

Gadolinium chelate functionalized gold nanoparticles for targeted NIR laser heating

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Introduction: Gold nanoparticles (Au NP) have been used for near-infrared (NIR) hyperthermia and ablation therapy of tumors (1). At our institution, we are interested in developing targetable gadolinium-chelate functionalized gold nanoparticles (Gd-Au NP) as theranostic agents that can be detected by MRI to guide NIR laser therapy. By tuning the optical properties of Gd-Au NP to absorb in NIR, where tissue penetration of light is optimal, one can selectively heat tumor tissue that contain nanoparticles. Although various formulations of Gd-Au NP have been proposed, mainly for multimodality MR/CT contrast agents (2, 3), modification of Au NP surface properties results in alteration of its optical absorption properties due to sensitivity of surface plasmon resonance (SPR) effect to local chemical environment at the metal surface (4, 5). We have taken this into consideration and used a Au NP that normally does not absorb in NIR and modified it to become a good NIR absorber that doubles as a very good T1-contrast agent.

Materials and Methods: Stock 50-nm spherical solid Au NP with SPR-absorption peak of 530 nm were obtained from Nanospectra (Houston, TX).

Synthesis of Gd-Au NP: N-hydroxysuccinimide was first added to thioctic acid in DCM. The mixture was cooled to 0 °C, and DCC was added as a coupling reagent. The mixture was stirred overnight and filtrated to remove solid precipitate. The organic phase was then washed by water, dried, and the solvent removed by rotary evaporation. Pure thioctic succinimide ester was obtained by flash chromatography with 81.5% yield. Next, p-NH₂-Bn-DTPA and DIPEA were added to thioctic succinimide ester in DMSO. The reaction mixture was heated with a microwave synthesizer at 90 °C for 30 min. Purification of the crude product was carried out on a semi-preparative reversed-phase HPLC system. Mass spectrum of the compound: m/z 687.1. DTPA-thioctic acid was then conjugated to Au NP by thiol-Au bond. In the final step, Gd³⁺ was chelated with DTPA-Au NP by incubating with GdCl₃ at room temperature. Free Gd³⁺ was removed by centrifuge, and the final Gd-Au NP product was resuspended in water.

MR Relaxivity: Phantoms were constructed using different concentrations of Au NP, Gd-Au NP, and Gd-DTPA control placed in sealed capillary tubes surrounded by water. All MR experiments were performed at 23 °C on a 3.0-T clinical whole-body MRI system (Signa, GE Healthcare, Milwaukee, WI) using a custom-made 3.5-cm diameter transmit/receive quadrature volume coil (InsightMRI, Worcester, MA). T1-, T2- and T2*-maps were generated using T1 IRSE, T2 FSE and GRE sequences, respectively, with the following parameters: T1 IRSE (TI = 500-2000 ms, TR = 2500 ms, TE = 15 ms), T2 SE (TR = 2500 ms, TE = 15-500 ms), and GRE (TR = 400 ms, TE = 5-100 ms, $\alpha = 30^\circ$). Geometric parameters were the same for all three sequences (slice thickness = 2 mm, gap = 0.2 mm, FOV = 5.0 cm, 256×256 matrix).

UV-Visible-NIR Spectroscopy: Spectroscopic analysis of Au NP and Gd-Au NP was carried out using a Varian Cary 50 UV-Vis spectrophotometer (Palo Alto, Ca).

NIR Laser Heating: Concentrated and 1:10-dilute 200- μ l samples of Au NP, Gd-Au NP, and water control were placed in glass test tubes surrounded by a water bath at 23 °C. NIR laser heating of the sample surface was performed using an 800-nm AlGaAs diode laser (Lumenis LightSheer ET, Santa Clara, CA) with the following parameters: peak power = 1600 W, fluence = 100 J/cm², spot size = 9 mm, pulse width = 400 ms, 20% duty cycle, applied pulses = 1-10. Change in temperature was measured from the center of the sample before and immediately after NIR laser heating with a calibrated thermal couple.

Results: Fig 1 shows a TEM micrograph of 50-nm Au NP, which has a fairly uniform diameter size distribution with occasional larger particles dispersed in the mix. A schematic of the Gd-chelating unit used in this study is illustrated in Fig 2. Each Gd-chelating unit has two thiol-Au bonds anchoring it to the Au NP.

MR Relaxivity: T1 SE images and T1-maps of phantoms containing 2×10^{10} to 2×10^{12} NP/mL concentrations of Au NP and Gd-Au NP are shown in Fig 3. Gd-Au NP with saturated amount of surface-bound Gd-chelate proves to be a very good T1 contrast agent. The measured r_1 and r_2 molar relaxivities of Gd-Au NP were 5.6×10^5 mM⁻¹s⁻¹ and 9.6×10^5 mM⁻¹s⁻¹, respectively (1 mM NP = 6.0×10^{17} NP/mL). As a comparison, measured r_1 and r_2 of Gd-DTPA were 3.0 mM⁻¹s⁻¹ and 4.8 mM⁻¹s⁻¹, respectively, which are consistent with literature values (6). No significant change in relaxivity was detected for the various concentrations of Au NP used.

UV-Visible-NIR Spectroscopy: With conjugation of Gd-chelate to Au NP, there is increasing spectral shift of SPR-associated absorption wavelength from a peak at 530 nm (green) to a broader distribution mostly between 600 nm and 900 nm (red-shifted) (Fig 4). A small absorption peak at 530 nm probably represents a subpopulation of non-functionalized Au NP. On visual inspection of reflected light, Au NP has a deep red hue, whereas Gd-Au NP is translucent violet-blue.

NIR Laser Heating: Fig 5 shows NIR laser heating results for Gd-Au NP and Au NP. Measured temperature change was linearly proportional to the number of laser pulses applied and concentration of NP used. Significant heating from selective laser absorption at 800-nm wavelength was demonstrated for both concentrated and 1:10-diluted samples of Gd-Au NP. After 6 laser pulses, there was almost a 40 °C change in temperature for the concentrated Gd-Au NP sample. The Au NP samples without Gd-chelate heated only minimally compared to water control.

Conclusion: We have synthesized a new Gd-DTPA-based chelate-linker for conjugation to Au NP. The Gd-chelating unit (Fig 2) has two thiol-Au binding sites and a longer linker segment than the one proposed by Moriggi, *et al* (3), which should allow for better immobilization and increased number of Gd-chelate conjugation to Au NP as a high-relaxivity T1-contrast agent. We have demonstrated that our Gd-Au NP is a very good T1-contrast agent that is detectable by MRI at nanomolar concentration. From expected spectral red shift of SPR-associated absorption with surface functionalization, the Gd-Au NP was also designed to be a good absorber in the NIR wavelength. We have demonstrated that our Gd-Au NP can be selectively heated using a clinical 800-nm AlGaAs diode laser. By selecting an appropriate size (as in our case with 50-nm solid Au NP) and/or core-shell dielectric properties, a variety of Gd-Au NP can be constructed for targeted MRI-guided NIR laser therapy.

References:

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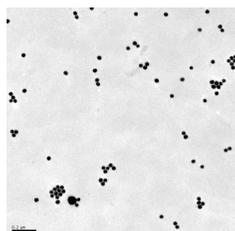


Fig 1. TEM of Au NP

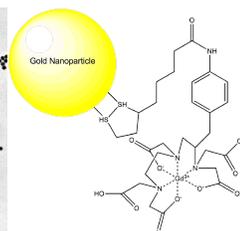


Fig 2. Gd-chelate unit

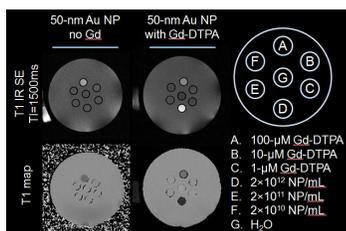


Fig 3. T1 SE and T1 maps of phantoms

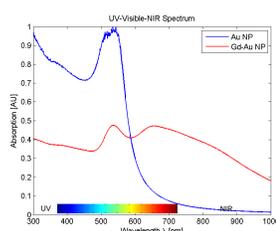


Fig 4. Absorption spectrum of Au NP and Gd-Au NP

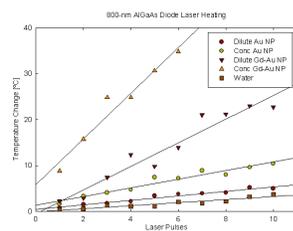


Fig 5. NIR laser heating of Au NP and Gd-Au NP