Supporting Information

Small interfering RNA delivery by polyethylenimine-functionalised porous silicon nanoparticles

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SEM imaging of multilayer pSi film

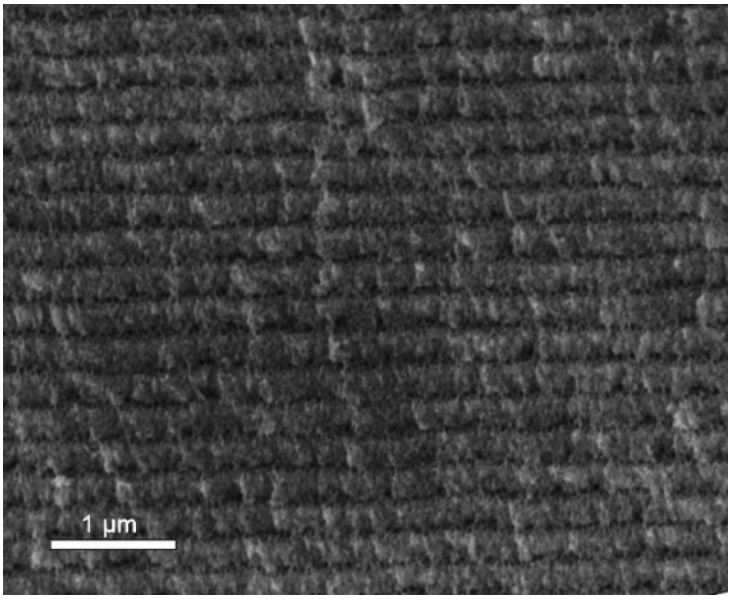

Fig. S1. Representative SEM image of a multilayer pSi film fabricated by the pulsed electrochemical etching.

TEM imaging of THCpSiNPs and PEI/siRNA/THCpSiNPs

The morphology of THCpSiNPs and PEI/siRNA/THCpSiNPs (Fig. S2) was investigated by means of TEM (JEM-2100F TEM, JEOL USA, Inc., MA, USA) with 20–120 kV beam energy under high vacuum 1×10^{-5} Pa. Both samples were prepared by allowing a single drop of the nanoparticle suspension (in EtOH) to dry overnight at room temperature on 200-mesh copper grids (ProSciTech Co., Thuringowa, Qld, Australia).

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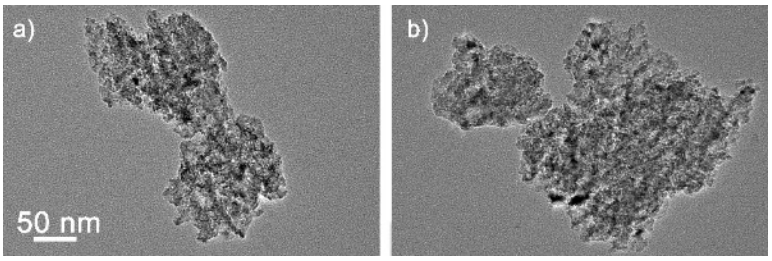

Fig. S2. Representative TEM images of (a) THCpSiNPs, (b) PEI/siRNA/THCpSiNPs.

Interferometric Reflectance Spectroscopy (IRS)

To measure stability of the surface modifications (THC and hydride terminated), interferometric reflectance spectra were acquired using an Ocean Optics USB2000 spectrometer equipped with a tungsten halogen lamp (Ocean Optics, LS-1). Light from the lamp was focused onto the surface of the sample using a collimating lens. pSi membranes (THC and hydride terminated) were clamped into a custom-built closed flow cell, through which PBS solution at 37 ^{o}C was circulated via a peristaltic pump (LongerPump BT100-1J, Baoding Longer Precision Pump Co. Ltd., Hebei, China) with a flow rate of 3.5 mL min^{-1}. Initial effective optical thickness (EOT) readings (obtained from interferometric reflectance spectra by fast Fourier transformation using IGOR software from Wavemetrics Inc., Portland, OR, USA) were recorded every 1 min for a period of 5 h (Fig. S3).

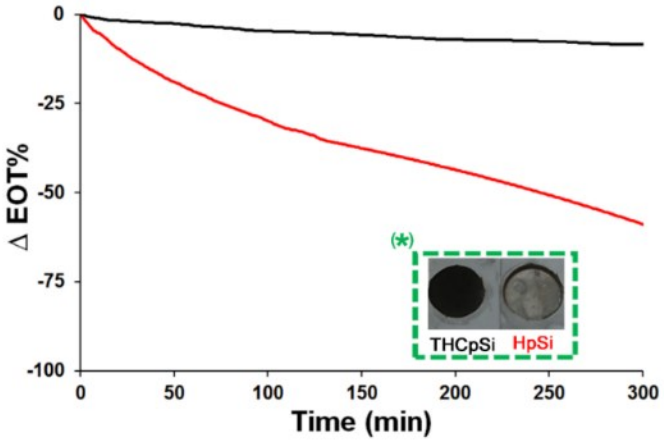

Fig. S3. Stability of HpSi (red line) and THCpSi (black line) films as a function of time in PBS as investigated by IRS. (*) Image of HpSi and THCpSi films (representatives of the surface modifications) fixed into a flow cell with a constant flow rate (3.5 mL min^{-1}) of PBS (pH 7.4 and temperature 37 ^{o}C) after 5 h incubation.

Equilibrium adsorption capacity

To load the siRNA into the THCpSiNPs (0.1 mg/mL, 0.050 mL, suspended in EtOH) for the batch adsorption experiments, the solution (initial concentrations 28, 57, 114 and 229 μg/mL) was dispersed by sonication for 1 min, then incubated at ambient temperature overnight (300 rpm). After the incubation, the supernatant was removed by centrifugation (5000 rpm, 5 min). The loaded pSiNPs were then collected. The amount of absorbed siRNA was calculated via UV-Vis spectrophotometry at 260 nm from three replicates. The amount of absorbed siRNA onto the THCpSiNPs was calculated based on Equation S1. The adsorption capacity was then examined by means of Langmuir isotherm given as

$$P = \frac{P_{\max} K_f C}{1 + K_f C} \quad \text{(S1)}$$

where P_{max} (μg/mg) and K_f (mL/μg) are the maximum amount of adsorption and the sorption equilibrium constant, respectively. C (μg/mL) and P (μg-solute/mg-THCpSiNPs) are the concentration and amount of adsorption.

Table S1. Langmuir isotherm constants for the adsorption of siRNA from EtOH/water solution to THCpSiNPs.

K_f (mL/mg)	P_{max} (μg/mg)	R^2
0.0172	**72.46**	**0.9943**

Adsorption kinetics

siRNA solution was added to 0.1 mg/mL THCpSiNPs suspension (50 μL, EtOH) at a volumetric ratio of 48/2 THCpSiNPs suspension/siRNA solution. Subsequently, the suspension solution was well dispersed by sonication for 1 min before the incubation (see Table S2) at ambient temperature for different predetermined incubation times (30, 60, 120, 180, 300 and 480 min). Afterward, the loaded THCpSiNPs were collected by centrifugation (5000 rpm, 5 min). The amount of the absorbed siRNA was measured by UV-Vis at 260 nm from three replicates. The amount of siRNA absorbed onto the THCpSiNPs, P (mg/mg), was calculated based on the Equation S1.

Table S2. Characteristics of THCpSiNPs for siRNA adsorption.

Characteristic	**Sample**
Functionalisation	THCpSiNPs
Agitation rate (rpm)	300
Type of oligonucleotide	siRNA
Average diameter (nm)	145.9
Diffusion coefficient (m^2/s)[*1, 2]	$\approx 8.39\times10^{-11}$
Initial oligonucleotides concentration (μg/mL)	230
Porosity (%)	54
Solid density (g/cm^3) [3]	≈2.33
pSiNPs concentration (mg/mL)	0.1
Solution bulk density (g-pSiNPs/cm^3)	≈1
Density of solvent (g/cm^3)	≈1
Temperature (°C)	25

Modeling of siRNA adsorption

Pseudo first and second-order models

The pseudo first and second-order models were used to assess the experimental data in order to evaluate the mechanisms involved in the adsorption of siRNA into THCpSiNPs (Table S2). The first-order rate expression of Lagergren is given as:

$$\log(P_e - P) = \log P_e - \frac{k_1 t}{2.303} \quad \text{(S2)}$$

Where P_e and P (μg/mg) are the amounts of siRNA adsorbed into the THCpSiNPs at equilibrium and at time t, respectively, and k_1 (1/min) is the rate constant of pseudo first-order model.[4] The measured data are able to fit to a straight line that is calculated by drawing log (P_e-P) versus t.

The second-order kinetic model can fit a range of sorption data without input parameters beforehand:[4,5]

$$\frac{t}{P} = \frac{1}{k_2 P_e^2} + \frac{t}{P_e} \quad \text{(S3)}$$

where k_2 (μg/mg min) is the rate constant of pseudo second-order model. Accordingly, there is a linear relationship in the plot of t/P versus t in the agreement with the second-order model. The slopes and intercepts of the plots are indicating the second-order rate constant k_2 and P_e. To determine the deviations among data, σ_{RMSD} (Root Mean Square Deviation) was applied:

$$\sigma_{RMSD} = \left[\frac{1}{n} \sum_{i=1}^{n} \left(\frac{P_{Exp} - P_{Cal}}{P_{Exp}} \right)_i^2 \right]^{0.5} \times 100 \quad \text{(S4)}$$

.

Table S3. Pseudo first and second-order adsorption rate constant and $P_{e,}$value.

Sample	Pseudo second-order kinetic model			Lagergren pseudo first-order kinetic model		
	K_1 (1/min)	$P_{e,Cal}$ (μg/mg)	R^2	K_2 (μg/mg·min)	$P_{e,Cal}$ (μg/mg)	R^2
	0.0005	**78.74**	**0.996**	**1.68**	**71**	**0.889**

Table S4. Calculated σ_{RMSD} of the kinetic models.

Kinetic models	σ_{RMSD} of sample
Pseudo first-order kinetic model	0.1487
Pseudo second-order kinetic model	**0.0223**

Human plasma protein adsorption

The behavior of nanoparticles in the circulating blood is subjected to the adsorption of proteins of the plasma.[6] The adsorption of human plasma proteins towards THCpSiNPs (0.1 mg/mL) before and after the PEI coating (with 0.1% w/v) was evaluated by measuring hydrodynamic diameter, PDI and ζ-potential. Thus, PEI coated and uncoated THCpSiNPs were suspended in 1 mL of human plasma. The samples were sonicated (30 s) to be well-dispersed, and then incubated at 37 °C for 0, 30, 60, and 120 min. NPs were then sedimented by centrifugation (5000 rpm, 5 min) and washed with de-ionised water. For DLS measurement, the treated NPs were resuspended in de-ionised water (1 mL) to measure their hydrodynamic diameter, PDI and ζ-potential. The sorption of serum proteins on the surface led to increase of the primary size and aggregation (PDI) of THCpSiNPs and PEI/siRNA/THCpSiNPs, and also changed the primary ζ-potential (Fig. S4). These changes occurred within 30 min of incubation.

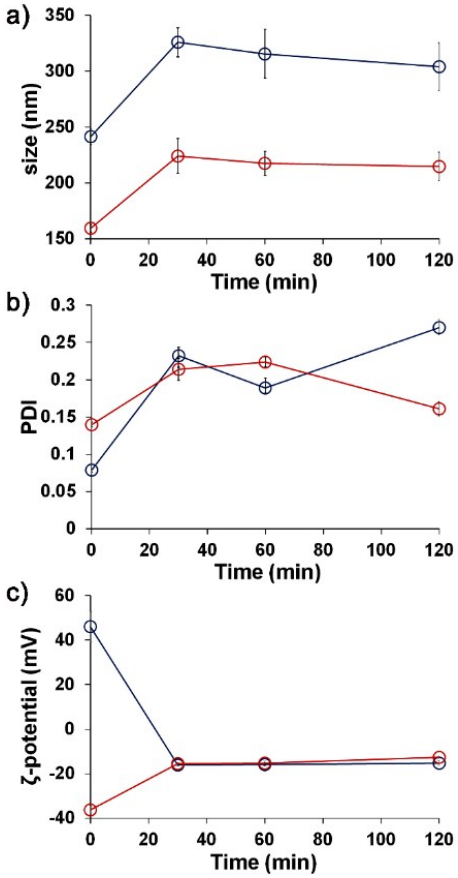

Fig. S4. Effect of human plasma protein adsorption on (a) the average hydrodynamic diameter, (b) PDI and (c) ζ-potential of THCpSiNPs (red line) and PEI/siRNA/THCpSiNPs (blue line) as a function of time.

Cell apoptosis by Annexin V analysis

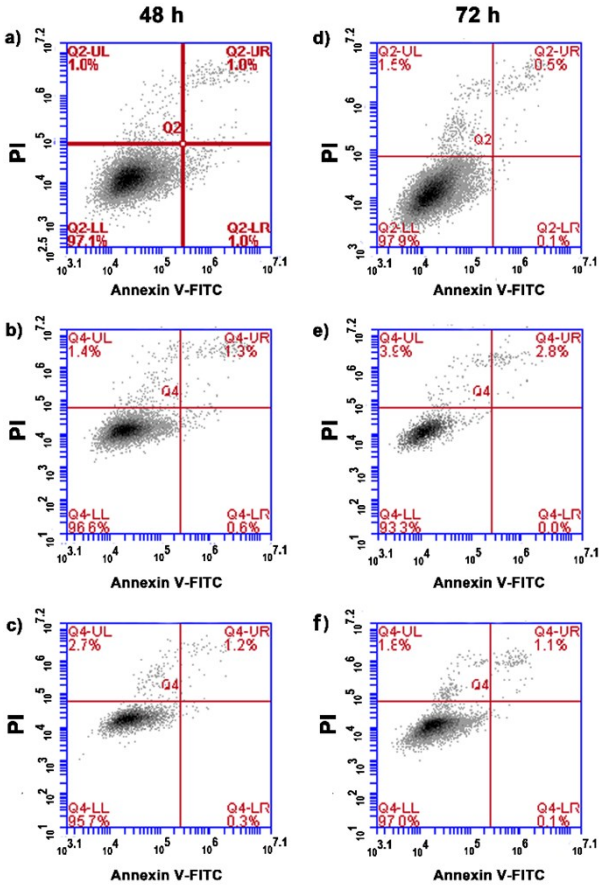

Fig. S5. T98G cell apoptosis at 48 and 72 h incubation with (a and d) Lipofectamine/siRNA, (b and e) PEI/THCpSiNPs and (c and f) PEI/NC-siRNA/THCpSiNPs at a concentration of 0.1 mg/mL. (n = 3; mean ± standard deviation shown). T98G cells line was analysed with Annexin V-FITC/PI assay after 48 h (a-c) and 72 h (d-f) of treatment with PEI/NC-siRNA/THCpSiNPs, PEI/THCpSiNPs and siRNA/lipofectamine. (Representative data, n = 3).

Cell proliferation in the presence of siRNA/THCpSiNPs

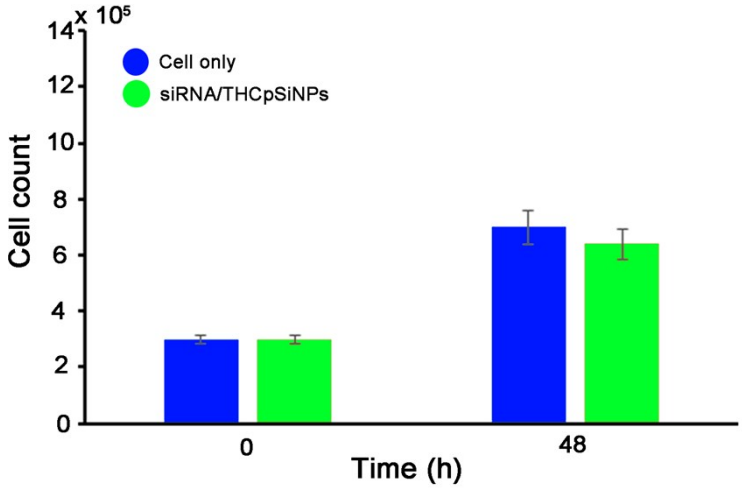

Fig. S6. Cell numbers of T98G cells transfected with the siRNA/THCpSiNPs and control (cells only) at 0 and 48 h time points. Proliferation was measured by means of the Trypan Blue assay. (n = 3; mean ± standard deviation).

Cellular uptake of PEI/siRNA/THCpSiNPs

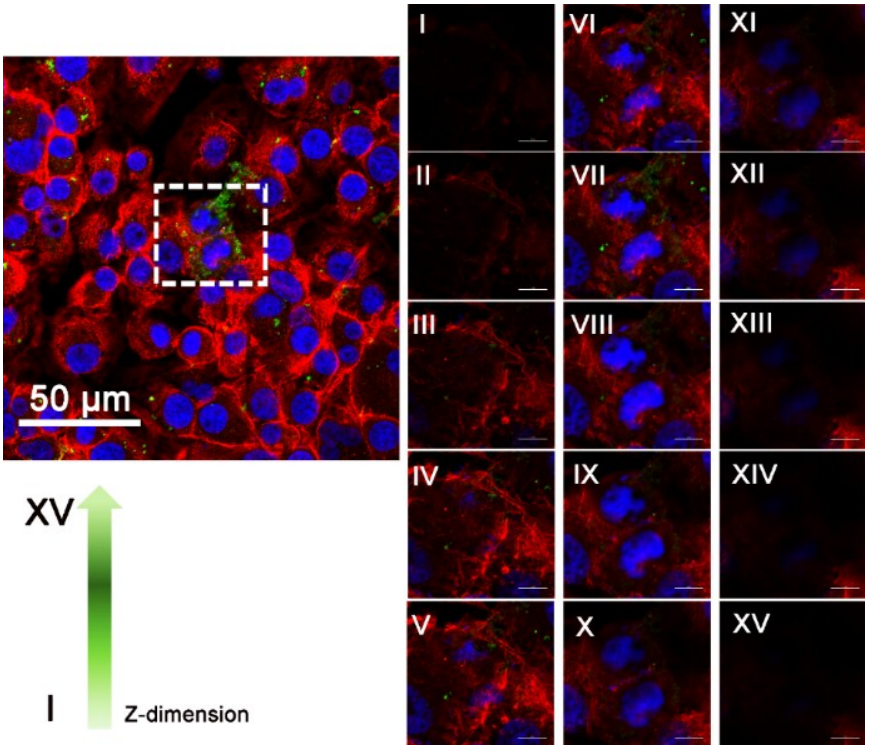

Fig. S7. Progressive Z-stack laser-scanning confocal microscopy image series for GBM cells incubated with PEI/Block-iT™/THCpSiNPs (0.1 mg/mL). Cell nuclei were stained with Hoechst 33342 (blue), the cell membranes were stained with phalloidin-TRITC (red) and Block-iT™/THCpSiNPs emit green fluorescence. The roman numbers correspond to images at different planes (height interval: 300 nm; down to up). I and XV are representative of the bottom and top plane of the GBM cell, respectively. Scale bars related to the images from I to XV are 50 µm.

References

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