Gold Nanoparticles Coated with Gadolinium Chelates as Multifunctional Contrast Agents

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Introduction

The development of contrast agents for use across multiple imaging modalities such as PET/MRI, PET/CT, SPECT/CT, and MRI/CT is a versatile area of research gaining lot of interest in recent times. X-ray CT imaging is widely used for diagnosis since X-rays can go across the human body. Recently, novel nanoparticle-based CT contrast agents have emerged to overcome the shortcomings of iodine agents [1]. In this regard, gold nanoparticles(AuNPs) were efficiently applied *in vivo* as X-ray contrast agents. Combining MRI CAs to a nanoparticle surface can make an extremely attractive multi-modal contrast agent. We anticipated the use of thiol-functionalized DTPA-bis(amide) complexes of gadolinium to coat gold surfaces as bimodal contrast agents. Here in, the synthesis, relaxivity and CT properties of gold nanoparticles coated with Gd-complex of DTPA-bis(amide) conjugate of cysteine are reported.

Material and Methods

DTPA-bis(amide) conjugate of cysteine (L) : To a stirred suspension of L-cysteine methylester (6.37 mmol) in dry DMR was added DTPA-bis(anhydride) (3.18mmol). The mixture was stirred at 80 ℃ for 6 h. The solution was passed through a short column of silica gel(60 mesh) with methanol as an eluant and the product was precipitated with acetone. ***Gadolinium(III) complex (GdL)*** : Gd_2O_3 (0.92 mmol) in water was placed in a 50mL round bottom flask and L(1.84 mmol) was added and stirred for 6 h at 90 ℃ after which any solid impurities were removed by filtration through celite. The residue was triturated with a mixture of acetone and diethylether (30 : 70 v/v, 100mL). The solid product was isolated by filtration and dried. ***Functionalized gold nanoparticles (Au NPs)*** : Citrate-coated gold nanoparticles were prepared by reducing $HAuCl_4$ with sodium citrate at 100 ℃. For a 100mL solution of as-prepared AuNP, GdL (150 mg) or L (150 mg) was added and stirred for 20 h. An equal amount of acetone was added and the solution was further stirred for 4 h. The nanoparticles were collected by centrifugation and washed successively with water, acetone, and ether.

Results and Discussion

The GdL-functionalized Au NPs are characterized by various techniques such as XRD, IR, UV-visible, TGA, TEM, DLS and ICP. Figure 1 (a) and (b) show the TEM images of well-dispersed spherical particles of citrate-coated and GdL-coated Au NPs, respectively. The particles have a mean size 12-15nm with a narrow size distribution. Based on TGA and ICP, the total number of GdLs per Au NP was found to be about 2.9×10^3 which is much higher than an analogous system [2]. Figure 2 shows the X-ray attenuation in HU units for Au@L, Au@GdL and Ultravist®. At the concentration of 200 mM in gold, the X-ray attenuation is much higher due to the attenuation effect of Au leading to marked difference from the iodine-based contrast agents [3]. This result clearly indicates that the GdL-coated Au NPs have a high potential for use in *in vivo* CT imaging. Also, the CT enhancement was compared among the Au NPs with and without gadolinium. Although the gadolinium K-edge energy (50.2keV) is largely lower than the one of gold, gadolinium ions immobilized on each nanoparticle ([Gd] = 1.8 mM for [Au] = 100 mM) seem to contribute to a small contrast enhancement of the CT phantom images, as shown in the Figure 2. But the main role of Gd^{3+} is to increase the positive contrast of the MR images. Table 1 shows relaxivities (R_1, R_2) of GdL, Au@GdL and Omniscan® at 1mM concentration at 293K and 1.5T. GdL exhibits R_1 and R_2 relaxivities approximately two times higher than those of Omniscan®. The relaxivity of Au@GdL at 1mM [Gd] dramatically increase to ~ 18 $mM^{-1}s^{-1}$. Such high relaxivities with our system may be rationalized in terms of not only the intrinsic nature of monomeric GdL but also a greater number of GdL loading per Au NP because of higher degree of polymerization and a more rigid framework, leading to slower tumbling of the Gd-chelates. In terms of Au NP concentration, relaxivities are in the order of 10^5 $mM^{-1}s^{-1}$. Figure 3 shows T1-weighted MR images of a mouse before and after intravenous injection of Au@GdL (0.1 mmol/kg). The MR images of the tumor in the brain become brighter after injection, as shown by the relative contrast enhancement. This study confirms that the Au NPs can be used to image brain tumors *in vivo*. Since a huge number of GdLs are coated on the Au NP surface, this CA can be used at much lower concentration. The inner core gold is then tested for the CT attenuation *in vivo*. As expected, the follow-up of MRI of Au@GdL by CT in mice yielded identical results. Figure 4 shows the CT image of rats following heart injection of Au@GdL (2 mg/g). The tumor in brain can be distinguished on the Au@GdL-enhanced CT image with higher contrast. The much longer half-live of Au@GdL in the body than iodine-based contrast agent may be due to the higher molecular weight and should help improve the diagnosis of the disease. The extremely high relaxivities in the order of 10^5 $mM^{-1}s^{-1}$ combined with CT capability of these Au NPs demonstrate the possible application as a bimodal contrast agent for clinical use. Further work is underway to develop target-specific MRI CAs based on Au NPs.

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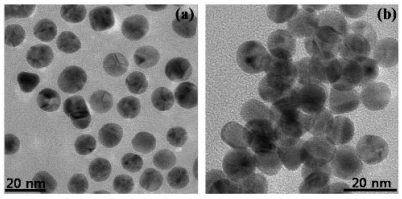

Figure 1. TEM image of (a) citrate-coated gold nanoparticles and (b) Au@GdL.

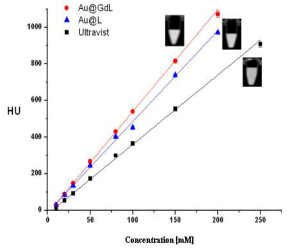

Figure 2. X-ray attenuation in HU units for Au@L, Au@GdL and Ultravist® at various concentrations. Phantom images are shown along with the plots.

Table 1. R_1 and R_2 values for the GdL, Au@GdL and Omniscan®

Sample	$R_1(mM^{-1}s^{-1})$	$R_2(mM^{-1}s^{-1})$
Omniscan®	3.30 ± 0.03	3.80 ± 0.06
GdL	7.50 ± 0.08	12.3 ± 0.21
Au@GdL[Gd]	17.9 ± 1.1	28.2 ± 1.0
Au@GdL[Au NP]	4.65×10^5	7.2×10^5

Each value is presented as a mean value (±SD)

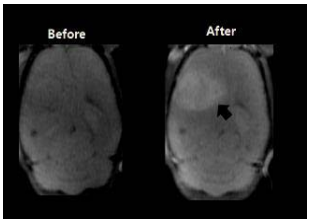

Figure 3. *In vivo* MR images of brain of the mouse injected with Au@GdL at 0.1 mmol/kg body weight. The arrow indicates brain tumor

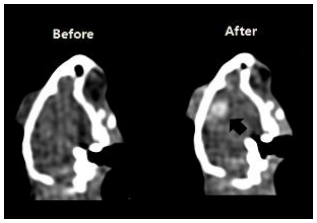

Figure 4. *In vivo* CT images of brain of the mouse injected with Au@GdL at 2 mg/g body weight. The arrow indicates brain tumor.