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Nanoparticle-mediated photothermal therapy: A comparative study of heating for different particle types

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Abstract

Introduction—Near infrared absorbing plasmonic nanoparticles enhance photothermal therapy of tumors. In this procedure, systemically delivered gold nanoparticles preferentially accumulate at the tumor site and when irradiated using laser light, produce localized heat sufficient to damage tumor cells. Gold nanoshells and nanorods have been widely studied for this purpose, and while both exhibit strong NIR absorption, their overall absorption and scattering properties differ widely due to their geometry. In this paper, we compared the photothermal response of both nanoparticle types including the heat generation and photothermal efficiency.

Methods—Tissue simulating phantoms, with varying concentrations of gold nanoparticles, were irradiated with a near-infrared diode laser while concurrently monitoring the surface temperature with an infrared camera. We calculated nanoshell and nanorod optical properties using the Mie solution and the discrete dipole approximation, respectively. In addition, we measured the heat generation of nanoshells and nanorods at the same optical density to determine the photothermal transduction efficiency for both nanoparticle types.

Results—We found that the gold nanoshells produced more heat than gold nanorods at equivalent number densities (# of nanoparticles/mL), whereas the nanorods generated more heat than nanoshells at equivalent extinction values at the irradiance wavelength. To reach an equivalent heat generation, we found that it was necessary to have ~36x more nanorods than nanoshells. However, the gold nanorods were found to have two times the photothermal transduction efficiency than the gold nanoshells.

Conclusion—For the NPs tested, the nanoshells generated more heat, per nanoparticle, than nanorods, primarily due to their overall larger geometric cross section. Conversely, we found that the gold nanorods had a higher photothermal efficiency than the gold nanoshells. In conclusion, the ideal choice of plasmonic nanoparticle requires not only per particle efficiency, but also the *in vivo* particle targeting ability under study.

Keywords

Cancer; Gold Nanorods; Gold Nanoshells; Infrared Imaging; Photothermal Therapy; Photothermal Efficiency

Introduction

Thermal therapy has shown potential as a tool for the treatment of solid tumors, due to cancerous cells having a low thermotolerance, relative to noncancerous tissue, because of their poor blood supply (1–2). Current hyperthermia-based procedures, including microwave and radio frequency heating, use elevated temperatures (ranging from 42–47° C) to damage diseased tissue, such as cancerous tumors (3–4). Both techniques provide a broad heating of the tumor; however, the heating is not specific to the tumor, elevating temperatures in surrounding normal tissue. Accordingly, it is desirable to focus the heat to within the tumor periphery.

Photothermal therapy, a targeted approach of cancer hyperthermia therapy, specifically directs heat into the tumor. In this procedure, an endogenous or exogenous chromophore is excited by laser radiation resulting in an emission of thermal energy. Recent developments in nanotechnology have produced a class of metal plasmonic nanoparticles with strong near-infrared (NIR) absorption. Plasmonic nanoparticle-mediated photothermal therapy has been shown to kill cancer cells *in vitro*, by such processes as protein denaturation and cellular membrane disruption, and reduce tumor size *in vivo*. (5–12) The plasmonic nanoparticles primarily utilized in photothermal therapy are gold nanoparticles including nanospheres, nanoshells, nanorods, and nanocages. (5,7,10,13–17)

Gold nanoparticles (GNPs), a class of plasmonic nanoparticles, preferentially accumulate within solid tumors due to their design. The enhanced permeability and retention effect (EPR) is a property found in most solid tumors, which maintains that molecules within a certain size range accumulate in the tumor site at a higher rate than normal tissue. (18–20) Tumors enhance proliferation by inducing angiogenesis to increase the number of nutrients that reach the cancer cells. The new blood vessels develop abnormally due to their rapid formation. Consequently, there are fenestrations (0.5 – 1.5 μm) that form between the endothelial cells lining the blood vessel. GNPs, designed with size dimensions smaller than these fenestrations, “leak” through the newly formed tumor blood vessels into the tumor tissue at a much higher rate than normal tissue. (21) Furthermore, the gold surfaces provide facile conjugation of targeting antibodies to actively target cancerous cells. Several cancer biomarkers, including anti-EGFR and anti-HER2 antibodies, have been used to target cancer cells successfully *in vitro*. (10,22–24)

GNPs provide desirable optical properties for photothermal therapy. Gold, when fabricated on the nanoscale, exhibits enhanced surface plasmon resonance (SPR). Surface plasmons are the electron oscillations on the particle surface that resonate at the plasmon resonant frequency. The SPR frequency strongly depends on the GNP geometry, and can be tuned within a broad range from the visible to the NIR of the electromagnetic spectrum. The tissue optical window is in the NIR at which there is minimal absorption of endogenous molecules (hemoglobin, melanin, water) and largest penetration depth of the laser light. As a result, the absorption maximum of many gold nanoshells and nanorods is tuned to the NIR. (25–26)

The significant optical properties for comparing the photothermal abilities of single absorbers, such as GNPs, include the absorption efficiency and absorption cross-section. The absorption cross-section (σ_a) is related to the geometric cross-section (σ_g) by the absorption efficiency (Q_a):

$$\sigma_a = Q_a * \sigma_g \quad (1)$$

Both of these values indicate the absorption capabilities with the absorption cross-section taking into account the absorber size.

The geometry of GNPs dictates their optical properties. Nanoshells are spherical GNPs composed of a silica core and gold shell, usually larger than nanorods as shown in Figure 1. The SPR peak is tuned by varying the silica core radius and gold shell thickness. Due to their size, we expect nanoshells to have a larger absorption cross-section than nanorods. Nanorods, however, are smaller pill-shaped nanoparticles (Figure 1) and have significantly different optical properties. Owing to its pill shape, gold nanorods have surface plasmons in both the longitudinal and transverse directions, allowing for two SPR frequencies and two absorption peaks. The peak in the visible region correlates to the smaller, transverse SPR frequency in the visible light range and is relatively untunable. The second peak, in the NIR, corresponds to the larger, longitudinal SPR frequency and is tuned by varying the nanorod aspect ratio (ratio of length to width). (26–28)

Comparisons between nanoshells and nanorods have been studied previously (29–32). They have determined that nanorods have a higher temperature increase per mass gold (29) and that the absorption cross-section of nanorods is two times higher than nanoshells (30). Furthermore, several studies have been published regarding the photothermal efficiency of GNPs (31–35). Both Cole et al. and Cheng et al. have compared gold nanoshells with nanorods but come to differing results. The Cheng study has a different formulation for determining the photothermal efficiency than we utilize in the current study, which is based on number density of the GNPs as opposed to optical density and finds that nanoshells are more efficient (32). Whereas, the Cole study follows a similar efficiency definition as we discuss in the current study, utilizing an energy balance equation to derive the efficiency based on previous work, to find that nanorods were more efficient than nanoshells (31,33–34).

However, these studies disregard the multiple methods of comparison used to evaluate nanoparticles, as well as the size factor that greatly influences the GNP optical properties and the photothermal properties. In this study, we aim to compare the capability of nanoshells and nanorods to generate heat based on their number density, mass gold and optical density for photothermal therapy. We simulated the optical properties of multiple sizes of nanorod and nanoshell, including our sized GNPs, using the discrete dipole approximation (DDA) and Mie theory, respectively. The heating profiles were measured of GNPs suspended in tissue simulating phantoms during a photothermal heating cycle and compared to determine the heat generation when nanoshells and nanorods were at equivalent number densities. We determined that nanoshells generated more heat than nanorods at equivalent number densities and that it was necessary to have ~36 times the number of nanorods as nanoshells to generate the same amount of heat. However, at equivalent optical densities we determined that nanorods generate ~2 times as much heat as nanoshells and calculated that the nanorods have twice the photothermal transduction efficiency as nanoshells. We believe that these results can be a comprehensive aid in determining the GNP for photothermal therapy.

Experimental Methods

Nanoparticle Fabrication

Nanospectra Biosciences, Inc. (Houston, TX) provided concentrated nanoshell and nanorod solutions. The fabrication process for both GNPs has been discussed in detail previously. (36–37) To determine the GNP size, we imaged (Figure 1) dilute solutions using transmission electron microscopy (TEM). The dimensions were averaged over 300 individual particles, for both GNPs, measured in the TEM image using ImageJ (NIH Bethesda, MD). The nanoshells were found to have a total outer diameter of $145 \text{ nm} \pm 11.7 \text{ nm}$, with a silica core diameter of 120 nm (Figure 1). The nanorod dimensions were measured to be $25.8 \text{ nm} \pm 3.8 \text{ nm}$ by $7.4 \text{ nm} \pm 0.9 \text{ nm}$, with an aspect ratio of 3.5 and an

effective radius of 6.18 nm (Figure 1). The effective radius, r_{eff} , is defined as the radius of a sphere with an equivalent volume (V) as the nanorod:

$$r_{eff} = \left(\frac{3V}{4\pi} \right)^{\frac{1}{3}} \quad (2)$$

During the fabrication process, Nanospectra Biosciences modified the GNP surface with polyethylene glycol (PEG) to mimic the GNPs that are used *in vitro* and *in vivo*. To measure the extinction spectra and optical density for the nanoparticle solutions, we used a UV-Vis Spectrophotometer (Beckman Coulter DU720, Brea, CA).

Nanoparticle Optical Simulations

Using Mie theory and the discrete dipole approximation (DDA), we simulated nanoshell and nanorod optical properties, respectively. The dielectric function for bulk gold, determined by Johnson and Christy (J&C), was used for the nanoshell and nanorod simulations. (38) The dielectric function for bulk gold was corrected based on the small size of both the nanoshells and nanorods. (39) The size correction includes an additional damping term in the dielectric function due to collisions of conduction electrons to the GNP surface. (39–40)

We used a DDA program, DDSCAT 7.0, to model nanorod optical properties using the lattice dispersion relation. (41) The DDA program approximates the scattering and absorption of any arbitrary geometry by modeling the target as a series of polarizable points. The nanorod geometry was modeled as a cylinder surrounded by water. (27–28)

The DDSCAT program divides the particle into I_d polarizable points (dipoles), which are on a lattice described by an interdipole distance d , based on the particle volume, V :

$$V = I_d d^3 \quad (3)$$

The number of dipoles is chosen by the following accuracy criterion:

$$|m|kd < 0.5 \quad (4)$$

where m is the particle's complex refractive index and k is the angular wave number

($k = \frac{2\pi}{\lambda}$), which all simulations performed in the DDSCAT software satisfy.

For the nanoshell optical properties, we utilized the Mie solution, because it is an exact solution to Maxwell's equations for concentric spheres. The nanoshells were modeled as an individual, gold-coated silica particle surrounded by water. To perform the calculations we used Maetzler's MATLAB Mie Theory code for coated spheres. (42) The nanoshell dielectric function was divided in two parts: (1) the inner core of silica was derived by Malitson and (2) the same size-corrected dielectric function for the gold shell as derived by J&C. (38,43)

With the nanorod and nanoshell absorption and scattering efficiency from DDA and Mie Theory we were able to determine the expected absorption and scattering coefficient (μ_a and μ_s):

$$\mu_a = \sigma_a N = \sigma_g Q_a N \quad (5)$$

$$\mu_s = \sigma_a N = \sigma_g Q_a N \quad (6)$$

where σ_a and σ_g are the absorption and scattering cross-sections (cm^2) and N is the GNP number density ($\#/\text{mL}$). The concentration of GNPs is known; thus, only the absorption cross-section needs to be determined, which is equivalent to the multiplication of σ_g , the geometric cross-section (cm^2), and Q_a , the absorption efficiency (Eq. 1). The absorption efficiency represents the ratio of the absorption and geometric cross-sections.

Experimental Procedures

We performed two sets of experiments to determine the concentration equivalency factor (ξ) in tissue phantoms and the photothermal transduction efficiency, respectively (defined later in this study). To simulate the environment within tissue, the GNPs in the experiments for the concentration equivalency factor were suspended in tissue phantoms at multiple concentrations. To calculate the photothermal transduction efficiency, it was necessary to have the sample of GNPs suspended in a non-absorbing and non-scattering media to simplify the solution for the photothermal transduction efficiency. As shown in Equation 8, the OD was included to correspond to the extinction of specifically the GNPs and not the sample as a whole. Additionally, scattering processes would increase the fluence at the solution surface, but also would attenuate the fluence deeper in the solution, which could cause artificial increases or decreases in photothermal efficiency. As a result, we conducted the experiments on GNPs in a 10% trehalose solution, which has the same optical characteristics as water in the wavelength range of interest.

Concentration Equivalency Factor Experimentation

The concentration equivalency factor defines the relationship between the concentrations of nanorods and nanoshells when heat generated from both solutions are equivalent. To determine this value we have to find the amount of heat generated from nanorods and nanoshells at multiple concentrations, to find the concentration of nanoshells and nanorods at which the generated heat is equivalent. The temperature change at the end of the heating cycle for the nanoshells, dT_{NS} , and nanorods, dT_{NR} , indicate the heat generated by each particle type during the procedures. The ratio of the temperature change at a fixed concentration of nanoshells, dT_{NS} , ($1x$ or $2x$) to the temperature change of varying concentrations of nanorods, dT_{NR} , ($5x - 80x$). Thus, the concentration equivalency factor ($\xi = dT_{NS}/dT_{NR}$) is the ratio of the determined concentrations, respectively.

For this experiment, we prepared tissue phantom solutions composed of diluted 10% intravenous fat emulsion (Liposyn II 10%, *Abbott Laboratories*, North Chicago, IL) and 90% dH_2O , which mimicked the scattering properties of tissue. This dilution resulted in a solution with similar scattering values to normal human epithelial tissue, $\mu_s' = 1 \text{ mm}^{-1}$, at the irradiation wavelength.(44) The experimental control was a sample with the intravenous fat emulsion dilution without GNPs. The GNPs were suspended in the tissue phantom solution at multiple concentrations including: $1x$, $2x$, $5x$, $10x$ for the nanoshells and $5x$, $10x$, $20x$, $40x$, and $80x$ for the nanorods, where x is the nominal physiological concentration. The physiological value, $x = 1.14 \times 10^9 \text{ GNPs/mL}$, is based on previous biodistribution studies of nanoshells in tumors in mice after intravenous injection. (45–46) The corresponding concentration for nanorods is unknown; hence, in this study we determined the concentration of nanorods at which the same amount of heat is generated as nanoshells at the physiological concentration.

Photothermal Transduction Efficiency Experimentation

The photothermal transduction efficiency denotes a value for the efficacy of gold nanoparticles converting absorbed light radiation into thermal energy. For determining the photothermal transduction efficiency, we prepared a set of different samples. In this study, the GNPs solutions were suspended in 10% trehalose at a set optical density of 0.25 at the irradiation wavelength (808 nm). The control for this experiment was a solution of 10% trehalose. All GNP samples were pipetted into a 96-well plate (BD Falcon, Franklin Lakes, NJ).

The calculation of the photothermal transduction efficiency for both nanoshells and nanorods was conducted in a similar method to Roper and coworkers.(33) Using an energy balance for our experimental system, we determined the photothermal transduction efficiency. The total energy balance is:

$$mC_p \frac{dT}{dt} = E_{in,np} + E_{in,sample} - E_{ext} \quad (7)$$

where m and C_p are the solvent (10% trehalose) mass and heat capacity and T is the sample temperature. $E_{in,np}$ is the energy input based on the heat dissipated by the nanoparticles in the solution:

$$E_{in,np} = I(1 - 10^{-OD})\eta \quad (8)$$

where I is the laser power, OD is the sample optical density at the excitation wavelength of 808 nm, and η is the photothermal transduction efficiency, fraction of absorbed light converted into heat. $E_{in,sample}$ is the energy input based on the heat generated by the solvent and sample well, which was measured independently following the same method of solving for E_{ext} with the experimental control of trehalose solution. E_{ext} is the energy output based on the external heat flux between the sample and the surroundings:

$$E_{ext} = hA(T_{sample} - T_{amb}) \quad (9)$$

where h is the heat transfer coefficient, A is the sample well surface area, T_{sample} is the temperature of the sample, which in our case is the steady state maximum temperature, and T_{amb} is the ambient room temperature.

We determined the quantity, hA , by measuring the sample cooling after the laser was turned off. When the laser is turned off Equation 7 becomes:

$$mC_p \frac{dT}{dt} = -E_{ext} = -hA(T_{sample} - T_{amb}) \quad (10)$$

Rearranging Equation 10:

$$dt = -\frac{mC_p}{hA} \frac{dT}{(T_{max} - T_{amb})} \quad (11)$$

After integrating Equation 11:

$$t = -\left(\frac{mC_p}{hA}\right) \ln(T_{max} - T_{amb}) \quad (12)$$

From this equation, we can determine the characteristic rate constant:

$$\tau_{out} = \frac{mC_p}{hA} \quad (13)$$

such that:

$$T_{max} - T_{amb} = \exp\left(-\frac{t}{\tau_{out}}\right) \quad (14)$$

Thus, from the cooling data, we can determine the characteristic rate constant and solve for the heat transfer coefficient. At the steady state temperature, the rate of heating is equal to the rate of heat transfer out of the system:

$$E_{in,np} + E_{in,sample} = I(1 - 10^{-OD})\eta + E_{in,sample} = E_{ext} = hA(T_{max} - T_{amb}) \quad (15)$$

As a result, the photothermal transduction efficiency can be solved for directly from the steady-state temperature experiments:

$$\eta = \frac{hA(T_{max} - T_{amb}) - E_{in,sample}}{I(1 - 10^{-OD})} \quad (16)$$

Instrumentation

We used an InSb infrared (IR) camera (FLIR Systems SC4000, Boston, MA) with a spectral range of 3–5 μm , to measure the heating characteristics of the sample. The IR camera was placed directly above the 96-well plate and a laser was mounted at an angle of approximately 30° such that the sample was irradiated completely while not interfering with the imaging (Figure 2). We used a fiber-coupled NIR ($\lambda = 808 \text{ nm}$) diode-laser (ThorLabs L808P1WJ, Newton, NJ) rated for up to 1 W of power. Using a biconvex lens (Newport Corp., Irvine, CA) the laser spot was focused to 6 mm in diameter, approximately the same diameter as the well.

Photothermal Experiments

Each sample was illuminated at a constant fluence rate of 2 W/cm². Prior to each experiment: we sonicated and mixed the sample thoroughly to create a uniform distribution of GNPs, and confirmed the laser power using a standard power meter (Newport Corp., Irvine, CA). We selected a region of interest at the laser spot center and solution to measure the sample heating and the cooling after the laser was turned off, using the ThermoVision software (FLIR Sys., Boston, MA). For the tissue phantom experiment, we heated all samples for 5 minutes, to mimic current *in vitro* and *in vivo* photothermal studies, and let the sample cool to room temperature for 10 minutes after the laser was turned off. For the photothermal transduction efficiency experiment, we heated the samples to steady state, which occurred after approximately 25 minutes of irradiations and let the samples cool to room temperature for around 20 minutes. All comparisons and analyses were performed in MATLAB (The Mathworks Inc., Natick, MA).

Results

The GNP optical properties were determined using simulations with the measured size parameters as input. Figure 3a illustrates the simulated optical efficiency spectra and

measured extinction spectrum for GNS. The resonance peak wavelength was found to be the same for both the simulated and measured extinction spectra (780 nm) indicating the validity of the Mie theory simulation. The simulation (Figure 3a) shows the nanoshells are highly scattering (~75% of the extinction efficiency), and they have an absorption efficiency of 1.9 at the laser wavelength of 808 nm. Given the absorption efficiency, the calculation for the absorption cross-section follows from Equation 1, with a value of $3.1 \times 10^{-10} \text{ cm}^2$.

Figure 3b illustrates the simulated absorption efficiency spectra as compared to the measured gold nanorod extinction spectrum. The simulated absorption efficiency shows a resonance peak wavelength that is red-shifted (812 nm) in comparison to that wavelength from the measured extinction spectrum (770 nm). We varied the nanorod length to change the aspect ratio from 3.5 to 3.0, resulting in a peak resonance blue shift. The aspect ratio that best matches the measured value was approximately 3.3. In all cases, the absorption efficiency accounted for approximately 99.8% of the extinction efficiency. These results suggest that nanorods in this size range are completely absorption dominated, and after simulating multiple aspect ratios we have found an absorption efficiency that is a good indicator of the individual nanorods used in this study. We found the absorption efficiency value to be 4.9 for the nanorods, and utilizing Equation 1 the absorption cross-section was calculated as $8.7 \times 10^{-12} \text{ cm}^2$.

We measured the heating response of both gold nanoshells and nanorods in tissue simulating phantoms to determine the ξ factor. Figure 4 shows the temperature profiles for the nanoshells and nanorods for 5 minutes of heating and the subsequent cooling cycle. Both GNP types exhibit a significant temperature rise in comparison to the experimental control (0x) when illuminated by NIR light. The temperature change (after 5 minutes of heating) ranged from approximately 12°C to 42°C for concentrations of 1x to 10x of gold nanoshells. However, for the gold nanorods, the temperature change (after 5 minutes heating) ranged from approximately 3°C to 17°C for concentrations of 5x to 40x. The temperature change increased as the concentration increased.

To determine the ξ factor, interpolation was performed for all nanorod concentrations between 5x and 80x using the power law (Figure 5). For the 1x nanoshell concentration, it is shown that the nanorod concentration when the ratio is equal to one is approximately 36. For the 2x nanoshell concentration, the nanorod concentration for heat generation equivalence is shown to be approximately 72. Therefore, we can conclude for these sized GNPs, to generate the equivalent heat, it is necessary to have a nanorod concentration 36 times the concentration of nanoshells. As with the per GNP heating abilities, the ξ factor is as a result of the larger nanoshell absorption cross-section ($3.1 \times 10^{-10} \text{ cm}^2$). In fact, when compared to the nanorod absorption cross-section, the nanoshell absorption cross-section is approximately 35 times larger ($3.1 \times 10^{-10} \text{ cm}^2 / 8.7 \times 10^{-12} \text{ cm}^2$).

To determine the photothermal transduction efficiency we fit equation 14 to the cooling data to determine the characteristic rate constant and the heat transfer coefficient (Figure 6) based on Equation 13. Both samples of GNPs were diluted to an optical density of 0.25 (inset of Figure 7) at 808 nm in a 10% trehalose solution. Using Equation 16, the photothermal transduction efficiency for nanoshells and nanorods was determined to be ~25% and ~50%, respectively (Figure 7). Thus, nanorods are shown to be twice as efficient as nanoshells at converting the light radiation into thermal energy.

Discussion

In this study, we simulated the optical properties of our sized nanoshells with Mie theory and determined an approximation of our sized nanorod optical properties with DDA (Figure

3). The nanoshells are scattering dominated and have a much lower absorption component than nanorods, whereas the nanorods were completely absorption dominated. However, to accurately compare the absorption properties of both GNPs, the overall geometric cross-section needs to be incorporated to determine the absorption cross-section. We found the nanoshell absorption cross-section to be approximately 35 times larger than the nanorod absorption cross-section. Thus, we would expect the nanoshells to generate more heat than nanorods per particle when illuminated with NIR light, despite their lower absorption efficiency.

Based on this study and the study performed by Ungureanu and coworkers, it is apparent that DDA does not provide an accurate approximation to the optical properties of nanorods. (40) In their study, they compared the simulated results with the measured extinction spectra of four different sized nanorods. The authors used four different shapes (cylindrical, hemispherically-capped cylinder, ellipsoid, and rectangular) and three different size-corrected dielectric functions for gold (J&C, Palik and Weaver) to model the gold nanorods. (38,47–48) Based on their results, the authors found that the size-corrected J&C and cylindrical shape gave the best results, but, it still was not completely accurate. However, we still performed the DDA in this study with the size-corrected J&C dielectric and modeling the nanorod as a cylinder as shown in Figure 3b to show that the nanorod optical properties are absorption dominated.

There are a few areas of loss that are not accounted for in our model and experiment. Possible losses of heat generation include the metal distribution around the GNP surface. When heated, the particle may elongate or compress, modifying the GNP geometry, which in turn may alter the optical properties and heating characteristics slightly. Additionally, since the temperature at the GNP surface cannot be determined, it is possible that there are heating effects in the direct surroundings of the GNPs. Even though the steady-state temperature is lower than boiling point due to losses to convection between the solution and air, the GNP surface temperatures can possibly reach boiling point. Consequently, phenomena such as localized evaporation could take place, which can cause cavitation bubbles. These steam pockets and cavitation bubbles may affect the GNP surroundings optically and physically, which is not taken into account in the experiment.

In our photothermal tissue phantom experiments, the heat generated by different concentrations of both nanoparticle types was recorded. From these results, it was determined that the temperature increase was substantial and, most likely, would cause damage to cells as in all previous photothermal studies. Additionally, we observed that per individual GNP, the nanoshells, indeed, had the larger temperature change.

However, if we compare the GNP heating based on their respective mass of gold, we see a different result. The mass per GNP of nanoshell is $1.34 \times 10^{-8} \mu\text{g}$ and for nanorod is $1.91 \times 10^{-11} \mu\text{g}$. At the 80x concentration of nanorods there is 0.58 μg gold in solution, corresponding to a $\sim 25^\circ\text{C}$ temperature change. Compare this data with the 1x concentration of nanoshells, which corresponds to a mass of 5.03 μg gold in solution, accounting for only the shell, and a temperature change of $\sim 14.8^\circ\text{C}$. Even at approximately 10 times smaller than the sample with the lowest mass gold of nanoshells used in this study, the sample with the highest mass gold of nanorods has a significantly greater increase in temperature. Therefore, the nanorods can generate more heat per μg gold in comparison to nanoshells.

This study was based on the known physiological dose of nanoshells that reach the tumor site *in vivo*. The heat generated by this concentration of nanoshells has been shown to be enough to cause cell death and a significant reduction in tumor size. In current literature, the same value is unknown for nanorods. As a result, the heat generation based on the

physiologic dose of nanoshells was chosen as the low end threshold for determining the equivalent concentration of nanorods necessary to cause tumor ablation. According to the observed, to generate the equivalent amount of heat it is necessary to have 36 times the concentration of nanorods as the concentration of nanoshells, for these specific sizes of nanoshells and nanorods. When compared to the ratio of the simulated absorption cross-sections, it is remarkably similar.

Our obtained data suggests that to achieve the same heating, a substantially larger number of nanorods would need to be present at the tumor site. Since the two GNPs have different sizes and shapes, likely there will be differences in the number of GNPs that reach the tumor sites. The comparative targeting efficiency of nanoshells and nanorods is unknown; therefore, it is possible that at similar doses to nanoshells, more particles can reach the tumor site. Effectively, if nanorods can reach the tumor site at a rate of 36 times more than nanoshells, then this would negate the lower heat generation, and it would be more efficient in converting the available laser power into heat. The choice of GNP may be an application dependent process. For example, it may be desired to have smaller GNPs, for more effective targeting or larger GNPs, for more heating with fewer particles. Further studies need to be performed to determine the targeting efficiency of both GNP types.

Another important finding in this study was the photothermal transduction efficiency. For this experiment, we heated both GNPs at the same optical density (at the illumination wavelength) to steady state and let cool to room temperature. The nanorods had a much larger temperature increase than nanoshells in this experiment as predicted by the simulations. The cooling data was modeled with a single exponential fit to determine the sample characteristic rate constant. Using this value, we determined the heat transfer coefficient, which in this model considers energy losses to the surroundings. Furthermore, we also modeled the control sample cooling data, which was heated to steady state, to determine the energy dissipated by the sample well. For our sized GNPs, it is important to note that these nanorods were found to be twice as efficient at transducing light radiation into thermal energy as nanoshells.

Conclusion

Nanoparticle heating dynamics depend on a combination of factors that include their overall geometry and optical absorption efficiency. Ultimately, the appropriate choice of nanoparticle will depend on the application and the particle's targeting kinetics. In this study, we determined the optical absorption efficiency for nanorods and the adjusted optical absorption efficiency for nanoshells per particle. Yet, due to the larger size of nanoshells, their absorption cross-section is much larger than nanorods. As a result, the nanoshells have a much larger increase in temperature than the nanorods on a per particle basis. Additionally, a higher nanorod concentration is required for equivalent heating of nanoshells, for the GNP sizes tested. However, in comparing the effectiveness of converting light radiation into thermal energy, the nanorods are twice as efficient. Both GNPs have utility in photothermal therapy depending on each particle's ability to reach the tumor site. Therefore, with the information from this study, an educated choice can be determined on which GNP to use for photothermal therapy *in vivo* based on the respective GNP targeting efficiency.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.