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Size and Concentration Effect of Gold Nanoparticles on X-ray Attenuation As Measured on Computed Tomography

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Gold nanoparticles (GNPs) have been used widely in imaging and therapeutics due to their surface plasmonic properties.[1–5] Recently, GNPs are also demonstrated to have great potential as contrast agents for computed tomography (CT) imaging applications.[6,7] Multidetector CT has become an essential medical examination tool for evaluating nearly all organ systems.[8] To increase contrast resolution, iodine-containing molecules are commonly used as CT contrast agents due to the high X-ray attenuation of iodine.[9] Since gold element provides almost three times greater X-ray attenuation per unit weight than iodine,[10] GNPs could provide much-needed contrast enhancment in CT imaging.[7] These X-ray and plasmonic characterics, plus the ease of the surface functionalization, make GNPs promising multifunctional probes for simultaneous imaging and drug or gene delivery applications.[11]

Here we report the size effect of GNPs on X-ray attenuation measured by CT. The size, shape, and concentration of GNPs have been demonstrated to influence cell uptake[12] and to induce cell toxicity at sizes smaller than 1.4 nm.[13] The appropriate GNPs for clinical imaging purpose should have sufficiently large size for optimal imaging quality and biocompatibility. Despite the recent demonstration of GNPs as a possible contrast agent for CT,[6,7] there have no studies about the effect of GNP size on CT attenuation. By investigating size (4, 20, 38, and 60 nm) and concentration (up to 1 mg/mL) effect of GNPs on cell uptake, cell viability, and the CT attenuation, we demonstrate that GNPs are nontoxic and the smaller GNPs show greater X-ray attenuation than the larger ones. The X-ray attenuation effect of GNPs over the iodine-based contrast agent, Omnipaque, is much enhanced at higher concentrations.

GNPs were prepared following the previous publications.[14] The 4 nm GNPs were made by borohydride reduction of

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(1) Hu, M.; Chen, J. Y.; Li, Z. Y.; Au, L.; Hartland, G. V.; Li, X. D.; Marquez, M.; Xia, Y. N. *Chem. Soc. Rev.* **2006**, *35*, 1084–1094.
(2) Huang, X. H.; Jain, P. K.; El-Sayed, I. H.; El-Sayed, M. A. *Nanomedicine* **2007**, *2*, 681–693.
(3) El-Sayed, I. H.; Huang, X. H.; El-Sayed, M. A. *Nano Lett.* **2005**, *5*, 829–834.
(4) Huang, X. H.; El-Sayed, I. H.; Qian, W.; El-Sayed, M. A. *J. Am. Chem. Soc.* **2006**, *128*, 2115–2120.
(5) Chen, J.; Saeki, F.; Wiley, B. J.; Cang, H.; Cobb, M. J.; Li, Z. Y.; Au, L.; Zhang, H.; Kimmey, M. B.; Li, X. D.; Xia, Y. *Nano Lett.* **2005**, *5*, 473–477.
(6) Kim, D.; Park, S.; Lee, J. H.; Jeong, Y. Y.; Jon, S. *J. Am. Chem. Soc.* **2007**, *129*, 7661–7665.
(7) Hainfeld, J. F.; Slatkin, D. N.; Focella, T. M.; Smilowitz, H. M. *Br. J. Radiol.* **2006**, *79*, 248–253.
(8) Krause, W.; Schneider, P. W. *Topics in Current Chemistry*; Springer: Heidelberg, Germany, 2002.
(9) Krause, W. *Adv. Drug Delivery Rev.* **1999**, *37*, 159–173.
(10) NIST Physics Laboratory Home Page. http://physics.nist.gov/PhysRefData/XrayMassCoef (accessed March 7, 2008).
(11) Son, S. J.; Bai, X.; Lee, S. *Drug Discovery Today* **2007**, *12*, 657–663.
(12) Chithrani, B. D.; Ghazani, A. A.; Chan, W. C. W. *Nano Lett.* **2006**, *6*, 662–668.
(13) Pan, Y.; Neuss, S.; Leifert, A.; Fischler, M.; Wen, F.; Simon, U.; Schmid, G.; Brandau, W.; Jahnen-Dechent, W. *Small* **2007**, *3*, 1941–1949.
(14) Supporting Information.

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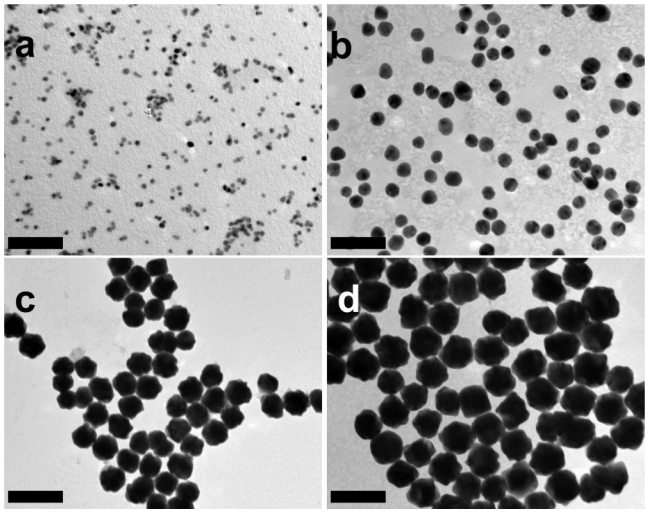

Figure 1. TEM images of (a) 4 nm; (b) 20 nm; (c) 38 nm; and (d) 60 nm of the MSA-coated GNPs (scale bar = 100 nm).

gold salts in the presence of sodium citrate.[15] The 20 nm GNPs were synthesized by Fens method. These 20 nm GNPs were used as seeds for growing 38 and 60 nm GNPs in the presence of 2-mercaptosuccinic acid (MSA) that acts both as a reducing and as a capping agent.[16] The 4 and 20 nm GNPs were stabilized with citrate and the 38 and 60 nm GNPs were protected with MSA. The citrate on the two smaller particles was replaced with MSA to ensure the same surface chemistry in these four groups of GNPs. The ligand replacement was performed by incubating citrate-coated GNPs with excessive MSA for 6 h, followed by washing with deionized water to remove the excessive citrate/MSA.[14] NMR spectra of the GNPs before and after the ligand exchange (Supporting Information, Figure S1) show that the original citrate is replaced by MSA. Analysis on the transmission electron micrioscopy (TEM) images of the GNPs indicates that the ligand replacement does not lead to any noticeable particle morphology change. Figure 1 gives TEM images of the MSA-coated GNPs with diameters at 4 ± 1 nm, 20 ± 1.5 nm, 38 ± 4 nm, and 60 ± 5 nm, respectively. These GNPs are also optically active, and their plasmonic absorptions locate from 510 to 540 nm (Supporting Information, Figure S2).

Dispersion stability of the GNPs is an important factor for imaging applications. Undesired aggregation can cause the loss of imaging conspicuity and induce thrombosis.[17] To examine the stability of the MSA-coated GNPs, we dispersed each of them in cell culture media, diluted the dispersion into 1 × PBS buffer plus 10% FBS, and incubated it under 37 °C. The dispersion was sampled by dynamic light scattering (DLS). Figure S3 (Supporting Information) is the change in hydrodynamic sizes of the GNPs over a 24 h period. It can be seen that there is no obvious size increase. The overall size for each group of GNPs is larger than those GNPs in the pure PBS. This size increase is likely caused by adsorption of FBS on the GNP surface.

(15) Jana, N. R.; Gearheart, L.; Murphy, C. J. *Adv. Mater.* **2001**, *13*, 1389–1393.
(16) Niu, J. L.; Zhu, T.; Liu, Z. F. *Nanotechnology* **2007**, *18*, 325607.
(17) Radomski, A.; Jurasz, P.; Alonso-Escolano, D.; Drews, M.; Morandi, M.; Malinski, T.; Radomski, M. W. *Br. J. Pharmacol.* **2005**, *146*, 882–893.

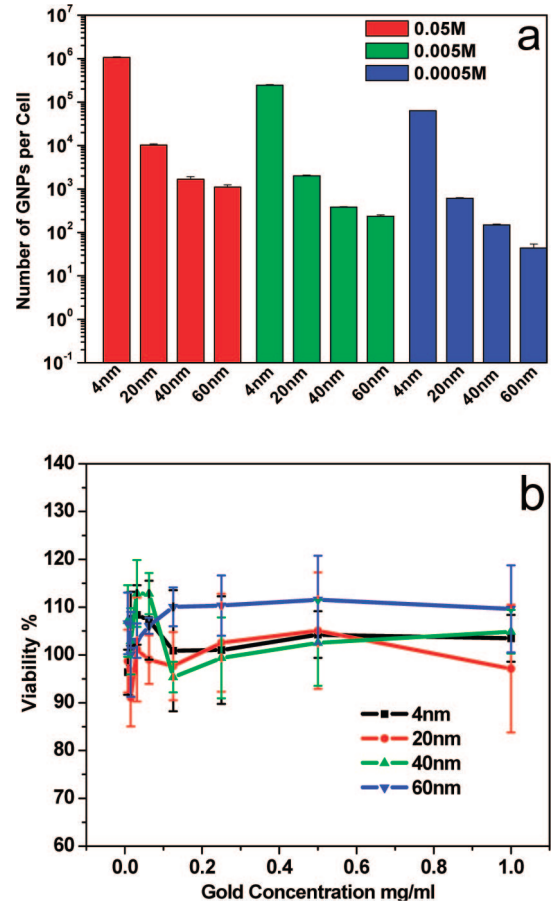

Figure 2. (a) Size and concentration dependent uptake of GNPs by HeLa S3 cells and (b) HeLa S3 cell viability with GNPs in different sizes.

The uptake of the stable GNPs by HeLa S3 cells was studied by incubating these cells with GNPs. Gold compositions within each cell were analyzed by inductively coupled plasma atomic emission spectroscopy, (ICP-AES)[14] and the number of GNPs in each cell was calculated from this composition and the size of the GNPs. Figure 2a shows the size and concentration dependent uptake of GNPs in each cell. A higher concentration results in more uptake. More importantly, at the same gold concentration, a larger number of small GNPs penetrate cellular membrane. Cell viability study demonstrates that the GNPs within the cells are not toxic. This is shown in the size and concentration dependent cell viability data (Figure 2b) from which we can conclude that the viability of HeLa S3 cells is not affected by either the size of the GNPs (4−60 nm) or the gold concentration (up to 0.05 M studied in this paper).

The X-ray attenuation of GNPs at different concentrations was compared with a commercial iodinated-contrast agent, Omnipaque. Gold has higher theoretical X-ray attenuation (at 100 keV: gold, 5.16 cm^2/g; iodine, 1.94 cm^2/g; water, 0.171 cm^2/g),[10] but experimentally this attenuation effect from gold and iodine is not easily detected in dilute aqueous solution. As shown in Figure 3a, the 4 nm GNPs and Omnipaque show similar X-ray attenuation at low concentration (0.02 mol/L). But at 0.1 mol/L concentration, the attenuation of the 4 nm GNPs in Hounsfield unit (HU) is 25% higher than that of Omnipaque. This concentration dependent effect is caused by the change in mass ratio

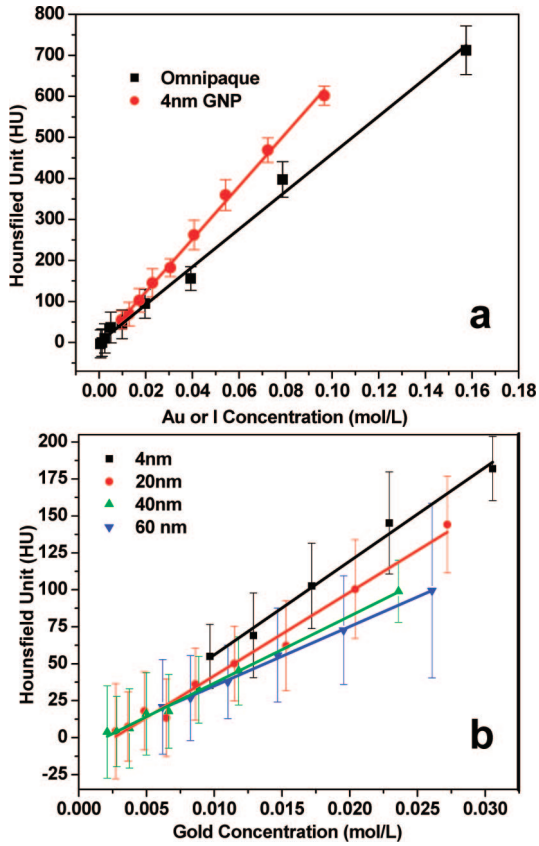

Figure 3. (a) X-ray attenuation and concentration relation between 4 nm GNPs and Omnipaque; (b) size and concentration dependent X-ray attenuation of GNPs.

between gold (or iodine) and water molecules. For example, the ratio of gold/water is only 1:253 when the gold concentration is 0.02 mol/L. However, the ratio is increased to 1:49 at 0.1 mol/L concentration. As the concentration of GNPs increases, gold dominates the X-ray attenuation due to the high X-ray attenuation effect of gold, leading to the marked attenuation difference between GNPs and Omnipaque. Note that when measured in mass concentration, the GNPs have slightly smaller attenuation effect than the Omnipaque (Supporting Information, Figure S4).[14]

The GNP size effect on X-ray attenuation was also studied. At the same gold concentration, the smaller GNPs show greater X-ray attenuation than either the larger ones (Figure 3b) or Omnipaque (Supporting Information, Figure S5). This can be attibuted to the surface area increase with the decrease in GNP sizes.[18] With the same amount of gold, the smaller GNPs offer larger gold surface area. Since X-ray attenuation is dependent on the target area, small GNPs with larger surface area should exhibit more dramatic X-ray attenuation and, therefore, serve as more efficient CT contrast agents.

In summary, we report that the MSA-coated GNPs with sizes ranging from 4 to 60 nm are stable in physiological conditions and are nontoxic to HeLa S3 cells. The 4 and 20 nm GNPs show higher X-ray attenuation than the commercial iodinated-contrast agent, Omnipaque, under the same molarity. The X-ray attenuation from GNPs is both size and concentration dependent with smaller GNPs at higher concentration exhibiting greater effect. Our work demonstrates that GNPs, especially small GNPs, may be a better alternative to Omnipaque as a robust contrast agent for CT scanning.

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Supporting Information Available: GNPs synthesis and characterization (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Rotello, V. M., Ed. *Nanoparticles: Building Blocks for Nanotechnology*; Springer Science: New York, 2004.