FE2O3/AGI CORE/SHELL NANOPARTICLES FOR DUAL MODAL COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING APPLICATIONS.

Anamaria Orza[1], Xiangyang Tang[1], Yi Yang[1], Hui We[1], Run Lin[1], Liya Wang[1], and Hui Mao[1]
[1]*Radiology and Imaging Sciences, Emory University, Atlanta, Georgia, United States*

PURPOSE

Nanomaterials are widely used for developing tumor targeted molecular imaging probes, such as magnetic resonance imaging (MRI),[1] computed tomography (CT),[2] fluorescence imaging,[3] and photoacoustic tomography (PAT)[4], and image-guided drug delivery systems has been reported. Furthermore, multimodal imaging probes combining the strengths from different methods, such as CT which has high spatial and temporal resolution but poor soft-tissue contrast and MRI which offers excellent soft-tissue contrast. Therefore, a targeted multimodal contrast agent with complementary diagnostic information is highly desirable. This work reports the development of a facile preparation of a new MRI/CT dual modality targeted contrast agent made of AgI/Fe_2O_3 core/shell nanoparticle dimmer (Fe_2O_3-AgI). The developed Fe-AgI dimmer enables excellent biomarker targeted molecular imaging with high contrast enhancement in both MRI and CT.

MATERIALS AND METHODS:

Synthesis of Fe_2O_3/AgI Core/Shell Dimmers (Fe_2O_3-AgI): A two-step seed mediated synthesis was used as shown in the ***Scheme. 1.*** Briefly, onto the seed solution of 10 nm water soluble **Fe_2O_3** nanoparticles (IONPs), 8 ml ascorbic acid AA (100 mM) was added under vigorous stirring. Further, 50 ml KI (100 mM) and $AgNO_3$ (100 mM) solution was prepared and slowly added onto the seeds at a rate of 0.75 ml of solution per minute. A color change from dark-brown to light-brown was observed. The resulting particles were purified by using a SuperMag Separator, washed three times with DI H_2O and re-dispersed in deionized water. The morphology, size and composition of the Fe_2O_3/AgI Core/Shell dimmers were studied using transmission electron microscope (TEM, HitachiH-7500 accelerating voltage 75 kV), HR-TEM (Jeol), STEM and EDX.

Preparation of Antifouling polymer protected Fe_2O_3/AgI Core/Shell Dimmers (Fe_2O_3-AgI): In order to apply an amphylilic coating on the surface of Fe_2O_3-AgI, the nanoparticles were transferred from water solution in THF by using a thioleted styrene linker. Then, the poly ethylene glycol (PEG)-*b*-poly allyl glycidyl ether (PAGE) copolymer, synthesized as we previously reported,[5] was applied to replace the hydrophobic styrene molecules from Fe_2O_3-AgI surface. Briefly, a 5 mg/ml styrene coated Fe-AgI a PEG-*b*-AGE solution in THF (18 mL, 5 mg/mL) was added and stirred for 24 hours at room temperature and then it was added drop-wise in 200 ml DI water. This aqua's mixture was dialyzed against water for 48 hours in order to remove the THF and the unreacted polymer molecules, and then was further centrifuged at 3000 rpm for 5 min. The resulted supernatant contains the PEG-*b*-AGE coated Fe_2O_3-AgI with –NH_2 groups available for further conjugation of targeting ligands.

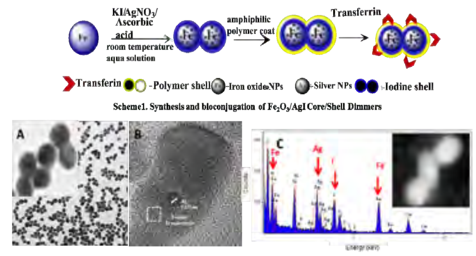
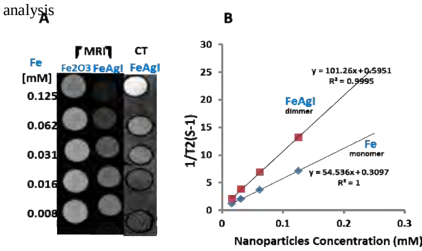


Figure 1. Schematically illustrate the AgI/Fe_2O_3 core/shell dimers synthesis and conjugation along with the electron microscopy images such as (A) TEM image of the AgI/Fe_2O_3 core/shell dimmers (B) HR-TEM Fe-AgI dimemrs (C) STEM and EDX analysis

Conjugation of Targeting Ligand Transferrin (Tf) on Fe_2O_3/AgI Core/Shell Dimmers (Tf-Fe_2O_3-AgI): A solution of 2 mg/mL Fe_2O_3-AgI dimmers in PBS was incubated with a linker, Sulfo-SMCC, at molar ratio of 1:4000 for one hour. In order to remove the unreacted linker and the free nanoparticles the solution was purified using a PD-10 column. On the other hand, transferrin (Tf) was supported to a thiotion reaction by mixing it overnight with Traut's Reagent (in a molar ratio of 1:15) in 0.1 M borate buffer, pH8.5, and then was purified by using a desalting spin column. Further, the Sulfo-SMCC-IONP and Thioleted-Tf were then mixed in PBS, and incubated at room temperature for four hours. Tf conjugated Fe_2O_3-AgI dimmers (Tf-Fe_2O_3-AgI) were purified by using both differential centrifugation and magnetic separation. The number of Tf conjugated to the Fe_2O_3-AgI dimmers was determined by using BCA protein assay and the Fe contained quantified using 1,10-phenanthroline colorimetric method.



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Probe [Mm]	Fe-AgI 0.125	Fe-AgI 0.062	Fe-AgI 0.031	Fe-AgI 0.016	Fe-AgI 0.008	SHP1 0 0.125
Contrast (HU)	506.6	231.1	170.1	87.6	18.0	0

Figure 3. CT, MRI images and quantitative measurements: (A) CT and MRI images of the solutions corresponding to various concentrations, (B) transverse relaxation rates ($1/T_2$, s^{-1}) of Fe_2O_3-AgI dimmers and Fe monomer as a function of the Fe concentration (mM). (C) CT number of the solutions corresponding to various concentrations

Cytotoxicity and Targeting capability Evaluation The cytotoxicity of the Fe_2O_3-AgI dimmers were examined using D556 meduloblastoma cell line. The cells seeded at a confluence of 80%in a96-well plate with were treated with different concentrations of dimmers 24 hours and 48h. After the incubation, the solutions were removed and cells were washed three times with PBS. Cell viability was then estimated using the MTT conversion test. For testing the targeting capability, 80 % confluent D556 meduloblastoma cells, which over express Tf receptors were cultured in 8-well chamber slide and incubated with 0.2 mg/mL, then were used to examine the targeting of Tf-Fe_2O_3-AgI dimmers and non-targeted Fe_2O_3-AgI dimmers (as a control). After incubation at 37 ^oC or 4 ^oC, the cells incubated at 37 ^oC were then rinsed by PBS for three times, and then fixed with 4% paraformaldehyde, followed by Prussian blue staining for iron, and counterstaining using nuclear fast red solution. The cells incubated at the cells incubated at 4°C were exposed to an antifade medium containing 4,6-diamidino-2-phenylindole (DAPI) in order to observe nuclei. The slides were examined using an inverted phase microscope with filters 488, 546, and 340/360 nm.

Measurement of Relaxivities*:* The transverse relaxation time (T_2) and relaxivity (r_2) were determined using a 3 Tesla MR scanner (Tim/Trio, Siemens, Erlangen, Germany). Solutions of Fe_2O_3/AgI core/shell dimmers of different iron concentrations ranging from 0.0045 to 0.0700 mM were used. A multi-echo spin echo (SE) sequence was performed with TR of 2520 ms and 20 TEs, starting at 12.2 ms with increments of 12.2 ms. A mean signal intensity values from all ROIs was calculated using ImageJ (National Institutes of Health, Bethesda, USA). The transverse relaxation time T_2 was determined by fitting the MRI signal intensities at different echo times (n = 20) exponentially. The transverse relaxivity (r_2) was calculated from the slopes of the linear correlation between the relaxation rates ($1/T_2$) at different iron concentrations.

Computed Tomography Imaging*:* Solutions of **Fe_2O_3/AgI Core/Shell Dimmers** at iron concentration from 0.008 to 0.125 mM are put into PCR tubes, which are installed in 50 ml lab tube filled with water, for micro-CT scanning at peak voltage 45keV. The micro-CT is consisted of a micro-focus (12 μm) x-ray tube and a flat panel x-ray detector at 48×48 μm^2 detector cell size. Tomographic images of the samples are reconstructed at 0.192×0.192 mm^2 pixel size, corresponding to a spatial resolution of 2.6 lp/mm. The CT contrast (i.e., CT number in Hounsfield Unit) of each **Fe-AgI** solution is defined as Contrast = $mean_{target}$ – $mean_{background}$, where $mean_{target}$ and $mean_{background}$ are the averaged CT number gauged in the ROI placed in the target and background (water) areas, respectively,kV)).

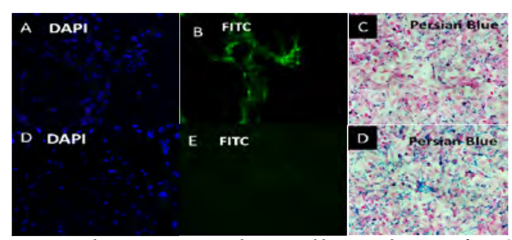

Figure 2. Florescence images and Prussian blue stained images of D556 medulloblastoma cell incubated with: *upper panel*-Tf-Fe_2O_3-AgI dimmers such as: (A) DAPI staining and (B) FITC staining at 4 °C for two hours and (C) Persian Blue staining at 37 °C for three; L*ower panel*- non-targeted antifouling Fe- cells such as: (D) DAPI staining (E) FITC staining at 4 °C for two hours and (F) Persian Blue staining at 37 °C. The Fe concentration was 0.2 mg/mL for both 37 °C and 4°C experiments. Scale bar, 20 μm.

RESULTS AND DISCUSSIONS

Transmition Electron Mycroscopy showed synthesized Fe_2O_3-AgI core/shell nanoconstruct has a dimmer like structure (**Figure 1. A, B**) and a hybrid composition such as Fe_2O_3 core covered by a layer of silver iodine composite (**Figure 1 C**). The size of individual components and overall morphology of the prepared nanoparticle determined by TEM and DSL analysis showed a 10 nm Fe_2O_3 core and 4 nm smooth composite AgI shell. The polymer coated Fe_2O_3-AgI core/shell nanoconstructs have a high stability in physiological condition and exhibit antifouling properties with –NH_2 functional groups that were able to conjugate targeting ligand of Tf. Tf- Fe_2O_3-AgI dimmers exhibited strong targeting effect to that over expressing Tf receptors as shown in florescent images (**Figure 2** A-B, D-E) and Persian blue iron staining (**Figure 2 C-F**). At 4°C, it can be clearly seen that the internalization of Tf-Fe_2O_3-AgI dimmers was higher compared with non-targeted Fe_2O_3-AgI dimmers. Since the endocytosis is blocked at this temperature, Tf- Fe_2O_3-AgI dimmers are likely internalized into the cells through transferrin receptor. The MRI and CT scans of the solution phantom made of Fe_2O_3-AgI dimmers at iron concentration from 0.008 to 0.125 mM showed dual modal CT/MRI imaging capability of Fe_2O_3-AgI dimmers with T2 weighted contrast and enhanced CT signals comparing to the water (**Figure 3A**). Furthermore, the transverse relaxivity (r_2) of the Fe_2O_3-AgI dimmer was determined at 108 $mM^{-1}s^{-1}$, comparing to 54 $mM^{-1}s^{-1}$ for the IONP monomer used as the core (**Figure 3B**), while their CT number against the background (water) are listed in the table presented in **Figure. 3C**. It is observed that the CT number corresponding to the lowest concentration (0.008 mM) is 18 HU, while that to the highest (0.125 mM) is 506.8 HU. Note that a contrast of approximately 10 CT number is the contrast lelve sufficient to make diagnostic decision in the clinic.

CONCLUSION

We have developed a novel core/shell Fe_2O_3-AgI dimmer with high CT and MRI contrast enhancement and biomarker targeting capability, and potential for targeted dual modal CT and MRI applications.

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