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Gadolinum-gold core-shell nanocrystals: Potential contrast agents for molecular MRI with high T_1 relaxivity

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ABSTRACT

Gold-coated gadolinium nanocrystals have been synthesized and their relaxivities, R1 and R2, found to be 19.7 and 21.7 s^{-1} mM^{-1} respectively at a 9.4 T applied field. R1 is more than 3 times higher than commercial Gd^{3+} chelate contrast agents on a per Gd atom basis and over 3000 times higher on a per nanocrystal (molecule) basis. Furthermore, the R2/R1 ratio, 1.1, is near ideal for a T1 contrast agent. Colloids of the PVP coated Gd@Au nanocrystals are stable in solution at physiological pH for more than a week. The Au shell protects the Gd from reaction over even in low pH solution. These results indicate that Gd@Au is an exceptionally promising candidate for development as an MRI T1 contrast agent for molecular imaging.

1. Introduction

All of today's commercial MRI contrast agents can be classified as either a T1 (or T_1-weighted) or T2 (or T_2-weighted) contrast agent. T1 agents are generally preferred as they render "positive" or "bright" contrast to areas of an MRI image by decreasing T1 (longitudinal or spin-lattice) solvent (H_2O) proton relaxation times while T2 agents decrease T_2 (spin-spin) relaxation times darkening areas of the image. All but one of the commercial T1 agents are paramagnetic Gd^{+3} chelates (a single Mn^{+2} agent has been approved for use) while T2 agents are superparamagnetic iron oxide nanoparticles. T2 (ferromagnetic or superparamagnetic) agents work by distorting the applied magnetic field; the inhomogeneous magnetic field surrounding the agent causes more rapid spin dephasing or T_2 relaxation. Spin flips and physical rotation of T1 (paramagnetic) agents create fluctuating magnetic fields that can couple to nearby protons and provide an energy transfer path, increasing the rate of T1 relaxation (i.e. decreasing T_1). Gd^{+3} has 7 unpaired electrons and, with its symmetric S state configuration, slow electron spin relaxation times, allowing efficient coupling to the slow relaxation of protons.

Commercial T1 agents have relatively low relaxivities and thus require the injection of gram ($\sim 10^{-4}$ mol/kg) quantities of Gd [1,2]. The relaxivity is too low for molecular imaging, where one would deliver the contrast agent only to target sites enabling far greater selectivity and resolution. For instance, the concentration of receptors is too low (10^{-9}–10^{-13} mol/g tissue) to be imaged by Gd^{+3} chelates [2,3]. The problem is exacerbated by the steady progression of MRI's to higher static magnetic field strength (for increased signal to noise ratio, spatial and temporal resolution), with 3 T scanners now becoming widely available and 9.4 T scanners being used for animal studies, as the R1 relaxivity of Gd^{+3} chelates decreases and R2 increases at high field [2,4]. Thus, increasing the relaxivities of contrast agents is of great interest.

Essentially, there are two ways to increase the efficiency of the Gd-based contrast agents to allow for molecular imaging, increasing their relaxivity or increasing the Gd loading of each complex (or any alternative delivery species). Large increases in the relaxivity of current agents are difficult. For example, slowing molecular rotation, decreasing f-electron relaxation times and increasing the exchange rate while decreasing the distance between the Gd^{+3} center and the H_2O molecule bound to the complex (in the first coordination sphere, or inner sphere) or the number of inner sphere H_2O molecules can all increase the relaxivity, but each presents challenges and can't overcome the theoretical limit to Gd^{+3} relaxivity ($\sim$100 $mM^{-1}s^{-1}$) [2,5]. The number of paramagnetic centers (e.g. Gd^{+3}) per contrast agent delivery species (chelate or otherwise) is a far less limited manner to increase relaxivity.

Recently, there has been great interest in Gd based nanoparticle MRI contrast agents [6]. The relaxivity of Gd based nanoparticles such as Gd_2O_3, GdF_3 and Gd^{+3} metal organic frameworks (MOF's) can be significantly higher than traditional Gd chelate MRI contrast agents due to their higher concentration of Gd^{3+} ions per particle,

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even though Gd^{+3} chelates have higher per ion T_1 relaxivities. However, there is increasing concern that currently used Gd chelate contrast agents show in vivo instability and release toxic Gd^{+3}, concerns that apply equally to nanoparticulate based Gd agents. Surface modification of Gd^{+3} based nanoparticle contrast agents that provides water solubility, prevents aggregation and the release of toxic Gd^{+3} is an outstanding problem that prevents their application. In addition, the surface modification should provide for appropriate bio-recognition and other functions such as targeting and therapeutic delivery [7,8].

Magnetic nanoparticles (T2 agents) are of interest as MRI contrast agents that avoid the toxicity concerns of Gd^{+3} based agents. Superparamagnetic iron oxide nanoparticle based agents are of particular interest due to their low toxicity and the high magnetic moment of iron, with a number of such agents approved for clinical use such as Feridex,® Resovist® and GastroMARK®, the later used as an oral agent for abdominal tissue imaging. However, as negative contrast agents, their utility is limited.

Recently, the solution synthesis of nearly monodisperse nanocystals of gold-coated gadolinium metal, not oxide, (Gd@Au) by alkalide reduction was reported [9]. In that report of the only method to date to be shown capable of producing these or similar metal nanocrystals, it was first suggested that they could serve as platforms on which therapeutic agents might be built; antitumor weapons that can be actively monitored and guided to a tumor for precise treatment delivery. The Au shells could serve critical roles in addition to providing water (over a wide pH range) and air stability; they might provide a protective layer to address Gd toxicity, a surface for which methods of chemical modification for targeting and drug delivery have been developed, a SERS substrate for chemical diagnostics and potentially be used for efficient photo thermal and photo acoustic therapy. Multiple therapy options could be available including photon activation therapy [10], synchrotron stereotactic radiotherapy [11], ^{159}Gd radionuclide therapy [12] and ^{157}Gd neutron capture therapy. Gd@Au is particularly interesting as neutron capture therapy agent because ^{157}Gd has the largest neutron absorption cross section of any nucleotide, 66 times greater than the currently used ^{10}B [13]. Finally, Gd@Au could serve as a molecular MRI contrast agent because elemental Gd, like Gd^{3+} ions, has 7 unpaired electrons in a symmetric S state configuration, but each nanoparticle has a vastly greater spin density than currently used Gd chelates.

Bulk Gd metal is one of the four elements that display ferromagnetism at room temperature, and as such one might assume that the MRI contrast properties of its nanocrytals would be similar to those of superparamagnetic agents. However, Gd@Au synthesized by alkalide reduction is a simple paramagnet at room temperature, showing ferromagnetic or superparamagnetic behavior only below ~20 K due to dimensional reduction to the nanoscale and lattice expansion [9]. Thus, Gd@Au is expected to be a T1 contrast agent. These Gd^0 based nanocrystals may have a number of advantages over Gd^{+3} based nanoparticle MRI contrast agents. First, the leaching of Gd^{+3} may be completely prevented by the crystalline Au shell, unlike Gd^{+3} based chelates and nanoparticles, perhaps even allowing oral rather than intravenous delivery [7]. Second, functionalization of Au surfaces for biocompatibility, water solubility, biological camouflage, targeting and therapeutic delivery has been the subject of intense research for more than a decade, generating a huge cache of knowledge that can be applied to the functionalization of the Au surface that surrounds the Gd metal nanocrystals [14]. Third, the density of Gd in the metal nanocrystals is much greater than in the most highly effective Gd^{3+} based contrast agents, offering the possibility of very high per particle relaxivity, enabling molecular imaging.

2. Experimental details

Gd@Au nanocrystals were prepared with the previously published method and functionalized with PVP to give water solubility [9]. $GdCl_3$ (99.99%) and 15-crown-5 (98%) were purchased from Alfa Aesar. Polyvinylpyrrolidone (average MW 55,000, 99%), $AuBr_3$ (99.99%), toluene (99.99%) and 1-Decanethiol (96%+) were purchased from Aldrich. All were used without further purification. Tetrahydrofuran (THF; Aldrich, 99.9+% HPLC grade, inhibitor-free) was purified by stirring over KNa alloy until a persistent blue solution was obtained, then vacuum transferred to storage bottles. Solution preparation and alkalide reduction was performed in open vessels at room temperature in an N_2 filled glovebox (<1 ppm O_2, H_2O). Alkalide ($K^+(15\text{-crown-}5)_2Na^-$) solution (100 mL, 9.56 mmol/L) was formed by stirring stoichiometric quantities of 15-crown-5 over KNa alloy in THF. $GdCl_3$ solution (100 mL THF, 0.948 mmol/L) was added to the rapidly stirred alkalide solution at a rate of 0.28 mL/s. After waiting 2.0 min, $AuBr_3$ solution (100 mL THF, 1.28 mmol/L) was added at a rate of 0.28 mL/s. To prepare samples for relaxivity measurements, a 50 mL aliquot of the product was surface modified by mixing with a solution of 0.560 g of PVP in 50 mL distilled water with vigorous stirring. The final solution was allowed to stay overnight undisturbed, and purple fine powders were formed. The surface modified nanoparticles were then collected by centrifuge and dried in the air. The absence of free Gd^{3+} was confirmed by washing the product with 0.1 M HCl, collecting it by centrifuge and the analyzing the wash by ICP-AE. Finally, the product was redispersed in ultra pure water (HPLC grade, Fisher Scientific) for relaxivity measurements. A portion of the dried sample was dispersed in phosphate buffer saline (PBS, 0.01 M phosphate buffer, 0.0027 M KCl and 0.137 M NaCl, pH 7.4 at 25 °C, Fisher Scientific) solution for dynamic light scattering (DLS) measurements.

A FEI Talos 200X TEM with a Super X electron dispersion spectroscopy (EDS) system was used for high-resolution transmission electron microscopy (HR-TEM) and elemental analysis. Bulk elemental analysis was obtained by EDS (Kratos Analytical EDX-700) and ICP Atomic Emission Spectroscopy (ICP-AE, Shimadzu ICPE-9820), magnetic characterization with Quantum Design MPMS XL, and powder XRD using a Rigaku Miniflex + diffractom eter. The population distribution of hydrodynamic radii was measured with at 25 °C with a Wyatt Dynapro Nanostar DLS System. Relaxivity measurements were obtained at 9.4 T on an Agilent 400 MR DD2 NMR regulated at 25 °C.

3. Results and discussion

The final product of PVP-capped Gd@Au nanocrystals is a black, free-flowing powder. Powder X-ray diffraction shows the superposition of nanocrystalline Gd and Au, consistent with previously reported Gd@Au nanocrytals, with no evidence of oxide (Fig. 1) [9]. The Gd pattern is consistent with a hexagonal structure with $a = b = 0.517$ nm, however, the principal axis is slightly larger than found in the previous report, with $c = 0.497$ nm. TEM images are also consistent with those previously reported (Fig. 2), with an average nanocrystal diameter of 3.44(19) nm.

Elemental analysis of the material by X-ray florescence found it to be 39.4 at.% Gd and 60.6 at.% Au, in good agreement with the reaction stoichiometry (42.6 at.% Gd, 57.4 at.% Au). Similar results, 37.5 and 62.5 at.% Gd and Au, respectively, were found by ICP-AE and used for relaxivity calculations.

Unlike bare gadolinium nanoparticles, which ignite immediately after exposing to the air, the PVP-coated Gd@Au nanocrystals are stable in the air and water. Furthermore, the Gd@Au nanocrys-