Supporting Information

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Efficient Functional Delivery of siRNA using Mesoporous Silica Nanoparticles with Ultralarge Pores

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Materials

Cetyl trimethyl ammonium bromide (CTAB) was purchased from Acros (New Jersey, USA). Ethanol was purchased from Merck (Darmstadt, Germany). 3-Aminopropyltriethoxysilane (APTES), tetramethyl orthosilicate (TMOS), toluene, dimethylsulfoxide (DMSO), and mesitylene (trimethyl benzene, TMB) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Sodium hydroxide was purchased from Junsei Chemical Co. (Tokyo, Japan). All the reagents were used as received without further purification. DMEM (Dulbecco's Modified Eagle's Medium), FBS (fetal bovine serum), and PBS (pH 7.4) were purchased from WelGENE Inc. (Daegu, Korea). RNase, heparin, and 3,3'-Dithiodipropionic acid di(N-hydroxysuccinimide ester) were purchased from Sigma Aldrich (St. Louis, MO, USA) and CCK-8 (Cell Counting Kit-8) was purchased from Dojindo Molecular Technologies, Inc. (Rockville, MD, USA), respectively. An siRNA targeting human VEGF (sense; 5′-GGAGUACCCUGAUGAGAUCdTdT-3′, antisense; 5′-GAUCUCAUCAGGGUACUCCdTdT-3′) and siRNA targeting green fluorescence protein (GFP) (sense; 5'-GGCUACGUCCAGGAGCGCACC-3', antisense; 5'- UGCGCUCCUGGACGUAGCCUU-3') were obtained from Bioneer Inc. (Daejeon, Korea).

Physicochemical analysis of siRNA-MSN interactions

Zeta potential: Around a positively charged MSN in solution, negative counterions form an electric double layer to screen the effect of bare charges of the nanoparticle. The zeta potential is an effective electric potential evaluated at the Debye screening length (κ^{-1}) away from the surface of a spherical particle (MSN) with radius R_{MSN}. The electrostatic potential around such MSN is determined by solving linearized Poisson-Boltzmann (PB) equation, $\nabla^2\phi = \kappa^2\phi$, with boundary conditions $\left.\frac{\partial\phi}{\partial r}\right|_{r=R_{\mathrm{MSN}}} = \sigma_{eff}/\varepsilon$ and $\phi(r\to\infty)=0$:

$$\phi(r) = \frac{\sigma_{eff}}{\varepsilon\kappa}\left(1+\frac{1}{\kappa R_{\mathrm{MSN}}}\right)^{-1}\frac{e^{-\kappa(r-R_{\mathrm{MSN}})}}{r/R_{\mathrm{MSN}}}. \quad (2)$$

with $r > R_{\mathrm{MSN}}$. Here, ε and σ_{eff} are the dielectric constant of the solution and the effective charge density of the MSNs, respectively. Note that linearizing the PB equation is justified in our experimental condition because $O(\kappa R_{\mathrm{MSN}}) \sim 10^3 \gg 1$ and $\zeta < k_B T / e \approx 25$ mV. For all MSN with varying R_p, $\sigma_{eff} \approx 10^{-2}\, C/m^2 = 10\ fC/\mu m^2$ (see Table I). σ_{eff} can be linked to the zeta potential, ζ, as [1, 2]

$$\zeta = \phi(R_{\mathrm{MSN}} + \kappa^{-1}) \approx \sigma_{eff} e^{-1} / \varepsilon\kappa , \qquad (3)$$

where we made an approximation since $R_{\mathrm{MSN}} \gg \kappa^{-1}$.

Electric potential for the pore: Entrance of the siRNA into the pores of MSN can be described by a charged semi-infinite cylindrical model. In this model, we set *z* as a position coordinate along the pore axis and the semi-infinite cylindrical pore of radius R_p occupies region of $z > 0$ for a monovalent electrolyte solution of concentration c and a uniform surface charge density σ over the pore walls and outer surface of the MSN. Since the values of the zeta potential are all smaller than thermal energy as the above description, one can obtain the equilibrium electrostatic potential via solving a linearized PB equation [3, 4]

$$\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial \phi}{\partial r}\right) + \frac{\partial^2 \phi}{\partial z^2} = \kappa^2 \phi , \qquad (4)$$

with boundary conditions

$$\left.\frac{\partial \phi}{\partial r}\right|_{r=0} = 0 , \left.\frac{\partial \phi}{\partial r}\right|_{r=R_p} = \frac{\sigma}{\varepsilon} H(z) , \text{ and } \phi(r, z \to -\infty) = 0 ,$$

where $\kappa = \sqrt{8\pi l_B c}$ is the inverse Debye length. The Bjerrum length, $l_B \approx 0.7$ nm in water at T = 300 K. The solution to above PB equation is easily obtained [4]:

$$\phi(r,z) = \frac{\sigma}{2\varepsilon\kappa}\frac{I_0(\kappa r)}{I_1(\kappa R_p)} + \frac{\sigma}{\varepsilon\kappa}\int_0^\infty d\lambda \frac{\sin(\lambda\kappa z)}{\pi\lambda}\frac{\kappa I_0(\kappa_\lambda r)}{\kappa_\lambda I_1(\kappa_\lambda R_p)} , \qquad (5)$$

where $\kappa_\lambda \equiv \kappa\sqrt{1+\lambda^2}$ and $I_\nu(\ldots)$ is the modified Bessel function of first kind [5]. The asymptotic solution for this potential far inside the pore ($z \to \infty$) is $\phi_\infty(r) = \lim_{z\to\infty}\phi(r,z) = \frac{\sigma}{\varepsilon\kappa}\frac{I_0(\kappa r)}{I_1(\kappa R_p)}$. The electrostatic energy between the siRNA and the pore of the MSN is simply given by $E(r,z) = -Z_{eff}e\phi(r,z)$. Note that $\phi_\infty(R_p) > \sigma/\varepsilon\kappa$.

References

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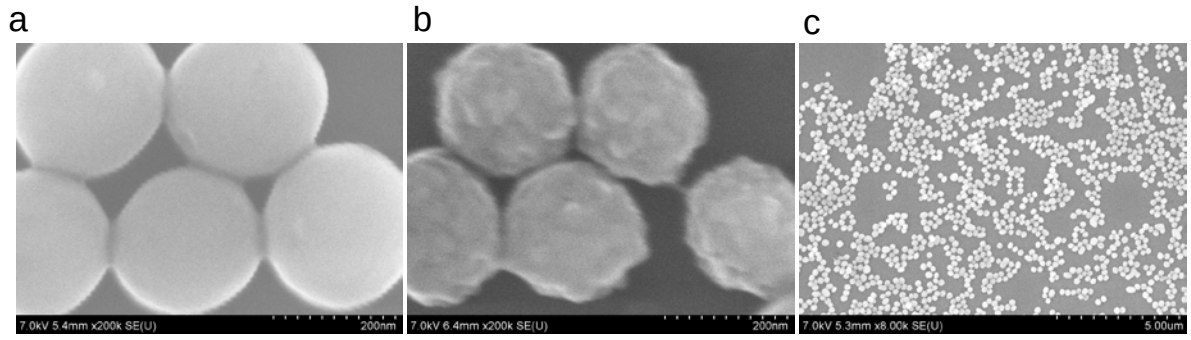

Figure S1. SEM images of MSN2 (a) and MSN23 (b and c).

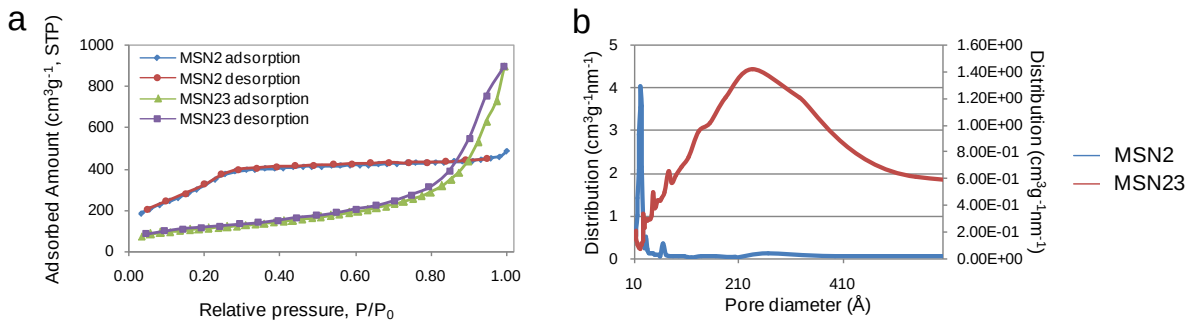

Figure S2. Nitrogen sorption data. a, adsorption/desorption isotherm **b**, pore size distribution of the synthesized MSNs

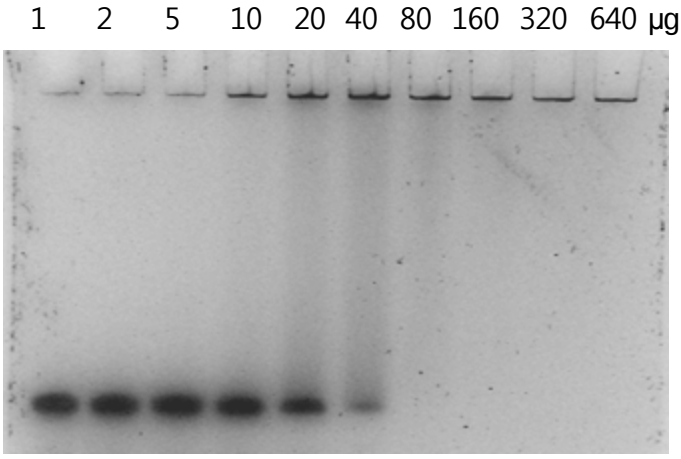

Figure S3 . SiRNA loading capacity of synthesized P-T-MSN23. Various amounts of P-T-MSN23 (1 μg to 640 μg) were incubated with 100 pmol of siRNA before loading. Unbound siGFP was not observed when 80 μg or more amounts of silica material was used.

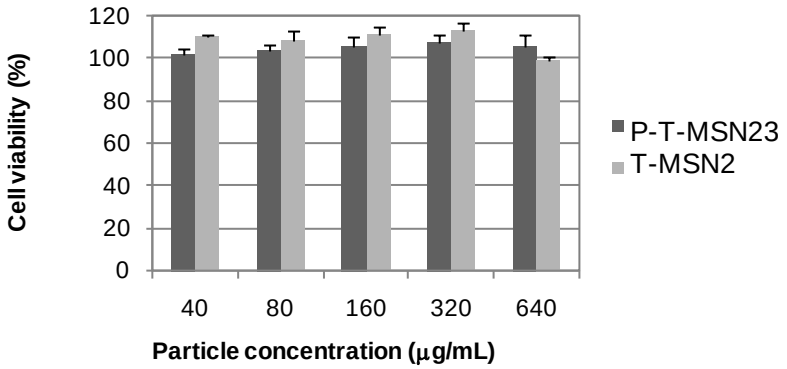

Figure S4. Cell viability was estimated based on CCK8 assay by incubating HeLa cells with various concentrations of of particles.

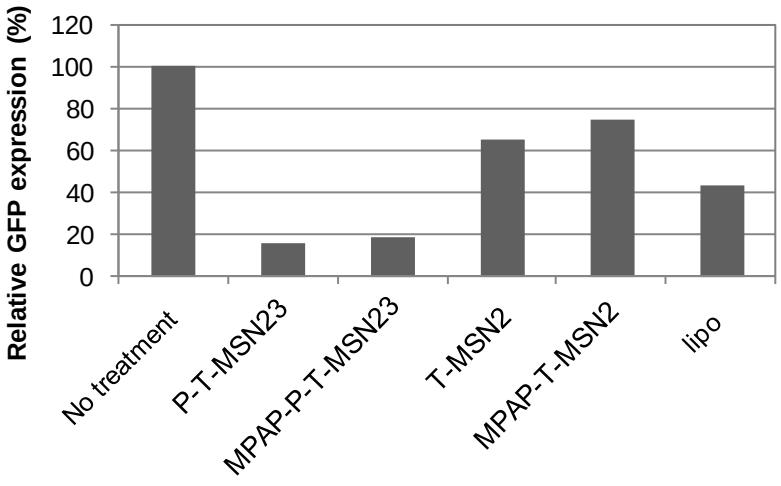

Figure S5. GFP knockdown efficiency of siRNA-MSN complex prepared by using T-MSN2 and P-T-MSN23 with or without MPAP conjugation (MPAP: myristoylated poly-arginine peptide, one of cell-penetrating peptide). P-T-MSN23 without MPAP conjugation showed highest knockdown of GFP. It is notable that there was no significant difference between MPAP conjugated P-T-MSN 23 and P-T-MSN23.

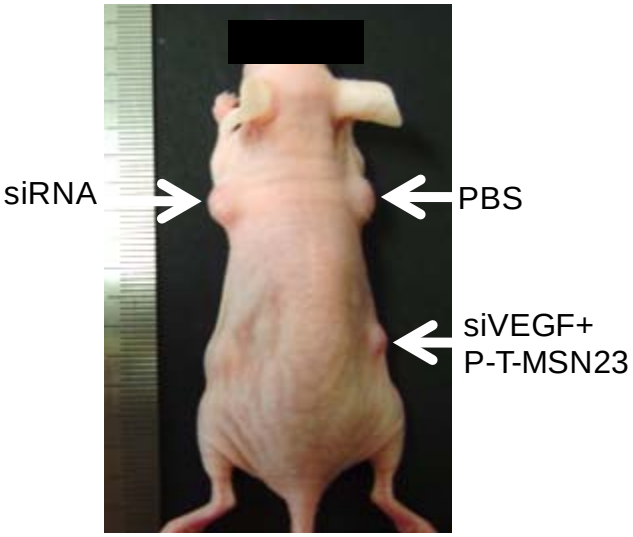

Figure S6. An image of a mouse bearing MDA-MB-231 tumors after the treatment of PBS, naked siVEGF and siVEGF-P-T-MSN23.