

Superparamagnetic Iron oxide-Gold Core-Shell Nanoparticles for Biomedical Applications

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ABSTRACT

Iron oxide nanoparticles are used for contrast enhancement in magnetic resonance imaging (MRI). We have prepared gold-coated iron oxide nanoparticles that show no change in their superparamagnetic behavior as a consequence of coating. Their potential use as MRI contrast agents was investigated by monitoring their T2 relaxation time with concentration. Cytotoxicity of these nanoparticles was also studied. The results of these studies are presented.

INTRODUCTION

Nanoparticles are increasingly being used in biomedicine [1]. A few of the applications suggested for magnetic nanoparticles are its use as sensing agents, in separation assays using their magnetophoretic properties, as drug delivery agent, in hyperthermia treatments [2] and in magnetic resonance imaging (MRI) contrast enhancement [3,4]. MRI technique is used to image soft tissue and utilizes contrast enhancing agents to improve imaging. Gadolinium containing compounds are used as positive contrast agents and signal enhancement is achieved through T1 relaxation. Use of positive contrast agents in the form of superparamagnetic iron oxide particles results in T2 relaxation and this results in signal enhancement.

Superparamagnetic iron oxide-based particles are generally small, in the range of nanometers, and are functionalized with various ligands such as TAT peptide [5], transferrin, and folate [6] for detection of tumor cells. Functionalized magnetic nanoparticles provide a unique platform for molecular imaging as they can be detected by conventional MRI methods, can be "tailored" for specific signal recognition, and can be targeted to the disease site based on the enhanced permeability and retention effect of the tumor vasculature. When the size of the nanoparticles are very small (<300 nm), in addition to T2 relaxation, T1 relaxation is also seen. To further improve the specificity of these iron oxide nanoparticles to target a site in the body, such as a specific tumor, it is important to have control on the surface properties of the nanoparticles. The surface of the nanoparticles should be flexible enough to be able to modify with a unique antibody to target only the site of interest.

We have used a gold shell on the iron oxide nanoparticle to achieve this. Conjugation of gold nanoparticle surfaces to various antibodies is tunable, highly controlled and very well established. Thus introducing a thin shell of gold on iron oxide nanoparticles to exploit the magnetic property of the iron oxide and site-specificity of gold shell, through suitable antibodies, will open a new class of MRI contrast agents. Additionally, polyethylene glycol (PEG) spacers can be introduced to help improve flexibility and the circulation in plasma [7].

EXPERIMENTAL METHODS

Synthesis and characterization of iron oxide-gold core-shell nanoparticles

The Fe₃O₄ nanoparticles was prepared by first dissolving the Fe (II) and Fe (III) chloride salts in a dilute acid solution [8]. The precipitation of Fe₃O₄ nanoparticles was then carried out by reduction of this salt solution by sodium hydroxide. The Fe₃O₄ nanoparticles were then oxidized in boiling diluted acid to form the core γ -Fe₂O₃ nanoparticles. Iterative hydroxylamine seeding procedure was followed to coat the nanoparticles with gold. In this method, to form the gold coating, sodium citrate solution was added to a small portion of iron oxide nanoparticles. The solution was diluted and aliquots of HAuCl₄ and excess hydroxylamine were added incrementally. This process was repeated until a uniform coating of gold was achieved on the iron oxide nanoparticles. Only the gold-coated iron oxide nanoparticles were separated by repeated centrifugation and magnetic separation from the solution containing a mixture of unreacted, excess material and gold nanoparticles. The gold-coated iron oxide nanoparticles were characterized by UV-vis absorption spectroscopy and transmission electron microscopy. Samples for TEM were prepared by allowing a drop of the nanoparticle suspension to be air-dried on a carbon-coated copper TEM grid.

Magnetic Susceptibility Measurement

Magnetic susceptibility was measured using Quantum Design MPMS XL-5 SQUID magnetometer both under field cooled and zero field cooled conditions.

Cytotoxicity Studies

The *in vitro* cytotoxicity of the γ -Fe₂O₃ and gold-coated Fe₂O₃ nanoparticles was measured using a commercially available CellTiter 96[®] Aqueous Non-Radioactive Cell Proliferation Assay kit, purchased from Promega (Madison, WI). According to the manufacturer's instructions, the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, (MTS) is bioreduced by viable cells into a formazan product. Approximately 5,000 NIH 3T3 cells, suspended in Dulbecco's minimum essential medium (DMEM) modified with 10% (v/v) fetal bovine serum and other essential nutrients, were seeded per well into 96-well plates and allowed to adhere for 24 hours at 37°C and 5% CO₂ atmosphere. The medium was replaced with graded concentrations of polyethyleneimine (MW ~ 10,000) as a positive control; γ -iron oxide nanoparticles and gold-coated iron oxide nanoparticles in serum free medium (SFM) and incubated for 12 hours. An additional control wells received only SFM. After the incubation time, the cells were washed once with sterile phosphate buffered saline (PBS, pH 7.4), followed by addition of 180 μ l of DMEM per well. The plates were incubated for 6 hours after the addition of 20 μ l of MTS solution to each well and the absorbance of the formed formazan product was read at 490 nm using a microplate reader. The absorbance values were converted to percentage viability against control and reported as mean and standard deviation.

Nuclear Magnetic Resonance Measurement

T1 and T2 relaxation times of the core-shell nanoparticles were measured at 11.7T (1H frequency 500 MHz) using a Varian Unity-INOVA 500 MHz NMR spectrometer. Aqueous dispersions of varying concentrations of gold-coated Fe₂O₃ nanoparticles were analyzed.

Magnetic Resonance Imaging

MRI of gold coated iron oxide samples were carried out in 4.7 Tesla, 40 cm bore actively shielded Bruker Biospec Magnet System. Different concentration of nanoparticles, suspended in water was used for the analysis. Various samples were placed inside sealed vials and introduced simultaneously in the MRI instrument. The signal intensity from each sample was plotted against concentration.

In both the NMR and MRI, the T2 value was converted to relaxivity in s-1/mM Fe using the Fe concentration.

RESULTS AND DISCUSSION

Aqueous dispersions of iron oxide/gold core/shell nanoparticles were synthesized. The iron oxide core, maghemite (γ -Fe₂O₃) was first synthesized by the oxidation of Fe₃O₄ nanoparticles formed by co-precipitation of acidified aqueous solution of Fe(II) and Fe(III) salts in an alkaline medium. The size of the core, as determined by

transmission electron microscopy (TEM) and Coulter particle size measurement is about 10 nm. The gold shell was introduced by the iterative hydroxylamine seeding process. HAuCl₄ was reduced on the surface of Fe₂O₃ nanoparticles in the presence of hydroxylamine to form a uniform coating on the iron oxide core. The average size of

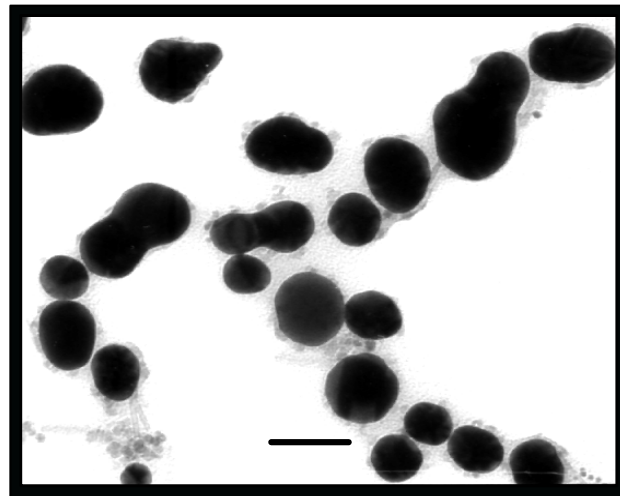


Figure 1. Representative TEM image of gold-coated iron oxide nanoparticles. [Scale bar is 50 nm].

the gold-coated iron oxide nanoparticles, as shown in Figure 1, is about 60-75 nm. The spherical shape suggests uniform coating of gold on individual nanoparticles. Also noticed was the formation of few clusters of coated iron oxide nanoparticles.

Gold nanoparticles, due to collective oscillations of free electrons, have a characteristic absorption peak known as the surface plasmon. The sharp band at 525 nm in the absorption spectrum for solution containing gold nanoparticles corresponds to this surface plasmon. Since iron oxide nanoparticles do not have any observable signal in the UV-vis absorption, the surface plasmon of gold was used to characterize the core-shell morphology. As seen in Figure 2, the gold-coated iron oxide nanoparticles have an absorption band at 532 nm and is close to the 525 nm band of gold nanoparticles. This clearly indicates the uniform coating of gold on the nanoparticles.

The effect of gold coating on iron oxide core was monitored through SQUID magnetometry. The *M vs. T* measurement was carried out for both uncoated as well for coated maghemite nanoparticles. In the case of γ -Fe₂O₃, at lower temperature, the particles are induced by the applied magnetic field to form aggregates. In the case of gold-coated iron oxide nanoparticles, this aggregation is not seen. In addition, the blocking temperature was significantly lowered in the case of gold-coated Fe₂O₃ nanoparticles. These measurements indicate that coated γ -Fe₂O₃ nanoparticles exhibit superparamagnetism above the blocking temperatures.

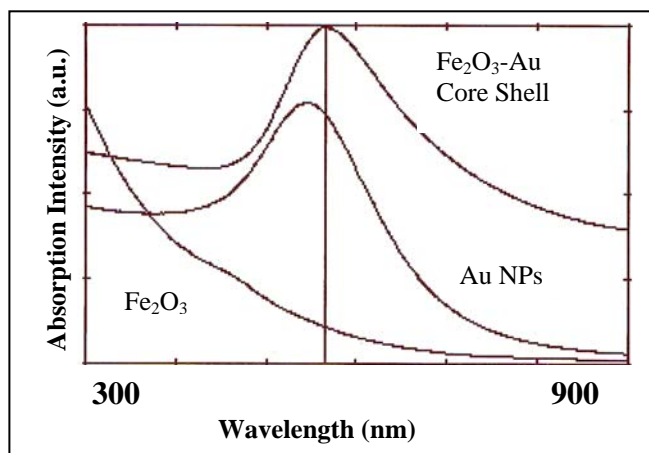


Figure 2. UV-vis absorption spectra of iron oxide core, gold-coated iron oxide and gold nanoparticles.

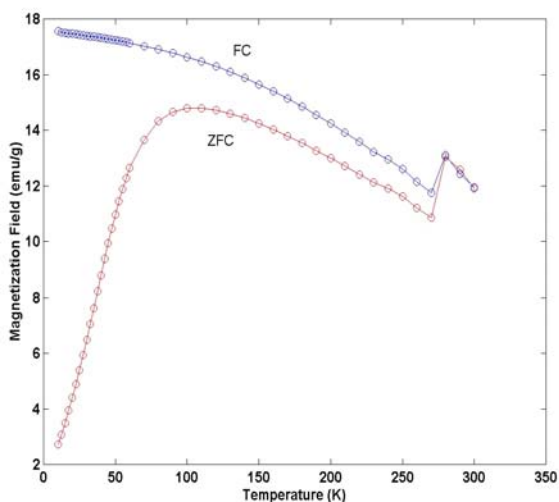


Figure 3. Magnetic moments as a function of temperature for gold-coated iron oxide nanoparticles

One important criteria for use of nanoparticles in biomedicine is their compatibility with cellular material. One method is to evaluate their in-vitro cellular toxicity. Cytotoxicity of nanoparticles was studied by monitoring cell viability and proliferation of NIH 3T3 cell line in the presence of nanoparticles. MTS assay was then performed to calculate percent viability. Percent viability vs. concentration of nanoparticles is plotted in Figure 4 and shows that neither the γ -Fe₂O₃ nor the gold-coated Fe₂O₃ nanoparticles are cytotoxic at the concentrations studied. Cell viability dropped to 65 % in the case of polyethyleneimine, a known cytotoxic compound that was used as reference.

Based on the magnetic moment and non cytotoxic behavior results for gold-coated iron oxide, experiments were done to measure the T2 relaxation in these

nanoparticles using NMR techniques. T2 relaxation shows a linear dependency with increasing concentration of nanoparticles.

Similar results were obtained by MRI studies. There was a linear dependency of magnetization with increasing concentration.

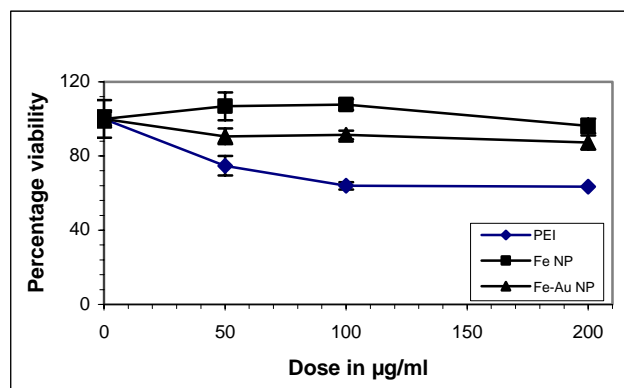


Figure 4. Cytotoxicity analysis for iron oxide core, gold-coated iron oxide nanoparticles using MTS assay in NIH 3T3 cells. Polyethyleneimine (MW 10,000) was used as control.

Preliminary results from NMR and MRI show that there is considerable suppression of T2 relaxation time with increasing concentration of gold-coated iron oxide nanoparticles. There is a linear relation between T2 and concentration as seen in Figure 5. This is consistent with

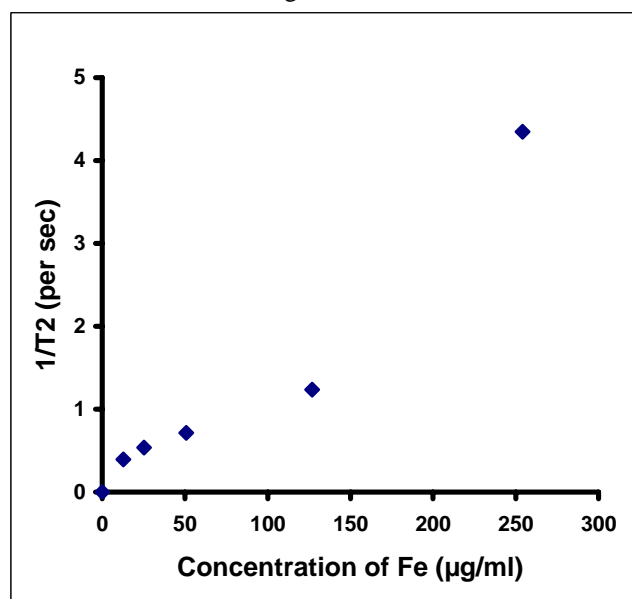


Figure 5. T2 relaxation vs. concentration for gold-coated iron oxide nanoparticles measured by NMR.

the data available in the literature for similar iron oxide nanoparticles. The gold coating on the iron oxide nanoparticles has no effect on its magnetic property. Thus, these results set the path to explore new types of site-specific MRI contrast agents.

CONCLUSIONS

Gold-coated iron oxide nanoparticles were synthesized for use as a MRI contrast enhancing agent. The thin coating of gold does not effect the magnetization moment of the iron oxide core. The gold shell offers a facile method to modify the surface of iron oxide nanoparticles for targeted delivery to specific sites in the body. Non cytotoxic behavior of these core-shell nanoparticles opens the door for a variety of applications in the field of nanobiotechnology.

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