Near-Infrared-Resonant Gold/Gold Sulfide Nanoparticles as a Photothermal Cancer Therapeutic Agent

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The development and optimization of near-infrared (NIR)-absorbing nanoparticles for use as photothermal cancer therapeutic agents has been ongoing. This work exploits the properties of gold/gold sulfide NIR-absorbing nanoparticles (≈35–55 nm) that provide higher absorption (98% absorption and 2% scattering for gold/gold sulfide versus 70% absorption and 30% scattering for gold/silica nanoshells) as well as potentially better tumor penetration. The ability to ablate tumor cells in vitro and efficacy for photothermal cancer therapy is demonstrated, and an in vivo model shows significantly increased long-term, tumor-free survival. Furthermore, enhanced circulation and biodistribution is observed in vivo. This class of NIR-absorbing nanoparticles has the potential to improve upon photothermal tumor ablation for cancer therapy.

1. Introduction

Photodynamic therapy is a mode of treatment whereby laser light at a particular wavelength is combined with exogenous chromophores to accomplish a therapeutic effect. These exogenous chromophores have often included sensitizers such as protoporphyrin and indocyanine green, which absorbs the laser energy converting it to heat.[1–3] Photothermal therapy with near-infrared (NIR)-absorbing nanoparticles has gained great attention and focus in recent years as an improvement to these methods.[4–7] The size, shape, and chemical structure of gold nanoparticles dictates their optical properties due to interaction of light with the free electrons of the gold surface, a phenomenon referred to as localized surface plasmon resonance.

A variety of different types of gold nanoparticles have been explored that can be “tuned” to have high absorption characteristics in the NIR region, including gold/silica nanoshells, nanorods, and nanocages.[4,5,7–14] The focus on the use of NIR-responsive nanoparticles is due to the low absorption and high transmission of light in this wavelength region for the majority of tissue components, thus making it an ideal pairing to use for biomedical applications.[15] Furthermore, the biocompatibility of gold and its ability to conjugate biologically relevant molecules to its surface through the sulfur–gold interaction, such as polyethylene glycol (PEG) for stealth capabilities and antibodies for targeting, make these nanoparticles ideal as therapeutic and diagnostic tools.[4–6,10,16–20] Scattering theory, or Mie theory, is an analytical solution to Maxwell’s equation for electromagnetic waves incident on a sphere; using it one can calculate the absorption and scattering cross-section profiles of small particles.[21,22] By use of simulations based on scattering theory, the optical properties of gold nanoparticles can be predicted based on the type and shape of the particle, thus allowing the design of particles with strong light absorption and/or scattering properties at particular wavelengths.[23–26]

Gold nanoparticles with an interior composed of gold sulfide, or gold/gold sulfide composite structures, were first produced by self-assembly by Zhou et al. and shown to have strong...
NIR-absorbing properties. The optical properties of these materials were later explained to be due to a dielectric-core/metal-shell structure. Although some controversy continues over the precise structure of these particles, the core/shell model appears to fit the data well and X-ray diffraction shows a gold surface and gold sulfide composite structure. Moreover, for work in biomedical applications, the particles appear to have a contiguous gold coating, which allows for the surface conjugation of molecules as discussed above. The NIR-resonant gold/silica nanoshells used in therapeutic and imaging applications have an average diameter of 120–140 nm, whereas these NIR-absorbing gold/gold sulfide nanoparticles typically have a diameter between 35 and 55 nm.

Using a Mie scattering theory simulation program, we calculated the absorption efficiencies of the gold/gold sulfide nanoparticles to be closer to 96–99% based on the size measured by transmission electron microscopy (TEM); the properties are summarized in Table 1. For the gold/silica nanoshells used in our previous work with imaging and therapy, the particle size of 143 nm resulted in a calculated 33% scattering of the light energy or only 67% absorbed for conversion to heat for photothermal therapy. Previous imaging results were based on the use of optical coherence tomography (OCT) with scattering-mode detection; however, an absorption-based mode of OCT detection would most likely have to be employed for imaging of the gold/gold sulfide nanoparticles.

Ideally, these smaller particles should provide additional benefits as a cancer therapeutic agent due to their smaller size, increased absorption efficiency, and ease of manufacture. It is well known that the leaky vasculature of tumors allows extravasation of macromolecules and nanoparticles, which allows therapy based on nanotechnology platforms. Our postulation herein is that these gold/gold sulfide nanoparticles will provide additional benefits for the treatment of cancer tumors based on their size, thus allowing better biodistribution and effective therapeutic benefits for the treatment of fast-growing tumors. Modeling work by Decuzzi et al. suggests that particles with radii of less than 50 nm and high relative density (density of a particle relative to the blood) will have a greater ability to move closer to the endothelium layer, potentially enhancing the movement of these particles into the tumors. Furthermore, experimental evidence suggests that nanoparticle uptake into cells is size dependent, with the maximum uptake occurring for gold particles of diameters between 30 and 50 nm.

| Table 1. Properties of gold/gold sulfide nanoparticles compared to gold/silica nanoshells. |
|-----------------|-----------------|
| Overall diameter [nm] | 35–55<sup>a</sup> | 120–160<sup>a</sup> |
| Core diameter [nm] | 30–40<sup>b</sup> | 100–120<sup>a</sup> |
| Shell thickness [nm] | 3–6<sup>b</sup> | 7–20<sup>b</sup> |
| Predicted absorption efficiency [%] | $>98$ | $<85$ |

<sup>a</sup> Measured by SEM and confirmed with Mie scattering theory.  
<sup>b</sup> Calculated based on Mie scattering and measured spectra.

2. Results and Discussion

2.1. Gold/Gold Sulfide Nanoparticles for Therapeutic Applications

To use gold/gold sulfide nanoparticles for therapeutic applications, the smaller gold-colloid contaminants formed during the self-assembly process have to be removed. Removal of this contaminant provides several benefits. Firstly, it reduces the total number of particles delivered to the body, which could overwhelm the phagocytes of the liver or the innate defense mechanisms such as the reticuloendothelial system (RES). Secondly, the gold-colloid particles would easily bind with the same targeting mechanism as the NIR-absorbing gold/gold sulfide nanoparticles, thus increasing the use of targeting agents such as antibodies or aptamers. Finally, the colloidal gold has a resonance near that of hemoglobin and thus provides no benefit for the NIR photothermal therapy being evaluated. We synthesized gold/gold sulfide nanoparticles with bare gold surfaces to allow the conjugation of stealth agents or antibodies onto the gold surface as previously described.

Gold/gold sulfide nanoparticles were synthesized by self-assembly as described by others using chloroauric acid and sodium sulfide. The steps of the self-assembly process yielded a variety of particles in addition to the gold/gold sulfide nanoparticles: nanoprisms and rods are often seen in TEM images (Figure 1A). However, after separation, the majority of particles were spherical gold/gold sulfide nanoparticles with small amounts of remaining gold colloid (Figure 1B). Figure 2 shows the spectral data following a three-step sequential centrifugation process to purify the gold/gold sulfide nanoparticles from gold colloid. The sample curves after the first and second separation steps show a maximum peak at $\approx 530$ nm, which is the gold colloid peak. However, the enrichment of the NIR fraction of the particles is evidenced by the increase in absorbance values between step 1 and step 2. After step 3, the NIR-absorbing fraction actually dominates the spectra and shows only a small amount of gold colloid resonance. Gold/gold sulfide nanoparticle suspensions purified by this centrifugation method were used in further studies to evaluate the heating, in vitro photothermal ablation properties, targeting abilities, and in vivo efficacy for photothermal therapy.
2.2. Gold/Gold Sulfide Nanoparticles Efficiently Absorb NIR Energy

Mie scattering theory predicts that the smaller size of the gold/gold sulfide nanoparticles will yield higher absorption efficiencies than the larger gold/silica nanoshell counterparts. Calculations based on software developed by others using scattering theory and TEM size analysis give predicted values of absorption efficiencies for both types of nanoparticles (see Table 1).\[26,38\] The temperature of suspensions of gold/gold sulfide nanoparticles was measured as described in the Experimental Section by using a thermocouple. Nanoparticle suspensions were used based on their total extinction (absorption + scattering) or optical density (OD) at the peak wavelength. Heating data for gold/gold sulfide nanoparticles were obtained with an OD of 1.0 and 2.0 at 800 nm (see Figure 3). Figure 3A shows the maximum temperatures attained and Figure 3B shows the initial rate of temperature increase. Temperatures quickly rose to a maximum of 100 °C (samples were suspended in water) at total powers of 5 and 7 W, regardless of concentration. The final temperature difference shows a statistically significant difference between OD values of 1.0 and 2.0 at 2 and 5 W; however, the differences were not significant at the higher laser power of 7 W. These parameters allow for the ability to better tune the laser power for treatment purposes when combined with in vivo nanoparticle concentration information. Indications are that future work with these nanoparticles could include a lower laser power for an OD of gold/gold sulfide nanoparticles equivalent to that of gold/silica nanoshells.

2.3. Photothermal Ablation in vitro

By using human prostate cancer cell lines and bare nanoparticles (not coated with PEG), we were able to demonstrate binding and subsequent heating via laser application of the nanoparticles, followed by the death of cells receiving exposure to the nanoparticles and laser. Figure 4A shows human prostate cells (PC3) that were incubated without nanoparticles and exposed to a NIR laser, which were killed within the laser spot, thus demonstrating the efficacy of gold/gold sulfide nanoparticles in vitro. This result is consistent with previously seen photothermal damage obtained with bare gold/silica nanoshells\[11\] and other NIR-absorbing nanoparticles with in vitro tests.\[14,39\]

2.4. Stabilization of Gold/Gold Sulfide Nanoparticles with PEG for in vivo Applications

For in vivo applications, it is necessary to surface coat the particles with PEG to stabilize the suspension and increase the biocompatibility to avoid RES clearance. The interaction of
the gold particles with physiological liquids will cause 1) agglomeration of the particles through ionic interaction with the charged gold surface, resulting in loss of particle resonance, and 2) adsorption of proteins on the gold surface, which will allow the RES to identify and remove the particles from circulation.

Prior to in vivo application, gold/gold sulfide nanoparticles were PEGylated and assessed for stability in a 1% NaCl solution at room temperature, to mimic physiological ionic conditions. The peak resonance is reduced as nanoshells aggregate in a strong ionic solution and can thus be easily monitored by spectroscopy. Similarly, gold/silica nanoshells were prepared and stabilized exactly as described previously by O’Neal et al.[19] and the reduction of the peak was monitored over 4 h. The results shown in Figure 5 indicate that gold/gold sulfide nanoshells are equally as stable as PEG-coated gold/silica nanoshells under equivalent conditions, and thus allowed in vivo therapeutic testing. Samples that had no NaCl added showed no difference in aggregation of the nanoshells after 4 h.

For the controls, gold/silica nanoshells were made as previously described, PEGylated, and sterilized for in vivo biodistribution testing.[7,19] The nanoshells were surface-coated with PEG, as above, to enhance circulation times and reduce the immune response. PEGylation was accomplished by adding 5 μM PEG-SH (20 μL) to 1.5 × 10^10 nanoshells mL⁻¹ in deionized (DI) water for a minimum of 8 h. PEG-modified nanoshells were sterilized by filtration and subsequently concentrated by centrifugation and rediluted with sterile phosphate-buffered saline (PBS) for injections.

2.5. Enhanced Biodistribution and Tumor Reduction with Increased Survival in vivo

We compared the in vivo distribution of PEGylated gold/gold sulfide nanoparticles and PEGylated gold/silica nanoshells, injected at equal OD, in mice with tumors after 24 h of accumulation. The 24 h time period was based on previous testing with gold/silica nanoshells by our group; photothermal therapy with gold/silica nanoshell-injected animals was followed after an accumulation time of less than 24 h.[7,19] Figure 6A illustrates the accumulation of gold/gold sulfide nanoparticles and gold/silica nanoshells. Figure 6B is an expansion of the y axis, to better visualize the distribution.

We can further compare the difference in tumor uptake relative to RES removal by looking at the ratio of gold in the tumor over gold in the spleen and liver (see Figure 6C). The data show that a larger proportion of injected gold accumulates in the tumor compared to accumulation in liver and spleen (p < 0.02) from the gold/gold sulfide nanoparticle-injected animals when compared with animals injected with gold/silica nanoshells. In conjunction with the data in Figure 6B, which show a higher gold content in the blood, gold/gold sulfide nanoparticles appear to avoid the RES more effectively, while allowing a higher accumulation in the tumor. To better evaluate the gold/gold sulfide nanoparticles in photothermal therapy, the accumulation time was increased to 48 h based on the high availability of nanoparticles in the blood pool after 24 h. Prior to therapeutic photothermal ablation, the tumor site was shaved and swabbed with glycerol for index matching to aid laser application.

During the photothermal ablation study, external tumor surface temperatures were spot checked during treatment with a handheld, noncontact infrared thermometer (OS 553XCF; Omega Instruments, Stamford, CT). These measurements
showed that the gold/gold sulfide nanoparticle treatment group achieved a maximum tumor temperature of 46.1 ± 2.7 °C after an accumulation time of 24 h. When the circulation/accumulation time was increased to 48 h, there was increased heating of the tumor, presumably due to increased accumulation of nanoshells within the tumor. A statistically greater external tumor temperature of 60.6 ± 2.4 °C was obtained for the group with an accumulation time of 48 h versus the 24 h treatment group (p < 0.05). Kaplan–Meier survival curves are shown in Figure 7 for the 8-week period of the study. Survival of the gold/gold sulfide nanoparticle-treated mice with 24 h accumulation time was 71%, and for the 48 h accumulation group, survival was statistically greater at 82%. Upon comparing the 48 h treatment using gold/gold sulfide nanoparticles and the 24 h treatment using gold/silica nanoshells, the survival was statistically equivalent based on Kaplan–Meier analysis.

3. Conclusions

We have shown that gold/gold sulfide nanoparticles can be used as a photothermal therapeutic agent for cancer therapy in much the same way as the gold/silica nanoshells currently in clinical trials. In this work, we demonstrate that gold/gold sulfide nanoparticles could be manufactured and purified to allow for both in vitro and in vivo applications. Heating profiles at varying laser powers show temperatures high enough to effect tumor ablation by hyperthermia at relatively low concentrations and low laser powers. The smaller gold/gold sulfide nanoparticles could offer additional advantages compared to the gold/silica nanoshells. The smaller size yields a particle with higher absorbing cross-sectional area ratio than the gold/silica nanoshell. Mie scattering theory calculations predict that the gold/gold sulfide nanoparticles will absorb 98–99% of the incident energy compared to 67–85% for the gold/silica nanoshells currently being used. The implications for these smaller, more highly energy-absorbing nanoshells for therapy are that fewer particles could be used during treatment or alternatively a lower laser power or time could be utilized during therapeutic laser administration. Furthermore, the NIR-resonant nanoshells are produced by a single-step self-assembly process; additional steps prepare these particles for in vivo usage and are similar to those used in all metallic nanoparticle preparation processes. The single-step synthesis technique could potentially reduce the costs associated with the production of nanoparticles, thus allowing greater scale-up and flexibility of manufacture.

In vitro testing shows that gold/gold sulfide nanoparticles in combination with NIR-laser light can cause photothermal
destruction of tumor cells. Gold/gold sulfide nanoparticles can be stabilized in a saline environment by surface coating with PEG. In vivo testing provided biodistribution data, which support the finding that the gold/gold sulfide nanoparticles can remain in circulation longer than gold/silica nanoshells: greater than 24 h based on neutron activation analysis (NAA) and longer based on dynamic light scattering (data not shown). Accumulation in liver, spleen, and tumors showed that larger-dose gold/gold sulfide nanoparticles actually accumulate in the tumor rather than the RES and as compared to gold/silica nanoshells. Survival data show an effective photothermal therapy with survival greater than 80% for optimized accumulation times. With further optimization of photothermal parameters and nanoparticle concentrations, gold/gold sulfide nanoparticles could provide an alternative therapeutic option that could prove very effective. When combined with the gold/silica nanoshells currently being evaluated clinically, these particles could complement the treatment options for particular types of cancers.

4. Experimental Section

**Synthesis of gold/gold sulfide nanoparticles:** Gold in the form of hydrogen tetrachloroaurate(III) trihydrate (chloroauric acid, 99.99% purity) was purchased from Alfa Aesar (Ward Hill, MA) and diluted to a concentration of 2 mM. Sodium sulfide (3mM) was prepared, and these reagents were aged in darkness for 40–48 h prior to use.[23] The ratio of chloroauric acid to sodium sulfide was varied from 1.70:1 up to 2.10:1 by volume. Spectra were obtained every 15 min with a UV/Vis spectrophotometer (Carey 50 Varian, Walnut Creek, CA). Although the volumetric sweep produced particles with peak resonances from 750 through 950 nm, only conditions yielding gold/gold sulfide nanoparticles with an extinction peak near 800 nm were scaled up 200×. Gold/gold sulfide nanoparticles were sized by TEM (JEOL FasTEM 2010–TEM) at 100 kV.

**Synthesis of gold/silica nanoshells:** Briefly, silica cores were grown by the Stöber process, using tetraethyl orthosilicate (Sigma–Aldrich, Milwaukee, WI) in ethanol. Silica nanoparticles were sized by SEM (Philips FEI XL30) at 20 kV. The silica was functionalized with (3-aminopropyl)triethoxysilane (APTES, Sigma–Aldrich) to allow for adsorption of gold colloid. Gold colloid was prepared to a size of 2–4 nm through the method of Duff et al.[40] and aged 2–3 weeks at 4°C. The colloid was then concentrated 20× by rotary evaporation and mixed with the functionalized silica particles. The gold shell was completed by the reduction of gold from chloroauric acid (HAuCl₄) in the presence of formaldehyde. The extinction characteristics of the nanoshells were determined using a UV/Vis spectrophotometer (Carey 50 Varian, Walnut Creek, CA).

Separation techniques resulting in a high yield of gold/gold sulfide nanoparticles were required to allow investigations in vitro as well as in vivo. As such, many forms of separation were investigated including size-exclusion chromatography, electrophoresis, and thin-layer chromatography, all with extremely low yield. Finally, an adequate yield was accomplished by using standard centrifugation steps with refinements based on particle size and density in a multistep procedure. Removal of gold colloid contaminant was accomplished by a three-step sequential centrifugation procedure. Gold/gold sulfide nanoparticles (20 mL) were centrifuged at 1100 g for 20 min. The pellet was saved and the supernatant was resuspended under the preceding conditions. The pellet from this second spin was combined with that from the first. The pellet was then resuspended via sonication and diluted in DI water (20 mL, 18 MΩ cm⁻¹). The centrifugation and collection procedure described above was repeated two more times to ensure the highest removal of colloidal gold.

**Temperature measurement of nanoparticle suspensions:** To measure the temperature changes in response to NIR absorption by gold/gold sulfide nanoparticles, we varied the output of a laser shone directly onto samples diluted to OD values of 1.0 or 2.0 at 800 nm. Laser irradiation was accomplished with an Integrated Fiber Array Packet (FAP-I System) at a wavelength of 808 nm (Coherent, Santa Clara, CA). Data were logged for 3 min 20 s, with 10 s collected prior to the laser being turned on and ≈10 s after it was turned off. The maximum temperature was recorded under each condition for each sample, and the heating rate during the first 60 s of laser heating was calculated and tabulated. The laser output was varied between 1 and 10 W. The laser was on for 3 min and data for the sample and the room temperature were recorded simultaneously using an Omega HHT system recorder system (Omega Instruments, Stamford, CT). Testing was repeated with n = 4 for statistical evaluation. The statistical significance was assessed by using Student’s t-test.

**Cell culture for in vitro ablation:** Human prostate carcinoma cells (PC3, ATCC; Manassas, VA) were grown at 37°C in 5% CO₂ F12K medium supplemented with 4 mM l-glutamine, 1% penicillin, 1% streptomycin, and 10% fetal bovine serum (FBS). Cells were harvested from culture flasks using trypsin (0.05%) and ethylene-diaminetetraacetate (EDTA; 0.02%) for well-plate seeding, and subsequent growth to a confluent monolayer for a cell ablation assay.

**In vitro photothermal ablation:** Bare nanoshells were used to allow binding to the cell surface by nonspecific protein absorption. Gold/gold sulfide nanoparticles were added to cells in 24-well plates to total extinction at a peak wavelength of OD = 1.0. This was accomplished by adding the suspension (500 μL) at extinction OD = 2.0 to medium (500 μL) in each well. Cells were incubated with nanoparticle suspensions for 6 h. The wells were rinsed twice with PBS to remove unbound nanoshells and fresh PBS was added for the laser irradiation step. In vitro illumination was accomplished with an FAP-I System (Coherent, Santa Clara, CA), at a wavelength of 808 nm, a power density of 80 W cm⁻², and a spot size 1.2 mm in diameter for 7 min on all samples except for “no laser” controls. Controls consisted of no nanoparticles with laser illumination as well as nanoparticles with no laser illumination were tested to verify the combined effects of the gold/gold sulfide nanoparticles plus laser. After irradiation, the cells were rinsed gently and the PBS replaced with medium. The cells were incubated for 4–6 h following irradiation before evaluation of the viability.

**Viability assessment:** The viability of cells was assessed using the Live/Dead Kit from Molecular Probes (Invitrogen, Eugene, OR). In this assay, calcein acetoxymethyl ester (calcein AM) enters the
cells and is cleaved in live cells by esterases to yield cytoplasmic green fluorescence. Dead cells, which have a compromised nuclear membrane, allow the ethidium homodimer-1 to enter and bind nucleic acids, thus rendering a red fluorescence. Dilutions recommended by the manufacturer were used: 2.5 μL calcein AM and 8 μL ethidium homodimer-1 per 10 mL PBS. Cells were incubated with the fluorescent stains for 45 min followed by imaging using an inverted Zeiss Axiovert 135 phase-contrast microscope (Carl Zeiss, Thornwood, NJ, USA) equipped with a Nikon digital camera.

**Stabilizing nanoparticle agglomeration with PEG:** Gold/gold sulfide nanoparticles were conjugated to PEG to enhance circulation times and prevent aggregation in vivo. PEGylation was accomplished by adding 5 μμμ PEG-SH (100 μμμL) of molecular weight 5 kDa (Nektar, Huntsville, AL) to a nanoshell suspension (20 mL) with OD = 2.0 (≈4.3 × 10^11 particles mL^-1) in DI water for a minimum of 8 h at 4 °C. PEG-modified nanoshells were sterilized by filtration with a 0.22-μm filter and subsequently lyophilized. To facilitate injection in vivo, gold/gold sulfide nanoparticles were resuspended in sterile PBS (pH 7.4) to OD = 50 (7.7 × 10^11 particles mL^-1).

**Stability testing:** Sodium chloride (NaCl) salt solutions were added to nanoparticle suspensions to a salt concentration of 1% to simulate slightly above physiological salt concentrations. Particles that were PEG-coated were mixed with and without salt and the aggregation state of the particles was monitored by measuring the absorbance at peak resonance over a 4-h period using a UV/Vis spectrophotometer (Carey 50 Varian, Walnut Creek, CA). Aggregation leads to a reduction of the peak absorbance at peak resonance over a 4-h period using a UV/Vis spectrophotometer (Carey 50 Varian, Walnut Creek, CA). Aggregation leads to a reduction of the peak absorbance at peak resonance over a 4-h period using a UV/Vis spectrophotometer (Carey 50 Varian, Walnut Creek, CA). Aggregation leads to a reduction of the peak absorbance at peak resonance over a 4-h period using a UV/Vis spectrophotometer (Carey 50 Varian, Walnut Creek, CA).

**Cell culture for in vivo biodistribution and survival study:** Murine colon carcinoma cells (CT-26, ATCC; Manassas, VA) were grown at 37 °C, 5% CO_2 in RPMI medium supplemented with 4 mM l-glutamine, 1% penicillin, 1% streptomycin, and 10% FBS. Cells were detached from culture flasks with trypsin (0.02%) and EDTA (0.02%). The cells were resuspended in sterile PBS for inoculation into BALBc mice (Charles River, Willington, MA).

**In vivo studies:** BALBc mice were used under an approved protocol of the Institutional Animal Care and Use Committee at Rice University (Houston, Texas). 150,000 CT-26 cells suspended in PBS (25 μL) were injected subcutaneously in the right flank. Tumors were allowed to grow to a diameter of approximately 5 mm (=8–10 days). For biodistribution studies, mice with tumors (n = 3) were injected intravenously with approximately 1.0 × 10^10 PEGylated gold/silica nanoshells mL^-1 or 7.7 × 10^11 PEGylated gold/gold sulfide nanoparticles mL^-1, corresponding to an equal OD at the time of injection. Mice were euthanized via CO_2 asphyxiation 24 h after nanoshell injection. Tissue and blood were removed, processed, and sent to the Texas A&M (College Station, Texas) Nuclear Science Center 1 MW TRIGA research reactor for NAA.

**Gold concentration analysis:** To quantify the gold concentration, blank and tumor samples were irradiated along with precise calibration standards for 14 h. The irradiation position used in this study has an average neutron flux of approximately 1 × 10^13 s^-1 cm^-2. High-purity germanium detectors with nominal resolutions (FWHM) of 1.74 keV were used to quantify the 412 keV gamma line from ^199Au. The Canberra Industries OpenVMS alpha processor-based Genie-ESP software was used for acquisition and computation of gold concentrations.

**In vivo photothermal ablation of tumors:** Transdermal irradiation was accomplished by using an FAP-I System with a wavelength of 808 nm at a power density of 4 W cm^-2 and a spot size 5 mm in diameter for 3 min. To evaluate the efficacy of photothermal treatment using gold/gold sulfide nanoparticles, animals with tumors were injected through the tail vein with PEGylated gold/gold sulfide nanoparticle suspension (75 μL) at OD = 50, equivalent to approximately 7.7 × 10^11 particles mL^-1. Animals with tumors were placed in four different groups: gold/gold sulfide nanoparticles (treatment at 24 h, n = 7); gold/gold sulfide nanoparticles (treatment at 48 h, n = 6); gold/silica nanoshells; and an untreated group (n = 6). The control cohort of seven animals was treated with gold/silica nanoshells using the procedures outlined by O’Neal et al.

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