Supplementary Material (ESI) for *Soft Matter*

Supplementary information

Tumor Microvasculature Targeting with Dendrimer-Entrapped Gold Nanoparticles

Rameshwer Shukla[a], Elliott Hill[b], Xiangyang Shi[a], Jinkoo Kim[b], Maria C. Muniz[a], Kai Sun[c] and James R. Baker, Jr[a]

General: G5-PAMAM dendrimer was prepared at the Michigan Nanotechnology Institute for Medicine and Biological sciences, University of Michigan, and were analyzed extensively by ^{1}H and ^{13}C NMR, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, high-performance liquid chromatography (HPLC), gel permeation chromatography (GPC), and polyacrylamide gel electrophoresis (PAGE). Pre-packed Sephadex G-25 PD-10 columns were purchased from Amersham Pharmacia Biotech (Piscataway, NJ) and equilibrated with degassed eluting buffer before sample introduction.

A JEOL 2010F analytical electron microscope was performed at 200 kV with an energy dispersive spectroscopy (EDS) system attached. Aqueous solution 5 μL of Au DENPs (1 mg/mL) was dropped onto a carbon-coated copper grid and air dried before measurements.

Synthesis of Au DENPs: The procedure to synthesize Au DENPs is adopted from those previously reported in the literature, but with slight variations.[1-3] The Au DENPs were prepared using sodium borohydride reduction chemistry with the dendrimer/gold atom

molar ratio at 1:74. G5-FI-RGD was synthesized as described previously.[4] An aqueous $HAuCl_4$ solution (0.005 g, 0.012 mmoles, 1 mL) was added into a G5-FI-RGD aqueous solution (0.006 g, 0.00017 mmoles, 2.0 mL) while stirring. After 30 min, $NaBH_4$ solution (0.010 g) dissolved in water was slowly added to the gold salt/dendrimer mixture while stirring. The reaction mixture turned a deep-red color within a few seconds after addition of the $NaBH_4$ solution. 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 $\{(Au^0)_{74}$-G5-FI-RGD} DENPs.

Cell Cultures: L1210 cell lines were grown in RPMI medium (with folate), supplemented with 10% FBS, 1% penicillin/streptomycin and 50 nM L-glutamine. These cells were grown in suspension and the media was changed every other day. HDMEC and HUVEC cells were grown in EGMV-2 media supplemented with: 5% FBS, 1% gentamycin, 1% VEGF, 1% ascorbic acid, 0.5% hydrocortisone, 3% hFGF, 1% IGF, and 1% hEGF. HUVEC and HDMEC cells were incubated as a monolayer at 37°C and 5% CO_2 and grown in 75 cm^3 flasks until 85% confluent ($2x10^6$ cells/flask). Approximately $0.5x10^6$ cells were seeded onto six well culture dishes (Matek Corp., Ashland MA) and cultured in 3ml of medium at least 24 h before the initiation of experiments. Samples were examined quantitatively for the $\{(Au^0)_{74}$-G5-FI-RGD} DENP uptake via flow cytometry (Coulter-Epiflow) at time points ranging from 1 h to 7 days. The data were normalized to controls that were prepared for each time point. The cell suspension (100 mL) was counted.

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Cell Targeting and Binding Assay: The cellular uptake of {$(Au^0)_{74}$-G5-FI-RGD} DENPs was measured in human dermal microvessel endothelial cells (HDMEC), human vascular endothelial cells (HUVEC) and Mouse lymphocytic leukemia cell line (L1210) cells. Endothelial cells were cultured in ECM-2 media supplemented with fetal bovine serum, EGF, VEGF, IGF and IL-8. All other cell lines were cultured in RPMI media (GIBCO) supplemented with 10% FBS and 1% P/S. A time course for {$(Au^0)_{74}$-G5-FI-RGD} DENPs (100nM) binding was done and results were analyzed via flow cytometric analysis (BectonDickinson FACScan analyzer, Fullerton, CA). The mean fluorescence of 10,000 cells was quantified. Confocal microscopic analysis was performed on HDMEC cells cultured in 35 mm glass bottomed culture dishes (MatTek Corp. Ashland MA) using a Carl Ziess confocal microscope. Fluorescence and differential interference contrast (DIC) images were collected simultaneously using an argon laser and Fluoview software. To determine the effects of binding of Au DENPs to HDMEC cells, cells were grown to 80% confluence ($1x10^6$ cells) in six-well plates and maintained in DMEM medium containing 10% FBS and 1% P/S. These cells were then cultured separately under one of the following conditions: 1) Untreated negative control, 2) Treated with {$(Au^0)_{74}$-G5-FI-RGD} DENPs for 6 h, 3) Pre-treated for 1h with free RGD peptide, and then incubated with {$(Au^0)_{74}$-G5-FI-RGD} DENPs for 6 h. After treatment, all cells were rinsed (3x) with PBS, and trypsinized (0.025% Trypsine, Worthington Biochemical, Lakewood, CA) for 5 min. Trypsin was neutralized with cold normal culture media (50/50), and cells were centrifuged at 1200 rpm for 5 min to form pellets and lysed using an aqua regia

solution (0.1 mL). The Au uptake was measured with a Finnigan™ ELEMENT2 ICP-MS instrument (Thermo Electron Corporation, Waltham, MA 02454).

References:

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