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Synthesis and testing of modular dual-modality nanoparticles for magnetic resonance and multi-spectral photoacoustic imaging

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Abstract

Magnetic resonance (MR) and photoacoustic (PA) imaging are being currently investigated as complementing strategies for applications requiring sensitive detection of cells *in vivo*. While combined MR/PAI detection of cells requires biocompatible cell labeling probes, water-based synthesis of dual-modality MR/PAI probes presents significant technical challenges. Here we describe facile synthesis and characterization of hybrid modular dextran-stabilized gold/iron oxide (Au-IO) multimetallic nanoparticles (NP) enabling multimodal imaging of cells. The stable association between the IO and gold NP was achieved by priming the surface of dextran-coated IO with silver NP resulting from silver (I) reduction by aldehyde groups, which are naturally present within the dextran coating of IO at the level of 19-23 groups/particle. The Au-IO NP formed in the presence of silver-primed Au-IO were stabilized by using partially thiolated MPEG5-gPLL graft copolymer carrying residual amino groups. This stabilizer served as a carrier of near-infrared fluorophores (e.g. IRDye 800RS) for multispectral PA imaging. Dual modality imaging experiments performed in capillary phantoms of purified Au-IO-800RS NPs showed that these NPs were detectable using 3T MRI at a concentration of 25 μ M iron. PA imaging achieved approximately 2.5-times higher detection sensitivity due to strong PA signal emissions at 530 and 770 nm, corresponding to gold plasmons and IRDye integrated into the coating of the hybrid NPs, respectively, with no “bleaching” of PA signal. MDA-MB-231 cells pre-labeled with Au-IO-800RS retained plasma membrane integrity and were detectable by using both MR and dual-wavelength PA at 49 ± 3 cells/imaging voxel. We believe that modular assembly of multi-metallic NPs shows promise for imaging analysis of engineered cells and tissues with high resolution and sensitivity.

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INTRODUCTION

Ongoing progress in the development of 3D-bioprinting technologies intended for organ/tissue engineering applications will fuel the need for new patterned complex biomaterials that can be seeded with human cells^{1, 2}. Assessing the viability and usefulness of these cell-seeded biomaterials will require the use of imaging modalities and specialized imaging probes capable of providing detailed information about the 3-D spatial distribution and molecular characteristics of the cells within engineered tissue matrix^{3, 4}. Thus, an ideal imaging strategy should allow the assessment of cell distribution in any given volume and throughout the depth of the engineered material and would be safe enough to allow repeated imaging / longitudinal monitoring of the above parameters at high resolution. These prerequisites essentially preclude the use of high-energy ionizing radiation (such as PET/CT). Hybrid photoacoustic (PA)/Magnetic Resonance Imaging (MRI) affords both unlimited penetration depth (e.g. MRI), and superior sensitivity (in picomolar range of particle concentration) with high spatial and temporal resolution (PA)⁵. PA imaging relies on the detection of light-absorbing particles that produce photoacoustic emissions upon irradiation with light pulses^{6, 7}. In biological tissue, the sensitivity of PA can be increased with use of probes that absorb in the near-infrared (NIR) range since in mammals fewer endogenous molecules absorb light in the NIR range than in visible range. In addition, PA imaging is well suited for imaging superficial tissue layers that can sometimes cause artifacts in MR images. The diagnostic usefulness of combined PA/MRI is being investigated and results suggest that it may offer unique signatures of specific pathologies^{8–10} and may also be useful as a platform for the development of other novel imaging strategies¹¹. Unfortunately, the sensitivity of PA lessens as the depth of the particles in the tissue increases, thus necessitating an alternate imaging modality with higher deep tissue contrast such as MRI. Towards the goal of producing dual MR/PA imaging probes, attempts to use monometallic iron oxide (Resovist) as a PA contrast agent have been made. Results showed that PA imaging was successful with contrast-to-background ratios of 15.7 dB, however, the concentration of iron needed to achieve this sensitivity was approximately 12 mM, i.e. a concentration not suitable for imaging in living tissue¹². As an alternative, hybrid multimetallic nanoparticles such as iron oxide (IO)-core based gold (Au) or cobalt nanoparticles (NP) have been previously explored for development of dual PA/MRI probes^{5, 13, 14}. However, water-based synthesis strategy of NP synthesis that would enable facile modification of NPs for multi-spectral detection with PA and MRI is not available. Here we report the strategy of modular iron oxide (IO)/gold nanoparticle (Au-IO-NP, Fig.1) synthesis and their initial characterization with an emphasis on multispectral PA imaging.

RESULTS AND DISCUSSION

Core-to-shell synthesis of IO-based contrast agents for dual modality PA/MR imaging is technically challenging and requires one to either deposit thick layers of gold on the surface of IO, or alternatively to synthesize gold shells separated by a gap between the IO core and gold shell¹⁵. In addition, since particle separation by the diamagnetic shells limit magnetic susceptibility of IO cores and susceptibility effects contribute significantly to the transverse relaxivity of protons (r_2) caused by the presence of superparamagnetic iron, core-to-shell synthesis may result in particles that produce less sensitive T2-weighted MR images.

A modular design approach to the synthesis of multi-functional NPs appeared to be less intuitive than core-to-shell design approach^{16–18} due to potential difficulties in controlling the resultant particle size. However, modular NP design may result in greater clustering of IO on gold NP thus increasing the magnitude of T2-weighted MR signal due to r_2 increase^{19, 20}. With this in mind we designed hybrid IO- gold NPs that are held together via anchoring provided by silver NPs deposited using Ag (I) reduction by aldehydes which are present in the carbohydrate coating of a typical stabilized USPIO. We then tested the obtained hybrid NPs for multiplexed PA that was enabled by linking NIR dyes, i.e. organic fluorophores with very high extinction coefficients, to the stabilizing layer of a biocompatible copolymer on the surface of hybrid NPs.

Hybrid nanoparticle synthesis

The synthesis of NPs was made feasible due to silver-reducing aldehydes available on the surface of dextran-stabilized ultra-small IO-NP as anchoring groups for silver nanocolloids. First, we determined the concentration of aldehydes using AF568H fluorescence measurements after linking to IO NPs. We assumed that fluorescence of AF568 fluorochrome is not sensitive to the presence of IO conjugated via dextran since in our experiments dextran-coated IO did not exhibit any concentration-dependent quenching effect on AF568H fluorescence. We determined that 19–23 aldehyde groups were present on the coating layer of an IO particle assuming that each particle has a core with an average diameter of 4.6 nm and 25 dextran molecules on its surface^{21, 22}. The formation of a layer of silver nanoparticles on the surface of IO was induced by a short incubation of IO-NPs with diamminesilver(I) nitrate solution at 75°C followed by a purification of obtained silver-coated IO-NPs (Ag-IO, Fig. 1 step 1). The obtained NPs showed a characteristic absorbance peak at 405 nm that was not present in IO NP preparations (Fig. 2A) and were stable during the storage in the absence of chloride anions. The purified Ag-IO contained nearly equal amounts of silver and iron by weight (i.e. the resultant molar ratio Fe/Ag was 2:1, Table 1).

Accordingly, scanning electron microscopy (SEM) of Ag-IO samples revealed the presence of particles that had a much stronger back-scattering signal than the initial IO and particle size distribution consistent with typical SEM appearance of similar dextran-coated IO NP²³ (Fig. 3A,B). If desired, purified Ag-IO NPs could be stabilized by treating NPs with an excess of MPEG5000/poly-l-lysine graft copolymer (MPEG5-gPLL) resulting in non-covalent adsorption of a stabilizing layer of copolymer on the surface of NPs, which caused a neutralization of the negative surface charge of Ag-IO (Table 1). Stabilized NPs (Ag-IOs) could be separated from MPEG5-gPLL by using density step-gradient ultracentrifugation. Although the Z_{av} value of purified MPEG5-gPLL stabilized Ag-IOs was lower than average diameters of the initial non-stabilized Ag-IO, the 2.5-fold increase of numerical average diameters and the concomitant increase of r_2 (Table 1) both point to NPs that were comprised of micro-aggregated Ag-IOs. Such microaggregation could have been induced by precipitation during the ultracentrifugation and was evident on TEM images (Fig 4, A).

The gel permeation chromatography-purified silver-primed, non-stabilized Ag-IO were further used as seeds for AuNP synthesis by incubating them in the presence of diluted tetrachloroauric acid (3 μ M) and various concentrations of trisodium citrate (3–6 mM) at pH

4.5–6.0 at the constant Fe: Au molar ratio of 200:1 (Fig. 1, step 2). After 24 h incubation at RT, we observed a color change of the solution to bright-red indicating the formation of small AuNPs. A control experiment that involved incubating IO NPs that were not pre-treated with diamminesilver nitrate with citrate and tetrachloroauric acid at the same ratio of Fe: Au resulted in large gold particles that did not form stable colloidal solutions, suggesting that Ag-IO NPs were efficiently limiting the formation of large gold NPs. Unlike Au-IO2 that were formed at higher concentrations of citrate, Au-IO1 NPs that formed at lower citrate concentrations and lower pH of 4.5 had smaller mean hydrodynamic diameters, higher iron content and higher transverse relaxivity (r_1) at 0.47 T (Table 1). Au-IO1 also showed higher r_1 and r_2 at 3T (supporting Figure S1). The broadening of AuNP plasmon peak in the case of Au-IO2 also pointed to the presence of larger gold NPs in Au-IO2 compared to Au-IO1 (Fig. 2B). The broader and red-shifted absorbance band may also have been a result of interparticle plasmon coupling that occurs between larger particles due to particle clustering²⁴. Measurements of dynamic light scattering (Table 1) indicate the possible occurrence of sub-micro clusters of Au-IO2 particles in solution. The results of transmission electron microscopy also showed that purified stabilized Au-IO (Fig. 4C) did not contain free (i.e. not associated with highly electron dense gold cores) silver-primed IOs that were present in NP solutions in large numbers before the ultracentrifugation (Fig. 4 A–B). Unlike silver nanoparticle-primed IO, Au-IO exhibited very strong back-scattering of electrons on SEM (Fig. 3C) confirming the presence of gold nanoparticles. Transmission EM of ultracentrifugation-purified MPEG5-gPLL-stabilized Au-IO demonstrated the absence of large aggregates of gold cores although nano-assemblies of gold NPs, which were surrounded by nets of smaller and less dense IO NPs (Fig. 4C).

To stabilize Au-IO NPs against aggregation and to simultaneously provide functionalization of NPs with amine groups we linked the third modular component, i.e. MPEG5-gPLL to NP surface. This component functioned primarily as a carrier of NIR dye for multispectral PA. The cooperative binding of amino groups of MPEG5-gPLL to gold surfaces is usually sufficient for AuNP stabilization and results in charge-neutral amine/gold surface interactions comparable to weak covalent bonds^{3, 25}. However, to further improve the stability of MPEG5-gPLL-coated Au-IO, we thiolated MPEG5-gPLL with 2-iminothiolane to convert approximately 10% of PLL amino groups into mercaptobutyrimidates. This enabled stable bonding with gold NPs after a simple co-incubation of diluted Au-IO with thiolated MPEG5-gPLL. Removal of excess free MPEG5-gPLL and IO that were not incorporated into the hybrid NPs was facilitated by IO's lower density during the ultracentrifugal rate separation procedure²⁶ (see supporting figure Fig. S2). To covalently conjugate NIR fluorophores Au-IO NPs were recovered in 50% cushion of Iodixanol density centrifugation medium and treated with NHS esters of IRDye 800RS or QC1 quencher that resulted in dye conjugation to the NPs as evidenced by recording the absorbance spectra of the NPs (Fig. 1B).

Dual modality imaging of Au-IO-based NPs

The investigation of imaging properties of the obtained fluorophore-modified and stabilized Au-IO1 NPs was accomplished by assembling phantoms that consisted of water-immersed 2 mm (1.9 mm ID) sealed plastic capillaries that were filled with degassed solutions of NPs

prior to dual MRI/PA imaging. Initially, T1 and T2 maps were acquired using a combined spin-echo and inversion recovery (IR) pulse sequence at 3T. By using solenoid RF coils and small field-of-view/thin imaging slice of 1 mm we succeeded in acquiring images of Au-IO1 NPs (50 μ M iron), with the dilution of NPs resulting in a loss of specific MR signal intensity due to the noise. The same phantoms were then exposed to dual 530/770 nm laser irradiation (i.e. multi-spectral PA) and images were reconstructed from the photoacoustic emissions (Fig. 5). PA imaging showed a characteristic photoacoustic signature of cylindrical phantoms, i.e. double PA signals corresponded to imaging of a homogeneous cylindrical absorber²⁷. PA signals of gold NPs recorded at 530 nm were similar in all three samples, while 770 nm signal was specific for both IRDye 800RS and QC1. The weaker PA signal in the case of Au-IO conjugated QC1 quencher a consequence of a 2-times lower extinction coefficient and blue-shifted absorbance peak of QC1 as compared to IRDye 800RS fluorophore. The limit of NP detection using PA was lower than that of T2-w 3T MRI and was estimated as 10 μ M Fe when using 1 mJ/cm² excitation fluence. Note that 20 mJ/cm² is the permissible laser skin-exposure limit²⁸ so PA imaging at higher fluence should potentially achieve sub-micromolar detection sensitivity for these particles.

Both types of Au-IO modified with IRDye 800RS (“smaller” Au-IO1 and “larger” Au-IO2), as well as non-modified MPEG5-gPLL stabilized Au-IO2 (which showed relatively high absorbance at 750–770 nm) were compared in a phantom imaging experiment at the same 770 nm absorbance to compare their relative strength as multimodality imaging agents (Fig. 6). The dye linked to MPEG5-gPLL in the absence of Au-IO showed negligible contribution to absorbance in the 530 nm channel, resulting in nearly absent PA signal. Imaging showed similar PA signal generated by either Au-IO1-800 or Au-IO2-800 after irradiating at 530 nm, while PA emission at 770 nm was higher for Au-IO2-800 due to higher IRDye 800RS content. Due to high iron content that was approximately 3-times higher in Au-IO1 than in Au-IO2 a shorter transverse relaxation time (and consequently, a lower T2-weighted imaging signal) was measured by 3T MRI (Fig. 5). Although Au-IO2 absorbed light at 770 nm in the absence of NIR fluorophore only a very weak PA signal was detected at 770 nm, which may result from interparticle plasmon coupling between neighboring nanoparticles²⁴.

It should be noted that we observed no acoustic “bleaching” of signal produced with irradiation at 530–550 nm. This suggests, as expected, that Au-IO that contain solid gold NPs were not collapsing into smaller NPs as happens in the case of gold nanoparticles with high aspect ratios, such as gold nanorods, nanocages, or nanoshells²⁹.

Cellular uptake of multifunctional Au-IO nanoparticles

Cell uptake and imaging detection sensitivity were tested initially by incubating two types of Au-IO-800 (Table 1) at various concentrations with MDA-MB-231 human mammary adenocarcinoma cells (MAC). Non-spherical NPs are known to exhibit toxicity in cell culture and this effect strongly depends on particle coating with various stabilizing components³⁰. Therefore, we performed Au-IO NP toxicity testing by following LDH release from normal epithelial cells, as well as MAC. We demonstrated that Au-IO-800RS nanoparticles did not induce appreciable plasma membrane integrity loss in both normal epithelial MCF10A and MAC at concentrations of iron below 100 μ M and that both Au-

IO1-800 and Au-IO2-800 appeared marginally more toxic to MAC than to normal cells. In the past we observed a similar effect when toxicity of MPEG5-gPLL-stabilized AuNP was tested in normal and cancer cells³. Larger Au-IO2-800 nanoparticles were less toxic, on the average, than their smaller Au-IO1 counterparts. Analysis of imaging results obtained after incubating cells with two types of Au-IO-800 at various concentrations of iron showed that IRDye 800RS fluorescence intensities were higher and concentration-dependent in the case of smaller Au-IO1-800 NPs. This suggested that IRDye 800RS fluorophores linked to Au-IO1 NPs could be easily cleaved-off and de-quenched after the uptake in cells resulting in fluorescence. In contrast, Au-IO2-800 NPs resulted in lower fluorescence intensity but a strong photoacoustic signal since then number of NIR dye per NP was higher in the case of Au-IO2-800 (Table 1) and PA emission correlates with absorbance of the dye and not with fluorescence intensity (Fig. 7). Cell phantoms by using PA at 530 and 770 nm showed that highly-absorbing larger Au-IO2-800 NPs resulted in a higher intensity PA signal and allowed imaging of 49 ± 3 cells/imaging voxel (0.034 mm^3). MR imaging enabled detection of approximately the same number of cells at the same concentration within similar total imaging volume. These results point to the ability of both imaging modalities to detect approximately the same number of cells labeled with hybrid nanoparticles and at the same time suggest the need of optimizing of modular structure of multifunctional nanoparticles.

Conclusions

We developed and tested a novel modular design for converting iron oxides into dual MRI/PA imaging probes by using Ag-IO as seeds for growing gold nanoparticles by using citrate-mediated reduction. Stabilization with MPEGylated polylysine enabled linking near-infrared dyes to the obtained hybrid Au-IO NPs. The dual modality NPs produced strong PA signals if irradiated in the visible and near-infrared regions of light and were detectable by MR imaging at 3T. Active internalization by cells results in strong PA and MRI signals with minimal acute toxic effects on cells suggesting the potential use of these newly synthesized compounds as cell-labeling imaging agents for multimodality tracking in various tissue engineering applications.

EXPERIMENTAL PROCEDURES

Iron oxide

DextranT10-stabilized iron oxides (IO) were synthesized as described in³¹, purified using ultrafiltration on UFP300 cartridges (GE-Healthcare Life Sciences), and stored in 10 mM trisodium citrate, pH 8. The particles were desalted by passage through water-equilibrated columns (PD-10, GE Biosciences). To determine the number of aldehyde groups on nanoparticles they were diluted to 25 mM [Fe] and 50 μl of IO were reacted with 0.5 mg/ml AlexaFluor568 hydrazide (AF568H final conc.) in water for 24h. After separating IO from non-reacted AF568H on Bio-Spin P-30 mini-columns (Bio-Rad) the fluorescence of IO-linked AF568H was measured at λ_{ex} 560 nm/ λ_{em} 600 nm. The number of aldehyde groups per particle was estimated by using the calibration curve of fluorescence intensity and AF568H concentration. Iron concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS).

Dual-modality particle synthesis

IO (0.1 ml, 235–240 mM Fe in water) was combined with 0.9 ml of 50 mM diamminesilver(I) nitrate, heated at 75°C, pH 9 for 10 min and purified on Sephadex G25m-filled spin columns (PD-10, GE) that were pre-equilibrated with water. The purified silver-primed 0.25 ml Ag-IO-NPs (20 mM Fe) eluting in the void volume (flow-through) of the columns were combined with an equal volume of trisodium citrate (pH 8), injected into 10 ml of tetrachloroauric acid (3 μ M Au), mixed and incubated overnight at RT. The final concentration of citrate in the obtained solutions of Au-IO was 3–6 mM.

Stabilization of nanoparticles

MPEG5-gPLL graft copolymer was synthesized and purified as described in³. Briefly, methoxy PEG5 succinimidyl carboxymethyl ester was used to modify 20% of TNBS-reactive amino groups of poly-L-lysine (m.w. 48,800 Da, DP 233, PDI 1.13, Alamanda Polymers) with subsequent purification of the product using UFP-100 ultrafiltration cartridges (GE Biosciences). Stabilization of Ag-IO was achieved by direct addition of MPEG5-gPLL to Ag-IO (final concentration 2 mg/ml MPEG5-gPLL) and incubating overnight at RT.

The stabilization of Au-containing NPs was performed by addition of 2-iminothiolane-modified MPEG5-gPLL. For thiolation of 10% of available amino groups, 1 ml of MPEG-gPLL solution (5 mg/ml, 0.05M Hepes, 1 mM EDTA, pH 7.5) was treated with a 3-molar excess of 2-iminothiolane (50 μ g) for 20 min; tris(2-carboxyethyl)phosphine (0.5 mM, final concentration) was then added and the solution was purified on a Sephadex-G25m-filled spin column equilibrated with degassed nitrogen-saturated 10 mM trisodium citrate, pH 7.0. The eluate was immediately added to Au-IO (10 ml), mixed and incubated overnight. The obtained MPEG5-gPLL stabilized solution was centrifuged at 4000 $\times$ g for 10 min to remove AgCl crystals and passed through a 0.22 μ m Millipore-PLUS membrane. The solution was concentrated to desired concentration of iron by using Amicon Ultra 4 YM-100 centrifuge membrane concentrators (EMD-Millipore, Billerica MA).

Purification of Au-IO

Free MPEG5-gPLL and non-incorporated IO were removed by ultracentrifugal rate separation in a step gradient consisting of 0.2 ml 50% Opti-Prep (60% Iodixanol solution in saline, Sigma-Aldrich, St. Louis MO) with an initial density of 1.32 g/ml, followed by 4 ml of 8% solution of Opti-Prep in 0.03M Hepes, pH 7.5. MPEG5-gPLL-stabilized or non-stabilized Au-IO were concentrated using Amicon Ultra 4 YM-100 and loaded in a volume of 1 ml on top of the step-gradient. The samples were centrifuged in an SW55.1 Ti rotor (Beckman) at 40,000 $\times$ rpm (rcf 152,000 $\times$ g) for 30 min. The bottom 0.5 ml fraction was collected after aspirating the top fractions of the gradient and washed 3 $\times$ with 20 mM Hepes, 0.1 M NaCl, pH 8.0 in Amicon Ultra 4 YM-100 centrifuge membrane concentrators as suggested by the manufacturer.

Modification of stabilized Au-IO with NIR fluorophores

Stock solutions of NIR fluorophore IRDye 800RS or QC-1 quencher hydroxysuccinimide esters (NHS, Li-Cor) were prepared in DMSO. NIR dye (0.2 mg) was added to MPEG-

gPLL stabilized Au-IO (0.25 ml in 0.05 M sodium bicarbonate, pH 7.8; 2.5–3.5 mM iron). After a 2 h incubation, Au-IO-800 and Au-IO-QC1 were purified using Ultra 4 YM-100 concentrators.

Cell culture experiments

MDA-MB-231 cells were grown in L15 medium, 2mM l-glutamine, 15% FBS and split after they reached sub-confluency. Cells were incubated with Au-IO (0.025–0.3 mM [Fe], final concentration) in 6-well plates for 24h at 37°C. Cells were trypsinized, washed 2 times by centrifugation through a cushion of 30% Histopaque-1077 (Sigma-Aldrich)/PBS at 800×g for 10 min³², counted, fixed with 2% paraformaldehyde in PBS for 10 min and re-suspended at a concentration of $1.44\text{--}0.08\cdot 10^6$ cells/ml in transparent PCR tubes for imaging using an Odyssey Near-Infrared imaging system (Li-COR, Lincoln NE). The same samples were then used for imaging in capillary phantoms as described below.

Photoacoustic imaging

Phantoms were built using 2 mm (1.9 mm inner diameter, 0.036 in ID, WW. Grainger Inc.) thermo-seal transparent tubing. The volume of each capillary sample was approximately 70 μl . The sealed capillaries were immersed in non-reflecting black rubber foam chambers (6×4×2.5 cm) filled with degassed water and sealed with optically transparent film using silicon-based adhesive. Photoacoustic images were acquired as previously described³³. Briefly, samples were broadly illuminated by 5 ns duration laser pulses produced by a pulsed optical parametric oscillator (OPO) laser (VersaScan OPO, Spectra Physics, Newport Corp.) and photoacoustic emissions were recorded by a L12-5 linear array transducer connected to a Verasonics V-1 programmable ultrasound imaging system (Verasonics, Inc, Kirkland, WA). The intersection of the acoustic sensing field and the optical excitation determined the sensing area, and off-target or off-axis optical absorbers had a negligible effect on the image. The estimated PA imaging voxel dimensions were $150 \times 300 \times 750 \mu\text{m}$ (axial, lateral, elevational), with the volume of 0.033 μl .

Magnetic Resonance Imaging

Quantitative T_1 -imaging experiments were performed using a magnetization-prepared T_1 -imaging sequence³⁴, which had two well-separated timing segments: a leading inversion-recovery pulse sequence segment and a subsequent spin echo (SE) imaging segment in which all timings were kept constant. The leading T_1 contrast segment had seven increments (0.05, 0.15, 0.3, 0.6, 1, 2.5, 5 s). Similar to quantitative T_1 -weighted imaging experiments, quantitative T_2 imaging experiments were performed using a magnetization-prepared SE T_2 pulse sequence³⁴. The echo times used in the leading contrast segment were 22, 50, 75, 100, 200, and 500 ms. Both the T_1 and T_2 relaxation times were calculated by fitting of the magnitude images mono-exponentially on a pixel-by-pixel basis³⁵. The repetition time (TR) of the imaging experiment was 5 s and 8 s for T_1 w and T_2 w imaging, respectively. Other imaging parameters were: echo time (TEi)- 17 ms; field of view (FOV) – 20×40 mm; imaging matrix size - 200×400; slice thickness - 1 mm; 8 NEX. Imaging phantoms consisted either of 0.25 ml (0.5 cm diameter) tubes (Supporting Fig. S2) or 1.9 mm diameter capillaries as described above.

Electron Microscopy

Transmission EM of nanoparticles was performed by incubating IO, Ag-IO, or Au-IO NPs diluted 1:10 with PBS on formvar coated grids stabilized with evaporated carbon film without negative staining and further examined under EM (FEI Tecnai 12 Spirit, at 100–140K magnification). Scanning EM experiments were performed using a FEI Quanta 200 FEG MKII scanning electron microscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

MPEG5-pPLL	covalent graft copolymer of methoxy poly (ethylene glycol) 5000 and polylysine
IO	iron oxide
IO-Ag	silver-primed iron oxides
Au-IO-NP	hybrid modular dextran-stabilized iron-oxide/gold nanoparticles
Au-IO-NP800	IRDye 800Rs-labeled hybrid modular dextran-stabilized iron-oxide/gold nanoparticle
ICP-MS	inductively coupled plasma mass spectrometry.

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Figure 1.

A scheme of Au-IO synthesis: 1) dextran-stabilized iron oxides bearing aldehyde groups were treated with an excess of diamminesilver(I) and the resultant IO-Ag NPs were purified; 2) IO-Ag seeds were used for hybrid Au-IO NPs synthesis in the presence of tetrachloroauric acid and trisodium citrate. 3) The obtained NPs were stabilized by using thiolated MPEG5-gPLL; 4) for multiplexed photoacoustic imaging the MPEG5-gPLL coat was modified by conjugating near-infrared dye 800RS.

Figure 2.

Normalized absorbance spectra of NPs and their mixtures: A – comparative spectra of iron oxide (IO), silver- primed IO (IO-Ag); non-purified Au-IO1 synthesized in the presence of 3 mM trisodium citrate (pH 4.5); MPEG5-PL-stabilized Au-IO1 (Au-IOs) and control citrate-stabilized gold NPs (AuNP); B – purified MPEG5-PL-stabilized nanoparticles – Au-IO1 synthesized in the presence of 3 mM trisodium citrate (pH 4.5); Au-IO2 synthesized in the presence of 6 mM trisodium citrate (pH 6.0); and corresponding purified Au-IO1 and Au-IO2 NPs conjugated with IRDye 800RS.

Figure 3. Back-scatter scanning electron microscopy of **A**- iron oxide (IO), **B**- silver-primed iron oxide (IO-Ag) and **C**- MPEG5-gPLL stabilized gold-IO nanoparticles (Au-IOs). Bar= 1 µm.

Figure 4.

Transmission electron microscopy of **A** – silver-primed IO; **B**- non-purified MPEG5-gPLL stabilized Au-IO1; **C**- purified MPEG5-gPLL stabilized Au-IO1. In panel C arrows point to iron oxide component of NPs and arrowheads point to gold nanoparticle component. White scale bars = 100 nm, black bar = 50 nm.

Figure 5. Dual modality imaging of Au-IO1

A- axial 3T T1- and T2- weighted MR images (top) and PA images (bottom) of a phantom consisting of three 2mm-diameter capillary tubes filled with Au-IO1 ($T_2=339\pm43$ ms), Au-IO1-800 ($T_2=225\pm23$ ms), and Au-IO1-QC1 ($T_2=229\pm17$ ms) and immersed in water.

Intensity scale on PA images is in dB (log-compressed scale);

B- quantification of PA signals shown in PA images generated at dual wavelength irradiations by using ROI statistics (shown as signal-to-noise ratio). All samples are diluted to $[\text{Fe}]=50\text{ }\mu\text{M}$. 5 ns width laser pulses were used at 530/770 nm, $1\text{ mJ}/\text{cm}^2$ fluence, PA emissions were detected using a linear array ultrasound transducer (9 MHz center frequency).

Figure 6.

Dual modality (MR and PA) imaging of a phantom consisting of samples with equal absorbance at 770 nm. Phantom consisted of four 1.9mm-I.D. capillary tubes filled with MPEG5-gPLL-800 (1) Au-IO2-800 (2), Au-IO2-800 (3), Au-IO2 (4) and diluted to $A_{770nm}=0.35$. Tubes were immersed in water. A - axial 3T T1- map of samples 1–4; B- PA images were acquired using 5 ns duration laser pulses at 530 nm (fluence =1 mJ/cm² or 770 nm (fluence=1 mJ/cm²). PA emissions were detected using a linear array ultrasound transducer (9 MHz center frequency). PA signals (shown as mean $\pm$ SD signal-to-noise ratio) is plotted against the mean transverse relaxivity values in each sample as measured by using 3T MRI.

Figure 7.

A- toxicity of IRDye 800RS-labeled Au-IO1 (red) and Au-IO2 (blue) in normal human epithelial cell line MCF10A (closed symbols) and in MDA-MB-231 mammary adenocarcinoma (open symbols) cultures measured by using LDH test; **B**- dual channel (gold- (Au, 530 nm, 1 mJ/cm² fluence) and near-infrared – (NIR, 770 nm, 0.5 mJ/cm² fluence) photoacoustic imaging of a phantom containing 1.9 mm ID capillaries filled with MDA-MB231 cells after 24h uptake of Au-IO NPs (0.1 mM Fe). In the right column the corresponding 3T MR (T2w SE) images of cell samples ($0.8\text{--}1.0 \cdot 10^5$ cells/tube) and T2 values are shown. Comparative near-infrared fluorescence intensity images of cell suspension samples acquired using identical settings (Odyssey NIR Imaging system, Li-Cor) are shown in the middle column.

Table 1

Properties of purified multimetallic nanoparticles and their derivatives.

NP	Description of nanoparticles	Z-Average (number-average diameter nm, fraction %)	Zeta potential (mV)	Molar transverse relaxivity, r_2 ([mMs] ⁻¹) ^{a)}	IRDye800RS mol/g Au	Fe/Ag/Au (molar ratio) ^{b)}
IO-Ag	Silver-primed dextran-stabilized iron oxide NP	131.3 (34.7, 96%)	−21.6	20.4±0.4		2:1:0
IO-Ag-s	IO-Ag stabilized with thiolated MPEG5-gPLL	97.68 (96.2, 72.6%)	−5.3	72.2±3.2		-
Au-IO1	Gold NP formed in the presence of 3.5 mM citrate (pH 4.5) and IO-Ag	1085 (87.9, 100%)	−8.0	70.4±1.6		9:3:1
Au-IO1s	Au-IO1 stabilized with thiolated MPEG5-gPLL	43.67 (48.4, 98%)	−0.8	77.2±1.3		9:3:1
Au-IO1-800	Au-IO1 modified with IRDye 800RS	60.0 (34.3)	−5.7	69.3±3.4	70	9:3:1
Au-IO2-800	Au-IO2 were synthesized in the presence of 6 mM citrate (pH 6.0) and modified with IRDye 800RS	87.5 (55.6)	−6.5	61.5±2.8	200	6:2.5:1

^{a)} measured at 0.47T, 40°C^{b)} ICP-MS data was used to calculate molar ratios.