Synthesis and characterization of multi-layered hybrid gold-iron oxide superparamagnetic nanoparticles.

Alexei Bogdanov, Jr[1], Mohammed S Shazeeb[1], Lejie Zhang[2], and Surong Zhang[1]

[1]*Radiology, UMASS Medical School, Worcester, MA, United States,* [2]*Biochemical Pharmacology, UMASS Medical School, Worcester, MA, United States*

Target audience: Physicians and researchers that are interested in molecular imaging, contrast agents, and MR cell tracking.

Purpose: to develop a synthesis of a convenient hybrid nanoparticle synthesis route to achieve a goal of combining the properties of a) superparamagnetic iron oxide for MRI tracking; and b) gold nanoparticle for photoacoustic imaging and as a platform for bioconjugation.

Methods: The synthesis of gold-iron oxide nanoparticles was accomplished in water milieu. First, we treated ultrasmall dextran T10-stabilized iron oxides (30 mM iron) in the presence of Tollens regent (50 mM $Ag(NH_3)_2NO_3$), which induced the formation of the nanocolloidal silver layer on the surface of iron oxide particles at 60°C, pH 9. We isolated silver-modified IO using size-exclusion chromatography on Sephadex G25m. Hybrid gold-iron oxide nanoparticles (IO-Ag-Au NPs) were obtained after incubating the purified IO-Ag nanoparticles in diluted solutions of tetrachloroauric acid (3 µM $HAu(III)Cl_4$) in the presence of 10 mM sodium citrate as buffering and capping agent for 16 h at RT, pH 5.5 resulting in the change of the color of the solution to bright-red. The hybrid nanoparticles were purified using density ultracentrifugation using a step of 7.5% iodixanol IO-Ag-Au nanoparticles were stabilized using stable binding of MPEG-gPLL (MPEG5000/poly-l-lysine graft copolymer) followed by purification on 100 kD cut-off spin-concentrators. The obtained nanoparticles were analyzed using transmission and scanning electron microscopy and diluted in water prior to 3T MR imaging. The samples were imaged using T2w SE pulse sequence and the data was used for T2-mapping of he sample images.

Results: The synthesis was accomplished in water milieu without the need of hydrophobic adaptor molecules and similar intermediates 1,2. We used an advantage of the presence of multiple silver-reducing aldehydes that are available on the surface of freshly-prepared dextran-stabilized ultra small iron oxide nanoparticles to induce formation of the nanocolloidal silver layer after treating of 20.5±4.6 nm dextran-stabilized iron oxides with an excess of Tollens reagent (diamminesilver(I) nitrate). After size-exclusion chromatography we isolated 34.0±9.5 nm silver-containing nanoparticles with unimodal size distribution and characteristic absorbance peak at 405 nm (Fig. 2) that showed increased scattering of X-rays upon examining with a back-scattered electron detector. Incubation of these silver-tagged nanoparticles in diluted solutions of tetrachloroauric acid in the presence of citrate as buffering and capping agent resulted in the formation of small, electron dense single-core negatively charged nanoparticles (30.9±9.6 nm, zeta potential – 19 mV), Fig 2 that had further increased electron back-scatter. These nanoparticles could be additionally purified using density gradient ultracentrifugation that resulted in the separation of iron oxides that were not associated with dense gold nanoparticles. The hybrid gold-iron oxide nanoparticles (IO-Ag-Au NP) were stabilized using stable absorption of PEGylated poly amino acid MPEG-gPLL (graft copolymer of MPEG5000 and poly-l-lysine) which resulted in stable, weakly positively charged NPs that did not aggregate in the presence of phosphate-buffered solutions. The MPEG-gPLL- stabilized IO-Ag-Au NPs had an average hydrodynamic diameter of 65.7 and were weakly negatively charged (zeta potential – 1.1 mV) and showed a strong increase of iron molar transverse relaxivity (r2) – from 52.6 to 165 $[mMs]^{-1}$ at the field strength of 0.47T. While r2 of initial iron oxide nanoparticles changed little at 3T, the changes were dramatic with a 12-times increase of r2 for IO-Ag-Au NPs allowing the detection of nanoparticles at low concentrations of iron (Figure 3). The IO-Ag-Au NPs showed binding capacity for short oligonucleotide duplexes at the level of 0.3-0.6 µg (16-33 nmol)/µg NP that suggests their potential use for oligonucleotide delivery combined with imaging of their biodistribution. In addition, we explored the conjugation of targeting moiety, i.e. anti-EGFR $F(ab')_2$, to the termini of long-chain PEG residues using FMOC-protected linkers.

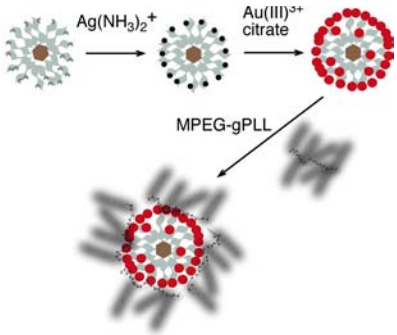

Fig.1. A schema of IO-Ag-Au nanoparticle synthesis and stabilization in the presence of MPEG-gPLL copolymer.

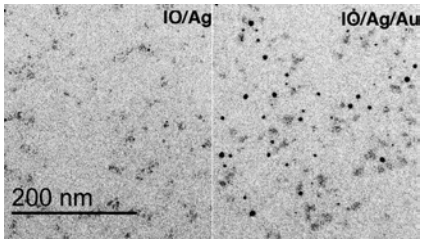

Fig. 2. Transmission electron microscopy of IO-Ag and IO-Ag-Au (magnification – 140Kx) shows the presence of the spherical electron-dense nanoparticles (diameter 6.8-9.6 nm) only after treating with $HAuCl_4$ solution.

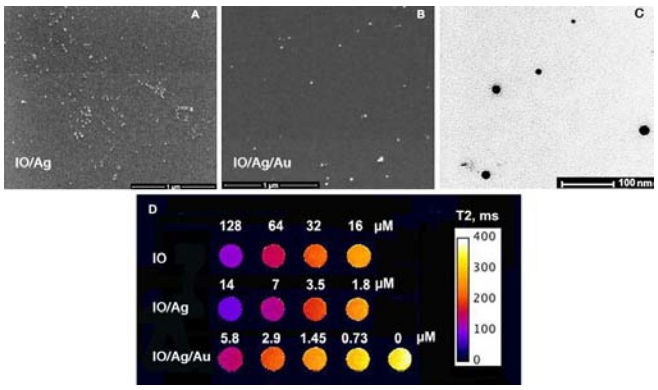

Fig 3. A- scanning electron microscopy of silver-treated IO (IO-Ag) and B- hybrid gold nanoparticle-iron oxide (IO-Ag-Au) (magnification – 50Kx) that shows increasing X-ray backscattering efficiency in the latter sample compared to IO/Ag indicating the presence of colloidal gold. C- TEM of gradient-purified IO-Ag-Au. D- A pseudo-color T2 map of IO, IO-Ag and IO-Ag-Au samples at various concentrations of iron showing that IO-Ag-Au NP could be detected at lower concentration of iron than the precursor NP that did not contain colloidal gold.

Discussion: The major applications of stabilized colloidal iron oxides include MR imaging of lymph nodes and cell labeling in cell trafficking after transplantation. The technology of incorporating iron oxides into gold nanoparticles or gold shells is of great interest due to the potential of combining magnetic, optical and surface plasmon resonance properties in a single nanoparticle entity. Such composite nanoparticles, due to feasibility of linking of targeting molecules (a consequence of large surface energy of colloidal gold) are expected to enable several future diagnostic and theranostic applications including multimodality photoacoustic and magnetic resonance imaging. We devised and tested an approach that included a greatly simplified synthesis of small gold nanoparticles with very high molar relaxivity enabling lowering the detection limit of iron oxides approximately by a factor of 10. Surface coating of gold nanoparticles with PEGylated poly amino acids resulted in a formation of protective barrier that can be further utilized for linking targeting molecules and by non-covalent loading with oligonucleotides and other polyanions. **Conclusion**: hybrid gold- dextran-stabilized iron oxide nanoparticles were obtained via a facile water-based synthesis using an accessory silver layer prior to depositing a surface layer of gold. The obtained particles had high molar relaxivity and were stabilized for better biocompatibility.

References: 1. Lim JK Majetich SA, Tilton RD. Stabilization of superparamagnetic iron oxide core-gold shell nanoparticles in high ionic strength media. Langmuir 2009, 25(23), 13384–13393. 2. Jin X, Liang J, Yang C et al. Facile deposition of continuous gold shells on Tween-20 modified Fe_3O_4 superparticles. J. Mater. Chem. B, 2013, 1, 1921–1925