroethylene **(**PTFE) filter. The products were stored at 4 ^oC until use. The pegylated MS42 nanoparticles without and with hydrothermal treatment were purified via centrifugation and are designated as MS42@PEG-c and MS42@PEG-hy-c, respectively.

4. Quantification of degraded free silicon from MS42-d and MS42@PEG-hy-c after 10-day aging in D.I. water and PBS at R.T. and 37 ^oC using a blue silicomolybdic assay: Surfactant-free MS42-d and MS42@PEG-hy-c nanoparticles were suspended in D.I. water and PBS at 1000 μg/mL concentration. These nanoparticle solutions were aged in PBS at R.T. and 37 ^oC for 10 days; then, the aged NPs were separated from the solution by centrifugation (66226g, 30 minutes). The degraded silicon concentration in the supernatant was determined using a blue silicomolybdic assay (SMA).[1] The silicon quantification was based on a calibration curve (0 ppm, 1 ppm, 5 ppm, 10 ppm, 20 ppm, 40 ppm, 50 ppm, and 60 ppm) made before sample measurements. The silicon quantification was performed in three independent experiments.

5. *In-vitro* cell viability (MTT assay) and hemolysis assay

(a) MTT assay: Human endothelial cells were purchased from American Type Culture Center (ATCC). Typically, 6×10^4 cells were seeded in 96-well plates and cultured in DMEM supplemented with 10% FBS and 1%PBS at 37 ^oC under 5% CO_2. After 24 hours, the cells were incubated with 100 μL of different concentrations of MS42@PEG-hy-c nanoparticle suspensions in DMEM+10% FBS media for 24 hours. After nanoparticle incubation, the cells were washed with 100 μL of serum-free DMEM two times and incubated with 100 μL of 0.5 mg/mL MTT media for 2 hours at 37 ^oC under 5% CO_2. Finally, the MTT media was removed and purple formazan crystals produced by live cells were dissolved in 200 μL of DMSO. Optical density of the produced stain was monitored at 570 nm with 655 nm as a reference using a

microplate reader.

(b) Hemolysis assay: Fresh ethylenediamine tetraacetic acid (EDTA)-stabilized human whole blood samples were purchased from Memorial Blood Center. Typically, 5 mL of whole blood was added to 10 mL of PBS and centrifuged at 10016 g for 10 minutes to isolate red blood cells (RBCs) from serum. The RBCs were then washed five times with 10 mL of PBS and diluted to 50 mL with PBS. To test the hemolytic activity of MS42@PEG-hy-c NPs, 0.2 mL of diluted RBC suspension was added to 0.8 mL of nanoparticle solution at different concentrations ranging from 15.625 to 1000 μg/mL. D.I. water and PBS were used as positive control and negative control, respectively. All the samples were placed on a rocking shaker at 37 ^{o}C for 3 hours. After incubation, the samples were centrifuged at 10016 g for 3 minutes. The hemoglobin absorbance in the supernatant was measured at 570 nm with 655 nm as a reference.

6. *In-vitro* macrophage uptake: Mouse macrophage, Raw 264.7, cells obtained from ATCC were cultured in DMEM with 10% FBS and 1% PS under 5% CO_2 atmosphere at 37 ^{o}C. Before macrophage uptake experiments, cells were trypsinized and seeded into 24-well plates at $3x10^5$ cells per well. After 24-hour incubation, the cells were exposed to 1 mL of MS42-d and MS42@PEG-hy-c at a concentration of 200 μg/mL in DMEM+10% FBS media for 24 hours. Cells incubated without mesoporous silica nanoparticles were used as control. The cells were then washed with PBS two times and lysed with 0.5 mL of acid solution containing 2% HNO_3 and 0.1% HF for 20 hours at 37 ^{o}C. After digestion, the solutions were centrifugation at 10016 g for 10 minutes. The supernatant was separated for silicon quantification. The silicon uptake amounts by the macrophage cells were determined by the blue silicomolybdic assay described previously.

Table S1. Hydrodynamic diameter of as-synthesized MS NPs (MS42, MS42@PEG, and MS42@PEG-hy) and surfactant-free MS NPs (MS42-c, MS42-d, and MS42@PEG-hy-c) measured in D.I. water by DLS at R.T.

Particle	Hydrodynamic diameter (nm)[a]
As-synthesized MS42	57.1±1.1
MS42-c	129±5.6
MS42-d	58.6±0.8
As-synthesized MS42@PEG	59.4±1.1
As-synthesized MS42@PEG-hy	59.0±2.1
MS42@PEG-hy-c	56.8±1.4

[a] All the data were obtained from three independent experiments. Each measurement was taken three times with one-minute run duration.

Table S2. The Structural Properties of Surfactant-Free MS42-d, MS42@PEG-c, MS42@PEG-hy-c NPs, MS42-d after 10-day PBS aging at 37 ^{o}C, and MS42@PEG-hy-c after 10-day PBS aging at 37 ^{o}C

Particle	BET surface area, S_{BET} (m^2/g)	BJH pore size, D_{BJH} (nm)
MS42-d	1131	2.60
MS42@PEG-c	943	2.51
MS42@PEG-hy-c	731	1.94
MS42-d after 10-day PBS aging at 37 ^{o}C	491	□
MS42@PEG-hy-c after 10-day PBS aging at 37 ^{o}C	432	□

[a] S_{BET}: Specific surface area calculated from data in the range $P/P_0 < 0.3$ using BET equation; [b] D_{BJH}: pore diameter assigned from the maximum on the BJH pore size distribution.

Supplementary figures:

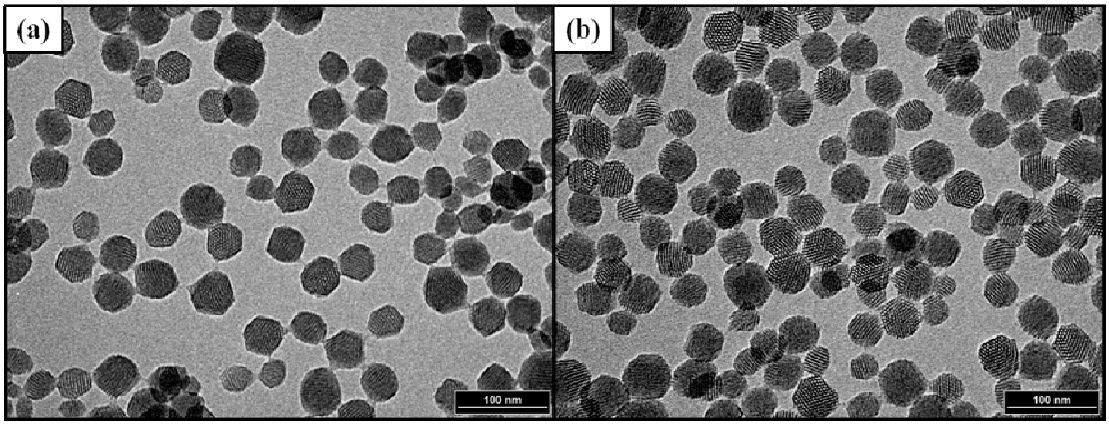

Fig. S1 TEM images of **(**a) surfactant-free MS42-c and (b) MS42@PEG-c NPs.

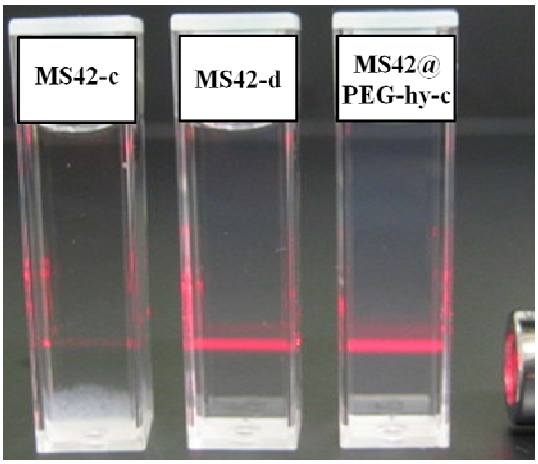

Fig. S2 A photograph of MS42-c, MS42-d, and MS@PEG-hy-c colloidal solutions after 30-minute aging in PBS at room temperature.

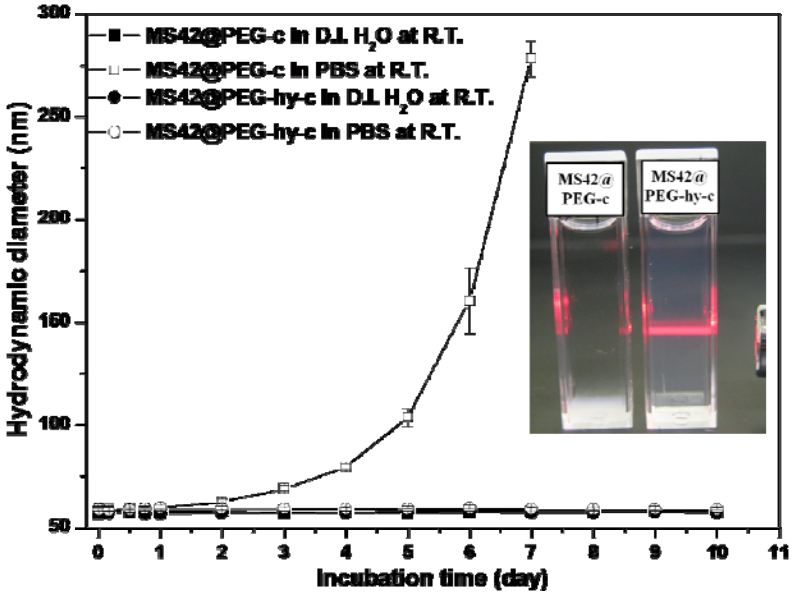

Fig. S3 Long-term particle stability of MS42@PEG-c and MS42@PEG-hy-c NPs in D.I. water and PBS at room temperature.

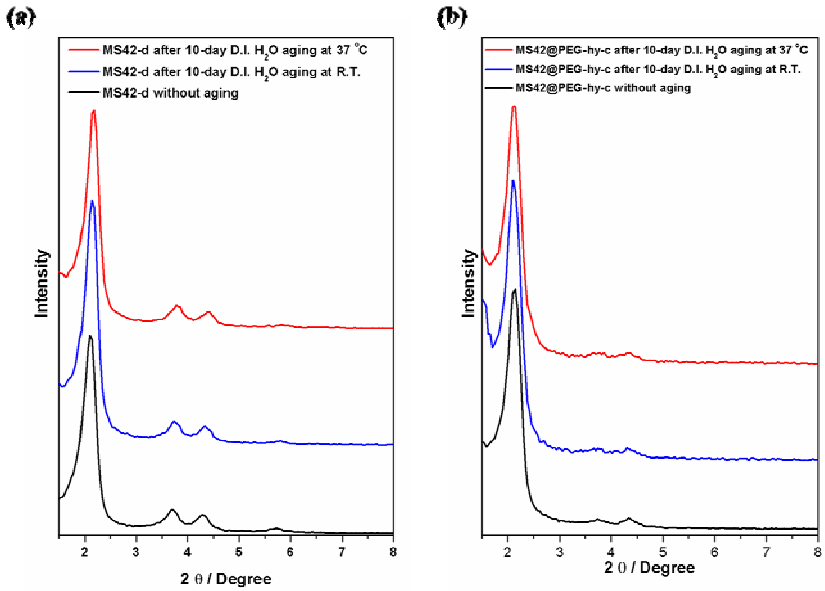

Fig. S4 XRD patterns from (a) MS42-d and (b) MS42-hy-c NPs after 10-day aging in D.I. H_2O at R.T. and 37 ^oC.

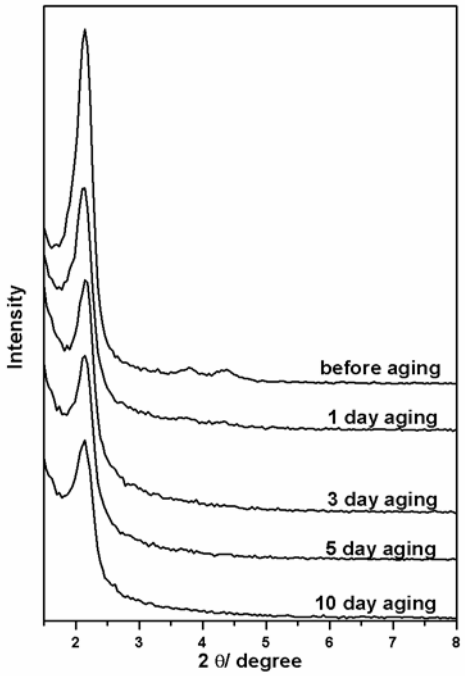

Fig. S5 XRD patterns of MS42@PEG-hy-c after different aging time in PBS at 37 ^{o}C.

References:

1. Y.-S. Lin and C. L. Haynes, *J. Am. Chem. Soc.*, 2010, ***132***, 4834.

2. C. Urata, Y. Aoyama, A. Tonegawa, Y. Yamachi and K. Kuroda, *Chem. Commun.*, 2009, 5094.

3. T. Coradin, D. Eglin and J. Livage *Spectroscopy: Int. J.* 2004, ***18***, 567.