Supporting Information (SI)

On-Chip Evaluation of Shear Stress Effect on Cytotoxicity of Mesoporous Silica Nanoparticles

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Experimental details

A. Device fabrication and cell culture

1. Device Fabrication: The microfluidic channels were fabricated using standard photolithography techniques. Mask device designs were printed on transparent film (Cad/Art Service Inc., Bandon, OR), transferred onto a 5 x 5 chrome mask plate (Nanofilm, Westlake Village, CA), and then developed in 351 developer solution (Rohm and Hass Electronic Materials LLC, Marlborough, MA). For completion of the mask, the exposed chrome layer was etched down in a chrome etchant solution (Cyantek Corporation, Fremont, CA), and then, the plate was washed and dried. To fabricate a device master, a 4-inch silicon wafer was cleaned using 1:10 hydrofluoric acid (Avantor Performance Materials, Phillipsburg, NJ):DI water solution for 1 minute to remove potential oxides on the surface, dried on a

115^{0}C hotplate, and spin-coated with a negative photoresist, either SU-8 50 or SU-8 2100 depending on the channel height (Microchem, Newton, MA). Channel width and height used in this research are described in Table 1, and the channel length is 20 mm for all devices. After a baking step, the photoresist layer was exposed to UV light through the previously prepared mask to transfer the channel images onto the photoresist. After another baking step, the wafer was developed in SU-8 developer. Upon completion of the master, Sylgard 184 elastomer and curing agent (Ellsworth Adhesives, Germantown, WI) were mixed in 10:1 weight ratio, de-gassed, cast onto the master and cured overnight at 95^{0}C. The PDMS layer was then cut and punched for inlet and outlet holes. After cleaning, the PDMS layer was permanently bonded onto a glass coverslip by oxygen plasma treatment at 100 W for 10 seconds. Glass coverslips were prepared before this bonding process by cleaning them in 1:1 volume ratio of 30% hydrogen peroxide and 99.9% sulfuric acid (Avantor Performance Materials, Phillipsburg, NJ) for 5 min followed by acetone, methanol, isopropyl alcohol, and D.I. water rinsing.

The complete device was then brought into a biosafety cabinet and 20 μL syringe tips (Thermo Scientific, Rockford, IL) were inserted in both the inlet and outlet holes. The device was exposed to UV light for an hour and kept in the biosafety cabinet overnight to promote sterility. Then, the channels were washed using a 70 volume percent ethanol solution in sterilized Milli-Q water (Millipore, Billerica, MA) followed by sterile Milli-Q water rinsing three times. The device was dried and kept in

the biosafety cabinet until use. This use of micropipette tips is trivial but worth noting as the tips addressed several practical issues. They facilitated exchange of the syringe without damaging the inlet and outlet holes as well as cell loading and feeding using standard culture procedures with minimal inducement of shear stress in the channel.

2. Endothelial Cell Culture: Human endothelial cells {Hy926} were purchased from American Type Culture Collection (ATCC, Manassas, VA) and kept frozen in a liquid nitrogen tank. Upon thawing, endothelial cells were expanded into Dulbecco's Modified Eagle Medium (DMEM) with high glucose (formula: 4mM L-glutamine, 4.5g/L L-glucose, and 1.5g/L sodium pyruvate (Gibco®, Carlsbad, CA)), supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin (Sigma Aldrich, Milwaukee, WI), and cultured in a T-flask in an incubator with 5% CO_2 at 37^0C (New Brunswick Scientific, Edison, NJ). Cells were fed every other day and, if needed, split once per week. Endothelial cells were used only between the third and tenth passages.

3. Endothelial coating of a microfluidic channel: First, 250 µg/mL of human fibronectin (Invitrogen, Carlsbad, CA) in sterilized Milli-Q water was injected through the channel and the device was incubated under 5 % CO_2 at 37 ^{0}C for an hour. Meanwhile, endothelial cells in a T-flask were washed with

phosphate buffered saline (PBS) (Invitrogen, Carlsbad, CA) solution 3 times, trypsinized (Invitrogen, Carlsbad, CA), and kept in the incubator for 4 min. The trypsin solution containing endothelial cells was collected and re-suspended into two-fold volume of DMEM with high glucose, 10% fetal bovine serum and 1% penicillin and streptomycin in a centrifuge tube and centrifuged at 150xg for 5 min. During the centrifugation, fibronectin solution was removed from each channel. The obtained endothelial cell pellet was then re-suspended into the same DMEM media again with the desired cell density (5 - 8 x 10^6 cells/mL), injected through the channel, and the device was kept in the incubator. After 2 hours, the media was exchanged to remove non-adherent cells, and the endothelial cells in the device were fed every twelve hours. Cells were cultured in the device for a minimum of 3 days and maximum of 5 days to ensure that endothelial cells cover the entire channel surface as different cell number would significantly affect the cytotoxicity results.

B. Mesoporous silica nanoparticle synthesis

1. Materials: All chemicals were used as received. n-Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), chlorotrimethylsilane (TMS) and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (Milwaukee, WI). 2-[Methoxy(polyethyleneoxy)propyl]-trimethoxysilane, (PEG-silane, MW 596-725 g/mol, 9-12 EO) was obtained from Gelest (Morrisville, PA). Absolute anhydrous 99.5% ethanol and 95% ethanol were purchased from Pharmco-Aaper (Brookfield, CT). The de-ionized (D.I.) water was generated using a Millipore Milli-Q system (Billerica, MA). Ammonium hydroxide (NH_4OH, 28-30 wt% as NH_3) was obtained from Mallinckrodt (Phillipsburg, NJ). Acetic acid was obtained from BDH (West Chester, PA).

2. Synthesis of unmodified fluorescent mesoporous silica nanoparticles (FMS NPs): The preparation of bare fluorescent MS nanoparticles was accomplished using our published procedure.[1] First, 1.9 mg of FITC was first dissolved in 1 mL of 99.5% ethanol. Two μL of APTES was then added

to the FITC ethanolic solution. The solution was stirred under dark condition for 18 hours at room temperature. Next, 0.29 g of CTAB was dissolved in 150 mL of 0.256 M NH_4OH solution at 50 ^oC. After one hour, 1 mL of FITC-APTES ethanolic solution and 2.5 mL of 0.88 M ethanolic TEOS solution were added simultaneously 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. The surfactant was removed from the as-synthesized FMS NPs 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 2 M 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 FMS NPs were filtered through a 0.8 μm filter and stored at 4 ^oC until use.

3. Synthesis of highly organo-modified fluorescent mesoporous silica nanoparticles (FMS@PEG/TMS NPs): The synthesis of FITC-MS@PEG/TMS NPs was based on a newly developed method by our group.[3] First, 0.29 g of CTAB was added to 150 mL of 0.256 M NH_4OH solution at 50 ^oC. Then, 1 mL of ethanolic FITC-APTES solution and 2.5 mL of 0.88 M ethanolic TEOS solution were added simultaneously 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 68 μL of TMS was added. After another 30 minutes, stirring was stopped and the obtained solution was aged at 50 ^oC for 20 hours. The as-synthesized modified MS 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. Finally, the surfactant-free FITC-MS@PEGTMS NPs suspended in 99.5% ethanol were filtered using a 0.2 μm polytetrafluoroethylene (PTFE) filter. The products were dried and

stored at room temperature until use. Full physical characterization of these NPs can be found in reference 3.

4. Nanoparticle 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. Hydrodynamic diameter data were measured at particle concentration of 200 μg/mL using dynamic light scattering (DLS) with a Brookhaven 90Plus/BIMAS particle analyzer (Holtsville, NY) 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.

C. Device operation and measurements

1. Shear stress control in microfluidic channels: Shear stress levels applied to the endothelial cells were controlled by adjusting channel dimensions and flow rate. Shear stress levels at given flow rate and channel dimensions were calculated using equation (1) and confirmed by computational fluid dynamics (CFD) modeling using COMSOL Multiphysics v4.1.

2. Fluorescence imaging: The FMS and FMS@PEG/TMS NPs used in this research were synthesized with incorporated fluorescein isothiocyanate (FITC) to facilitate easy characterization of cell/NP interaction. Following cell exposure to NPs, the cells were washed with PBS for 3 min and then fluorescence imaging was performed on an inverted Nikon TE3000 microscope (Melville, NY).

3. On-chip *in vitro* MTT assay: After visually insuring appropriate cell coverage of a microfluidic channel, the inlet and outlet tips were connected to syringes via 0.0038'' ID Teflon tubes. One experiment consisted of two channels with full coverage of endothelial cells. One channel was used for

the control purpose and exposed to media only, while the other was used for the experimental condition and exposed to the media containing nanoparticles. The presence of any bubbles compromises shear stress control in the device, so extra care was taken to avoid and identify bubbles in the tips and device. Then, each channel was washed with DMEM with high glucose and 1% penicillin and streptomycin (henceforth, DMEM without serum) for 3 minutes, and the syringe was exchanged to that with either DMEM without serum (control) or DMEM without serum but containing 200 μg/mL silica nanoparticles (experimental). After 2 hours of flow, the syringe was changed to that with DMEM without serum to remove non-adherent nanoparticles, and a 0.5 mg/mL MTT solution was injected through the channel for 1 minute. After 2 hours incubation, the tube was connected to a syringe filled with dimethyl sulfoxide (DMSO), and the channel contents were collected into an eppendorf tube. If needed, the total volume of DMSO in each eppendorf tube was balanced so that all tubes contained the same final volume. Absorbance of the collected DMSO solutions was measured at 570nm with 655nm measurement for background using a plate reader (Bio-Rad, Hercules, CA) in triplicate. Toxicity was assessed by taking the absorbance of the collected DMSO from the experimental condition channel (with nanoparticles) and divided by that of the absorbance of the collected DMSO from control condition channel (without nanoparticles).

D. Effect of shear stress without nanoparticles on endothelial cell viabilities

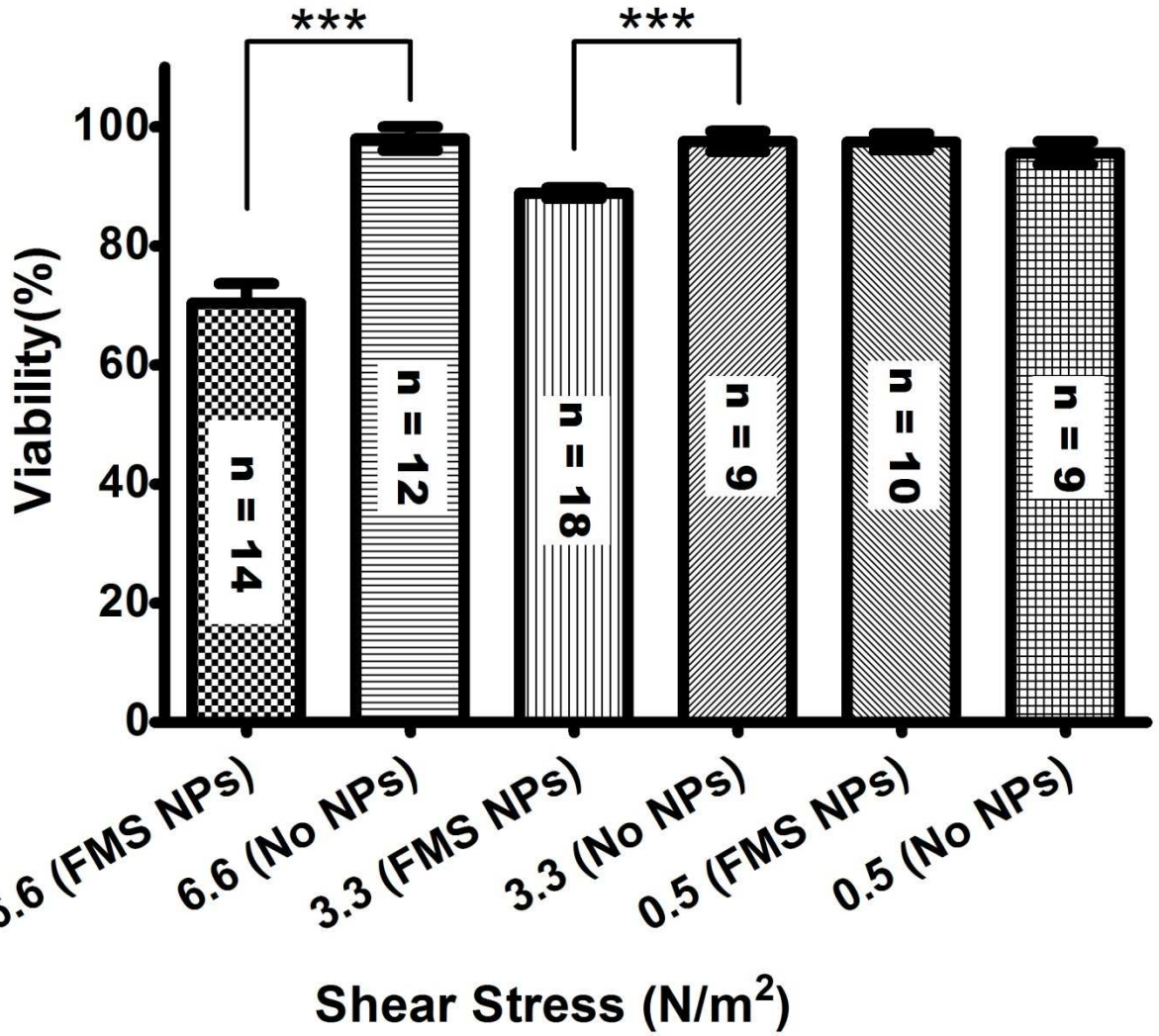

Figure S1. The effect of shear stress without nanoparticles on endothelial cell viabilities

E. Dose calculations for the flow conditions

1. The number of nanoparticles per gram:

$$Density\ of\ amorphous\ silica = 2.2\ \left(\frac{g}{cm^3}\right)$$

$$Primary\ pore\ volume = 0.81\ \left(\frac{cm^3}{g}\right)$$

$$Assumption: The\ nanoparticles\ is\ a\ hexagonal\ prism.$$

The total volume of a single nanoparticle:

$$= 6 \times \frac{\sqrt{3}}{4} \times L^2 \times H$$

where L is the length of a side of the hexagon and H is the height of the entire structure. Herein, the values for L and H are determined based on TEM imaging.

$$= 6 \times \frac{\sqrt{3}}{4} \times (21 \times 10^{-7})^2 \times (42 \times 10^{-7}) = 4.812 \times 10^{-17} \left(\frac{cm^3}{NP}\right)$$

Defining X_{42} as the number of nanoparticles per gram,

$$\left\{\left[X_{42} \times 4.812 \times 10^{-17} \left(\frac{cm^3}{NP}\right)\right] - \left[1 \times 0.81 \left(\frac{cm^3}{g}\right)\right]\right\} \times 2.2 \left(\frac{g}{cm^3}\right) = 1$$

$$\therefore X_{42} = 2.6 \times 10^{16} \left(\frac{No.NPs}{g}\right)$$

2. Potential volume of nanoparticle-containing fluid interacting with endothelial cell layer:

$$Diffusion\ length\ in\ z-axis\ (height) \times Channel\ width \times Total\ traveled\ length$$

$$= 1 \times 10^{-6}m \times 150 \times 10^{-6}m \times 0.2\frac{m}{sec} \times 7200seconds \times \frac{1000\ L}{m^3} = 2.16 \times 10^{-4}L$$

3. Number of nanoparticles exposed to endothelial cells:

$$Concentration \times Total\ volume\ of\ nanoparticle\ stream \times Number\ of\ nanoparticles\ per\ gram$$

$$= 200\ \frac{10^{-6}g}{10^{-3}L} \times 2.16 \times 10^{-4}\ L \times 2.6 \times 10^{16} = 1.2 \times 10^{12}\ \text{NPs}$$

Table S1. Dose comparison between static and the highest shear stress conditions

Shear Stress (N/m^2)	0	6.6
Flow Rate (μL/min)	0	75
Concentration	200	200
# of NPs	5.2×10^{11}	1.1×10^{12}

Table S2. Dose variation done in static conditions

Concentrations (μg/mL)	200	475	950
Shear Stress (N/m^2)	0	0	0
# of NPs	5.2×10^{11}	1.2×10^{12}	2.4×10^{12}

Table S3. Dose variation done in shear stress conditions

Channel Dimensions (μm)	150W80H	150W65H	300W45H
Flow Rate (μL/min)	75	50	45
Shear Stress (N/m^2)	6.6	6.6	6.2
Concentrations (μg/mL)	200	200	200
# of NPs	5.2×10^{11}	4.5×10^{11}	5.6×10^{11}

* W: Width, H: Height

F. Supplementary TEM images

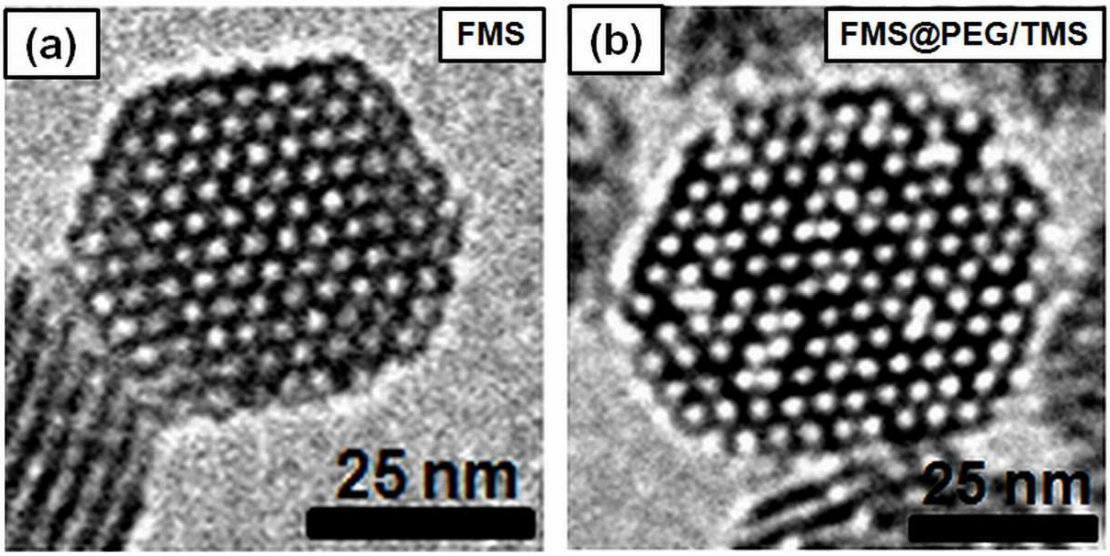

Figure S2. Enlarged TEM images of surfactant-free (a) FMS and (b) FMS@PEG/TMS

References:

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

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

3. Lin, Y.-S.; Abadeer, N.; Hurley, K. R.; Haynes, C. L. **2011**, *in preparation*.