Electronic Supplementary Information

Targeted cancer cell inhibition using multifunctional dendrimer-entrapped gold nanoparticles†

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Experimental Section

Materials

Ethylenediamine core amine-terminated PAMAM dendrimers of generation 5 (G5.NH_2) with a polydispersity index less than 1.08 were purchased from Dendritech (Midland, MI, USA). FA, acetic anhydride, triethylamine, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), and all other chemicals and solvents were obtained from Aldrich and used as received. FA- and MTX-functionalized generation 5 (G5-FA-MTX) PAMAM dendrimers were synthesized and characterized elsewhere.[1, 2] The molecular weight of the G5-FA-MTX dendrimers was determined to be 42,750 via size exclusion chromatography with a polydispersity index of 1.1. The number of FA and MTX moieties attached onto each dendrimer was determined to be 4.5 and 4.0, respectively. KB cells were from American Type Tissue Collection (ATCC, Rockville, Maryland). Penicillin, streptomycin, fetal bovine calf serum (FBS) were purchased from Sigma (St. Louis, MO, USA). Trypsin-EDTA, Dulbecco's phosphate buffered saline (PBS), RPMI 1640 medium (with or without FA), and bovine serum albumin was obtained from GIBCO-BRL (Gaithersburg, MD). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (China). Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ cm. Regenerated cellulose membranes with molecular weight cut-off (MWCO) of 10,000 were acquired from Fisher.

Synthesis of Multifunctional Au DENPs

The procedure used to synthesize multifunctional Au DENPs is shown in Scheme 1. In a typical synthesis, G5-FA-MTX (11.97 mg) was dissolved in 5 mL water. $HAuCl_4.3H_2O$ (5.51 mg) with 50 molar equivalents of dendrimers dissolved into 2 mL water was then mixed with the aqueous solution of G5-FA-MTX dendrimers under magnetic stirring. After 30 min, the G5-FA-MTX/$HAuCl_4.3H_2O$ mixture solution was added with a $NaBH_4$ solution (1.59 mg dissolved into 1 mL methanol/water (v/v =1:1) mixture solution) with 3 molar equivalents of the gold salt. The reaction mixture turned deep-red within a few seconds after addition of the $NaBH_4$ solution. The stirring was continued for 2 h to complete the reaction. The reaction mixture was extensively dialyzed against water (six times, 4 L) for 3 days to remove the excess reactants, followed by lyophilization to obtain the product [$(Au^0)_{50}$-G5-FA-MTX] DENPs.

General Characterization Methods

^1H NMR spectra were recorded using Bruker AV-400 NMR spectrometer. The [$(Au^0)_{50}$-G5-FA-MTX] DENPs (3-5 mg) were dissolved in 0.5 ml D_2O and DMSO-d_6, respectively before measurements. UV-Vis spectra were collected using a Perkin Elmer Lambda 25 UV-Vis spectrometer. The Au DENP sample was dispersed in water at a concentration of 1 mg/mL before measurement. Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer Nano ZS model ZEN3600 (Worcestershire, UK) equipped with a standard 633 nm laser. The hydrodynamic size of the formed Au DENPs dispersed in PBS was analyzed for a time period up to 7 d to check the colloidal stability of the particles. TEM was performed using a JEOL 2010F analytical electron microscope with an accelerating voltage of 200 kV. An aqueous solution of a sample (5 μL, 1 mg/mL) was dropped onto a carbon-coated copper grid and air dried before measurements. The size distribution histogram of each sample was measured using ImageJ software (http://rsb.info.nih.gov/ij/download.html). For each sample, 300 NPs

were randomly selected from different images to analyze their size. TGA measurements were performed using a Perkin-Elmer TGA-7 thermogravimetric analyzer with a heating rate of 48 °C/min in air.

Cell Culture and Nanoparticle Uptake Experiments

The KB cells were continuously grown in two 10-cm culture dishes, one in FA-free medium and the other in regular RPMI 1640 cell culture medium supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL), 10% heat-inactivated FBS, and 2.5 μM FA. The cells grown in FA-free medium express high-level FARs (for short, KB-HFAR cells), while the cells grown in FA-containing medium express low-level FARs (for short, KB-LFAR cells).

For cellular uptake experiments, both KB-HFAR and KB-LFAR cells were cultured in 6-well plates at a density of 800,000 cells per well one day prior to the experiment. The cells were incubated with 20 nM or 50 nM of [$(Au^0)_{50}$-G5-FA-MTX] DENPs in RPMI medium at 37 ºC, 5% CO_2 for 2 h. After the incubation, the cells were washed once with Versene (Gibco), twice with PBS, and three times with Hanks' Balanced Salt Solution (HBSS). The cells were then lifted with trypsin/EDTA, resuspended in 200 μL of PBS, and dispersed into 1.8 mL of 10% FBS in PBS. The cell suspension (100 μL) was counted. The remaining cells were centrifuged to form pellets and lysed using an aqua regia solution (0.1 mL). The Au uptake was measured with a Finnigan™ ELEMENT2 inductively coupled plasma-mass spectrometer (ICP-MS) (Thermo Electron Corporation, Waltham, MA 02454).

MTT Quantification of Cell Viability

MTT assay was performed to quantify cell viability. Both KB-HFAR and KB-LFAR cells were plated in 96-well plates in RPMI 1640 medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin for 2 d prior to experiment. Both KB-HFAR cells and KB-LFAR cells were plated at a density of 10,000 cells per well to accommodate faster cell growth. Then KB-HFAR and KB-LFAR cells were treated with 100, 200, 300, 400, 500, 1000 and 2000 nM of either [$(Au^0)_{50}$-G5-FA-MTX] DENPs or G5-FA-MTX dendrimers at 37 ºC for 2 h, followed by replenishing with fresh medium

and culturing the cells for another 48 h. The MTT assay was preformed according to the manufacturer's instruction. Mean and standard deviation for the triplicate wells were reported. One-way analysis of variance (ANOVA) statistical method was used to evaluate the differences of the viability of cells (KB-HFAR or KB-LFAR cells) treated by either [$(Au^0)_{50}$-G5-FA-MTX] DENPs or G5-FA-MTX dendrimers with different concentrations and the control cells treated by PBS. 0.05 was considered as a significance level.

X-Ray Attenuation Measurements

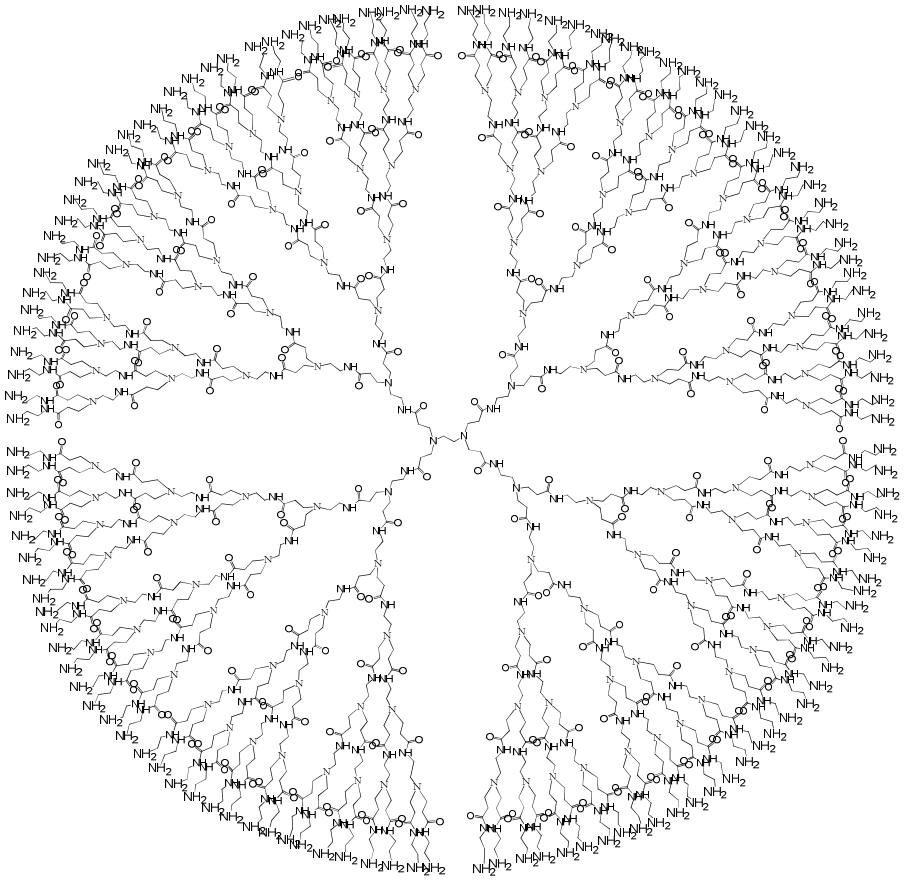
Solutions of [$(Au^0)_{50}$-G5-FA-MTX] DENPs and iohexol 300 (Omnipaque 300 mg I/mL, GE Healthcare) with different concentrations were prepared in 2.0 mL Eppendorf tubes and placed in a self-designed scanning holder. CT scans were performed using a GE LightSpeed VCT imaging system (GE Medical Systems) with 100 kV, 80 mA, and a slice thickness of 0.625 mm. Evaluation of the X-ray attenuation intensity was carried out by loading the digital CT images in a standard display program (GE Advantage Workstation AW4.4) and then selecting a uniform round region of interest on the resultant CT image for each sample. Contrast enhancement was determined in Hounsfield units (HU) for each concentration of [$(Au^0)_{50}$-G5-FA-MTX] DENPs and Omnipaque.

References

1. J. F. Kukowska-Latallo, K. A. Candido, Z. Cao, S. S. Nigavekar, I. J. Majoros, T. P. Thomas, L. P. Balogh, M. K. Khan and J. R. Baker, Jr., *Cancer Res.*, 2005, **65**, 5317-5324.
2. I. J. Majoros, T. P. Thomas, C. B. Mehta and J. R. Baker, Jr., *J. Med. Chem.*, 2005, **48**, 5892-5899.

Table S1. The hydrodynamic sizes of [$(Au^0)_{50}$-G5-FA-MTX] dispersed in PBS buffer at different time points.

Sample	Time (day)	Size in PBS (nm)	PDI
[$(Au^0)_{50}$-G5-FA-MTX]	1	285.3 ± 8.99	0.70 ± 0.010
	3	230.8 ± 4.84	0.54 ± 0.202
	7	288.8 ± 9.00	0.58 ± 0.064



Scheme S1. The molecular structure of the G5 poly(amidoamine) dendrimer.

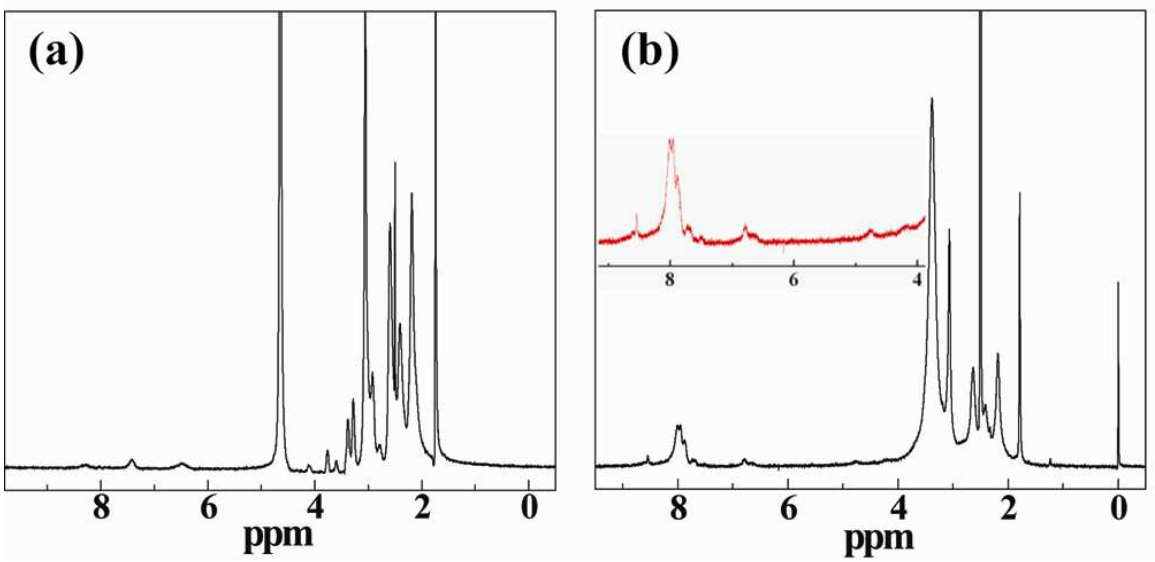


Figure S1. ^{1}H NMR spectra of [$(Au^{0})_{50}$-G5-FA-MTX] DENPs dispersed in D_2O (a) and DMSO-d_6 (b).

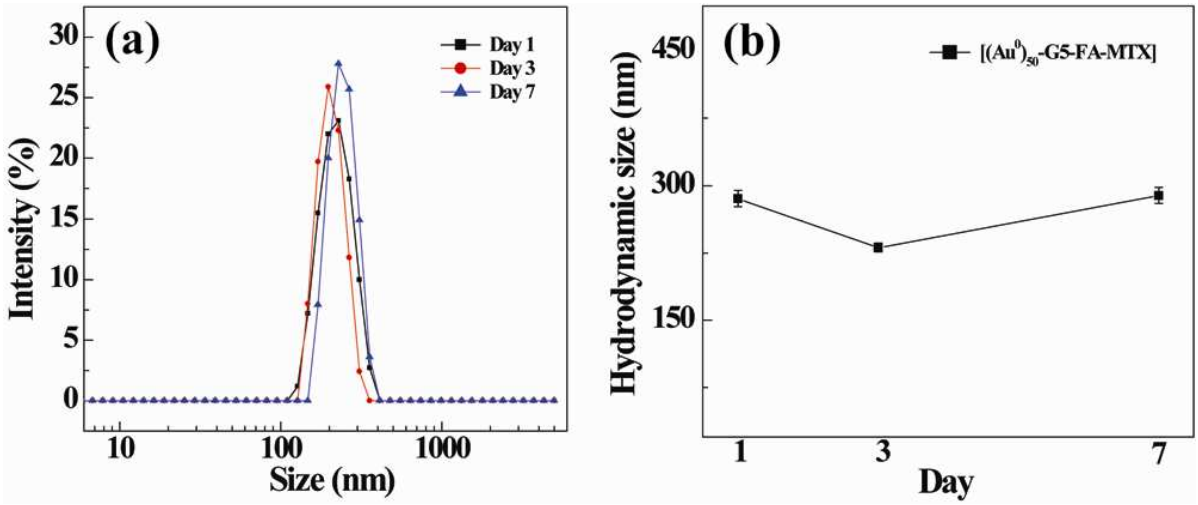


Figure S2. (a) The hydrodynamic size distribution graph of [$(Au^{0})_{50}$-G5-FA-MTX] DENPs dispersed in PBS at different time points. (b) The hydrodynamic size of [$(Au^{0})_{50}$-G5-FA-MTX] DENPs dispersed in PBS as a function of time.

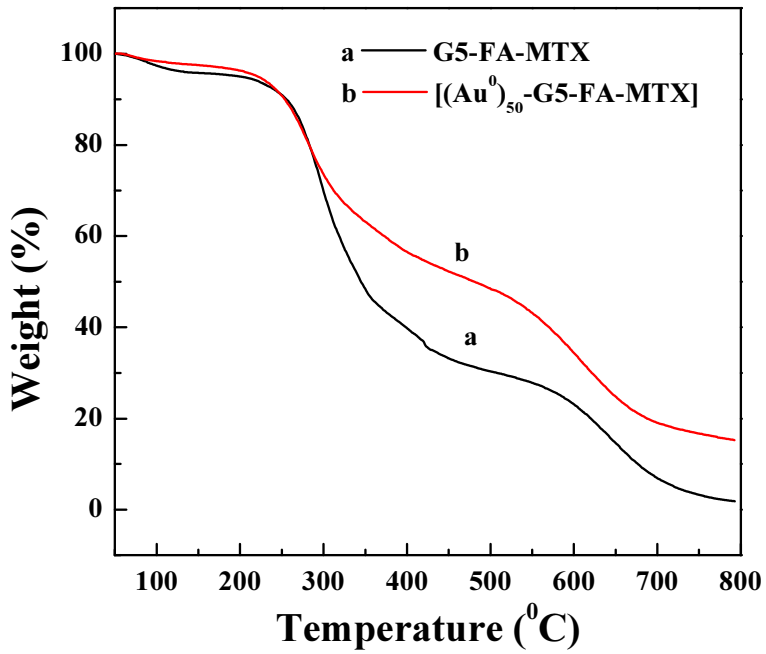


Figure S3. TGA curves of G5-FA-MTX dendrimers and $[(Au^0)_{50}$-G5-FA-MTX] DENPs.