

Supplementary Information for Publication

Gas-phase self-assembly of soft nanocomposites for efficient gene transfection and photothermal therapy

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Experimental Procedure

The size distributions of the gas-phase self-assembled nanocomposites are measured using a scanning mobility particle sizer (SMPS), consisting of an electrostatic classifier (3085, TSI, US), condensation particle counter (3776, TSI, US), and a charge neutralizer (4530, HCT, Korea). The SMPS system, which measures the mobility equivalent diameter, is operated at a sample flow of 0.3 L min⁻¹, a sheath flow of 3.0 L min⁻¹, and a scan time of 135 sec (measurement range: 4.61-156.8 nm). The SMPS measurements were performed after particles had exited the heated tubular reactor region. TEM (JEM-3010, JEOL, Japan) images were obtained at an accelerating voltage of 300 kV. Specimens were prepared for examination in the TEM by direct electrostatic aerosol sampling at a sampling flow of 1.0 L min⁻¹ and an operating voltage of 5 kV using a Nano Particle Collector (NPC-10, HCT, Korea). For FTIR analysis, samples were prepared using polytetrafluoroethylene (PTFE) media substrate (0.2 µm pore size, 47 mm diameter, 11807-47-N, Sartorius, Germany) by physical filtration (*i.e.* mechanical filtration mainly by diffusion of particles on the surfaces of the substrate), and the spectra were recorded on a IFS 66/S spectrometer (Bruker Optics, Germany). The IR spectra were taken of the samples in the range of 4000-400 cm⁻¹ in absorbance mode. The zeta potential of nanocomposites-pDNA complexes was determined using a zeta potential analyzer (Nano ZS-90, Malvern Instruments, UK). The samples were mixed with pDNA and incubated at room temperature for 30 min. The complexes were then diluted with double de-ionized water to an appropriate concentration. Measurements of the zeta potential were carried out at 25°C and calculated using the manufacturer's supplied software. A SPM system (NanoScope IIIa, Veeco, USA) was used for the topography of the samples. The drive frequency was 330 kHz, and the voltage was between 3.0 V and 4.0 V. The drive amplitude was ~300 mV, and the scan rate was 0.5-1.0 Hz. In order to observe individual particle in a SPM window, 45 s sampling time was chosen in the direct electrostatic gas-phase sampling for preparing SPM specimens.

For the preparation of the SiO₂ particles, Solution 1 was a tetraethoxysilane (Merck, US), and Solution 2 was a mixture solution of 4.8 mL ammonia (25%), 63 mL ethanol (EtOH), and 20 mL deionized water. Solutions 1 and 2 were injected drop by drop with the aid of a peristaltic pump

(323Du/MC4, Watson-Marlow Bredel Pump, US) at constant rates of 0.4 and 3.1 mL min⁻¹, respectively. Solutions 1 and 2 were mixed in a flask and an ultrasonic probe (VCX 750, 13 mm titanium alloy horn, 20 kHz, Sonics & Materials Inc., US) was then immersed into the mixture solution. The probe acted as an ultrasound irradiator (10 W mL⁻¹ input power density) and the active part of the probe was the planar circular surface, of area 1.3 cm², at the bottom of the probe.

The density (ρ) was estimated using equation, $\rho = m_p / (C_{pv} \times Q \times t)$, where m_p , C_{pv} , Q , and t are the measured particle mass using a microbalance, measured particle volume concentration (cm³ particle/cm³ gas) using a scanning mobility particle sizer, gas flow rate (cm³/min), and sampling time (min).

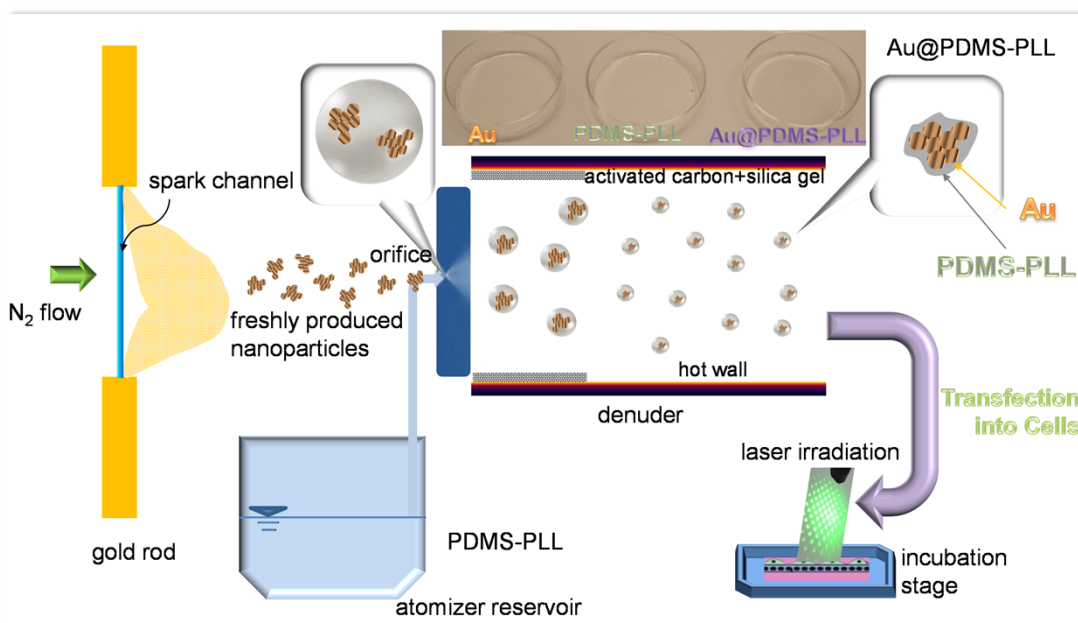
Before *in vitro* measurements, the sampled nanocomposites on a PTFE substrate were detached in an ultrasound bath for 10 s. 1×10^6 HeLa cells pre-incubation in a 24-well culture plate for 24 h were replaced separately with the nanocomposites and control samples. After being applied to the different samples, cells were replaced with a 2 mL culture medium containing an MTT assay reagent (4 mg mL⁻¹) and incubated for an additional 4 h. The resulting purple crystals were dissolved in 2 mL dimethyl sulfoxide (DMSO). 250 μ L of the DMSO solutions from the culture wells were loaded into a 96-well plate. Absorbances were measured at 570 nm by an ELISA plate reader (Thermo Multiskan Spectrum, US). The percentage of cell viability was related to untreated control cells.

HeLa cells was seeded in 24-well plates at a density of 1×10^5 cells:well in 1 mL of complete Dulbecco's modified eagle medium (DMEM) medium supplement with 10% fetal bovine serum at 37 °C, 5% CO₂, and 95% relative humidity, one night before plasmid transfection. The culture medium was replaced with serum free DMEM medium, and transfection complexes were added to the cells. The cells were incubated with the transfection complexes (PEI, SiO₂@PLL, or Au@PDMS-PLL and pDNA) at 37 °C for an additional 24 h after the medium was replaced by fresh complete medium. After incubation for 24 h, the medium was aspirated and washed with phosphate-buffered saline. Luciferase activity was measured with a luminometer (TD-20/20, Promega, US). The final luciferase activity was expressed as RLU mg⁻¹ of protein. An inverted fluorescent microscope (DMI 4000 B, Leica, Germany) was used to observe the EGFP expression of the complexes in the cells.

Synthesized Au@PDMS-PLL nanocomposites were evenly dispersed in 2% agar at concentrations

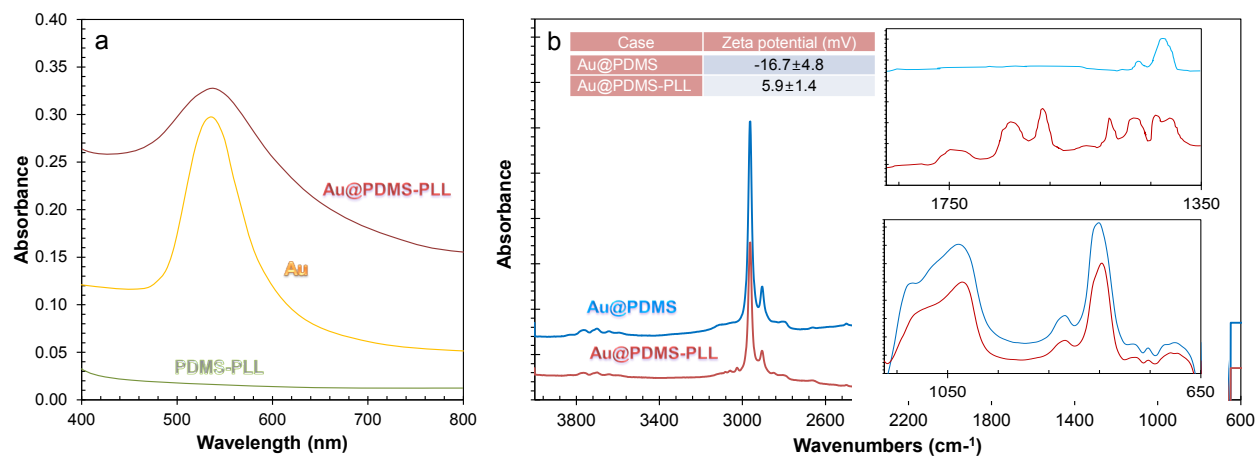
of 10, 30, 50, 70, and 90 $\mu\text{g mL}^{-1}$. The gels were formed in shallow, 35 mm diameter plastic petri dishes. For exposure, the gel phantom samples at room temperature were exposed to a 705 nm continuous wave IR laser beam (1.12 W cm^{-2} in power density) emitted by a solid state laser system (HL7001MG, Opnext, Japan). The gel samples were positioned in the laser beam and irradiated by the beam for fixed durations of 20, 40, and 90 sec. In order to evaluate the application to photothermal therapy, ATP assay was further employed, which is based on a highly sensitive firefly reaction to determine the level of cellular ATP as a surrogate marker for the number of live cells. After a 24 hr incubation with Au@PDMS-PLL nanocomposites, the cells were washed three times with Hank's buffered salt solution and 0.1 mL of CellTiter-Glo Luminescent (Promega, US) assay reagent was added to each well and the plate was then mixed using an orbital shaker for 2 min, followed by 10 min incubation to stabilize the luminescence signals. Luminescence was read using the luminometer.

SCHEME S1



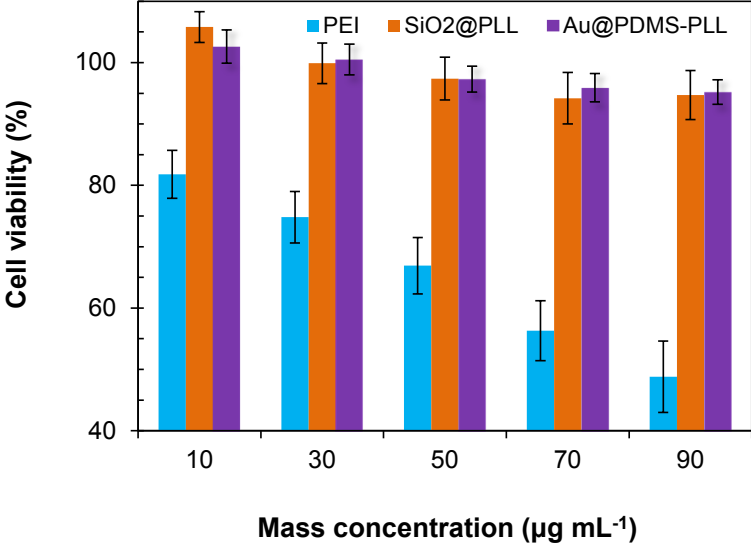
A schematic diagram of gas-phase self-assembly for fabricating Au@PDMS-PLL soft nanocomposites for their photothermal therapy application after transfection into cells. This scheme also noted photos of gas-phase self-assembled-collected Au, PDMS-PLL, and Au@PDMS-PLL samples on PTFE substrates.

FIGURE S1



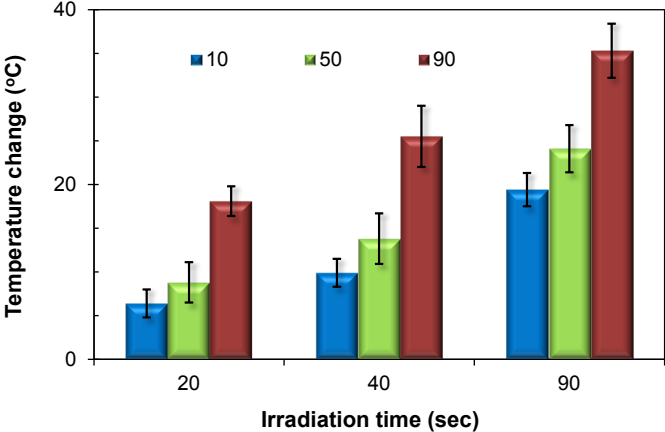
(a) UV-vis spectra of the gas-phase fabricated Au, PDMS-PLL, and Au@PDMS-PLL samples. (b) IR spectra of Au@PDMS and Au@PDMS-PLL samples. An inset table summarizes zeta potential data of both samples.

FIGURE S2



In vitro cytotoxicity of PEI, SiO₂@PLL, and Au@PDMS-PLL nanocomposites with different mass concentrations (10-90 µg mL⁻¹).

FIGURE S3



Temperature changes in growth medium containing Au@PDMS-PLL nanocomposites (10-90 $\mu\text{g mL}^{-1}$ in particle concentration) exposed to a continuous IR laser light fixed at 705 nm for 20, 40, and 90 sec.

TABLE SI A summary of the size distributions of spark-produced Au and collision atomized PDMS-PLL nanoparticles, and their incorporated structure (Au@PDMS-PLL) through the gas-phase self-assembly

Case	GMD (nm)	GSD (-)	TNC ($\times 10^7$ particles cm^{-3})
Au	18.0	1.50	0.96
PDMS-PLL	44.5	1.60	0.77
Au@PDMS-PLL	59.4	1.58	1.10