

## Supplementary Information

### Pluronic polymer capped biocompatible mesoporous silica nanocarriers

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We have provided experimental details and general procedures, particle size distributions, surface area and pore volume analysis, TGA and FTIR results, and effect of washing on average particle size.

## S1. Experimental Section

### Materials

Tetraethyl orthosilicate (TEOS), octyltriethoxysilane (OTS), sodium hydroxide, ammonium nitrate, and hydrofluoric acid (HF) were purchased from Merck (Germany), cetyltrimmoniumbromide (CTAB), pluronic polymer (F127), ethanol, hydrochloric acid (37%) (HCl), and doxorubicin hydrochloride (DOX) were purchased from Sigma-Aldrich (U.S.A.), dimethyl sulphoxide (DMSO), methanol, and isopropanol (IPA) were purchased from Carlo-Erba (Italy), and tetrahydrofuran (THF) was purchased from Labkim (Turkey). All chemicals were used as purchased.

### Synthesis of MSNs

To synthesize OMSN, first 200 mg CTAB and 5 mg F127 were dissolved in 96 mL of deionized water and 0.7 mL of 2 M NaOH was added. Then the reaction mixture was heated to 80 °C while stirring vigorously (600 rpm) and 1 mL of TEOS was rapidly added under vigorous stirring (600 rpm). After 90 min, to prepare the octyl containing shell, 0.25 mL of OTS was dissolved in 10 mL THF and slowly added to the reaction mixture. The mixture was further stirred for 3 h. Finally, reaction mixture was cooled down to the room temperature, particles were collected by centrifugation at 9000 rpm for 20 min and washed with ethanol twice. Surfactant molecules were extracted by stirring the particles in 50 mL of 20 g L<sup>-1</sup> ethanolic ammonium nitrate at 60 °C for 30 min. This treatment repeated twice to ensure complete surfactant removal. Particles were washed with methanol twice afterwards and dried at 50 °C overnight. MSN was synthesized without the addition of OTS; other parameters were same with the O-MSN synthesis.

### F127 capping of O-MSN

25 mg of O-MSN was dispersed in 50 mL of F127 solution in water (5 mg/mL) by sonication and stirring. The dispersion sonicated for 15 min and afterwards stirred vigorously for 1 h. F127 capped particles were precipitated at 9000 rpm for 20 min and redispersed in 50 mL of F127 solution in water (5 mg/mL). Aforementioned sonication and stirring steps were repeated. Finally, particles were precipitated and washed with water or phosphate-buffered saline (PBS) twice to remove the excess F127 molecules.

## Cell Culture

Human breast adenocarcinoma cells (MCF-7) were grown to confluence at 37 °C under 5 % CO<sub>2</sub> in Dulbecco's Modified Eagle Serum (DMEM) containing 1% penicillin/streptomycin, 10 % fetal bovine serum (FBS) and 2 mM L-glutamine.

## Cytotoxicity assay in vitro

Cells in the logarithmic growth phase were washed once with PBS, trypsinized and resuspended in fresh medium. The cells were seeded in 96-well plates at  $5 \times 10^3$  cells/well. After 24 h of culture, the medium was removed by aspiration and replaced with 100  $\mu$ L of fresh medium containing MSN or F127-OMSN at concentrations of 100, 250, 500 or 1000  $\mu$ g/mL and incubated for 24 or 48 h. The cytotoxicity of particles was determined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay (Sigma, Aldrich). At least two well columns containing cells without the particles were used as a negative control. MTT reagent (5 mg mL<sup>-1</sup>) containing medium was added to each well, and the plates were incubated in the dark for 4 h at 37°C. After incubation, medium was removed and the resulting purple formazan crystals were dissolved by adding medium. Then the optical density was measured at 570 and 680 nm by using a microplate reader (SpectraMax, M5). The optical density of wells containing untreated cells was considered as 100 %. All the experiments were performed in triplicate.

## Hemolysis assay

EDTA stabilized human blood samples were collected from volunteers at Bilkent University Health Center (Ankara, Turkey). Fresh blood samples (3 mL) were centrifuged at 1600 rpm for 5 min and RBCs were obtained after removing the blood plasma. Precipitated RBC pellet was washed five times with 6 mL of PBS and RBCs were dispersed in 25 mL of PBS. 0.2 mL of RBCs were placed in plastic vials and 0.8 mL of MSN or F127-OMSN solutions in PBS at different concentrations were added. Also, positive and negative control samples were prepared by adding 0.8 mL of water and PBS, respectively. The samples were incubated at room temperature for 2 h. Samples were slightly shaken once for every 30 min to resuspend the RBCs and MSNs. After incubation, RBC were precipitated at 1600 rpm and 200  $\mu$ L of supernatants was transferred to a 96-well plate to measure the absorbance of released hemoglobin, from damaged RBCs, with a microplate reader at 570 nm. Absorbance at 655

nm was recorded as reference. Hemolysis percentages of the RBCs were calculated using the following formula;

$$\% \text{ Hemolysis} = (\text{abs of sample} - \text{abs of negative control}) / (\text{abs of positive control} - \text{abs of negative control})$$

Percent hemolysis values were calculated from three separate experiments.

### **Blood clotting assay**

For PT and aPTT measurements, human blood samples were collected to citrate stabilized vials from volunteers. Plasma samples were freshly prepared from the blood samples and immediately used in the experiments. 50  $\mu\text{L}$  of MSN or F127-OMSN solutions in PBS were added to the 450  $\mu\text{L}$  of plasma samples, and incubated for 5 min at 37  $^{\circ}\text{C}$ . Final particle concentrations in the resulting solutions were 0.1 and 1 mg/mL. After incubation, particles were removed by centrifugation and 50  $\mu\text{L}$  portions of supernatants were used to measure PT and aPTT values using a semi-automatic blood coagulation analyzer (Tokra Medikal, Ankara, Turkey). Also, control measurements were performed using 50  $\mu\text{L}$  PBS. All PT and aPTT values were calculated from three separate measurements.

### **DOX loading and release experiments**

In order to load DOX into the pores of MSN or O-MSN, 10 mg of particles were dispersed in 1 mL of DOX solution (7.5 mg mL<sup>-1</sup>) in ethanol and shook at 300 rpm for 24 h. Then the particles were collected by centrifugation. DOX loaded MSNs were washed with water twice to remove the non-adsorbed DOX molecules. For O-MSN before precipitation 5 mg of F127 added into the solution. DOX loaded OMSN were transferred into the water as described above to produce DOX loaded F127-OMSN. The amounts of adsorbed DOX were determined by monitoring the fluorescence of DOX at 590 nm (excitation wavelength is 488 nm) after etching the silica in 1.2 % HF solution for overnight.

DOX release profiles of MSN and F127-OMSN was determined by dispersing 5 mg of particles in 8 mL of PBS at pH 7.4 or pH 5.5 and each solution were separated to 10 eppendorf tubes. The tubes were shaken at 37  $^{\circ}\text{C}$  for 12 h and at different time intervals one tube removed and centrifuged. The volumes of supernatants were completed to 20 mL and fluorescence peak of DOX at 590 nm was monitored using a fluorescence spectrophotometer

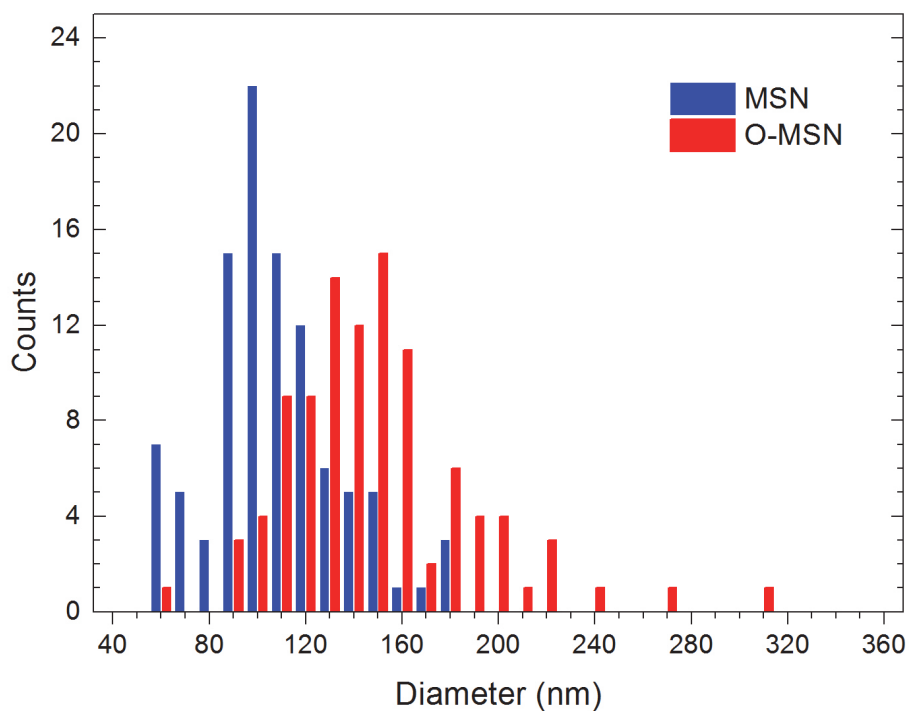
in order to determine the released DOX amount.

### **Characterization**

Transmission electron microscopy (TEM) images were taken using a Tecnai G2 F30 (FEI) microscope. Average particle sizes of MSNs were measured with Zetasizer Nanoseries (Malvern Instruments). Surface area and pore volume of the particles were determined using iQ-C (Quantachrome). Before measurements, all samples were degassed at 150 °C for 24 h. Thermal gravimetric analyses (TGA) were performed with Q500, (TA Instruments). Fourier transform infrared (FTIR) spectra of particles were recorded by using a Fourier transform infrared spectrometer (FTIR, Vertex 70, Bruker). Optical absorption measurements in cytotoxicity and hemolysis assays were carried out using a Microplate reader (Spectramax M5, Molecular Devices). Fluorescence of DOX was measured using a Fluorescence Spectrophotometer (Eclipse, Varian).

## S2. Particle size distributions

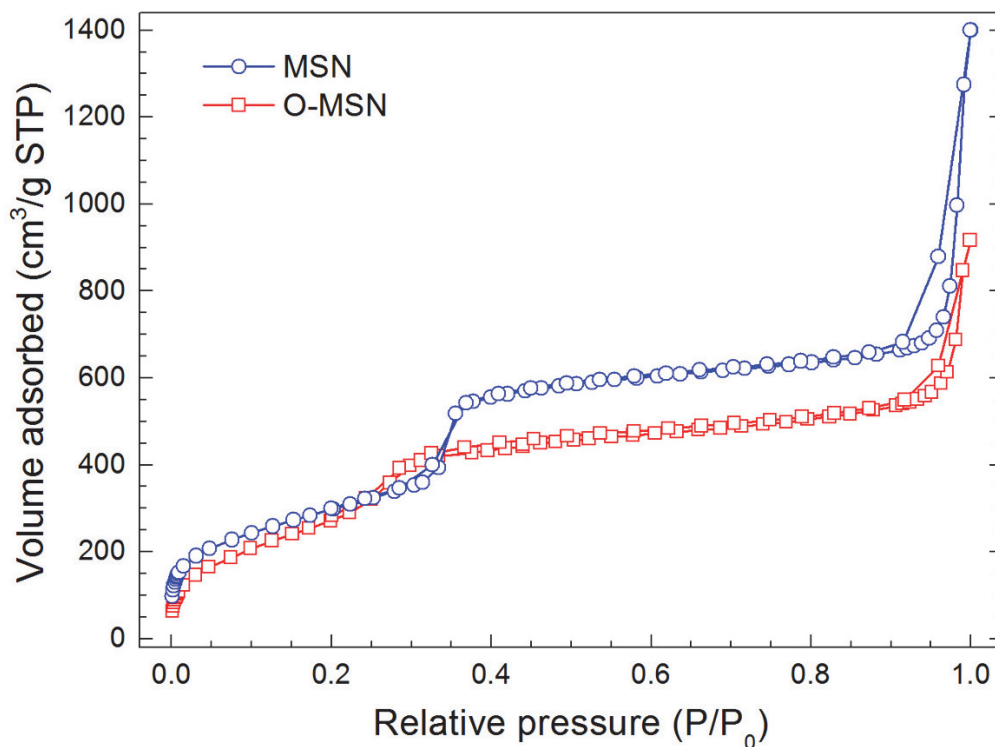
Average particle sizes of MSN and O-MSN was calculated to be  $107 \pm 27$  nm and  $149 \pm 39$  nm, respectively.



**Fig. S1** Particle size distributions of MSN and O-MSN which were calculated according to the TEM images. Octyl modification significantly increased particle size indicating octyl containing shell formation.

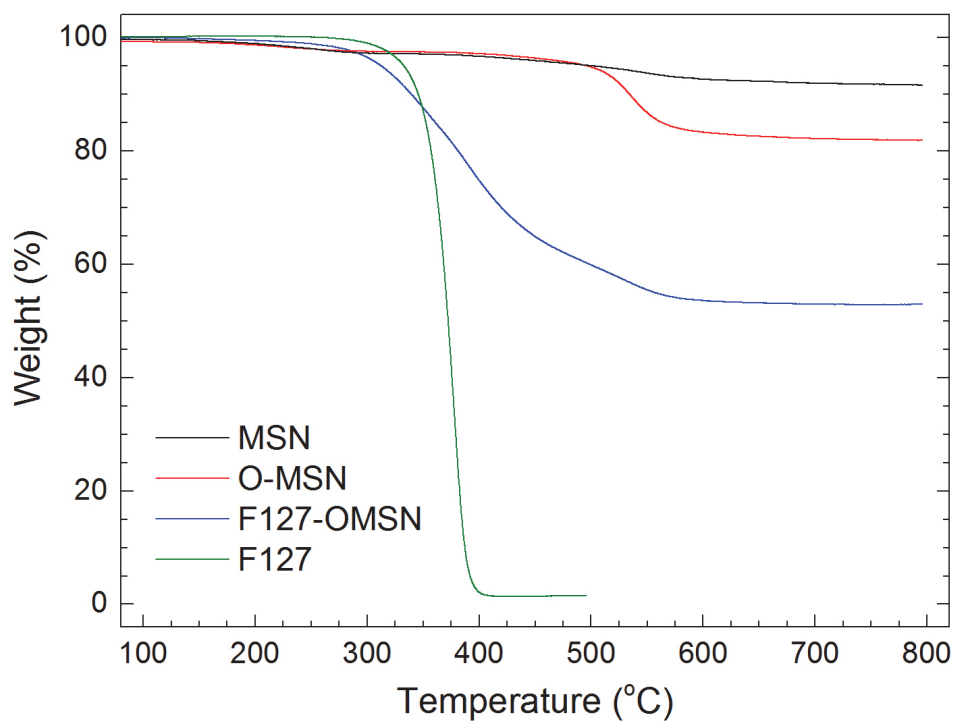
### S3. Nitrogen adsorption and desorption curves

Surface area and pore volume of the particles were calculated from nitrogen adsorption/desorption curves using Brunauer-Emmett-Teller (BET) theory and Density Functional Theory (DFT), respectively.



**Fig. S2** Nitrogen adsorption and desorption curves of MSN and O-MSN. Octyl addition slightly reduced the surface area and pore volume of the particles.

#### S4. TGA Analysis

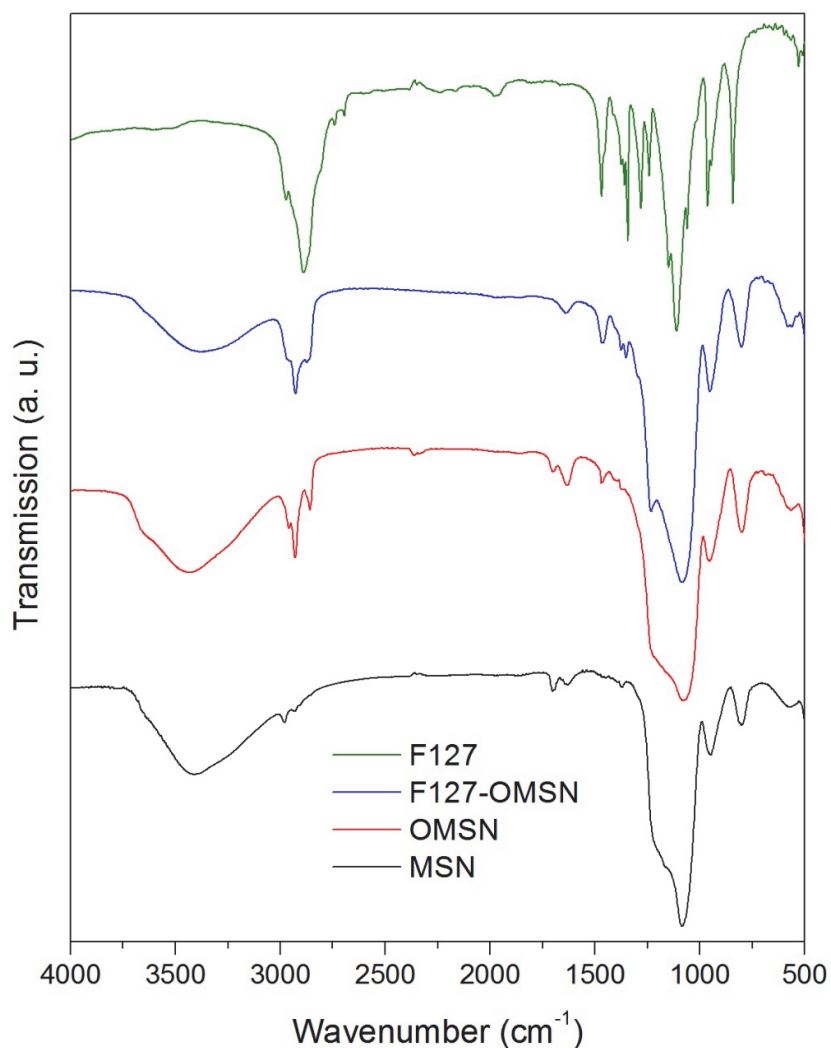


**Fig. S3** TGA spectra of MSN, O-MSN, F127-OMSN and F127 polymer. After octyl addition and F127 capping weight loss of the particles gradually increased proving the formation of octyl and F127 layer around MSNs.



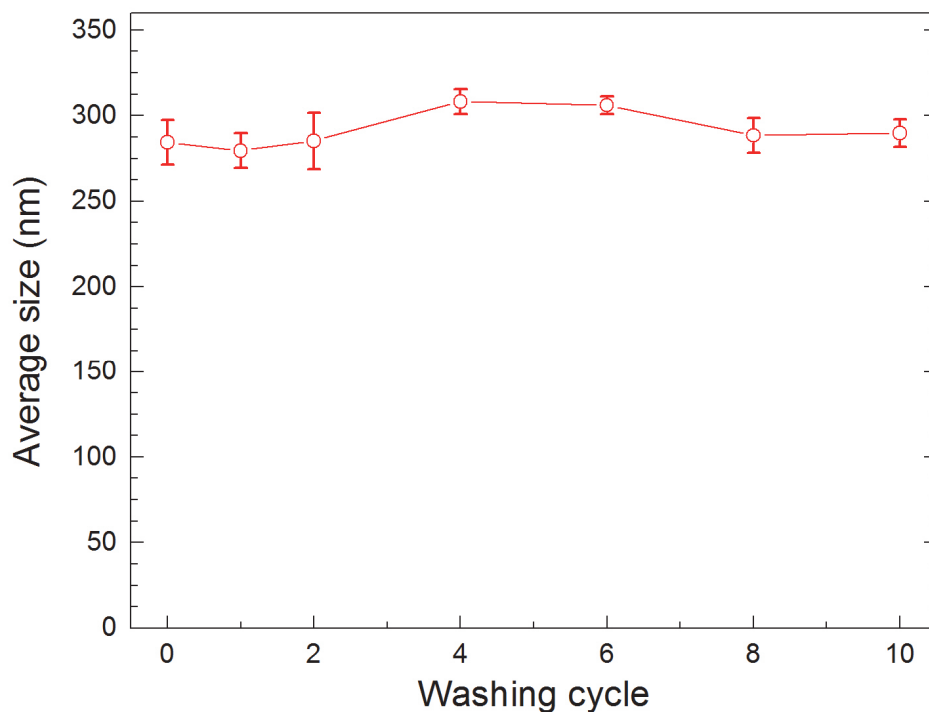
## S5. FTIR Analysis

Several infrared absorption bands of F127 polymer is clearly observable in the FTIR spectrum of F127-OMSN; CH<sub>2</sub> stretch (2880 cm<sup>-1</sup>), CH<sub>2</sub> scissor (1464 cm<sup>-1</sup>), CH<sub>2</sub> wag (2880 cm<sup>-1</sup>), CH<sub>2</sub> twist (1234 cm<sup>-1</sup>), proving the capping of OMSN with the F127 polymer (Fig. S4).<sup>1,2</sup> The broad absorption band around 2900 cm<sup>-1</sup> in the FTIR spectrum of OMSN indicating the presence of octyl groups. Also, some weak CH<sub>2</sub> absorption bands were observed in the bare MSN spectrum, which is due to the presence of residual surfactants; CTAB and F127.



**Fig. S4** FTIR spectra of MSN, O-MSN, F127-OMSN and F127 polymer showing successful octyl modification and F127 capping of particles.

## S6. Stability of F127 coating



**Fig. S5** The average zeta size of F127-OMSN in PBS after several washing cycles. Even after 10 washing cycles average particle size remained almost intact indicating the good stability of self-assembled pluronic layer.

## References

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- (2) Y. L. Su, J. Wang, and H. Z. Liu, *Langmuir*, 2002, **18**, 5370-5374.