Electronic Supplementary Information (ESI)

Synthesis of partially dextran coated gold nanoworms and anisotropic structure based dual-strategic cargo conjugation for efficient combinational cancer therapy

Hongje Jang[a], Young-Kwan Kim[b] and Dal-Hee Min[c,d,e]**

[a.] Department of Chemistry, Kwangwoon University, 20, Gwangwoon-ro, Nowon-gu, Seoul, Republic of Korea

[b.] Carbon Convergence Materials Research Center, Korea Institute of Science and Technology, Wanju-gun, Jeollabuk-do, Republic of Korea

[c.] Department of Chemistry, Seoul National University, Seoul, 151-747, Republic of Korea

[d.] Center for RNA Research, Institute for Basic Science (IBS), Seoul, 151-747, Republic of Korea

[e.] Institute of Nanobio Convergence Technology, Lemonex Inc., Seoul, 151-742, Republic of Korea

Materials.

Hydrogen tetrachloroaurate (III) hydrate was purchased from Kojima Chemicals Co. (Sayama, Saitama, Japan). 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethylsulfoxide and silver nitrate were purchased from Sigma (St. Louis, MO, USA). Epichlorohydrin (99%) was purchased from Aldrich Chemical Co., (Milwaukee, WI, USA). Dextran from leuconostoc spp. (Mr~ 15-25k) was purchased from Fluka (Milwaukee, WI, USA). Trisodium citrate dehydrate and ammonium hydroxide were purchased from Junsei (Tokyo, Japan). Phosphate-buffered saline (PBS, 10X), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from WelGENE (Seoul, Korea). Live/Dead viability/cytotoxicity assay kit was purchased from Molecular Probes Invitrogen (Carlsbad, CA, USA). Amicon ultra centrifugation filter device (100 kDa cutoff) was purchased from Millipore (Billerica, MA, USA). N-Succinimidyl-3-(2-pyridyldithio)propionate (SPDP) was purchased from Pierce (Rockford, IL, USA). The myristic acid-ARRRRRRRC (myristoylated polyarginine peptides, MPAP) as a cell penetrating peptide was synthesized by solid-phase peptide synthesis.

DNAzyme which was designed against HCV NS3 was prepared as follows by Genotech (Seoul, Korea):

FAM - 5`- AAT GGG GA**G GCT AGC TAC AAC GA**G GCT TTG C-3` - thiol

(* underlined letters: catalytic motif of DNAzyme)

All chemicals were used as received.

Synthesis of 50 nm sized dextran coated Ag nanoparticle (dAgNP).

12.0 g of cextran (Mr~15-25k) was fully dissolved in DI water (160 mL, 18.2 MΩ) by vortex to prepare 7.5 wt% homogeneous solution. Next, the prepared solution was heated until start to boil, then 864 μL of 250 mM silver nitrate fresh solution in DI water was directly injected into dextran solution. The mixture was heated for 30 min until the color turned into deep yellow. The product was cooled down to room temperature. The product was washed with DI water for four times using Amicon ultra centrifugation filter (100 kDa cutoff) at 4000 rpm for 15 min. Finally, obtained dAgNP was redispersed in 8 mL of DI water and characterized by UV-vis spectrophotometer for calculating molarity.

Preparation of dextran coated Au nanoworm (dAuNW).

4 mL of DI water was added to 1 mL of the prepared dAgNP and briefly inverted for several times to prepare diluted homogeneous dAgNP solution. To prepare dAuNS, 1000 μL of 1 mM hydrogen tetrachloroaurate (III) hydrate solution in DI water was directly added, then was quickly shaken for 10 s and incubated for 10 min at ambient condition. The prepared dAuNS was gathered and washed with DI water for at least four times by using Amicon ultra

centrifugation filter at 4000 rpm for 10 min each. The final product was characterized by UV-vis spectrophotometer and kept at 4 ℃.

Preparation of dextran coated Au nanoshells (dAuNS).

4 mL of DI water was added to 1 mL of the prepared dAgNP and briefly inverted for several times to prepare diluted homogeneous dAgNP solution. To prepare dAuNS, 150 μL of 1 mM hydrogen tetrachloroaurate (III) hydrate solution in DI water was directly added, then was quickly shaken for 10 s and incubated for 10 min at ambient condition. The prepared dAuNS was gathered and washed with DI water for at least four times by using Amicon ultra centrifugation filter at 4000 rpm for 10 min each. The final product was characterized by UV-vis spectrophotometer and kept at 4 ℃.

Synthesis of 50 nm sized citrate stabilized Au nanoparticle (cit-AuNP).

The 50 mL of 0.25 mM hydrogen tetrachloroaurate (III) hydrate solution in DI water was prepared and heated until boiling. When the solution started to boil vigorously, 300 μL of 34 mM trisodium citrate dehydrate solution in DI water was quickly added. The solution color changed into black within few second, and heated for additional 20 min until the color totally changed to deep-red. When the color did not exhibit any further changing, removed the heat source and cooled down to room temperature. The final product was characterized by UV-Vis spectrophotometer and kept at 4 ℃.

Crosslinking of dAuNS and dAuNW.

200 μL of 1M sodium hydroxide solution was added to the prepared 4 mL of each dAuNS and dAuNW. The mixture was inverted for 10 s, then 60 μL of epichlorohydrin was added to solution. After vigorous vortex, mixed solutions were allowed to incubate on table shaker at 600 rpm at room temperature for 12 hrs. The products were gathered and rinsed with DI water for at least four times by using Amicon ultra centrifugation filter at 3000 rpm for 10 min.

Amination of CL-dAuNS and CL-dAuNW.

Amination was achieved by simple addition of ammonium hydroxide to CL-dAuNS and CL-dAuNW respectively by final concentration of 3%. Mixed solutions were incubated for 12 hrs at room temperature on the table shaker with 600 rpm. The products were gathered and rinsed with DI water for at least four times by using Amicon ultra centrifugation filter at 3000 rpm for 10 min.

Conjugation of FAM-Dz-SH and MPAP to N-CL-dAuNS and N-CL-dAuNW.

FAM-Dz-SH conjugation was accomplished by simple addition of FAM-Dz-SH to N-CL-

dAuNW and N-CL-dAuNW. After the addition of designed amount of FAM-Dz-SH, each particles were incubated for 6 hrs at room temperature. The unbound FAM-Dz-SH was removed by centrifugation and discarding the supernatant. For MPAP conjugation, 200 eq. of SPDP in anhydrous DMSO was added to FAM-Dz-N-CL-dAuNW and incubated for 3 hrs at room temperature. After the purification to remove excess SPDP by centrifugation, 3 eq. of MPAP was added to SPDP conjugation FAM-Dz-N-CL-dAuNW and incubated for 12 hrs at room temperature. The final product was rinsed with DI water for several times and kept at 4℃. The successful conjugation of each biomolecules was observed by zeta potential measurement as FAM-Dz- (-34.4 mV) and MPAP/FAM-Dz- (-19.6 mV) for dAuNW and FAM-Dz- (-17.2 mV) and MPAP/FAM-Dz- (-2.6 mV) for dAuNS, respectively.

Characterization of prepared nanoparticles.

Energy-filtering transmission electron microscope LIBRA 120 (Carl Zeiss, Germany) was used to obtain images of nanoparticles. UV-Vis spectrophotometer S-3100 (Scinco, Korea) and SynergyMx (Biotek, UK) were used to obtain UV-Vis spectrum. Zeta potential measurement was accomplished by using a Zetasizer Nano ZS90 (Malvern, UK). 808 nm NIR irradiation was performed by surgical laser accessories OCLA (Soodogroup Co., Korea). Cell images were taken by using a Ti-inverted fluorescence microscope equipped with a 60X (1.4 numerical aperture) objective (Nikon Co., Japan) and a CoolSNAP cf charge-coupled device (CCD) camera (Photometrics, Tucson, AZ, USA).

Cell culture.

The HCV NS3 replicon RNA carrying human hepatocarcinoma Huh-7 was grown in DMEM containing 4.5 g/L D-glucose supplemented with 10% FBS, 100 units/mL penicillin, 100 mg/mL streptomycin and 500 μg/mL of G418 (A.G. Scientific Inc., USA) at 37 ℃, 5% CO_2.

AuNWs treatment to cell

To the NS3-Huh7 cells plated in a multi-well plate, each AuNW derivative dispersed in 1X PBS was added to final concentration of 50 pM. The AuNW-cell mixture was incubated at 37 ℃, 5% CO_2, and then, the supernatant was discarded and replaced with fresh cell culture media after a designated incubation time.

MTT assay for viability test.

NS3-Huh7 cells were seeded at 7,000 cells per well of a 96-well culture plate with 150 μL of culture media (about 50% confluency). After the incubation of cells with designed nanocomplex treatment condition, cells were rinsed with 1X PBS and incubated with 20 μL of 5 mg/mL MTT dissolved in 1X PBS solution for 2 hrs to detect metabolically active cells. Along with incubation, purple color was developed to indicate that MTT was to be metabolized. Then the media was discarded and 200 μL of DMSO was added to each well to

dissolve formazan salt. The optical density was measured at 560 nm wavelength and background absorbance at 670 nm was subtracted.

Live/Dead staining of cells.

After the designed *in vitro* experiments, cells were rinsed with 1X PBS then incubated with 50 μL of the live/dead cell staining solution (2 μM calcein AM and 4 μM EthidD-1 in 1X PBS). After 30 min of additional incubation, images were obtained using a microscope equipped with fluorescence light source and filters.

Therapeutic efficiency test

To investigate the combinational effect of NIR irradiation with MPAP/FAM-Dz- conjugated dAuNW platform, cell based viability test was performed. To the cultured NS3-Huh7 cells in 96 well-plate, 50 pM of dAuNWs derivatives in 1X PBS were added. In case of control experiment sets, free Dz and N-CL-dAuNW were treated as same manner. For the complete cellular uptake of each gene-nanocomplexes, the cells were incubated for 24 hrs at 37 ℃. Then, the supernatant was discarded and cells were rinsed with 1X PBS for 2 times. Against the samples which requires NIR irradiation, 0.5 W cm^{-2} of 808 nm laser was irradiated for 3 min. The cells were incubated for additional 12 hrs, then MTT assay and live/dead staining were performed in a same method.

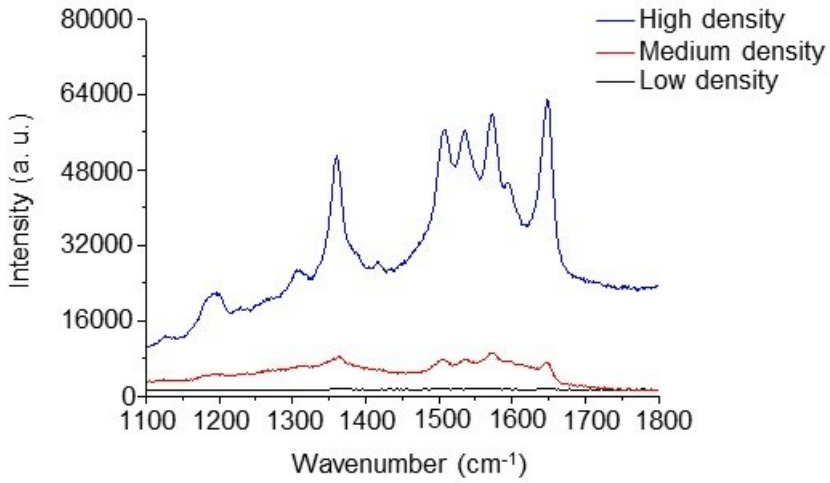

Figure S1. The dAuNW was immobilized on the 3-aminopropyltriethoxysilane (3-APTES) functionalized Si substrates by immersing the substrates into a dAuNW suspension for 30 min. After immobilization, the substrates were washed with water and ethanol to remove excess dAuNW, and dried under a stream of nitrogen. To confirm the feasibility of dAuNW as a surface-enhanced Raman scattering (SERS) platform, the dAuNW immobilized substrates were immersed in 1 mM rhodamine 6G (R6G) solution for 12h, washed with water and ethanol, and dried under a stream of nitrogen. Micro-Raman analysis of R6G-treated dAuNW substrates was carried out by Renishaw inVia Raman Microscope with Ar laser (514 nm) and HeNe laser (633 nm) at the power of 4.0 mW with laser spot size of 1 μm focused through a BXFM confocal microscope equipped with an objective (20×, numerical aperture = 0.50). The Raman spectra of R6G was obtained from three representative regions of R6G-treated dAuNW substrates with different density of dAuNW clusters. This result clearly indicated the applicability of dAuNW as a novel SERS platform.

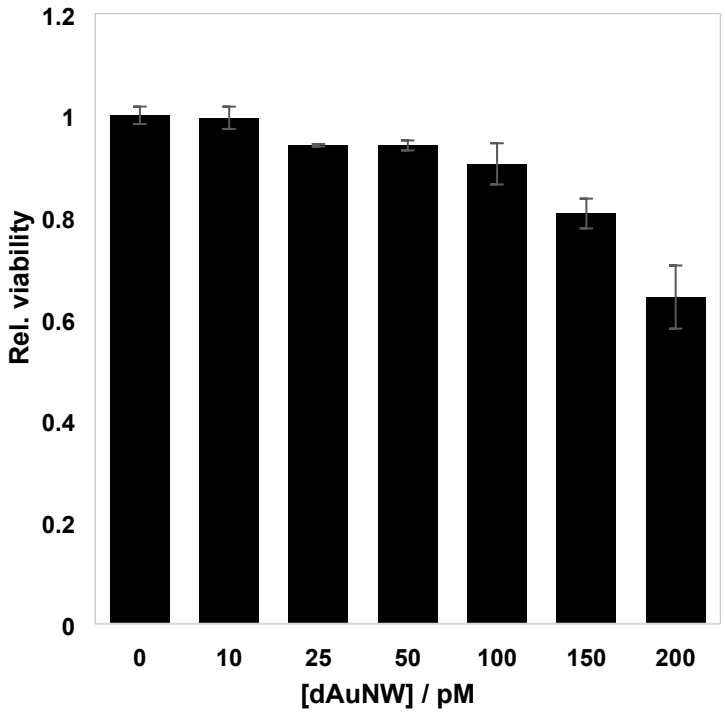

Figure S2. MTT cell viability test of prepared dAuNW against NS3-Huh7 cells. According to the MTT assay, dAuNW represented considerably low cytotoxicity up to 50 pM concentration.

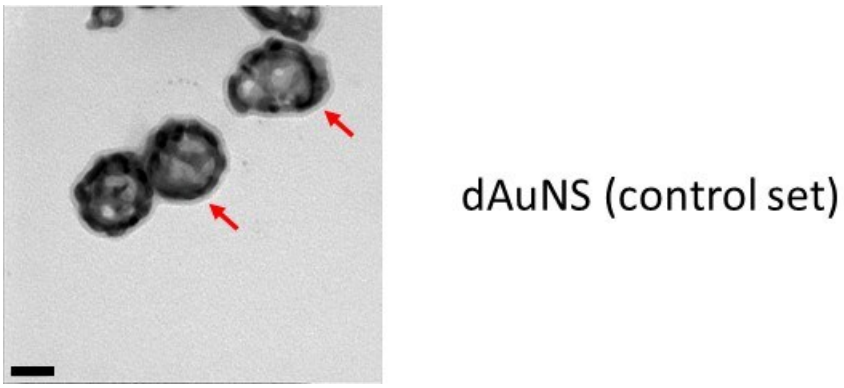

Figure S3. TEM image of prepared dAuNS. In case of dAuNS, surface dextran coating was clearly observed by TEM image (red arrows). The scale bar is 20 nm.