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

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Abstract

The development and optimization of near-infrared (nIR) absorbing nanoparticles for use as photothermal cancer therapeutic agents has been ongoing. We have previously reported on larger layered gold / silica nanoshells (~140 nm) for combined therapy and imaging applications. This work exploits the properties of smaller gold / gold sulfide (GGS) nIR absorbing nanoparticles (~35–55 nm) that provide higher absorption (98% absorption & 2% scattering for GGS versus 70% absorption & 30% scattering for gold/silica nanoshells) as well as potentially better tumor penetration. In this work we demonstrate ability to ablate tumor cells *in vitro*, and efficacy for photothermal cancer therapy, where in an *in vivo* model we show significantly increased long-term, tumor-free survival. Further, enhanced circulation and bio-distribution is observed *in vivo*. This class of nIR absorbing nanoparticles has potential to improve upon photothermal tumor ablation for cancer therapy.

Keywords

Near Infrared; nanoshells; gold / gold sulfide nanoparticles; photothermal therapy; 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 therapeutic effect. These exogenous chromophores have often included sensitizers such as protoporphyrin and indocyanine green (ICG) which absorbs the laser energy converting it to heat. [1–3]. Photothermal therapy via 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 the optical properties due to interaction of light with the free electrons of the gold surface, a phenomenon referred to as localized surface plasmon resonance, LSPR.

There have been a variety of different types of gold nanoparticles being explored which 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 use of nIR responsive nanoparticles is due to the low absorption and high transmission of light in this wavelength

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region for the majority of tissue components making it an ideal pairing to use for biomedical applications. [15] Further, the biocompatibility of gold and ability to conjugate biologically relevant molecules to its surface through the sulfur-gold interaction including polyethylene glycol (PEG) for stealth capabilities and antibodies for targeting make them 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] Using 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 one to design 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. [27] The optical properties of these materials were later explained to be due to a dielectric core / metal shell structure. [23] 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. [28–30] Moreover, for work in biomedical application, the particles appear to have a contiguous gold coat which allows for the surface conjugation of molecules as discussed above. Whereas the nIR-resonant gold / silica nanoshells used in therapeutic and imaging applications had an average diameter of 120 – 140 nm, these nIR-absorbing gold / gold sulfide nanoparticles typically have a diameter between 35 – 55 nm. [4,7–11,19] Using a Mie scattering theory simulation program, we calculated absorbing efficiencies of the gold / gold sulfide nanoparticles to be closer to 96 – 99% based on size measured by transmission electron microscopy (TEM); properties are summarized in Table 1. For gold / silica nanoshells used in our previous work with imaging and therapy the particle's 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. [7] Previous imaging results were based on use of optical coherence tomography (OCT) via scattering mode detection however, an absorption based mode of OCT detection would likely have to be employed for imaging with the gold / gold sulfide nanoparticles. [31–32]

Ideally, these smaller particles should provide additional benefits as a cancer therapeutic due to their smaller size, increased absorbing efficiency and ease of manufacture. It is well known that the leaky vasculature of tumors allow extravasation of macromolecules and nanoparticles, allowing therapy based on nanotechnology platforms. [33–35] Our postulate in this work is that these gold / gold sulfide nanoparticles will provide additional benefits for treatment of cancer tumors based on their size, allowing better bio-distribution and effective therapeutic benefits for the treatment of fast growing tumors. Modeling work by Decuzzi *et al.* suggests that particles with radii less of 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 [36] potentially enhancing the movement of these particles into the tumors. Further, experimental evidence suggests that nanoparticle uptake into cells is size dependent with the maximum uptake occurring for gold particles of diameters between 30–50 nm. [37]

2. Results & Discussion

2.1 Gold / gold sulfide nanoparticles for therapeutic application

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 reticulo-endothelial system (RES). Secondly, the gold colloid particles would easily bind with the same targeting mechanism as the nIR gold / gold sulfide nanoparticles thus increasing the use of such targeting agents like antibodies or aptamers, and 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 stealthing agents or antibodies unto the gold surface as previously described. [9–10]

Gold / gold sulfide nanoparticles were synthesized by self assembly as described by others using chloroauric acid and sodium sulfide. [23,27] The self-assembly process steps yielded a variety of particles in addition to the gold / gold sulfide nanoparticles; nanoprisms and rods are often seen in TEM, Figure 1-A. However, after separation, the majority of particles were spherical gold / gold sulfide nanoparticles and small amounts of remaining gold colloid, Figure 1-B. Figure 2 shows spectral data following a three step sequential centrifugation process to purify gold / gold sulfide nanoparticles from gold colloid. The sample curves after the first and second separation steps show the maximum peak at ~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, nIR absorbing fraction actually dominates the spectra and shows only a small amount gold colloid resonance. Gold / gold sulfide nanoparticles suspension purified by this centrifugation method was used in further studies to evaluate 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 gold / gold sulfide nanoparticles will yield a higher absorbing efficiencies than the larger gold / silica nanoshell counterparts. Calculations based on software developed by others using scattering theory and TEM size analysis shows the predicted values for absorbing efficiencies for both types of nanoparticles in Table 1. [26,38] Temperature of suspensions of gold / gold sulfide nanoparticles were measured as described in the methods section 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 was obtained with OD of 1.0 and 2.0 at 800 nm and are shown in Figures 3-A & B. Figure 3-A shows maximum temperatures attained and Figure 3-B shows initial rate of temperature increase. Temperatures quickly rose to a maximum of 100°C (samples were suspended in water) at 5 and 7 watts of total power, regardless of concentration. Final temperature difference shows statistical significant difference between OD = 1.0 and 2.0 at 2 and 5 W, although at 5 W the final temperature was close to the boiling point of water. Initial heating rates show significant increases for OD = 2.0 compared to OD = 1.0 at 2 and 5 W, however differences were not significant at the higher laser power of 7 W. These parameters allow for the ability to better tune laser power for treatment purposes when combined with *in vivo* nanoparticle concentration information. Indications are that future work with these nanoparticles could include lower laser power for equivalent OD of gold / gold sulfide nanoparticles as gold / silica nanoshells.

2.3 Photothermal Ablation *in vitro*

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 death of cells receiving exposure to nanoparticles + laser. Figure 4-A shows human prostate cells (PC3) that were incubated without nanoparticles and exposed to a nIR laser. The yellow circle shows the approximate location of the laser during exposure. The fluorescent stain confirms that the cells were all viable (green) after laser

exposure. Figure 4-B shows cells that were incubated with gold / gold sulfide nanoparticles and exposed to the laser, were killed within the laser spot, demonstrating the efficacy of gold / gold sulfide nanoparticles *in vitro*. This is consistent with previously seen photothermal damage reported by bare gold / silica nanoshells [11] and other nIR absorbing nanoparticles in *in vitro* tests. [14, 39]

2.4 Stabilization of Gold / gold sulfide nanoparticles with PEG for *in vivo* application

For *in vivo* applications, it is necessary to surface coat the particles with PEG to stabilize the suspension and increase biocompatibility to avoid RES clearance. The interaction of the gold particles with physiologic liquids will cause 1) agglomeration of the particles through ionic interaction with the charged gold surface, resulting in loss of particles resonance and 2) adsorption of proteins on the gold surface will allow 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 physiologic ionic conditions. The peak resonance is reduced as nanoshells aggregate in a strong ionic solution and can thus easily be monitored by spectroscopy. Similarly, and gold / silica nanoshells were prepared and stabilized exactly as described for previous O'Neal *et al.* [19] The reduction of the peak was monitored over four hours and the results shown in Figure 5 indicate that gold / gold sulfide nanoparticles are equally stable as PEG coated gold / silica nanoshells at equivalent conditions and thus allowed *in vivo* therapeutic testing. Samples which had no NaCl added showed no difference aggregation of nanoparticles after 4 hours.

For the controls, gold / silica nanoshells were made as previously described, PEGylated and sterilized for *in vivo* bio-distribution testing. [7,19] Nanoshells were surface coated with PEG, as above, to enhance circulation times and reduce immune response. PEGylation was accomplished by adding 20 μ L of 5 μ M PEG-SH to 1.5×10^{10} nanoshells / ml in DI water for a minimum of 8 hours. PEG-modified nanoshells were sterilized by filtration and subsequently concentrated by centrifugation and re-diluted with sterile phosphate buffered saline (PBS) for injections.

2.5 Enhanced bio-distribution tumor reduction with increased survival *in vivo*

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

We can further compare the difference of tumor uptake to RES removal by looking at the ratio of gold in the tumor over gold in the spleen + liver. This comparison is shown in Figure 6-C. The data shows 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 nanoparticles injected animals when compared against animals injected with gold / silica nanoshells. In conjunction with the data in Figure 6-B, showing higher gold content in the blood; gold / gold sulfide nanoparticles appear to avoid RES more effectively, while allowing a higher accumulation in the tumor. To better evaluate the gold / gold sulfide nanoparticles with photothermal therapy, accumulation time was increased to 48 hours based on the high availability of nanoparticles in the blood pool after 24 hours. Prior to therapeutic

photothermal ablation, the tumor site was shaved and swabbed with glycerol for index matching to aid the laser application.

During the photo-thermal ablation study, external tumor surface temperatures were spot checked during treatment with a handheld non-contact infrared thermometer OS 553XCF (Omega Instruments, Stamford, CT). These measurements showed gold / gold sulfide nanoparticles treatment group achieved a maximum tumor temperature of 46.1 ± 2.7 °C, after 24 hour accumulation time. When circulation/accumulation time was increased to 48 hours, 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 48 hour accumulation time vs. the 24 hr treatment group ($p=0.00005$). Kaplan - Meier survival curves are shown in Figure 7 for the 8 week period of the study. Survival of the gold / gold sulfide nanoparticles treated mice with 24 hour accumulation time was 71%, and for the 48 hour accumulation group, survival was statistically greater at 82%. Comparing the 48 hour survival using gold / gold sulfide nanoparticles and the 24 hour treatment using gold / silica nanoshells the survival was statistically equivalent based on Kaplan-Meier analysis.

3. Conclusions

Based on data presented above, gold / gold sulfide nanoparticles can be used as a photothermal therapeutic agent for cancer therapy in much the same way as 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 compared to the gold / silica nanoshell. Mie scattering theory calculation predicts 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 fewer particles could be used during treatment or alternatively lower laser power or time could be utilized during therapeutic laser administration. Further, the nIR resonant nanoparticles are produced by a single step self-assembly process, additional steps prepare these particles for *in vivo* usage and are similar to those use in all metallic nanoparticle preparation. The single step synthesis technique could potentially reduce costs associated with production of nanoparticles 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 bio-distribution data, which support the gold / gold sulfide nanoparticles can remain in circulation longer than gold / silica nanoshells, greater than 24 hours based on neutron activation analysis (NAA) and longer based on dynamic light scattering (DLS) (data not shown). Accumulation in liver, spleen and tumors showed that larger dose gold / gold sulfide nanoparticles actually accumulate in the tumor compared to the RES and as compared to gold / silica nanoshells. Survival data shows an effective photothermal therapy with survival greater than 80% for optimized accumulation times. With further optimization of laser power parameters and nanoparticle concentrations, gold / gold sulfide nanoparticles could provide an alternate therapeutic option that could prove very effective, combined with 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

For gold / gold sulfide nanoparticle synthesis, 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 concentration of 2 mM. 3mM sodium sulfide was prepared, and these reagents were aged in darkness 40–48 hours 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 minutes with a UV-Vis spectrophotometer (Carey 50 Varian, Walnut Creek, CA). Although the volumetric sweep produced particles with peak resonances from 750 through 950 only conditions yielding gold / gold sulfide nanoparticles with an extinction peak near 800 nm were scaled up 200X. Gold / gold sulfide nanoparticles were sized under transmission electron microscopy (JEOL FasTEM 2010 - TEM) at 100 kV.

Synthesis of Gold / silica Nanoshells—Briefly, silica cores were grown using the Stöber process, using tetraethyl orthosilicate (Sigma-Aldrich, Milwaukee, WI) in ethanol. Silica nanoparticles were sized under scanning electron microscopy (SEM; Philips FEI XL30) at 20 kV. The silica was functionalized with (3-aminopropyl) triethoxysilane (APTES, Sigma-Aldrich) allow for adsorption of gold colloid. Gold colloid was prepared to a size of 2–4 nm in the method of Duff *et al.* and aged 2–3 weeks at 4 °C. [40] The colloid was then concentrated 20X through 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 high yield of gold / gold sulfide nanoparticles was required to allow investigations *in vitro* as well as *in vivo*. As such, many forms of separation were investigated including size exclusion chromatography (SEC), electrophoresis and thin layer chromatography (TLC) all with extremely low yield. Finally, adequate yield was accomplished using standard centrifugation steps with refinements based on particle size and density in a multi-step procedure. Removal of gold colloid contaminant was accomplished by a 3 step sequential centrifugation procedure. 20 ml of gold / gold sulfide nanoparticles were centrifuged at 1100 g for 20 min. The pellet was saved and the supernatant was re-spun at the preceding conditions. The pellet from this second spin was combined with the first. The pellet was then re-suspended via sonication and diluted in 20 ml DI water (18 MΩ / cm). The centrifugation and collection procedure described above was repeated two more times to ensure highest removal of colloidal gold.

Temperature Measurement of Nanoparticle Suspensions—To measure temperature changes in response to nIR absorption by gold / gold sulfide nanoparticles, we varied the output of a laser directly shone onto samples diluted to different OD, 1.0 or 2.0 at 800 nm. Laser irradiation was accomplished using an Integrated Fiber Array Packet, FAP-I System, with a wavelength of 808 nm (Coherent, Santa Clara, CA). Data was logged for 3 minutes 20 seconds, 10 seconds collected prior to the laser being turned on and ~10 seconds after it was off. The maximum temperature was recorded at each condition for each sample and heating rate during the first 60 seconds of laser heating was calculated and tabulated. Laser output was varied between 1 and 10 W. The laser was on for 3 minutes and data for the sample and room temperature was recorded simultaneously using an Omegaette HH500 data recorder system (Omega Instruments, Stamford, CT). Testing was repeated with n=4 for statistical evaluation. Statistical significance was assessed by using a 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 media 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 EDTA (0.02%) for well plate seeding, and subsequent growth to confluent monolayer, for a cell ablation assay.

In vitro Photothermal Ablation—Bare nanoshells were used to allow binding to the cell surface by non-specific protein absorption. Gold / gold sulfide nanoparticles were added to cells in 24-well plates, to a total extinction at peak wavelength of OD = 1.0. This was accomplished by adding 500 µL of suspension at extinction OD = 2.0 to 500 µL of media to each well. Cells were incubated with nanoparticle suspensions for 6 hr. Wells were rinsed twice with PBS to remove unbound nanoshells and fresh phosphate buffered saline, PBS, added for the laser irradiation step. *In vitro* illumination was accomplished using an Integrated Fiber Array Packet, FAP-I System, with a wavelength of 808 nm (Coherent, Santa Clara, CA) at a power density of 80 W/cm² and a spot size of 1.2 mm diameter for 7 min on all samples except for “no laser” controls. Controls consisting of no nanoparticles with laser illumination as well as nanoparticles with no laser illumination were performed to verify the combined effects of the gold / gold sulfide nanoparticles + laser. After irradiation, cells were rinsed gently and PBS replaced with media. Cells were incubated for 4–6 hours following irradiation before evaluating viability.

Viability assessment—Viability was assessed using the Live/Dead Kit from Molecular Probes (Invitrogen, Eugene, OR). In this assay, calcein AM enters the cells and is cleaved in live cells by esterases to yield cytoplasmic green fluorescence. Dead cells, having compromised nuclear membrane, allow the ethidium homodimer-1 to enter and bind nucleic acids rendering a red fluorescence. Dilutions recommended by the manufacturer were used, 2.5 µL of calcein AM and 8 µL of ethidium homodimer-1 per 10 ml of PBS. Cells were incubated with the fluorescent stains for 45 minutes 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 100 µL of 5 µM PEG-SH, molecular weight 5 kDa (Nektar, Huntsville, AL) to 20 ml of a nanoshell suspension with an OD = 2.0 ($\sim 4.3 \times 10^{11}$ particles / ml) in DI water for a minimum of 8 hr at 4 °C. PEG-modified nanoshells were sterilized by filtration using 0.22 µm filter and subsequently lyophilized. To facilitate injection *in vivo*, gold / gold sulfide nanoparticles were re-suspended in sterile PBS, pH = 7.4, to an OD = 50 (7.7×10^{11} particles / ml).

Stability Testing

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

Cell Culture for In vivo Bio-distribution and Survival Study—Murine colon carcinoma cells (CT-26, ATCC; Manassas, VA) were grown at 37°C, 5% CO₂ in RPMI media supplemented with 4 mM l-glutamine, 1% penicillin, 1% streptomycin and 10% FBS.

Cells were detached from culture flasks using trypsin (0.05%) and EDTA (0.02%). Cells were re-suspended in sterile PBS for inoculation into BALBc mice (Charles River, Willington, MA) as described below.

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 25 μ L of PBS were injected subcutaneously in the right flank. Tumors were allowed to grow to a diameter of approximately 5 mm (~8–10 days). For bio-distribution studies, mice with tumors ($n = 3$) were injected intravenously with approximately 1.0×10^{10} PEGylated gold / silica nanoshells / ml or 7.7×10^{11} PEGylated gold / gold sulfide nanoparticles / ml, corresponding to equal OD at time of injection. Mice were euthanized via CO₂ asphyxiation 24 hours after nanoshell injection. Tissue and blood were removed, processed and sent to Texas A&M (College Station, Texas) Nuclear Science Center 1 MW TRIGA research reactor for neutron activation analysis NAA [41].

Gold Concentration Analysis—To quantify the gold concentration, blank and the tumor sample were irradiated along with precise calibration standards for 14 hours. The irradiation position used in this study has an average neutron flux of approximately $1 \times 10^{13} \text{ sec}^{-1} \text{ cm}^{-2}$. High purity germanium detectors with nominal resolutions (FWHM) of 1.74 keV were used to quantify the 412 keV gamma line from ¹⁹⁸Au. 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 using an Integrated Fiber Array Packet, FAP-I System, with a wavelength of 808 nm at a power density of 4 W/cm² and a spot size of 5 mm diameter for 3 min. To evaluate the efficacy of photo-thermal treatment using gold / gold sulfide nanoparticles, tumored animals were injected, through the tail vein, with 75 μ L of PEGylated gold / gold sulfide nanoparticles suspension at OD = 50, equivalent to approximately 7.7×10^{11} particles / ml. Animals with tumors were placed in four different groups: gold / gold sulfide nanoparticles (treatment at 24 hours, $n = 7$); gold / gold sulfide nanoparticles (treatment at 48 hours, $n = 6$); gold / silica nanoshells and an untreated group ($n = 6$). The control cohort of 7 animals were treated with gold / silica nanoshells using the procedures outlined by O’Neal [19]. For this group, 75 μ L of PEGylated gold / silica nanoshells at OD = 50 (approximately 1.0×10^{11} nanoshells) was injected, animals were treated with the laser following a 24 hour accumulation period. The survival of all animals was followed over eight weeks. Following therapeutic light administration, tumor dimensions were measured with digital calipers (Cen-Tech, Harbor Freight Tools, Camarillo, CA) every 2 – 3 days to monitor tumor growth or regression.

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Figure 1. TEM images of gold / gold sulfide nanoparticles, (A) before separation – note the presence of triangular nanoprisms, small colloid gold as well as the spherical gold / gold sulfide nanoparticles. (B), after separation using sequential centrifugation, there are very few colloidal gold particles remaining after separation.

Figure 2.

Nanoparticles made by self assembly show varying different peak resonances depending on concentration of reactants. A suspension made at a particular ratio of reactants (green curve) is subsequently separated by multiple centrifugation steps to produce a sample with minimal pure gold (530 nm resonance) contamination. Shown below are spectra of gold / gold sulfide nanoparticles after sequential centrifugation separation to remove gold colloid contaminants. The nIR peak of the as-made gold / gold sulfide nanoparticles shifts and narrows after each successive step and as similar species are collected during the process.

Figure 3. Temperature profiles of gold / gold sulfide nanoparticles obtained by heating with a laser and measured using thermocouple. (A) shows maximum temperature of samples after 3 minutes; (B) shows temperature rate increase during first 60 seconds, * $p < 0.05$.

Figure 4.

In vitro cellular ablation with bare gold / gold sulfide nanoparticles following incubation and laser application. Yellow circle indicates the laser spot; live/dead stain for viability shows dead cell as red while viable cells appear green. White bars = 200 microns.

Figure 5. Stability testing of PEGylated gold / gold sulfide nanoparticles in 1% saline (+NaCl), controls are without saline (−NaCl); a decrease from 100% indicates aggregation as the peak resonance diminishes during nanoparticle aggregation.

Figure 6.

(A) Bio-distribution of nanoparticles in tumored mice. Samples were taken after 24 hour circulation time. Data shows accumulation in the tumors for both types of nanoparticles and higher blood concentration after 24 hours for gold / gold sulfide nanoparticles; (B) Expansion of the y-axis, *p<0.05; (C) Proportion of gold in tumor compared to the liver and spleen for gold / gold sulfide nanoparticles and gold / silica nanoshells. There is a larger amount of gold in the tumor compared to spleen + liver for the gold / gold sulfide nanoparticles, showing better uptake in tumor compared to removal by the RES for these smaller nanoparticles, *p<0.05.

Figure 7.

Kaplan - Meier survival of mice following treatment with gold / gold sulfide nanoparticles and laser irradiation. There is a statistically significant increase in survival with 48 hour accumulation compared to 24 hour accumulation for the gold / gold sulfide nanoparticle treated mice and no difference for 48 hour gold / gold sulfide nanoparticle treated mice as compared to gold / silica nanoshell treated mice.

Table 1

Properties of gold / gold sulfide nanoparticles compared to gold / silica nanoshells.

	Gold / gold sulfide Nanoparticles	Gold shell / silica core Nanoshells
Overall Diameter	35 – 55 nm **	120 – 160 nm **
Core Diameter	30 – 40 nm *	100 – 120 nm **
Shell Thickness	3 – 6 nm *	7 – 20 nm *
Predicted Property	> 98 % absorbing efficiency	< 85 % absorbing efficiency

* calculated based on Mie scattering and measured spectra,

** measured by SEM and confirmed with Mie scattering theory.