Supporting Information

Gold, Poly (β-amino ester) Nanoparticles for Small Interfering RNA Delivery

Jae-Seung Lee[§], *Jordan J. Green*[‡], *Kevin T. Love*[†], *Joel Sunshine*[‡], *Robert Langer*[†], *and Daniel G. Anderson*[†,*]

[§]Department of Materials Science and Engineering, Korea University, Seoul, 136-713, Republic of Korea, [‡]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA, [†]The David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

Materials

Gold nanoparticle colloid (15 nm in diameter) was purchased from Ted Pella. (Redding, CA). Thiol-PEG-amine (HS-$(CH_2CH_2O-)_n$-NH_2, M_w 1000 Da) was purchased from Creative PEGWorks (Winston-Salem, NC). Phosphate buffered saline (PBS, 0.15 M NaCl, 10 mM phosphate, pH 7.4) 1X, Opti-MEM®, Lipofectamine™ 2000, Cell Titer96® AQ_{ueous} One Solution Cell Proliferation Assay and Quant-IT™ RiboGreen RNA reagent were purchased from Invitrogen (Carlsbad, CA). Dithiothreitol (DTT) and *N*-succinimidyl 3-(2-pyridyldithio)

propionate (SPDP) were purchased from Pierce Biotechnology and used as received. Dual-Glo™ Luciferase Assay System was purchased from Promega (Madison, WI). Amine and diacrylate monomers were purchased from Sigma-Aldrich (St. Louis, MO), Alfa Aesar (Ward Hill, MA), Molecular Biosciences (Boulder, CO), TCI America (Portland, OR), and Acros Organics (Pittsburgh, PA) and used without further purification. All the other chemicals were purchased from Sigma-Aldrich and used without further purification. The RNAse-free HPLC-purified HS-siRNA (sense: 5' HS-GAUUAUGUCCGGUUAUGUA-UU 3'; antisense: 5' UACAUAACCGGACAUAAUC-UU 3') was purchased from Dharmacon /Thermo Fisher Scientific Inc. (Lafayette, CO) based upon the sequence of Anti-Luc siRNA-1 (cat #: D-002050-01-20) with a thiol-modification.

Synthesis of Poly (β-Amino Ester)s

End-modified polymers were synthesized using a two-step process.[1-4] First, acrylate-terminated polymers were synthesized. Second, amine molecules were conjugated to the polymer ends post-polymerization. Acrylate-terminated poly(β-amino ester) C32-Ac was synthesized by mixing 3532 mg of 1,4-butanediol diacrylate (17.8 mmol) with 1533 mg of 5-amino-1-pentanol (14.8 mmol) for 24 hrs at 90°C. End chain capping reactions were performed by adding 9.1 g of THF to 5 g of C32-Ac, vortexing, and then transferring to a 100 mL flask with

a stir-bar. 40 mL of 0.25 M end-capping amine solution was then added, and the mixture was left stirring at room temperature in the dark for 24 hrs. Excess amine was used to fully end-modify the polymer without causing detectable cross-linking or aminolysis. End-modified polymers were precipitated by the addition of 10 volumes of diethyl ether and centrifugation at 2,500 rpm for 2 minutes. Polymers were washed twice and dried in a vacuum desiccator. Polymers were characterized by ^{1}H-NMR (300 MHz, DMSO-d_6) on a Varian mercury spectrometer. C32-Ac: δ (ppm) 1.3-1.6 (m, $-NCH_2(CH_2)_3CH_2OH$), 1.7 (bs, $-N(CH_2)_2COOCH_2CH_2-$ and $CH_2CHCOOCH_2CH_2-$), 2.3-2.5 (m, $-COOCH_2CH_2N-$ and $-NCH_2(CH_2)_4OH$), 2.7-2.8 (m, $-COOCH_2CH_2N-$), 3.6 (bs, $-N(CH_2)_4CH_2OH$), 4.1 (bs, $-N(CH_2)_2-COOCH_2CH_2-$), 4.2 (m, $CH_2CHCOOCH_2CH_2-$), 4.4 (bs, $-N(CH_2)_5OH$), 5.9 (m, $CH_2CHCOOCH_2CH_2-$), 6.1-6.2 (m, $CH_2-CHCOOCH_2CH_2-$), 6.4 (m, $CH_2CHCOOCH_2CH_2-$). End-modification of polymers was confirmed by the disappearance of the acrylate peaks at 5.9, 6.1-6.2, and 6.4. Organic phase Gel Permeation Chromatography (GPC) was performed using 95% THF/5% DMSO (v/v) + 0.1 M piperidine as the eluent at a flow rate of 1.0 mL/min in a Waters GPC system equipped with an autosampler (Waters Corporation, Milford, MA). A Phenogel (Phenomenex, Torrance, CA) MXL column (5 μm, 300 x 7.8 mm) and a Waters Styragel HR4 column were used in series and the molecular weights of the polymers are reported relative to monodisperse poly(2-vinylpyridine) standards. Gel permeation chromatography characterization of the newly

synthesized 200 series end-modified polymers is shown in Table S1.

Polymer	Mn	Mw	PDI
C32-117	4500	8300	1.84
C32-208	10800	33700	3.12
C32-210	5200	12400	2.38
C32-213	3100	8000	2.58
C32-218	3100	7300	2.35
C32-221	9100	19700	2.16
C32-228	7700	15700	2.04

Table S1. Gel permeation chromatography characterization of lead polymers

Synthesis of NH_2-PEG-AuNPs

Typically 100 mL of AuNP solution (15 nm in diameter, ~ 2.5 nM) was combined with ~ 20 mg of HS-PEG-NH_2 and incubated at 25 ^oC for 12 hours. The particles were washed four times by centrifugation, removal of the supernatant and redispersion in PBS containing 0.01 % Tween 20. The concentration of NH_2-PEG-AuNPs was obtained from the absorbance of the UV-vis spectrum (Cary100, Varian) of the solution at 525 nm at which the spectrum has the maximum intensity ($\varepsilon_{525nm} = 3.56\times10^8$ $cm^{-1}M^{-1}$).[5] The final concentration of NH_2-PEG-AuNPs was adjusted to 30 nM, and the solution was stored at 4 ^oC until use.

Synthesis of siRNA-AuNPs

The conjugation of HS-siRNA to NH_2-PEG-AuNPs is a two-step procedure beginning with the conjugation of SPDP to NH_2-PEG-AuNPs to form SPDP-PEG-AuNPs, followed by the conjugation of HS-siRNA to SPDP-PEG-AuNPs (Figure 1B in the main text). For the conjugation of SPDP to NH_2-PEG-AuNPs, typically 400 μL of NH_2-PEG-AuNPs (30 nM) in PBS (0.01 % Tween 20, pH 7.4) was combined with 400 μL of SPDP in PBS (3 mM, 10 % DMSO), and incubated at 25 ^oC for 40 min with vigorous vortexing. The unconjugated SPDP was removed by repeated centrifugation, removal of the supernatant and redispersion of the particles in PBS containing 0.01 % Tween 20 (four times). After the final removal of the supernatant, the particles were redispersed in 400 μL of borate buffer containing the HS-siRNA (15 μM HS-siRNA, 2.5 M NaCl, 30 mM borate, pH 8.5, 0.01 % Tween 20) and incubated at 25 ^oC for 40 hours with vigorous vortexing. The final product was washed by centrifugation, removal of the supernatant and redispersion of the particles in PBS (0.01 % Tween 20) four times and adjusted to the final concentration of 15 nM. To quantitatively analyze the number of siRNA strands per particle, the siRNA-AuNPs were incubated in 0.05 M dithiothreitol solution in PBS (pH 7.4, 0.01 % Tween 20) for 30 min at 30 ^oC to cleave the disulfide bonds, and the number of released siRNA strands were quantitatively analyzed by RiboGreen^{TM} RNA reagent following the manufacturer's protocol.

***in vitro* Transfection of HeLa Cells using PBAE-siRNA-AuNPs and the Assay for Firefly Luciferase Expression**

HeLa cells (human cervical carcinoma) were pre-transfected to stably express firefly and Renilla luciferases and plated in opaque 96-well plates (15000 cells/well). The cells were allowed to adhere at 37 ^{o}C, 5 % CO_2 overnight in growth medium (10 % FBS and 90 % phenol red-free DMEM). For the formation of PBAE-siRNA-AuNPs, each PBAE in DMSO (100 μg/μL) was diluted to 0.36 μg/μL in 25 mM acetate buffer (pH 5), and rapidly combined with 15 nM siRNA-AuNPs and growth medium to make 10.8 μg of PBAE and 300 fmol of AuNPs in 100 μL per well (0.108 μg/μL of PBAE and 3 nM of AuNPs, respectively). The mixed solution was allowed to form PBAE-siRNA-AuNPs for 10 minutes, transferred to the cells (100 μL/well), incubated for 4 hours for complete transfection, and replaced by fresh growth medium (100 μL). The cells were further incubated for one day at 37 ^{o}C, 5 % CO_2 and analyzed to measure both firefly and Renilla luciferase expressions, respectively, using Dual-Glo^{TM} Luciferase Assay System following the manufacturer's protocol. Dose response was measured in the similar way using decreased amount of the siRNA-AuNPs and PBAEs at the same ratio (1.5 nM siRNA-AuNP+5.4 μg/μL PBAE and 0.3 nM siRNA-AuNP+1.08 μg/μL PBAE, respectively).

Cell Viability Test

Cell viability test was performed by Cell Titer96® AQ_{ueous} One Solution Cell Proliferation Assay (Invitrogen) following the manufacturer's protocol (Figure S1).

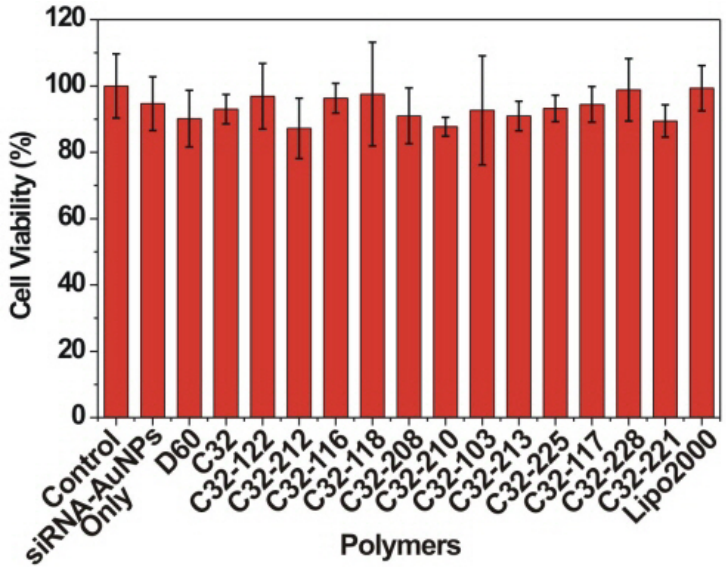

Figure S1. Cell viability test result.

Experimental Conditions for Lipofectamine 2000 (Lipo2000)

① Dilute 17.8 μL of Lipofectamine2000 in 513 μL of OptiMEM, and wait 5 min.

② Dilute 1.79 μL of siRNA (0.28 mg/mL, or 20 μM) in 3.33 mL of OptiMEM.

③ Combine 530 μL of the siRNA solution (②) with the Lipo2000 solution (①) and wait 20 min.

④ Add 20 μL of Lipo2000/siRNA mixture (③) to 130 μL of media of the positive control (The final [siRNA] = ~ 1 nM).

References

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5. The molar extinction coefficient is calculated from the measured UV-vis extinction of the colloid with the known particle concentration from the manufacturer.