Supporting information

Processing pathway dependence of amorphous silica nanoparticle toxicity - colloidal versus pyrolytic

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Synthesis, preparation and fluorescence labeling of silica nanoparticles

Synthesis of Stöber silica

Colloidal silica NPs were synthesized by base catalyzed hydrolysis of tetratethyl orthosilicate (TEOS) via a modification of the well-known Stöber process;[1] a solution of 10.0 g of TEOS in 32.0 g of ethanol was added as a continuous stream to a solution of 8.0 g of concentrated (28 weight %) NH_4OH in 158 g of ethanol under vigorous stirring (300 rpm) at room temperature. The combined solution was left stirring for 24 hours before further processing of the resulting silica particles (ca. 16 nm in diameter). Aggregates of amorphous silica were synthesized by addition of 0.5 mL of 1.0 M NaCl to 5.0 mL of Stöber silica solution inside a 15 mL centrifuge tube followed by vigorous shaking for 5 seconds; aggregates were then transferred to acid solution by alternate centrifugation (2000 rpm, 1 minute) and redispersion in 5 mL DI H2O (two cycles) and 0.01 M HCl (2 cycles). After 15 hours of aging in acid, the aggregates were transferred to DI H2O using the same process. DLS measurements yielded a similar hydrodynamic radius of the Stöber aggregates to fumed silica, within one particle diameter (171.5 vs. 185.5 nm, respectively).

Synthesis of mesoporous silica nanoparticles (MSNPs)

MSNPs were synthesized based on the modified sol-gel process. Typically, 120 mL of cetyltrimethylammonium bromide (CTAB) aqueous solution (5.4 mmol/L) was heated to 80 ℃, followed by addition of 875 μL of a NaOH solution (2 mol/L). After stirring for 15 min, 1.25 mL of tetraethylorthosilicate (TEOS) was added dropwise to the CTAB solution. After 15 min

[1] Stöber, W.; Fink, A.; Bohn, E. *Journal of Colloid and Interface Science* **1968**, *26*, 62.

stirring, 315 μL of 3-(trihydroxysilyl)propyl methylphosphonate aqueous solution (42% wt.) was added to above mixture and stirred for 2 hours at 80 ℃. The MSNP precipitates were collected by centrifuging at 7800 rpm for 15 min, washed three times with methanol and the CTAB extracted by washing in 60 mL of methanol mixed with 2.5 mL of hydrochloric acid (12.1 mol/L). The suspension was refluxed for 12 hours and the MSNPs were collected by centrifuging at 7800 rpm for 15 min, washed three times with methanol and dispersed in methanol.

Synthesis of silicalite nanoparticles

7.52 mL of TEOS (tetraethyl orthosilicate) was added to 10.0 mL of a 1M TPAOH in water (tetrapropylammonium hydroxide) and stirred vigorously for ten minutes. An amount of 2.08 mL of doubly-distilled water was added to the homogeneous mixture. The reaction vessel was sealed and stirred for 24 hours at room temperature, and transferred into an oil bath set at 80^{o}C for 48 hours. The resulting milky solution was separated by centrifuge (1 hour, 15000 rpm), washed with methanol until pH was neutral, and dried overnight to isolate around 1 g of silicalite-1. The crude product was characterized through TEM imaging, XRD and DLS. 1g of dried crude silicalite-1 nanoparticles were lightly crushed with a mortar and pestle, and heated to 550^{o}C for 3 hours in a ceramic crucible at ramp rate of 2^{o}C/min. A steady air stream was applied to the oven during the heating process. The calcinated particles were suspended in anhydrous methanol. Complete removal of the organic template was verified through TGA and IR spectroscopy.

Preparation of nano-sized quartz

Nano-sized quartz was prepared through isolation from commercially available micro-sized quartz product (Min-U-Sil 5 from US Silica, Frederick, MD, USA). Briefly, 1.6 g of Min-U-Sil 5

microparticles was suspended in 40 mL DI water contained in a 50 mL centrifuge tube (Fisher Scientific), followed with probe sonication in an ice bath for 10 minutes at 40% output power. The resulted suspension was allowed to sediment overnight at room temperature. After 12 hours, the top translucent layer (10 mL) in the suspension was gently extracted for further use. The concentration of nano-sized quartz in the suspension was determined as 8 mg/mL.

Fluorescence labeling of fumed and Stöber silica nanoparticles

2.5 mg of fluorescein isothiocyanate (FITC) was dissolved in 1.5 mL of dry toluene, and 1.87 μL of 3-aminopropyltriethoxysilane (APTES) was added and allowed to react under dry N_2 for 2 hours. The fluorescein/APTES mixture was added to 20 mL of 5 mg/mL fumed or Stöber silica nanoparticles suspension in dry toluene, and allowed to react overnight over dry N_2 at 80^{o}C. The solution containing FITC-labeled Stöber nanoparticles was transferred to a cellulose membrane dialysis tube (MWCO, 12 kD) for dialysis against ethanol for 6 hours and then against pH 5 HCl aqueous solution for 2 days with 4 buffer exchanges. Finally, the FITC-labeled Stöber nanoparticle suspension was achieved by dialysis against DI water for 24 hours. The FITC-labeled fumed silica was collected through centrifugation (2 minutes, 7000 rpm), washed twice with toluene and twice with methanol. Fluorescein attachment was verified through IR spectroscopy and UV-Vis spectroscopy.

Table S1. Hydrodynamic sizes and zeta potentials of silica nanoparticles

Silica nanoparticles	Size (nm)			Zeta potential (mV)		
	H_2O	BEGM (2 mg/mL BSA)	DMEM (10% FCS)	H_2O	BEGM (2 mg/mL BSA)	DMEM (10% FCS)
"As-prepared" Stöber silica	118.4 ±1.9	257.6 ±22.6	196.7 ±11.7	-39.2 ±1.4	-5.5 ±3.5	-25.2 ±14.1
Stöber silica calcined at 600 ^oC	156.6 ±3.2	282.6 ±3.2	227.8 ±0.9	-26.8 ±0.5	-8.5 ±4.1	-10.9 ±2.4
Stöber silica calcined at 800 ^oC	211.0 ±6.2	311.9 ±7.1	233.2 ±3.6	-34.9 ±1.6	-10.7 ±2.8	-5.7 ±2.8
Rehydrated Stöber silica	143.3 ±3.8	249.9 ±2.2	203.6 ±4.7	-36.8 ±2	-9.6 ±4.4	-11.0 ±6.9
Aggregated Stöber silica	148.1 ±2.1	532.8 ±27.8	231.2 ±2.3	-8.81 ±0.1	-9.04 ±1.7	-11.24 ±1.5
"As-received" fumed silica	131.5 ±0.6	627.6 ±69.9	256.3 ±19	-22.4 ±7.1	-4.5 ±3.1	-10.1 ±8.5
Fumed silica calcined at 600 ^oC	286.2 ±22.5	667.3 ±23.6	326.3 ±3.9	-33.2 ±3.7	-11.5 ±4.9	-4.9 ±2.2
Fumed silica calcined at 800 ^oC	310.1 ±11.2	676.7 ±32.8	339.0 ±9.9	-19.0 ±1.6	-7.1 ±1.2	-4.1 ±1.7
Rehydrated fumed silica	161.6 ±29	623.3 ±18.6	264.4 ±7.3	-40.5 ±2.2	-10.7 ±1.5	-6.5 ±2.1
Mesoporous silica	263.3 ±7.8	409.3 ±43.2	248.6 ±12.8	-58.0 ±1.3	0.9 ±7.9	2.1 ±10.9
Min-U-Sil quartz	235.0 ±7.2	364.8 ±128.4	362.9 ±51.1	-43.9 ±3.6	-18.4 ±8.7	-4.8 ±13.1
Silicalite	94.3 ±5.5	246.1 ±35.9	126.8 ±8.0	-15.5 ±0.3	-19.3 ±1.6	-7.74 ±0.3

Figure S1. Comparison of Raman spectra for different silica materials, with the position of the three-membered ring band marked with a dashed line.

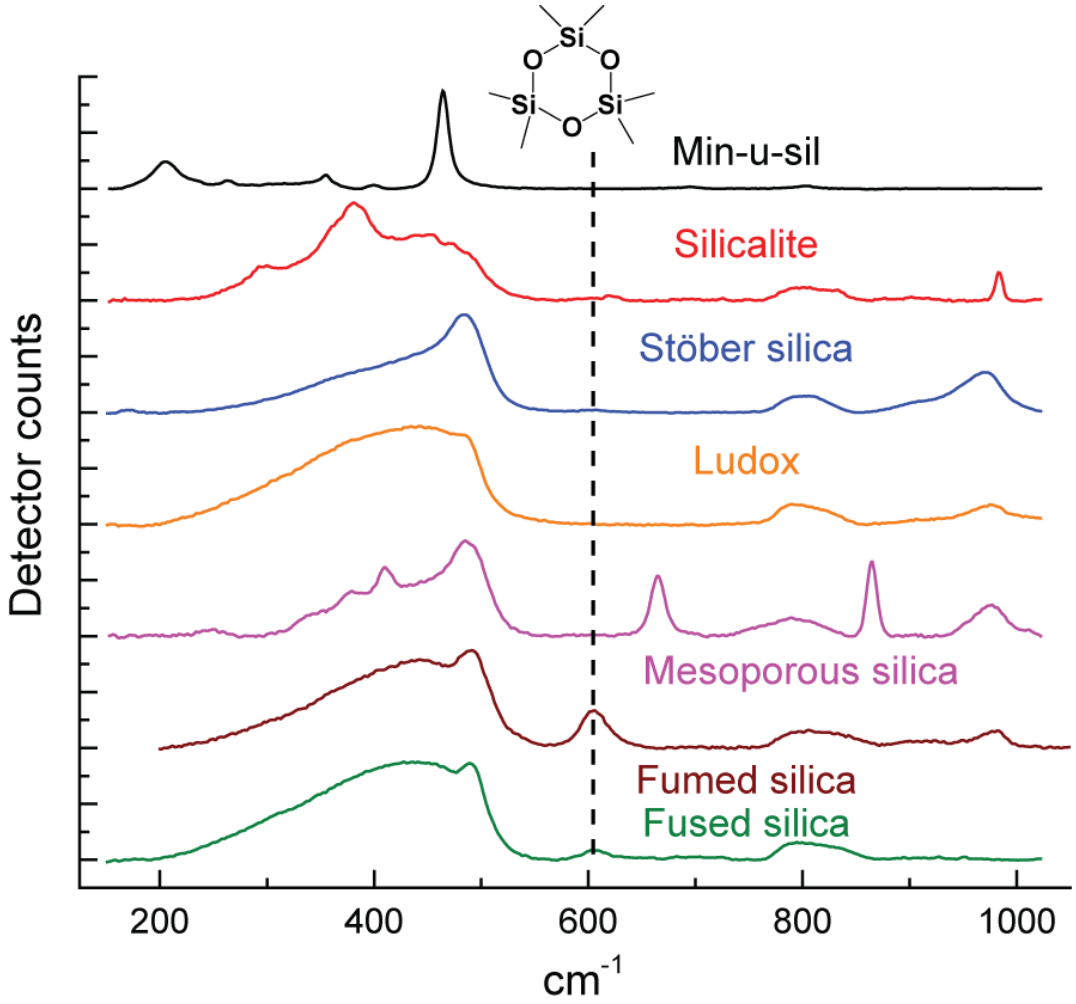
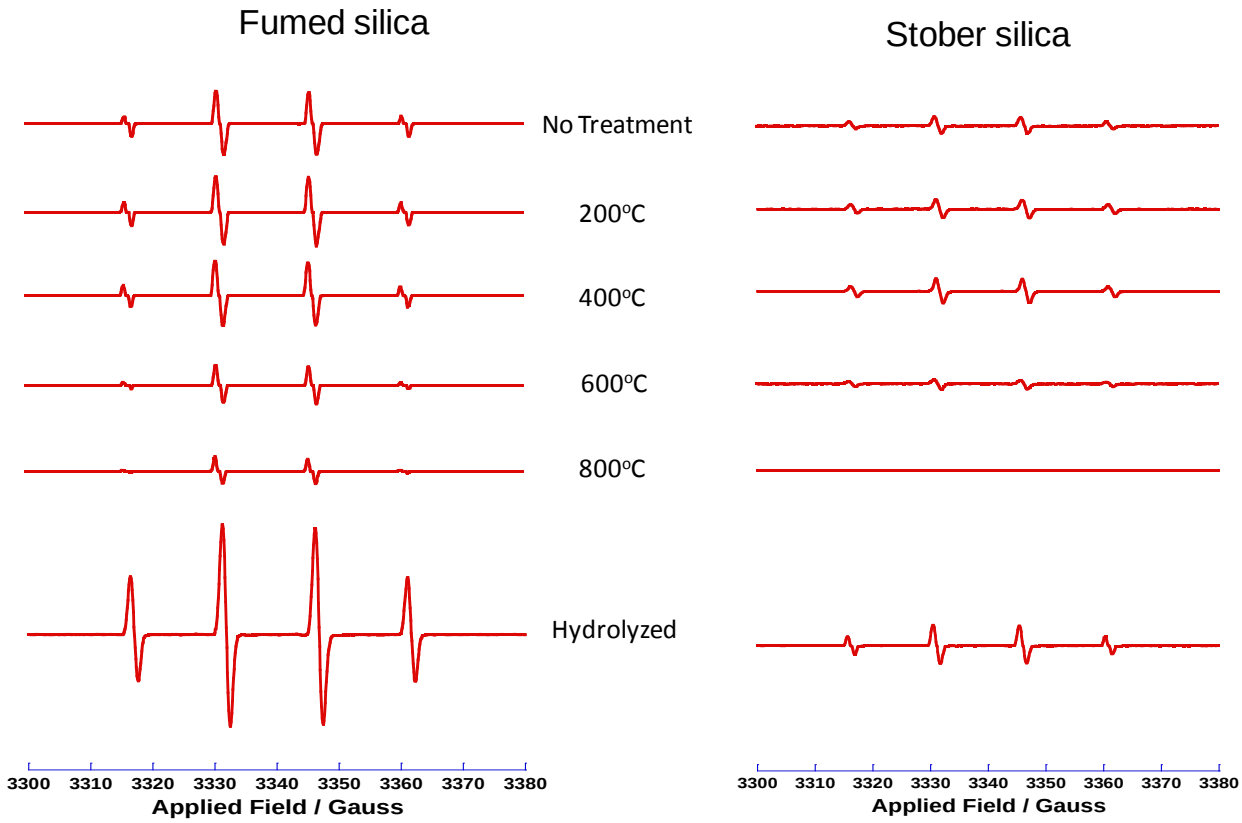


Figure S2. Derivative EPR data of fumed silica left and Stöber silica right as received or as synthesized (top) and (top to bottom) after heating to 200, 400, 600, 800˚C and rehydrated by reflux in DI water for 24 hours.



All data normalized to surface area

Figure S3. Cytotoxicity of silica nanoparticles assessed in RAW cells. Cell death, cell viability and ATP level were determined by LDH, MTS and ATP single-parameter assays and showed in left, middle and right hand panels, respectively. This experiment was performed by introducing a wide dose range (400 ng/mL - 200 μg/mL) of each material to 10,000 of cells grown in 96-well plates overnight and then performing the assays with commercial kits as described in Materials and Methods. *$p < 0.05$ compared with control.

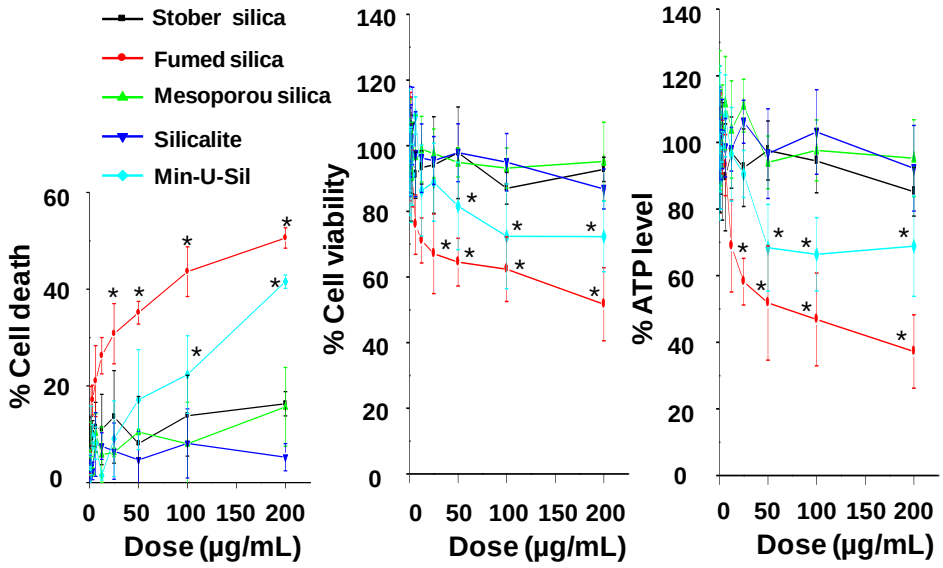


Figure S4. Heat maps to compare the toxic oxidative stress potential of silica nanoparticles in RAW 264.7 cells using the multi-parameter HTS assay. The heat maps were established using SSMD statistical analysis to evaluate the supra-threshold cellular responses by automated epifluorescence microscopy in the high throughput screening laboratory. The response parameters included measurement of intracellular calcium flux (Fluo-4), ROS generation (MitoSox Red and DCF) and mitochondrial membrane depolarization (JC-1). Cells were treated with a wide dose range of silica nanoparticles, beginning at 400 ng/ml and then doubling the dose up to 200 µg/mL. Epifluorescence images were collected hourly for the first 6 h and then again that 24 h.

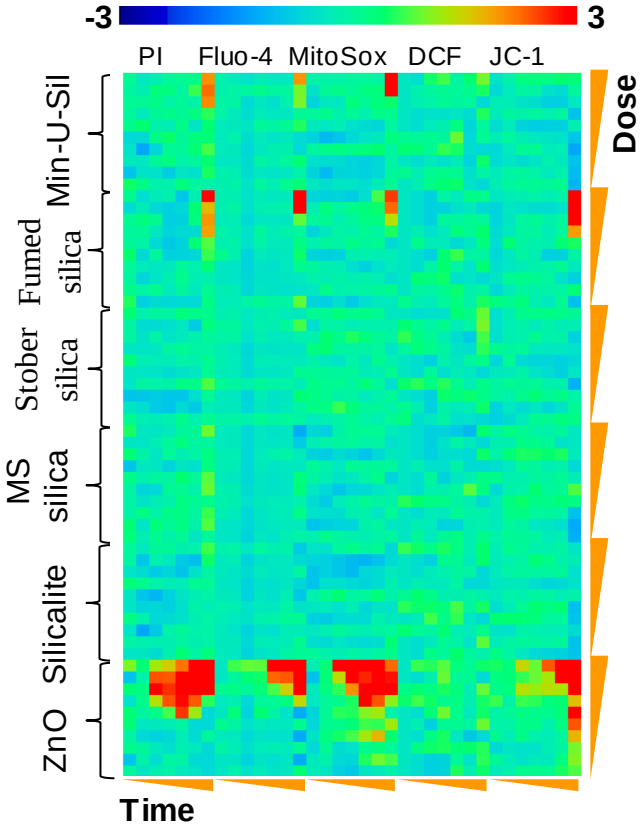


Figure S5. Cytotoxicity assessment of various fumed and Stöber silica in RAW 264.7 cells. A) Fumed silica series; B) Stöber silica series. Cells were treated with a wide dose range (400 ng/mL - 200 μg/mL) of nanoparticles for 24 hours, and cell viability was assessed by MTS assay. Calcined nanoparticles at 600 or 800 ^{o}C were obtained through heating the "as-prepared" fumed silica or Stöber silica at 600 or 800 ^{o}C for 6 hours, respectively. Rehydrated nanoparticles were obtained through refluxing the calcined nanoparticles (800 ^{o}C) in water for 24 hours. * and $^{\#}$ are defined as $p < 0.05$ compared with data of samples calcined at 600 or 800 ^{o}C, respectively, at the same doses.

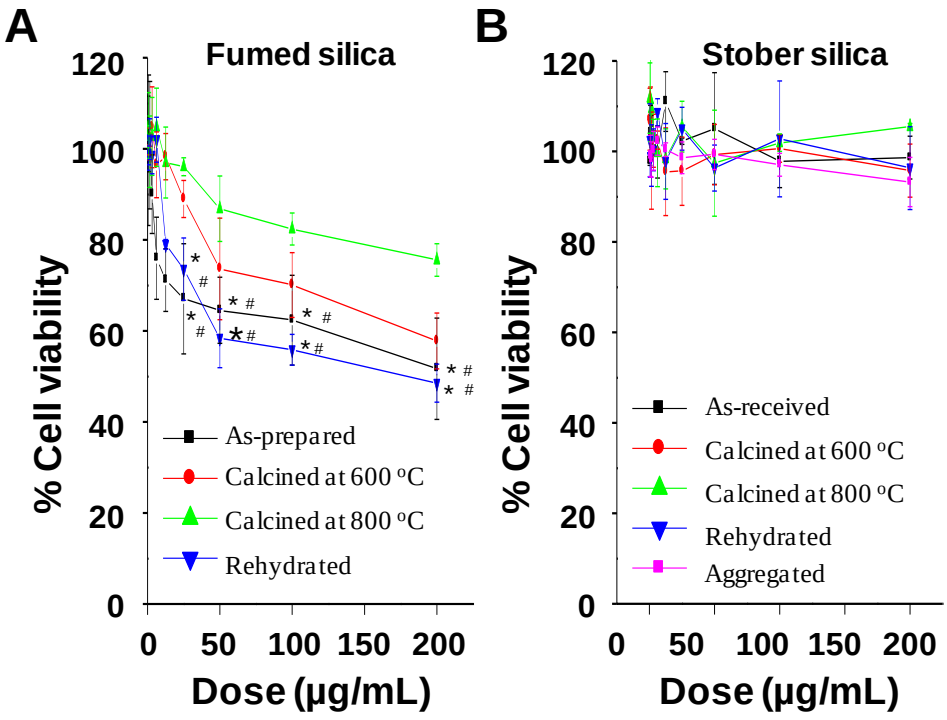


Figure S6. Differential cellular distribution of fumed and Stöber silica in THP-1 cells assessed by confocal microscopy. A) Fumed silica-treated cells; B) Stöber silica-treated cells. THP-1 cells were treated with 25 μg/mL FITC-labeled fumed or Stöber silica for 5 hours. After fixation, cell membrane was stained by Alexa Fluor 594-conjugated WGA to show red fluorescence while nuclei were stained with Hoechst 33342 to show blue fluorescence. Most green fluorescent FITC-labeled fumed silica NPs appear adherent to the cell membrane while most FITC-labeled Stöber silica NPs appear internalized.

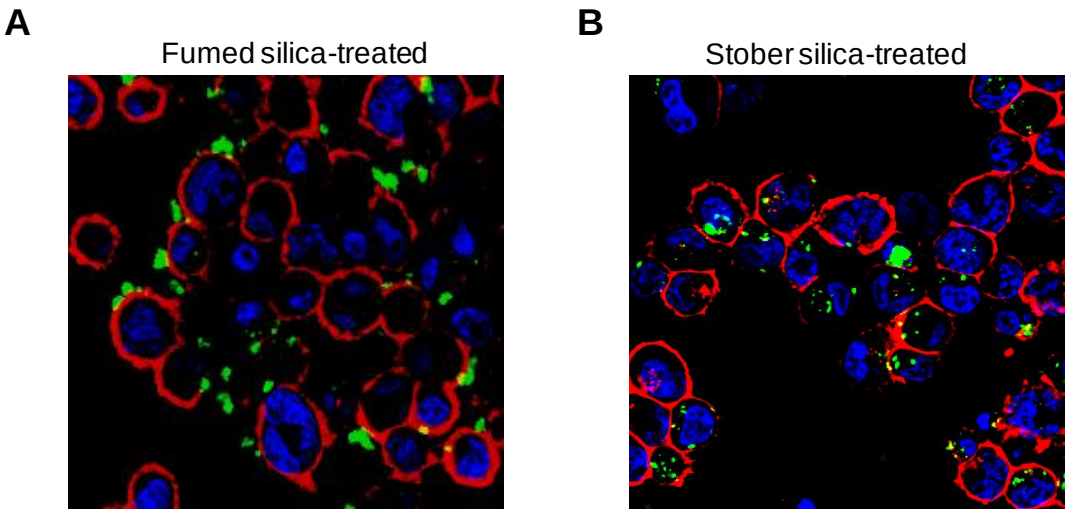


Figure S7. Kinetic analysis of sedimentation index of Stöber silica, fumed silica and aggregated Stöber silica nanoparticles in cell culture medium. A) in BEGM; B) in DMEM. The sedimentation index of silica nanoparticles (200 µg/mL) were expressed as the percentage of the initial absorbance (t=0) (λ=450 nm) for time periods of 1, 2, 3, 4, 5, 6 and 24h. 200 µg/mL of the silica nanoparticle suspensions were prepared in BEGM and DMEM as described in Materials and Methods (section 2.2). The absorbance measurements were performed using a UV-Vis spectrometer (SpectroMax M5, Molecular Devices Corp., Sunnyvale, CA).

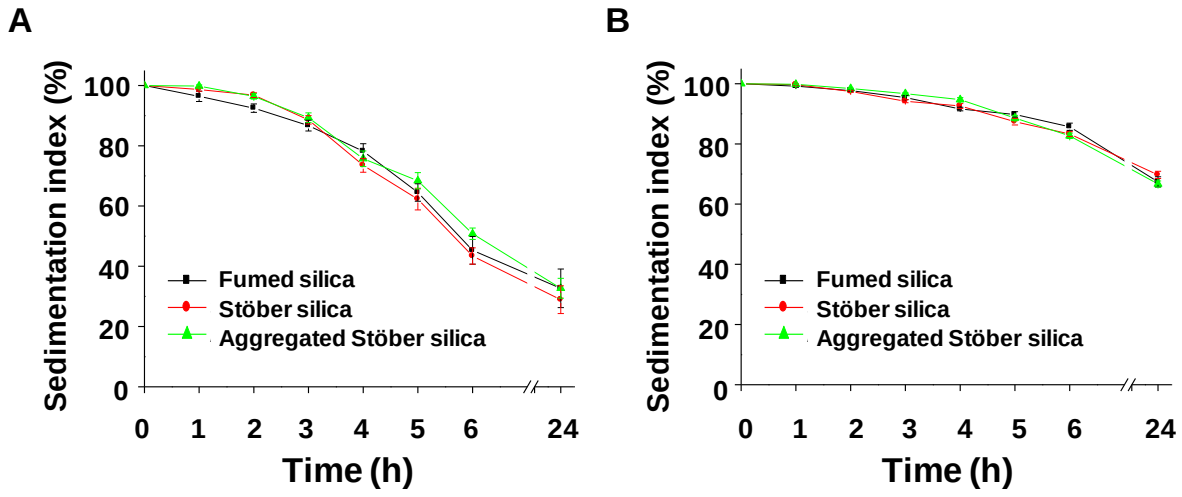


Figure S8. Cellular distribution of Stöber silica NP aggregates in BEAS-2B and THP-1 cells assessed by confocal microscopy. A) BEAS-2B cells; B) THP-1 cells. Cells were treated with 25 μg/mL FITC-labeled Stöber silica aggregates for 5 hours. After fixation, the cell membrane was stained by Alexa Fluor 594-conjugated with WGA to show red fluorescence, while nuclei were stained with Hoechst 33342 to show blue fluorescence. In both cell lines, most green fluorescent FITC-labeled Stöber silica aggregates appear to be internalized into cells.