Supporting Information

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Supplementary Information for the manuscript submitted to *Small*

Gold Nanoparticles Are Taken up by Human Cells but Do Not Cause Acute Cytotoxicity**

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Experimental

Materials

Hydrogen tetrachloroaurate (III) trihydrate (99.9+%, Aldrich), sodium borohydride (99%, Aldrich), sodium citrate dihydrate (99%, Aldrich), *L*-ascorbic acid (99+%, ACS reagent, Sigma-Aldrich), cetyltrimethylammonium bromide (Aldrich), *N*-[6-(Biotinamido)hexyl]-3'-(2'-pyridyldithio)propionamide (Pierce); sodium hydroxide (certified ACS, Fisher), *L*-cysteine (97 %, Aldrich), D-(+)-glucose (>99.5 %, Aldrich), osmium tetroxide (4%, Electron Microscopy Sciences), LR white embedding resin (medium grade, London Resin Company), ethyl alcohol (200 proof, Aaper Alcohol) were used as received. All solutions prepared should be assumed to be aqueous unless otherwise stated. All glassware was cleaned with aqua regia and rinsed with deionized water.

Synthesis of 18 nm Citrate-Stabilized Gold Nanoparticles

Gold nanoparticles, 18 nm in size, were synthesized using a modification of the Frens' method (Frens, G. *Nature* **1973**, *241*, 20). A pale yellow solution of 100 mL deionized water and 2.5 mL 0.01 mM tetrachloroaurate (III) trihydrate was heated to boiling, while stirring, in a covered Erlenmeyer flask. 3 mL 1% trisodium citrate was then added. The solution first turned pale blue, then wine red. Boiling was continued for an additional 15 - 30 min, while topping up the solution with hot deionized water. No further color change was observed. The final concentration of gold atoms was 2.5×10^{-4} M and the particles formed were ~18 nm spheres. The particles were used as prepared.

Synthesis of 18 nm Biotin-Modified Gold Nanoparticles

Citrate-stabilized gold particles, 18 nm, were synthesized as described above. These particles were then modified with biotin-HPDP using the method of Connolly *et. al.*(Connolly, S. C., S.; Fitzmaurice, D. J. Phys. Chem. B. **2001**, *105*, 2222-2226) To 50 mL of the gold nanoparticle solution, 0.372 μL of a 2 x 10^{-5} M biotin-HPDP solution was added and stirred for 30 min. The particles were used as prepared.

Synthesis of 4nm L-Cysteine-Modified Gold Nanoparticles

Gold nanoparticles, 4nm, were synthesized and modified using the method of Mayya *et al.*[16], which was similar to the method used for the preparation of the citrate-stabilized gold particles. Briefly, 100 mL of a 10^{-4} M hydrogen tetrachloroaurate (III) trihydrate solution was reduced by 0.01g sodium borohydride, producing 4nm gold particles. The particles were then modified by adding a solution of *L*-cysteine, yielding a final *L*-cysteine concentration of 10^{-4} and a solution pH of ~9. The particles were used as prepared.

Synthesis of 12 nm Glucose-Modified Gold Nanoparticles

Gold nanoparticles were synthesized and modified using an adaptation of the method of Gole *et al.*[17] A pale yellow solution of 18 mL 0.01 M hydrogen tetrachloroaurate (III) trihydrate and 2 mL 1 M *D*-glucose solution was heated to 60 °C. The solution turned red upon the addition of 40 μL 1 M NaOH. The particles were used as prepared.

Synthesis of 18 nm CTAB-Stabilized Gold Nanoparticles

CTAB stabilized gold nanoparticles were synthesized in a 3-step process developed by Jana *et al.*[18] Typically this seeding growth method first involves the synthesis of a 3.5 nm citrate stabilized gold seed solution as detailed previously.[15] Next, a growth solution is prepared wherein 100 mL of an aqueous solution of 2.5 x 10^{-4} M $HAuCl_4$ was taken in a conical flask. To this 3 g of solid CTAB (0.08

M final CTAB concentration) was added and heated while stirring. The solution was cooled to room temperature and used as the growth stock solution. Following the mixing of 9 mL of growth solution and 50 μL of 0.1 M ascorbic acid solution, 1.0 mL of the 3.5 nm seed solution was added while vigorously stirring. Stirring continued for an additional 10 min. The final color of the solution was dark red and the particles prepared by this method were 8.0 nm ± 0.8 nm. The particles prepared in this step were then used as seeds for preparing 18 nm particles. To 9 mL of growth solution was added 50 μL of 0.1 M ascorbic acid solution, followed by 1.0 mL of 8.0 nm seed (as synthesized above) that was added while vigorously stirring. After 10 min of stirring, the purple colored solution showed a particle size of 18 nm according to TEM.

Treatment of Cells and Examination of Cell Viability

Human K562 cells (ATCC, Manassas, VA) were maintained in Iscove's Modified Dulbecco's Medium (IMDM, ATCC) lacking antibiotics and supplemented with 10 % fetal bovine serum (FBS, ATCC) at 37°C and 5% CO_2.[19] Cell survival, as measured by the MTT assay, was determined following three days continuous exposure to the gold nanoparticles.[20] Log phase cells in fresh media were dispensed into a 96-well round bottom plate at a concentration of 10^4 cells/well in 90 μl volume. Filter sterilized gold solutions were diluted appropriately and added in a 10 μl volume, 6 wells per sample. The media was not changed during the incubation. Following three days incubation, cell viability was determined by the addition of 20 μl MTT (thiazolyl blue tetrazolium bromide, 5 mg/ml in sterile PBS). The plate was incubated for an additional 5 hours at 37°C and 5% CO_2, allowing viable cells to convert the pale yellow MTT to an insoluble purple dye. The insoluble dye was pelleted by centrifugation, 300 g for 5 minutes. The media was carefully removed and the pelleted dye was dissolved in 200 μl dimethyl sulfoxide (DMSO). Absorbance values at 595 nm wavelength were collected on a ELX808 Ultra microplate reader (Bio-Tek, Winooski, VT). Cell viability following

gold particle exposure was calculated as a percentage compared to untreated control cells. For the experiments to determine cell numbers, cells were seeded into flasks at a concentration of 3000 cells/ml and 18 nm citrate capped nanoparticles (25 μM) were added. Cell numbers were counted on days 2 through 5.

Determination of gold nanoparticle concentration in the cell culture media

Gold particle concentration in the media was determined by absorbance measurements at 526 nm. For these experiments, the human K562 cells were grown IMDM lacking phenol red (Gibco-Invitrogen, Carlsbad CA) supplemented with FBS. Cells were treated with a single concentration of gold nanoparticles, 2.5×10^{-5} M, in a 6-well plate (2 ml total volume). At the appropriate time, cells were removed from the media by centrifugation at 300 g for 5 minutes, a speed that pellets the cells while leaving the gold nanoparticles in solution. Electronic absorption spectra were collected with a CARY 500 Scan UV-Vis-NIR spectrophotometer. Samples were measured in a 1 mL quartz cuvette across a wavelength range of 800 – 200 nm. Data is an average of three independent experiments and is presented as a disappearance of gold particles from the media compared to a control sample of media and gold particles but lacking cells.

Preparation of Samples for Transmission Electron Microscopy (TEM)

Gold nanoparticle samples were examined by transmission electron microscopy (TEM) to confirm the shape and size distribution of the gold particles. The samples were prepared by placing 3 μl of the nanoparticle solution on the carbon-coated copper grids and drying at room temperature. Transmission electron micrographs (TEM) were obtained using a Hitachi H-8000 electron microscope. In order to observe gold particles within the human K562 cells, samples of cells treated with citrate-stabilized 18 nm gold particles were examined by TEM. Cells that were not exposed to nanoparticles

were also examined. Cells were treated with a single concentration of gold nanoparticles, 2.5 X 10^{-5} M, in a volume of at least 5 ml. Following incubation of the cells with gold particles for 1 hour, the cells were centrifuged at 300 g for 5 min, and the media was removed. The pelleted cells were then suspended in ~2 mL of a 4 % osmium tetroxide solution and incubated at room temperature for 1 hour. Following removal of the osmium tetroxide, the cells were washed once with 70 % ethanol and three times 100 % ethanol for 15 min each wash. The cells were then transferred to a beam capsule, LR white was added and the capsule was incubated at 60°C overnight to allow the polymer to harden. Using a Sorvall Porter-Blum MT2-B Ultra-Microtome machine, 200 micron sections were cut with a diamond knife and placed on carbon-coated copper TEM grids.

Results

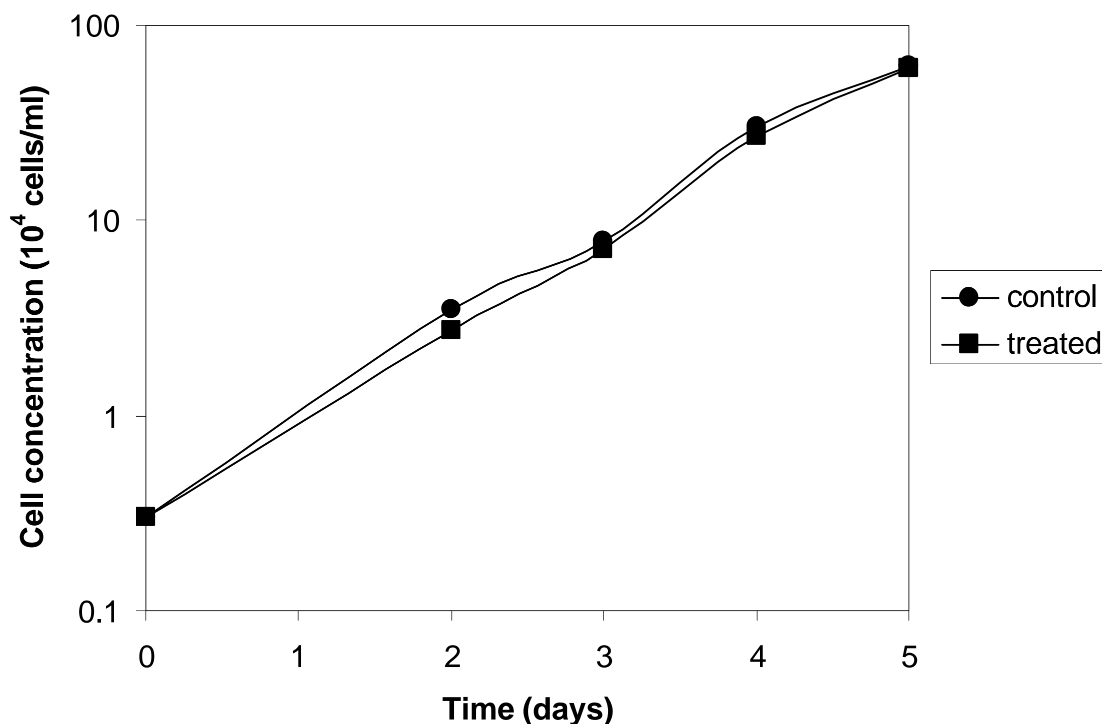

Figure S1. Measurement of cell growth in the presence or absence of 18 nm citrate capped nanoparticles. At day 0, the cells were seeded and 18 nm citrate capped nanoparticles (25 μM) were

added. Cell numbers were counted on days 2 through 5. No difference was seen in the growth of untreated control cells (?) and cells exposed to the nanoparticles (¦). Data points are an average of two experiments.