Supplementary Information

Efficient Delivery of siRNAs by Photothermal Approach using Plant Flavonoid-inspired Gold Nanoshells**

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Experimental Section

Characterization

XPS spectra were obtained to measure the surface atomic composition using an Omicron ESCALAB (Omicron, Taunusstein, Germany) with a monochromated Al Kα (1486.8 eV) 300 W X-ray source with an ultrahigh vacuum (< 10-9 Torr). The takeoff angle was fixed at 45° except as otherwise mentioned, and all spectra were calibrated using the hydrocarbon C1s peak (284.5 eV). The UV-Vis absorbance of the nanoparticles was measured using a UV-Vis spectrophotometer (Agilent HP8453, United States). TEM images of the nanoparticles were taken using a Zeiss Omega 912 transmission electron microscope (Carl Zeiss, Germany). The morphologies of the nanoparticles were confirmed by scanning electron microscopy (SEM, Magellan400, FEI, USA).

Pyrogallol 2-aminoethane surface modification on polymeric nanoparticles

Poly(methylmethacrylate) (PMMA), Poly(styrene) (PS), Poly(lactic-co-glycolic acid) (PLGA) nanoparticles were purchased from Polysciences (Polysciences Inc., United States). Pyrogallol 2-Aminoethane (PAE) and sodium tetrachloroaurate (III) hydrate were purchased from Sigma-Aldrich (Sigma-Aldrich, USA). For PAE coating, all nanoparticles were incubated at 1 mg/ml of PAE buffer solution (Tris-HCl 10 mM, pH 8.5) for 2 hrs. After PAE coating,

the nanoparticles were collected by centrifugation (15,000 rpm for 20 min) and washed three times with ultrapure water. White to yellowish color change was observed for all of polymeric NPs after PAE coating.

Preparation of Polymer Gold Nanoshells (PGNs)

For the growth of Au seeds, PAE-coated PS NPs were re-dispersed (5 mg/ml) in water, and 100 µl of the particle solution was added to a gold salt precursor solution (sodium tetrachloroaurate (III) hydrate, 0.5 mM) at pH 4.1. After 2 h incubation with gentle stirring, the color of the solution changed to brownish red, and the particles were collected by centrifugation followed by three washing steps with ultrapure water. For the gold shell formation, PAE-coated particles with Au seed on the surface were further reduced using a 1 mM gold salt solution with 5 µl of formaldehyde (37 wt % in H_2O) for 5 min. The color of the particle suspension changed from brownish red to blue, which confirmed the formation of the gold nanoshells. The gold nanoshells formed on PAE-coated particles were collected by centrifugation and washed three times with ultrapure water for further experimental analysis.

Photothermal Property of PGNs

The PGNs were diluted in 1 mL of deionized water (final concentration: 0.05 mg/ml) and irradiated using an 808 nm laser (diode laser, MDL-III- 808, 0–2.5W continuous wave output; Optoengine) with three different intensities of 0.5, 1.5, and 2.33 W/cm^2. During the NIR irradiation, the temperature of the solution was measured with a digital thermometer (Lutron Thermometer TM-917, Taiwan) at every 30 s for 20 min.

GSH mediated siRNA release from the siRNA-PGN conjugates

siRNA immobilization was conducted on the surface of PGNs as descried previously.[1] GSH mediated siRNA release was evaluated by PAGE. 10 % Polyacrylamide gel was used to visualize siRNA release from the surface of PGNs. Under 10 mM GSH condition, siRNA-PGN conjugates were incubated for 2 hrs and the gel electrophoresis was performed at 125 V for 20 min. All RNA bands were stained with the SYBR gold dye (Invitrogen) for gel imaging.

Cellular cytotoxicity of PGN-siRNA conjugates under photothermal condition

The cellular cytotoxicity of PGN-siRNA conjugates under NIR treatment was evaluated by the cell proliferation assay. HeLa cells were seeded onto 24-well plates at a density of 5×10^4 cells/well and incubated for 24 h. siRNA immobilized PGNs (200 nM) were treated to the cells for 2 hrs in 500 µL of serum containing media and then two different NIR laser

intensities (1.5 and 2.4 W/cm^2) were applied in the course of 20 min. After the 12 h incubation followed by a laser irradiation, the cytotoxicity was measured by the CCK assay (Dojindo Molecular Technologies).

Gene silencing studies

GFP-overexpressing HeLa cells were cultured in DMEM medium with 10 % FBS. For gene silencing test, GFP-HeLa cells were seeded at 1.0 × 10^5 cells/well in 12-well culture plates. After 24 h incubation, three different siRNA concentrations (25, 50, and 100 nM) of PGN-siRNA conjugates (n=3 for each group) were applied to the cells with/without exposure to the NIR-laser (1.5 W/cm^2) for 5 min. The GFP expression of treated cells was measured after 1 days by FACSCalibur system (BD biosciences) and the GFP gene silencing was analyzed using the flowJo program. For spatial GFP gene regulation on the cell monolayer, half of the cover-lid was photo-masked by applying aluminum foil on the top. The cell monolayer was similarly treated with 100 nM of siRNA concentration (PGN-siRNA conjugates) with 2 hr incubation and then exposed to the NIR-laser (1.5 W/cm^2) for 5 min to induce photothermal effects. Fluorescence images of cell monolayer were obtained after 24 hrs by the Zeiss Axiovert 200 microscope (Carl Zeiss).

Confocal Analysis of intracellular uptake of PGN-siRNA conjugates

For confocal images, HeLa cells were seeded in BD Falcon^{TM} CultureSlides (BD, USA) at a density of 2 × 10^4 and maintained for 48 hrs. The cultured cells were treated with 100 nM of PGN-siRNA conjugates and incubated for 2 hrs. After intracellular uptake of PGN-siRNA conjugates, NIR irradiation was applied with an intensity of 1.5 W/cm^2 for 5 min to induce the endosomal release of PGN-siRNA conjugates. After NIR treatment, the media were discarded and the cells were washed 2 times with PBS solution and fixed with 2 % paraformaldehyde solution. Confocal images were obtained using the LSM510 confocal laser scanning microscope (Carl Zeiss Inc., USA). To acquire a fluorescence signal of endosome entrapment, Lysotracker green (Invitrogen) has been utilized (ex/em: 488/520 nm). A fluorescence signal of cy5 labeled siRNA was detected at 670 nm with an excitation at 643 nm. Co-localization analysis has been performed using the Zeiss Imaging Software.

References

[1] D.S. Seferos, D.A. Giljohann, C.S. Thaxton, A.K.R. Lytton-Jean, M.S. Han, and C.A. Mirkin, **Science**, 2006, 312, 1027; S.J. Hurst, A.K.R. Lytton-Jean, C.A. Mirkin, **Anal. Chem.**, 2006, 78, 8313.

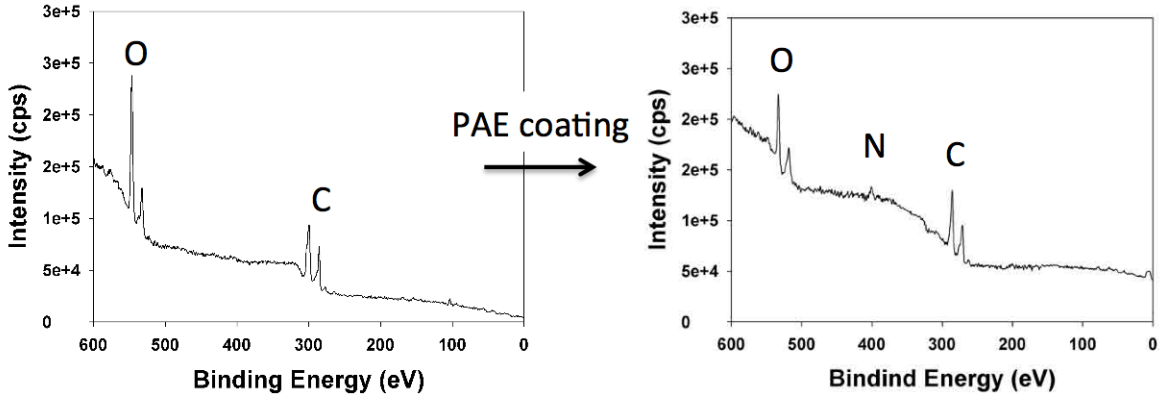
Table S1. List of oligonucleotides sequences used in this study. All oligonucleotides were obtained from Integrated DNA Technology (IDT)

Strands	Sequences (5′→ 3′)
AS GFP siRNA-polyA tail-thiol	rArArGrUrCrGrUrGrCrUrGrCrUrUrCrArUrGrUTTAAAAAAAAAAAAAAA-thiol
SS GFP siRNA	rArCrArUrGrArArGrCrArGrCrArCrGrArCrUrUTT

* r indicates ribonucleotide sequences.

Figure S1. XPS analysis of PAE coated PMMA, PS, PLGA NPs. (a) XPS spectrum of bare PLGA NPs and PAE coated PLGA NPs. (b) % surface composition (C, O, N) of three polymeric NPs by XPS analysis

a



b

	% C_{1s}	% O_{1s}	% N_{1s}
PAE-PMMA	62.62	29.34	8.04
PAE-PS	71.87	21.75	6.38
PAE-PLGA	64.30	27.58	8.12

Figure S2. Characterization of polymeric nanoparticles a) DLS/zeta potential measurement, and b) SEM images of polymer nanoparticles before and after the PAE functionalization (PMMA, PLGA).

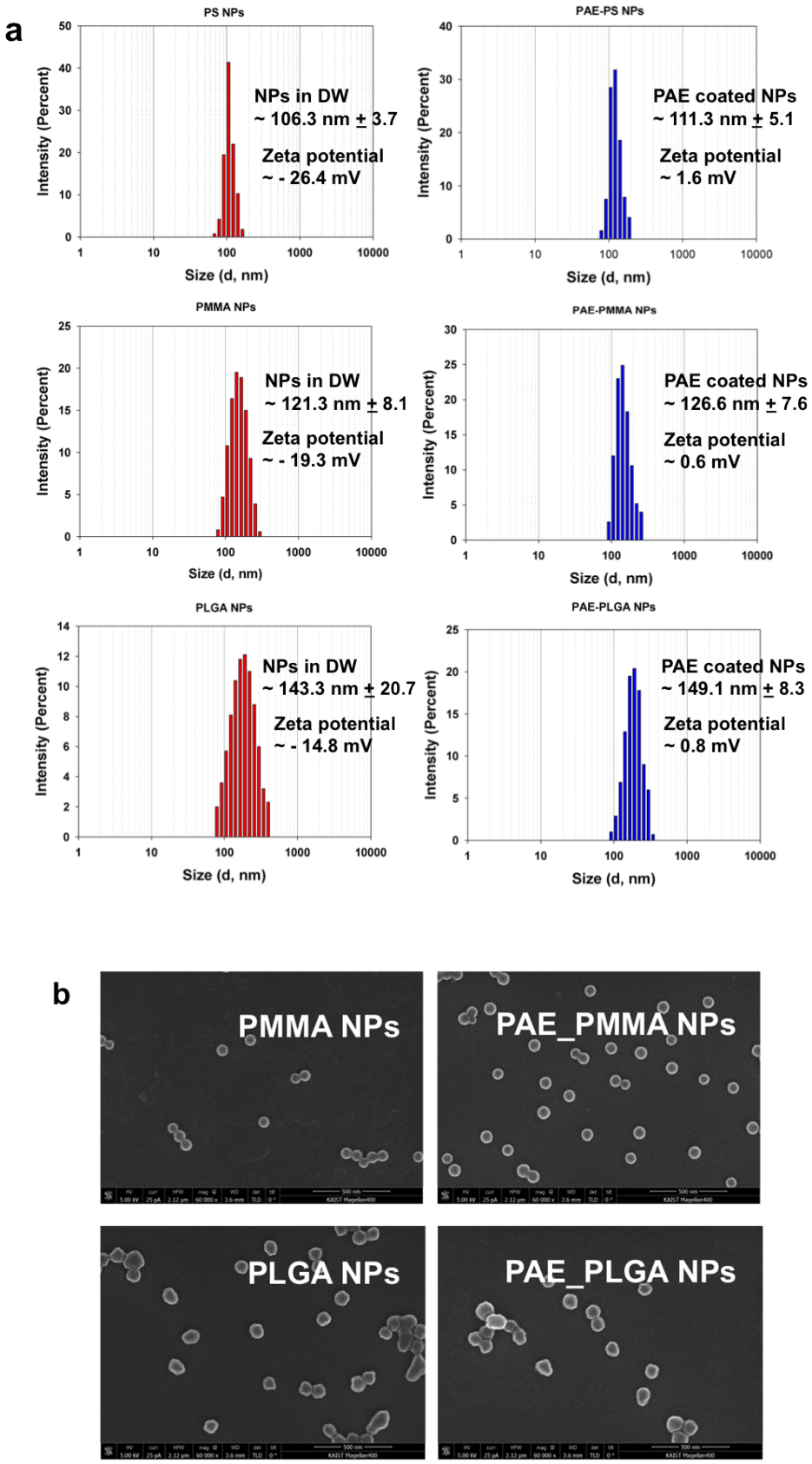


Figure S3. Cellular cytotoxicity data of PGN-siRNA conjugates (200 nM) under NIR irradiation

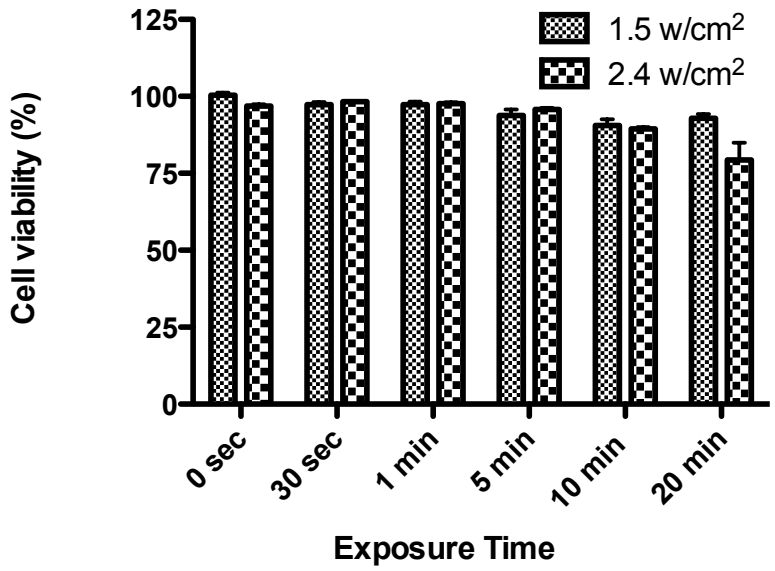


Figure S4. Intracellular uptake and co-localization analysis of PGN-siRNA conjugates under NIR exposure. (a) without NIR exposure, (b) with NIR exposure for 5 min at intensity of 1.5 w/cm^2. Endosome was labeled with Lysotracker Green (Green), while siRNA was labeled with Cy5 dye (Red). For nucleus staining, DAPI has been used. Co-localization analysis has been performed and listed right of the confocal images, respectively.