Gold Nanostructures with Tunable Photothermal Properties for Cancer Treatment

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Introduction:

Gold nanoparticles (AuNPs) have a variety of properties that make them favorable for biomedical applications, including the low reactivity and low cytotoxicity intrinsic to gold [1]. Photothermal therapy is based on the tunable electromagnetic absorption characteristics of AuNPs in a phenomenon known as localized surface plasmon resonance (LSPR). The absorption peak can easily be tuned into the near-infrared (NIR) region by controlling the shape of the NP. The NIR region, covering wavelengths from 650-900 nm, is a region of low absorption for both water and hemoglobin in soft tissues, allowing for photothermal therapy to be useful in biological settings [1]. We have synthesized and compared the photothermal properties of three different types of AuNPs: nanohexapods [2], nanocages, and nanorods. By studying the photothermal properties and experimental *in vitro* and *in vivo* capabilities of these three classes of nanostructures, we gain a sense for their potential as agents for cancer treatment.

Experimental Procedure:

Nanoparticle Synthesis, Characterization, and Use in Photothermal Solution Studies. In order to take advantage of the NIR region, gold nanohexapods, nanocages, and nanorods were synthesized using the reported methods with their absorption peaks tuned to 800 nm as measured with ultraviolet-visible (UV-Vis) spectroscopy (Figure 1). Solutions of nanohexapods, nanocages, and nanorods at various concentrations were irradiated with laser at a wavelength of 808 nm and data was captured with a thermal imaging camera.

Influx and Viability Studies ***in vitro*****.** Nanostructures were also prepared for application *in vitro* and *in vivo* by process of PEGylation. Melanoma cancer cells were cultured and then incubated with our three types of gold nanostructures. Experiments determined the rate of influx of gold nanoparticles into melanoma cells, as influx was halted and the gold concentration within cells determined by inductively coupled plasma mass spectrometry (ICP-MS) after various incubational time periods. In addition, experiments of viability were conducted examining the cytotoxicity of various concentrations of nanoparticles. After a 24-hour incubation period, estimates of viability were made using an MTT assay.

Photothermal Studies ***in vivo*****.** Preliminary photothermal studies *in vivo* were conducted by directly injecting a suspension of gold nanostructures into a tumor-bearing mouse. Tests compared an injection of nanohexapods with an injection of a saline control. In addition, the tissue distribution of

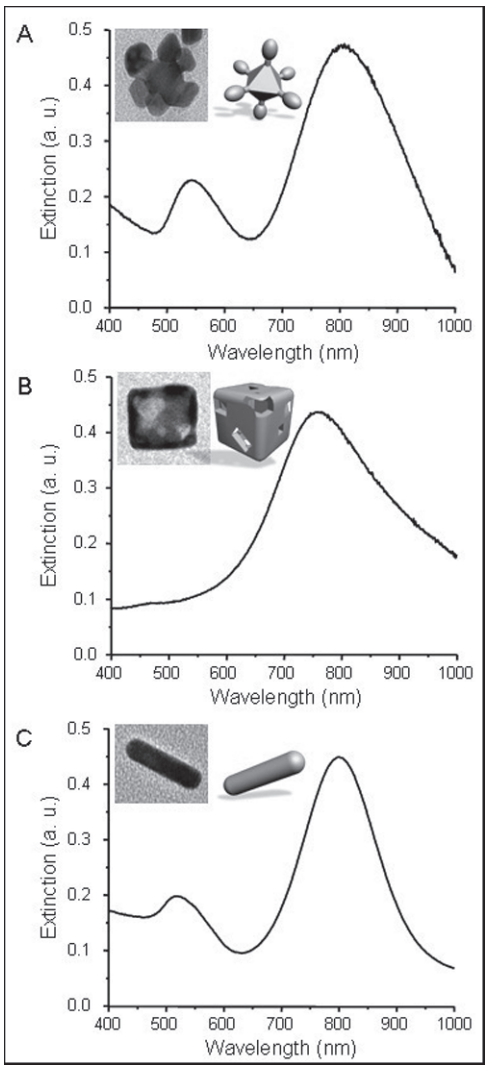

Figure 1: UV-Vis spectra, TEM images, and models of gold nanohexapods (A), nanocages (B), and nanorods (C).

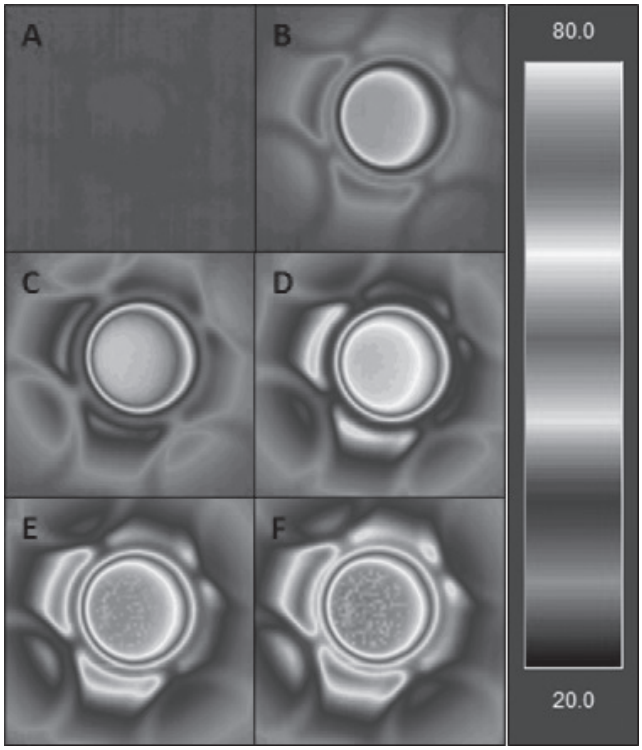

Figure 2: Thermal images of gold nanohexapods (0.4 nM in concentration) after 0 (A), 45 (B), 90 (C), 135 (D), 180 (E), and 225 s (F) of laser irradiation. The temperature is in degrees celsius.

AuNPs was measured 12 hours and 48 hours after tail-vein injection into a mouse with ICP-MS.

Results and Discussion:

Photothermal studies of gold nanohexapods showed that even a low concentration at 12.5 pM was sufficient to cause a rapid temperature increase of a few degrees. At a higher concentration of 0.4 nM, the temperature increased to 60°C after only 3 min of laser irradiation (Figure 2). In addition, photothermal characterization on all three types of gold nanostructures indicated comparable photothermal efficiency for nanohexapods, nanocages, and nanorods.

Our cellular uptake results demonstrated that gold nanohexapods had significantly higher levels of uptake than both nanocages and nanorods after an incubation time of 20 h (Figure 3). While the exact mechanism of influx is unknown, the shape of a nanoparticle largely determined the rate at which it was picked up by the cell. Also, our results indicated that gold nanohexapods displayed no apparent cytotoxicity even up to concentrations of 1 nM, while nanocages and nanorods affected a cell viability of approximately 20% lower than nanohexapods at a comparable concentration. We believe this was because the silver present in the gold-silver alloyed nanocages was toxic to living cells, while one of the reagents, hexadecyltrimethylammonium bromide (CTAB), involved in nanorod synthesis was particularly difficult to completely remove from the nanorod product and was also toxic to cells.

Our preliminary studies *in vivo* showed that only five minutes of laser irradiation raised the tumor temperature to over 60°C in our nanohexapod trial, while the temperature change recorded during our injection of saline was insignificant. Also, tissue distribution studies indicated that 48 h post injection, nanohexapods had the highest level of accumulation in tumor among the three types of nanoparticles at a rate of nearly 15%. While the largest proportions of nanohexapods were found in the liver and the spleen, nanoparticle accumulation in the tumor was significant and is based on the enhanced permeability and retention (EPR) effect.

Conclusions and Future Work:

Gold nanohexapods, when compared with nanorods and nanocages, were shown to have comparable or higher levels of photothermal efficiency, cellular uptake, viability, and accumulation in tumor. As such, they have great potential for use in cancer therapy. However, a large-scale method for nanohexapod synthesis still needs to be developed. In addition, a detailed investigation of the *in vivo* toxicity of gold nanohexapods and the photothermal efficiency of tail-vein injections would contribute greatly to our understanding of nanohexapods as photothermal agents.

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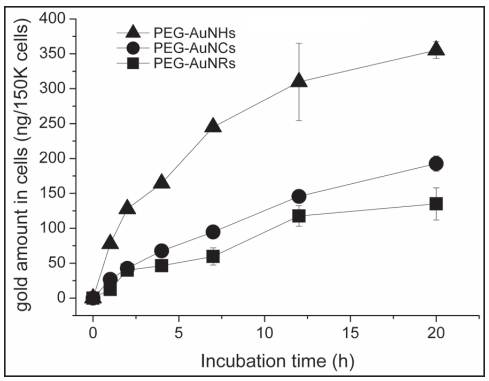

Figure 3: Influx results for PEGylated gold nanohexapods, nanocages, and nanorods.