

Multifunctional theranostic gold nanoparticles for targeted CT imaging and photothermal therapy

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Gold nanoparticles have emerged as some of the most extensively utilized nanoplatforms for the diagnosis, imaging, monitoring and treatment of malignant diseases. In particular, in computed tomography (CT) imaging and in therapy (PTT), the exploitation of the various, advantageous properties of gold nanoparticles have resulted in numerous advances in each of these fields. The purpose of this review is to assess the status of gold-nanoparticle mediated CT and PTT, highlight several promising outcomes and motivate the combination of these two functionalities in the same nanoparticle platform. The given examples of research based advances and the encouraging results of *in vitro* and *in vivo* studies provide much excitement and promise for future theranostic (therapy + diagnostic) clinical applications, as well as for image-guided therapy and/or surgery, and their monitoring. Copyright © 2014 John Wiley & Sons, Ltd.

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1. INTRODUCTION

One of the major aims in nanomedicine is the ability to perform multiple functions using the same nanovehicle, that is, the ability to target, image, diagnose and monitor cells using only a single nanoparticle. The unique physical and optical properties of gold nanoparticles, as well as the ability to attach multiple types of ligands to their surfaces, has led to varying schemes for developing multifunctional gold nanoparticles, with multiple capabilities within a single platform (1–3). Compared with other methods, treatment plans involving the use of multifunctional nanoparticles hold the promise of more accurately targeted treatment, with a higher likelihood of a successful outcome. So far, gold nanoparticles (GNPs) have been utilized for drug delivery, phototherapy and as a contrast-enhancing agent for optical, computed tomography (CT), Raman, X-ray, diffusion reflection and photoacoustic imaging applications (1,4–14). It is this multipronged approach on which many studies have been anchored, since it addresses many issues usually associated with the most aggressive aspects of disease, including multidrug resistance and recurrence of tumors.

This review addresses the potential simultaneous utilization of GNPs for diagnostics (CT being the most common medical imaging method) and for the emerging and highly promising treatment by photothermal therapy (PTT), thus providing a classical example for nanoparticle-based 'theranostics' (combined therapy and diagnostics).

In the following sections we first describe the basic design principles that enable gold nanoparticles to be utilized both as targeted CT contrast agents and as targeted PTT enablers. Next, we review recent CT imaging studies, studies in which gold nanoparticles expanded the role of the CT beyond its present structural imaging capabilities, endowing it with functional and molecular-based imaging capabilities as well. Finally, gold nanoparticle-mediated PTT will be described, focusing on

targeting the cell's nucleus. In the case of CT imaging contrast enhancement, GNPs attenuate X-rays more efficiently than the sometimes used iodine by orders of magnitude. This is due not only to the much stronger attenuation per atom (gold vs iodine) but also to the large number of atoms per gold nanoparticle (e.g. spherical 20 nm particle, containing $\sim 2 \times 10^5$ gold atoms, which is 8×10^{-14} mg gold per nanoparticle). Furthermore, it is easier to attach targeting moieties, such as peptides or antibodies, to GNPs, and there is a large targeting efficiency benefit owing to the multivalency targeting effect, that is, the large number of targeting ligands per GNP. Furthermore, there is better body tolerance towards GNPs compared with iodine. Finally, in case of theranostic treatment, that is, combined use of GNPs for both imaging and therapy, there is an obvious conservation of dose advantage. In the case of photothermal therapy, GNPs are advantageous because of their ability to strongly absorb light (orders of magnitude higher than organic dye molecules) (4), and then convert the absorbed light into heat via nonradiative processes. This process occurs on a time scale on the order of picoseconds, leading to intense localized heating and irreparable damage to the cell (15,16).

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Dr. Kopelman is the The Richard Smalley Distinguished University Professor of Chemistry, Physics, and Applied Physics; Professor, Biomedical Engineering; Professor, Biophysics; Professor, Chemical Biology; Member, MNIMBS (Michigan Nanotechnology Institute for Medicine and the Biological Sciences), The University of Michigan. He is an expert in photonics, laser and bioanalytical chemistry, chemical biology, molecular and nano-materials, with much recent work done on the nanofabrication of biocompatible opto-chemical sensors as well as of safe, biodegradable and bio-eliminable, medical nano-actuators, and their *in vitro* and *in vivo* applications. Dr. Kopelman is the author of over 600 scientific papers, patents and books. Dr. Kopelman coined the term "nanophotonics" and initiated the concept and use of targeted multifunctional nanoparticles for biomedical use, including contrast agents for MRI, CT and PAI.



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2. GOLD NANOPARTICLES: IDEAL CT CONTRAST AGENTS AND PTT MEDIATORS

Currently, CT is one of the leading radiology technologies applied in the field of biomedical imaging. CT provides superior visualization of bone structures owing to the inherent contrast between electron-dense bones and the more permeable surrounding soft tissues. CT, however, is limited in distinguishing between different soft tissues that have similar densities (17). CT contrast agents were introduced in order to improve vascular contrast and to enable better delineation of soft tissue structures with similar or identical contrast properties.

The ability of the CT to distinguish between different tissues is based on the fact that different tissues provide different degrees of X-ray attenuation, according to equation (1):

$$I = I_0 e^{-\mu x} \quad (1)$$

where I_0 is the incident X-ray intensity, I is the transmitted X-ray intensity, x is the thickness of the absorber medium and μ is the

mass attenuation coefficient. The most dominant factor impacting the mass attenuation coefficient is the photoelectric effect, which is proportional to the third power of the atomic number of the material (Z^3). Therefore, in order to provide good contrast in CT images, the key factor in the selection of materials as CT contrast agents is having a high atomic number.

The high- Z nanoparticle contrast agents could also address the important issue of relatively high radiation exposure of the CT. The new generation CT contrast agents that are based on high atomic number materials such as gold have great potential not only because of their ability to produce higher intrinsic contrast, but even more importantly because of the possibility of lowering the overall radiation exposure to patients.

Low to medium X-ray photon energy (25–120 keV) is used for diagnostic radiology, producing significant contrast between bone and other tissues and resulting in high-quality CT images. However, since most of this energy is being absorbed, it exposes the patient to a high dose of radiation. As the higher-energy photons in the energy spectrum produced by the X-ray tubes

will have a much lower interaction cross section for soft tissue than the nanoparticles, it is possible that, by filtering the X-ray spectrum, yielding a lower absorbed radiation dose to the patient, the uptake pattern of these particles can be visualized as distinct contrast relative to their soft tissue background (17). Therefore, high-Z nanoparticles as contrast agents may permit CT imaging at lower patient doses and with better sensitivity and good specificity.

The atomic number of gold (79) is much higher than that of the currently used CT contrast material – iodine (53), and therefore gold can induce stronger X-ray attenuation (18). In addition, the small size of iodine molecules allows only very short imaging times owing to rapid clearance by the kidneys. In contrast, GNPs can be designed so as to overcome biological barriers and to remain confined to the intravascular space for prolonged times (19–21). The optimal size (for spherical particles) should be larger than ~15 nm, so as to avoid rapid clearance by the kidneys or uptake in the liver, but smaller than ~200 nm to avoid filtration in the spleen (20).

Other than its high atomic number advantage, gold is consistently used in various forms for biological applications owing to ease of fabrication and surface modification, as well as gold's inherent biological compatibility (1,4,22,23). Using simple wet-laboratory techniques, gold nanoparticles have been fabricated in a variety of shapes and sizes, and used as the core or the shell for polymer-metal (22,23) and metal-metal (24,25) hybrid nanoparticles. Photosensitizers, dyes, drugs, genetic materials and targeting moieties can all be attached to the gold nanoparticle surface directly, via amine or thiol groups (23), or indirectly, using a molecule such as bovine serum albumin (26) or the penta-peptide with the amino acid sequence CALNN (27).

In many cases gold by itself has served as the photosensitizer. Consequently, gold nanoparticles have been most frequently exploited for photothermal therapy, in comparison to options involving other metal, dye-polymer or carbon-based nanoparticles. The exceptionally high extinction coefficient of gold ($1 \times 10^{19} \text{ m}^{-1} \text{ cm}^{-1}$ for 20 nm gold nanoparticles) (28) is orders of magnitude higher than that of strongly absorbing organic dyes (e.g. Coomassie blue, $4.3 \times 10^4 \text{ m}^{-1} \text{ cm}^{-1}$) (29), which makes gold an ideal photosensitizer. Also, the localized surface plasmon resonance of gold nanoparticles is highly dependent on the morphology of the nanoparticles and can be easily tuned during the fabrication process (28). Typically, the absorption of gold nanoparticles is tuned to be in the range between 600 and 1000 nm, the so-called therapeutic window in which the interaction of light with biological tissues is low, that is, attenuation and scattering effects are at a minimum.

These wide applications of GNPs and their potential for clinical implementation have led to substantial research regarding their *in vivo* chemical stability (30–32), pharmacokinetics (31), biodistribution (32–37) and bio-toxicity (31,33,38–41). The well-known biosafety of gold (42,43), along with the high degree of flexibility in terms of particles' size, shape and functional groups for coating and targeting, provide GNPs with high potential to become the next generation theranostic agents for cancerous diseases.

3. IMAGING APPLICATIONS

Nanoparticle-based CT contrast agents have been suggested for several medical imaging applications depending on multiple

parameters such as the particles' size, coating materials and targeting moieties. These parameters determine not only the efficacy of the contrast achieved in CT, but also their biodistribution and clearance mechanisms. Note that for CT imaging the total amount of gold per unit volume (voxel) is the only important parameter, regardless of the shape of the particles.

As blood pool contrast agents, GNPs extended the blood circulation time from several minutes (with the clinically used iodine compounds) up to 24 h (44) and show stronger X-ray attenuation than the currently used iodine-based compounds (under the same clinically relevant conditions) (44,45). By taking advantage of the progressive permeation through trans-endothelial pores on tumor blood vessels (the EPR effect), GNPs of a certain size range can passively accumulate on tumors. This nonselective 'passively targeting' approach was demonstrated for breast tumor detection in recent study (46). This study showed significant CT contrast enhancement caused by accumulation of nanoparticle contrast agent both within the tumor and in areas surrounding it.

Conjugation of antibodies, peptides or other ligands onto the nanoparticle surface produces active targeting agents, which can selectively accumulate on specific cells or tissues. Molecularly targeted nanoparticles reach tumor tissues through the EPR effect (as in passive targeting). However, the active targeting has additive values; the nanoparticles home selectively onto specific tumors and remain at the tumor site for extended durations, thereby increasing the local accumulation of the nanoparticles at sites of interest.

Specific targeting could be achieved through the conjugation of nanoparticles to a variety of ligands, including antibodies, peptides, aptamers or small molecules that possess high affinity toward unique molecular signatures found on diseased cells, such as cancer cells. Hainfeld *et al.* demonstrated molecular imaging of cancer with actively targeted CT contrast agents (47). They showed that gold nanoparticles can enhance the visibility of millimeter-sized human breast tumors in mice, and that active tumor targeting (with anti-Her2 antibodies) is 1.6-fold more efficient than passive targeting. They also showed that the specific uptake of the targeted gold nanoparticles in the tumor's periphery was 22-fold higher than in surrounding muscle tissue. In another study, Chanda *et al.* (48) reported enhanced CT attenuation of bombesin functionalized gold nanoparticles that selectively targeted cancer receptor sites that are overexpressed in prostate, breast and small-cell lung carcinoma. Recently, folic acid-modified dendrimer-entrapped GNPs have been suggested as targeted CT contrast agents of human lung adenocarcinoma (49). The study showed an increased CT signal in the tumor site after administration of these nanoparticles, as can be seen in Fig. 1. It has also been demonstrated *in vitro* (9,50) and *in vivo* (51) that the CT number of molecularly targeted head and neck cancer is over 5 times higher than the corresponding CT number of an identical but untargeted tumor, and that active tumor targeting is more efficient and specific than passive targeting (Fig. 2) (51). This specific interaction between antigen and antibody or receptor and its ligand was shown to be an effective strategy to improve the amount and residence time of contrast agents in tumors, as well as to provide specific molecular knowledge regarding the findings. This new approach of molecularly targeted CT contrast agents has changed the concept of CT from diagnosis based on anatomical structures to diagnosis according to molecular markers.

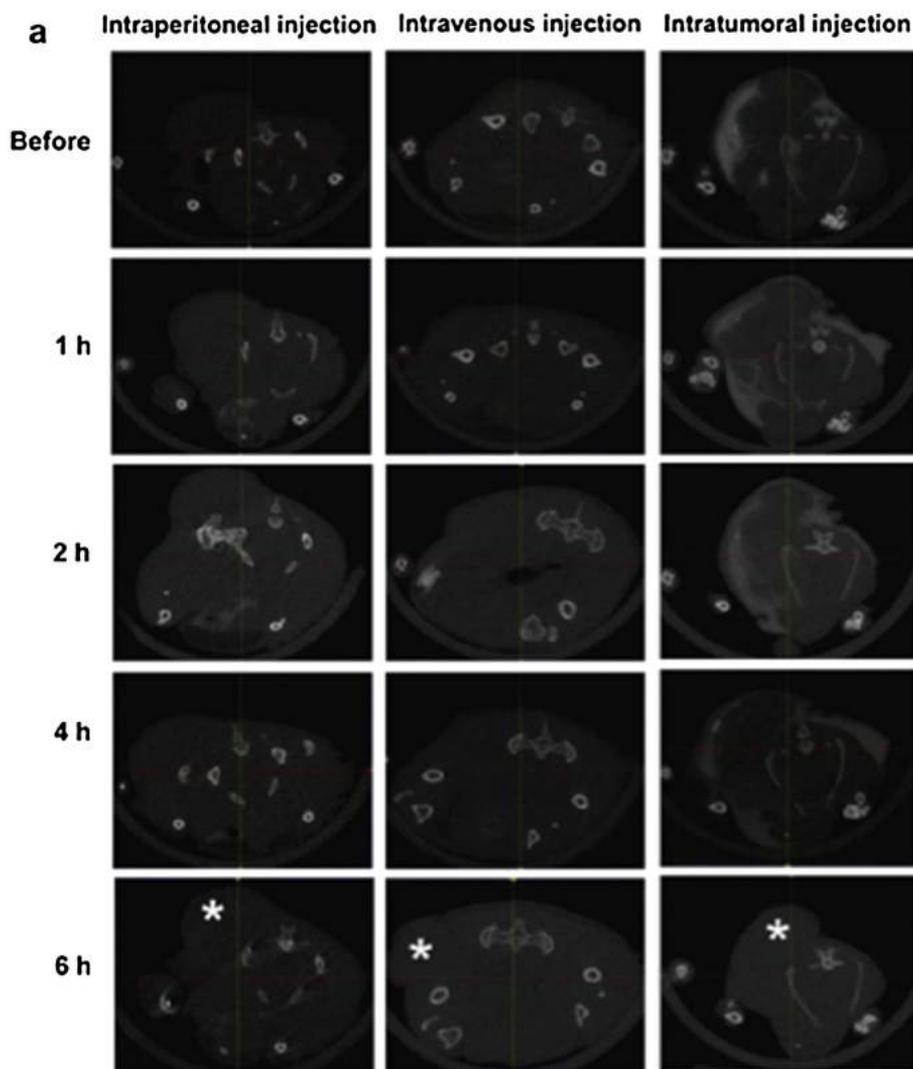


Figure 1. Representative axial micro-computed tomography (CT) images of the xenografts SPC-A1 tumor in nude mice before and after injection with DENP (dendrimer-entrapped gold nanoparticles) by different injection routes for 1, 2, 4 and 6 h. The white star in (a) indicates the location of the tumor. Reprinted with the permission of Wang *et al.* (49).

4. PHOTOTHERMAL THERAPY WITH TARGETED GOLD NANOPARTICLES

Photothermal therapy involves the use of light and a photosensitizer to generate heat for therapeutic purposes. In contrast to conventional photodynamic therapy, PTT does not require the presence of oxygen, which may be of importance when a tumor is large enough to have a hypoxic center. This form of therapy has many clinical applications in which a laser is utilized as the light source and certain *endogenous pigments* are exploited as photosensitizers. In dermatology, the wide use of PTT for the treatment of several skin diseases is enabled by the overexpression of certain endogenous pigments by the anomalous or diseased cells (52–55). Other clinical applications include biostimulation of wound healing (56–58) and the induced coagulation of blood vessels (55,59,60). On the other hand, *nanoparticle mediated-photothermal therapy* enables noninvasive delivery of *exogenous photosensitizers* to cells, for example, to cancer cells, in high enough concentrations (61). Gold nanoparticle mediated-

photothermal therapy involves the irradiation of a gold nanoparticle to generate localized heat so as to damage a region of interest. When excited with light at or near the absorption maximum (typically in the visible or near-IR range), the electrons in the gold nanoparticle absorb the incoming irradiation and, subsequently, are excited from the ground state to a higher energy state. Heat is then produced in the sample through the nonradiative de-excitation of the electrons from the upper state back to the ground state. Detailed calculations concerning the time scales and heat generation involved in the photothermal process have been previously reported in various works (28,62–64). In order to ensure effective nanoparticle-mediated photothermal therapy, the nanoparticles should be: (1) small in size (about 100 nm or less in diameter) (20); (2) biologically compatible; and (3) have no serious toxicity. Moreover, the ability to easily add targeting moieties to the gold nanoparticle surface enables preferential uptake into the malignant cells, for selective heating of the targeted cells, while leaving the neighboring cells intact.

Gold nanoparticle-mediated photothermal therapy can enable extracellular heating of the cells, by attaching them predominantly

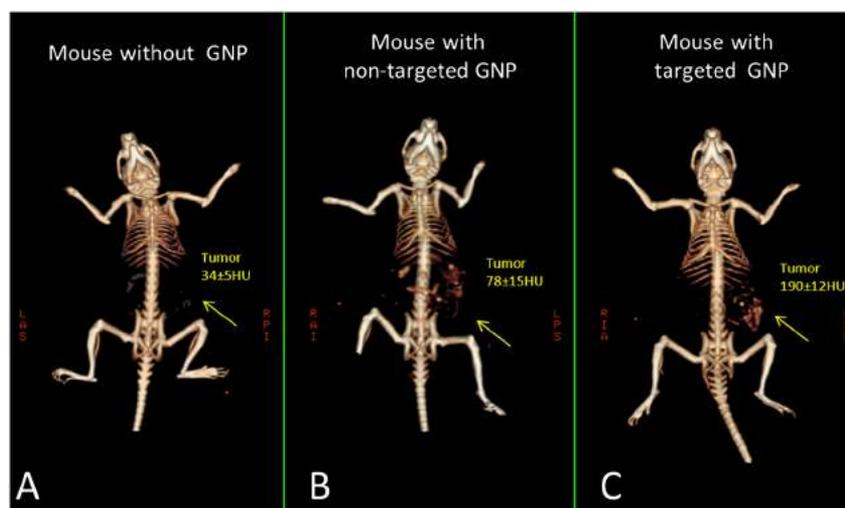


Figure 2. *In vivo* X-ray CT volume-rendered images of (A) a mouse before gold nanoparticle (GNP) injection, (B) a mouse 6 h post-injection of nonspecific Immunoglobulin G (IgG) GNP as a passive targeting experiment, and (C) a mouse 6 h post-injection of anti-epidermal growth factor receptor (EGFR)-coated GNPs that are specifically targeted to the Squamous-cell carcinoma (SCC) head and neck tumor. The anti-EGFR targeted GNPs show clear contrast enhancement of the tumor (C, yellow arrow), which was undetectable without the GNP contrast agents (A, yellow arrow). CT numbers represent the average HU of the whole tumor area. All scans were performed using a clinical CT at 80 kV_p, 500 mA s, collimation 0.625 × 64 mm and 0.521 pitch size (a 64-detector CT scanner, LightSpeed VCT, GE Medical Systems). Reprinted with the permission of Reuveni *et al.* (51).

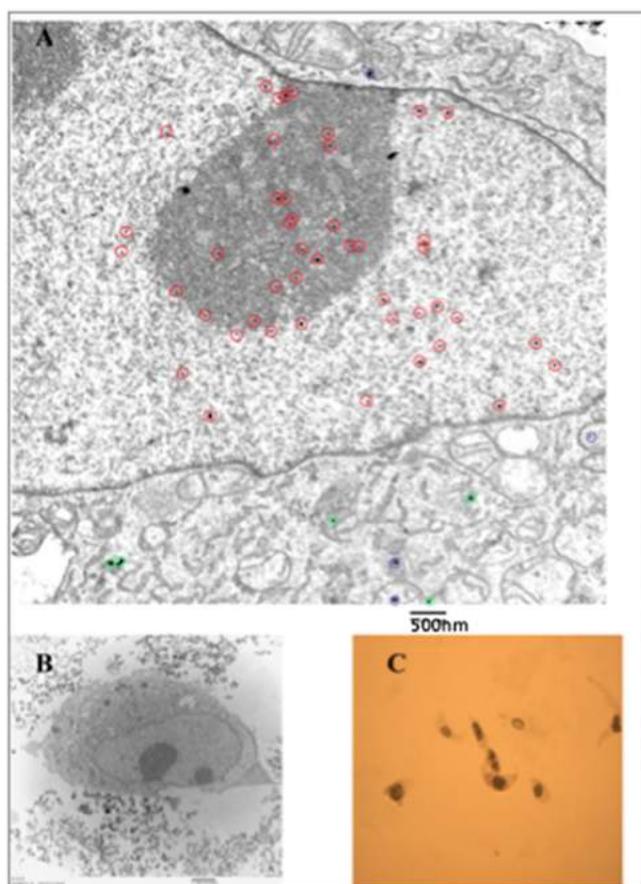


Figure 3. Transmission electron microscopy (TEM) Confirmation of nuclear delivery. (A) Surface-modified GNPs inside the cell's nucleus are denoted by red circles. Gold nanoparticles located in the cytosol (circled in green) and within the lysosome/endosome complex (circled in blue) are also noted. (B) Zoomed-out view of the same cancer cell. (C) Bright field image of cells before they were fixed. Reprinted with the permission of Curry (72).

to the target cells' external membranes, or intracellular heating, once the nanoparticle has been taken up within the cell. Successful nanoparticle-mediated photothermal therapy has been demonstrated with a variety of different types of nanoparticle matrices, encouraging continuous research efforts and applications (5,65). Nanoparticle-mediated photothermal therapy is especially well suited for treating cancer owing to the increased heat sensitivity associated with cancer cells, as well as a tumor's inability to efficiently dissipate heat as a result of its poor vasculature networks. Nanoparticle-mediated photothermal therapy has been used widely as both a primary mode of treatment as well as a preliminary treatment aimed at weakening otherwise resistant cells toward treatment with conventional therapies (65,66).

To date, various cancer cell lines, including breast (6), epithelial (67,68) and colon (2) cancers, have been successfully treated by gold nanoparticle-mediated photothermal therapy, both *in vitro* and *in vivo*. Studies involving photothermal therapy aimed at antibiotic resistant bacteria (69,70) have also been reported with positive results. Recently, a study that builds on work by Qian *et al.* (71), has successfully achieved controlled surface engineering of gold nanoparticles, using multiple ligands [PEG 5K, (RGD)₄ and (nuclear localization signal) NLS (nuclear localization signal) peptides] for efficient delivery into cell nuclei (72). Confirmation of such nuclear delivery is shown in Fig. 3. An added advantage of utilizing gold nanoparticles is that they can be clearly distinguished when cells are imaged using electron microscopy, as also demonstrated in Fig. 3. The nanoparticles were then utilized in PTT studies. One motivation for this study (72) was to investigate the likely advantages of directly heating the nucleus of cancer cells, advantages stemming from the fact that the nucleus has a smaller target volume, a lower heat capacity and an increased likelihood of causing irreparable damage to the cell's DNA. A stark reduction in the survival curves of the cells that had gold nanoparticle located predominantly in the nucleus, vs cells that had nanoparticles located predominantly in the

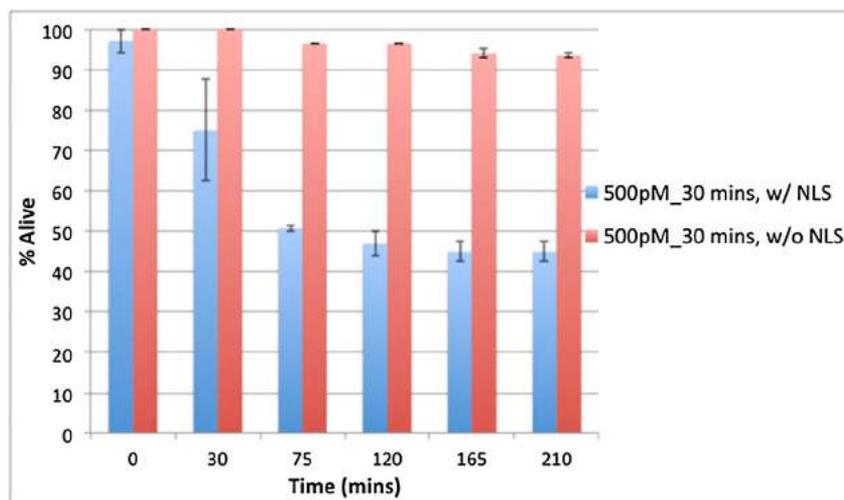


Figure 4. Viability of cells incubated with 500 pM concentrations of surface-modified GNPs and then illuminated at 514 nm for 30 min. Note the large effect of nuclear targeting (with nuclear localization signal (NLS)). All experiments were done in duplicates. Error bars denote standard error in the percentage of live cells at a given time point. Reprinted with the permission of Curry (72).

cytosol, was observed for cells that were treated under the same conditions, that is, the same gold nanoparticle concentration and treatment time (72), as shown in Fig. 4.

Another recent study involving both *in vitro* and *in vivo* PTT studies was reported by Yuan *et al.* (73). They detail the fabrication of *surfactant-free* gold nanostars and their use for both *in vitro* and *in vivo* studies of nanoparticle tracking, via plasmon enhanced two-photon photoluminescence, and for photothermal therapy of breast cancer cells. The 60 nm gold 'nanostars' were fabricated using a citrate gold seed-mediated method that produces a high yield in a short preparation time of 1 min. The resulting nanostars have a core diameter ranging from 20 to 25 nm, with approximately eight to 10 protruding branches (Fig. 5). The *in vitro* studies were carried out as follows: breast cancer cells were incubated with 0.2 nM of bare gold nanostars for 1 h, and then treated using a 980 nm diode laser (15 W cm^{-2} , spot size 8 mm^2) for 1–5 min. Yuan and his colleagues found that 3 min of treatment yielded successful cell kill, and 5 min of treatment gave the best results. Control measurements involving cells that were exposed to the laser, but had not been incubated with nanostars, show that these cells were still viable after 5 min of illumination. For the *in vivo* studies, PEGylated nanostars were injected into CD-1 nude mice that were previously implanted with small dorsal window chambers. After 10 min of PTT, visual confirmation of photo-ablation was indicated by the release of the gold nanoparticles out of the ruptured vessels; scarring occurred one week later (Fig. 6).

One major research area aimed at improving gold nanoparticle-mediated photothermal therapy is the utilization of gold clusters or aggregates (74–77) as the corresponding maximum absorption is red-shifted toward the near-IR range of the 'therapeutic window', resulting in less concern regarding unwanted interaction with the surrounding healthy tissues. It should be noted that, despite the multitude of promising outcomes, the variability among the results has continued to encourage further studies aimed at elucidating the mechanisms of heat generation and thermolysis [e.g. shockwaves, micro- and nano-bubbles and nanoparticle destruction (63,76,78)].

5. MULTIFUNCTIONAL APPROACH: COMBINING THERAPY AND DIAGNOSTICS (THERANOSTICS)

To illustrate the multifunctional approach, combining both imaging and therapy (i.e. theranostics), Fig. 6 shows a mouse model where both the photothermal therapy and optical imaging/monitoring thereof were performed with the aid of gold nanoparticles. For larger animals, such optical imaging would not work, but X-ray-based CT, enhanced by gold nanoparticles, would. Thus the emphasis here is on the combination of CT and PTT. The important point is that, for CT enhancement, it does not matter where in the cells the nanoparticles are located, while this can make a large difference with respect to PTT. The potential medical use of gold nanoparticles for 'theranostics' (therapy + diagnostics),

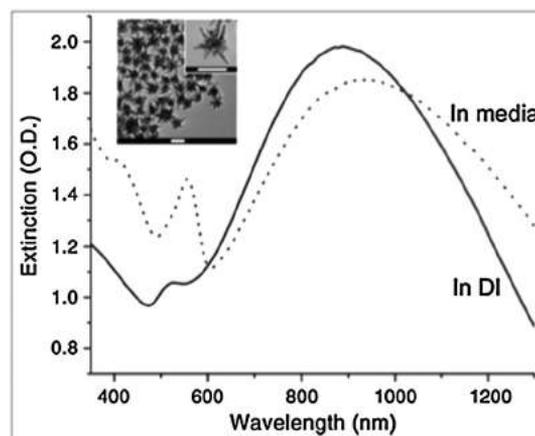


Figure 5. The extinction spectra of 0.2 nM nanostars used for *in vitro* photothermal therapy (PTT). In deionized water (DI; solid line) the plasmon peaks at around 890 nm with a small peak around 525 nm. In Fetal bovine serum (FBS) containing growth medium (dotted line), the nanostars' plasmon peak red-shifted and its intensity decreased. TEM image of nanostars (inset). Scale bars, 50 nm. Reprinted with the permission of Yuan *et al.* (73).

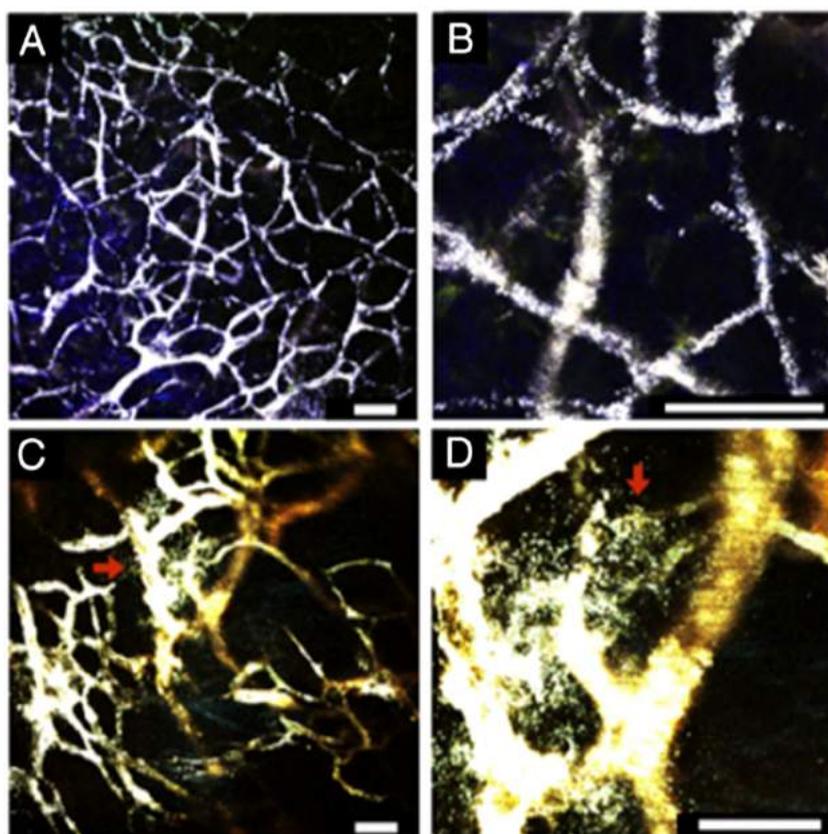


Figure 6. *In vivo* optical monitoring of PTT; multiphoton microscopy imaging through a dorsal window chamber (implanted in CD-1 nude mice), 30 min following the systemic injection of PEGylated nanostars (50 nm, 50 μ l). A clear vasculature pattern, delineated by the intravascular nanostars, is visible with minimal tissue autofluorescence under 3% transmission power (A); zoom-in (B). In some regions, extravasation of nanostars (numerous white dots outside the vessel; red arrows) is observed (C); zoom-in (D). Scale bars, 50 μ m. Reprinted with the permission of Yuan *et al.* (73).

that is, their utilization for a combination of therapy (PTT) and imaging/monitoring (CT), is the major message of this review.

6. SUMMARY AND OUTLOOK: IMAGE GUIDED THERAPY AND SURGERY

The promise of GNPs for optical and photo-acoustic imaging, including the utilization of nonlinear optics and enhanced Raman effects, is covered elsewhere (79). To this can be added tumor visualization and delineation for the surgeon's eyes, an important medical need. In addition to such image-guided surgery, one aims today at image-guided therapy, including image-monitored therapy (80). The latter has been mainly achieved with the use of targeted, multifunctional nanoplatforms (81). Notably, CT is still the most prevalent imaging method, and PTT is a particularly safe method, especially with the use of double targeting: targeted GNPs and target-focused photons. This minimizes potential side effects, owing to both the double targeting and the biocompatibility of GNPs, that is, their inertness in the dark. The overall result is a combination, with a high degree of safety, of imaging and therapy.

REFERENCES

- Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. *Chem Soc Rev* 2009; 38(6): 1759–1782.
- Huang HC, Barua S, Sharma G, Dey SK, Rege K. Inorganic nanoparticles for cancer imaging and therapy. *J Control Rel* 2011; 155(3): 344–357.
- Liu DB, Wang Z, Jiang XY. Gold nanoparticles for the colorimetric and fluorescent detection of ions and small organic molecules. *Nano-scale* 2011; 3(4): 1421–1433.
- Jain S, Hirst DG, O'Sullivan JM. Gold nanoparticles as novel agents for cancer therapy. *Br J Radiol* 2012; 85(1010): 101–113.
- Norman RS, Stone JW, Gole A, Murphy CJ, Sabo-Attwood TL. Targeted photothermal lysis of the pathogenic bacteria, *Pseudomonas aeruginosa*, with gold nanorods. *Nano Lett* 2008; 8(1): 302–306.
- von Maltzahn G, Park JH, Agrawal A, et al. Computationally guided photothermal tumor therapy using long-circulating gold nanorod antennas. *Cancer Res* 2009; 69(9): 3892–3900.
- Minati L, Antonini V, Torrenco S, et al. Sustained in vitro release and cell uptake of doxorubicin adsorbed onto gold nanoparticles and covered by a polyelectrolyte complex layer. *Int J Pharm* 2012; 438(1–2): 45–52.
- Rand D, Ortiz V, Liu Y, et al. Nanomaterials for X-ray imaging: gold nanoparticle enhancement of X-ray scatter imaging of hepatocellular carcinoma. *Nano Lett* 2011; 11(7): 2678–2683.
- Popovtzer R, Agrawal A, Kotov NA, et al. Targeted gold nanoparticles enable molecular CT imaging of cancer. *Nano Lett* 2008; 8(12): 4593–4596.
- Shilo M, Reuveni T, Motiei M, Popovtzer R. Nanoparticles as computed tomography contrast agents: current status and future perspectives. *Nanomedicine* 2012; 7(2): 257–269.
- Jakobsch K, Motiei M, Sinvani M, Popovtzer R. Towards real-time detection of tumor margins using photothermal imaging of immune-targeted gold nanoparticles. *Int J Nanomed* 2012; 7: 4707–4713.
- Ankri R, Peretz V, Motiei M, Popovtzer R, Fixler D. A new method for cancer detection based on diffusion reflection measurements of targeted gold nanorods. *Int J Nanomed* 2012; 7: 449–455.

13. Ankri R, Meiri A, Lau SI, Motiei M, Popovtzer R, Fixler D. Intercoupling surface plasmon resonance and diffusion reflection measurements for real-time cancer detection. *J Biophotonics* 2013; 6(2): 188–196.
14. Ankri R, Duadi H, Motiei M, Fixler D. In-vivo Tumor detection using diffusion reflection measurements of targeted gold nanorods – a quantitative study. *J Biophotonics* 2012; 5(3): 263–273.
15. Link S, El-Sayed MA. Spectral properties and relaxation dynamics of surface plasmon electronic oscillations in gold and silver nanodots and nanorods. *J Phys Chem B* 1999; 103(40): 8410–8426.
16. El-Sayed MA. Some interesting properties of metals confined in time and nanometer space of different shapes. *Acc Chem Res* 2001; 34(4): 257–264.
17. Yu SB, Watson AD. Metal-based X-ray contrast media. *Chem Rev* 1999; 99(9): 2353–2377.
18. Xu CJ, Tung GA, Sun SH. Size and concentration effect of gold nanoparticles on X-ray attenuation as measured on computed tomography. *Chem Mater* 2008; 20(13): 4167–4169.
19. Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. *Nat Rev Drug Discov* 2010; 9(8): 615–627.
20. Hallouard F, Anton N, Choquet P, Constantinesco A, Vandamme T. Iodinated blood pool contrast media for preclinical X-ray imaging applications – a review. *Biomaterials* 2010; 31(24): 6249–6268.
21. Allkemper T, Bremer C, Matuszewski L, Ebert W, Reimer P. Contrast-enhanced blood-pool MR angiography with optimized iron oxides: effect of size and dose on vascular contrast enhancement in rabbits. *Radiology* 2002; 223(2): 432–438.
22. Beija M, Li Y, Duong HT, et al. Polymer-gold nanohybrids with potential use in bimodal MRI/CT: enhancing the relaxometric properties of Gd(III) complexes. *J Mater Chem* 2012; 22(40): 21382–21386.
23. Xie MR, Ding L, You ZW, Gao DY, Yang GD, Han HJ. Robust hybrid nanostructures comprising gold and thiol-functionalized polymer nanoparticles: facile preparation, diverse morphologies and unique properties. *J Mater Chem* 2012; 22(28): 14108–14118.
24. Han SY, Guo QH, Xu MM, et al. Tunable fabrication on iron oxide/Au/Ag nanostructures for surface enhanced Raman spectroscopy and magnetic enrichment. *J Colloid Interface Sci* 2012; 378: 51–57.
25. Guo X, Zhang Q, Sun YH, Zhao Q, Yang J. Lateral etching of core-shell Au@metal nanorods to metal-tipped Au nanorods with improved catalytic activity. *ACS Nano* 2012; 6(2): 1165–1175.
26. Tkachenko AG, Xie H, Liu YL, et al. Cellular trajectories of peptide-modified gold particle complexes: comparison of nuclear localization signals and peptide transduction domains. *Bioconjug Chem* 2004; 15(3): 482–490.
27. Nativo P, Prior IA, Brust M. Uptake and intracellular fate of surface-modified gold nanoparticles. *ACS Nano* 2008; 2(8): 1639–1644.
28. Link S, El-Sayed MA. Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals. *Int Rev Phys Chem* 2000; 19(3): 409–453.
29. Chial HJ, Thompson HB, Splittgerber AG. A spectral study of the charge forms of Coomassie Blue-G. *Anal Biochem* 1993; 209(2): 258–266.
30. Ghosh P, Han G, De M, Kim CK, Rotello VM. Gold nanoparticles in delivery applications. *Adv Drug Deliv Rev* 2008; 60(11): 1307–1315.
31. Arvizo R, Bhattacharya R, Mukherjee P. Gold nanoparticles: opportunities and challenges in nanomedicine. *Exp Opin Drug Deliv* 2010; 7(6): 753–763.
32. Zhang GD, Yang Z, Lu W, et al. Influence of anchoring ligands and particle size on the colloidal stability and in vivo biodistribution of polyethylene glycol-coated gold nanoparticles in tumor-xenografted mice. *Biomaterials* 2009; 30(10): 1928–1936.
33. Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, et al. Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. *Biochem Biophys Res Commun* 2010; 393(4): 649–655.
34. Balasubramanian SK, Jittiwat J, Manikandan J, Ong CN, Yu LE, Ong WY. Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats. *Biomaterials* 2010; 31(8): 2034–2042.
35. Lipka J, Semmler-Behnke M, Sperling RA, et al. Biodistribution of PEG-modified gold nanoparticles following intratracheal instillation and intravenous injection. *Biomaterials* 2010; 31(25): 6574–6581.
36. Chanda N, Kattumuri V, Shukla R, et al. Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity. *Proc Natl Acad Sci U S A* 2010; 107(19): 8760–8765.
37. Kunzmann A, Andersson B, Thurnherr T, Krug H, Scheynius A, Fadeel B. Toxicology of engineered nanomaterials: focus on biocompatibility, biodistribution and biodegradation. *Biochim Biophys Acta, Gen Subj* 2011; 1810(3): 361–373.
38. El-Sayed IH. Nanotechnology in head and neck cancer: the race is on. *Curr Oncol Rep* 2010; 12(2): 121–128.
39. Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S, Stone V. A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. *Crit Rev Toxicol* 2010; 40(4): 328–346.
40. Cobley CM, Au L, Chen JY, Xia YN. Targeting gold nanocages to cancer cells for photothermal destruction and drug delivery. *Exp Opin Drug Deliv* 2010; 7(5): 577–587.
41. Zhang XD, Wu HY, Wu D, et al. Toxicologic effects of gold nanoparticles in vivo by different administration routes. *Int J Nanomed* 2010; 5: 771–781.
42. Sherman AI, Ter-Pogossian M. Lymph-node concentration of radioactive colloidal gold following interstitial injection. *Cancer* 1953; 6(6): 1238–1240.
43. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 2005; 1(3): 325–327.
44. Kojima C, Umeda Y, Ogawa M, Harada A, Magata Y, Kono K. X-ray computed tomography contrast agents prepared by seeded growth of gold nanoparticles in PEGylated dendrimer. *Nanotechnology* 2010; 21(24): 245–251.
45. Hainfeld JF, Slatkin DN, Focella TM, Smilowitz HM. Gold nanoparticles: a new X-ray contrast agent. *Br J Radiol* 2006; 79(939): 248–253.
46. Ghaghada KB, Badea CT, Karumbaiah L, et al. Evaluation of tumor microenvironment in an animal model using a nanoparticle contrast agent in computed tomography imaging. *Acad Radiol* 2011; 18(1): 20–30.
47. Hainfeld JF, O'Connor MJ, Dilmanian FA, Slatkin DN, Adams DJ, Smilowitz HM. Micro-CT enables microlocalisation and quantification of Her2-targeted gold nanoparticles within tumour regions. *Br J Radiol* 2011; 84(1002): 526–533.
48. Chanda N, Kattumuri V, Shukla R, et al. Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity. *Proc Natl Acad Sci U S A* 2010; 107(19): 8760–8765.
49. Wang H, Zheng LF, Peng C, Shen MW, Shi XY, Zhang GX. Folic acid-modified dendrimer-entrapped gold nanoparticles as nanoprobes for targeted CT imaging of human lung adenocarcinoma. *Biomaterials* 2012; 34(2): 470–480.
50. Sanz J, Fayad ZA. Imaging of atherosclerotic cardiovascular disease. *Nature* 2008; 451(7181): 953–957.
51. Reuveni T, Motiei M, Romman Z, Popovtzer A, Popovtzer R. Targeted gold nanoparticles enable molecular CT imaging of cancer: an in vivo study. *Int J Nanomed* 2011; 6: 2859–2864.
52. Weber RJ, Taylor BR, Engelman DE. Laser-induced tissue reactions and dermatology. In *Basics in Dermatological Laser Applications*, Allemann IB, Goldberg DJ (eds). Basel: Karger, 2011; 24–34.
53. Paithankar DY, Ross EV, Saleh BA, Blair MA, Graham BS. Acne treatment with a 1,450 nm wavelength laser and cryogen spray cooling. *Lasers Surg Med* 2002; 31(2): 106–114.
54. Majaron B, Verkruyse W, Tanenbaum BS, et al. Combining two excitation wavelengths for pulsed photothermal profiling of hypervascular lesions in human skin. *Phys Med Biol* 2000; 45(7): 1913–1922.
55. Heger M, Salles II, Bezemer R, et al. Laser-induced primary and secondary hemostasis dynamics and mechanisms in relation to selective photothermolysis of port wine stains. *J Dermatol Sci* 2011; 63(3): 139–147.
56. Basso FG, Pansani TN, Turrioni APS, Bagnato VS, Hebling J, de Souza Costa CA. In vitro wound healing improvement by low-level laser therapy application in cultured gingival fibroblasts. *Int J Dent* 2012; 2012: 719452.
57. Santos NRS, Sobrinho JbDM, Almeida PF, et al. Influence of the combination of infrared and red laser light on the healing of cutaneous wounds infected by *Staphylococcus aureus*. *Photomed Laser Surg* 2011; 29(3): 177–182.
58. Cafaro A, Albanese G, Arduino PG, et al. Effect of low-level laser irradiation on unresponsive oral lichen planus: early preliminary results in 13 patients. *Photomed Laser Surg* 2010; 28(suppl 2): S99–103.
59. Barton JK, Rollins A, Yazdanfar S, Pfefer TJ, Westphal V, Izatt JA. Photothermal coagulation of blood vessels: a comparison of high-speed optical coherence tomography and numerical modelling. *Phys Med Biol* 2001; 46(6): 1665–1678.

60. McMillan K, Perepelitsyn I, Wang Z, Shapshay SM. Tumor growth inhibition and regression induced by photothermal vascular targeting and angiogenesis inhibitor retinoic acid. *Cancer Lett* 1999; 137(1): 35–44.
61. Curry T, Smith R, Kopelman R. Photothermal therapy of cancer cells mediated by blue hydrogel nanoparticles. *Nanomedicine* 2013, doi: 10.2217/nnm.12.190.
62. Pustovalov V, Babenko V. Computer modeling of optical properties of gold ellipsoidal nanoparticles at laser radiation wavelengths. *Laser Phys Lett* 2005; 2(2): 84–88.
63. Letfullin RR, Joenathan C, George TF, Zharov VP. Laser-induced explosion of gold nanoparticles: potential role for nanophotothermolysis of cancer. *Nanomedicine* 2006; 1(4): 473–480.
64. Jain PK, Lee KS, El-Sayed IH, El-Sayed MA. Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *J Phys Chem B* 2006; 110(14): 7238–7248.
65. Iancu C, Mocan L. Advances in cancer therapy through the use of carbon nanotube-mediated targeted hyperthermia. *Int J Nanomed* 2011; 6: 1675–1684.
66. Kirui DK, Rey DA, Batt CA. Gold hybrid nanoparticles for targeted phototherapy and cancer imaging. *Nanotechnology* 2010; 21(10): 105105–105115.
67. Choi J, Yang J, Bang D, et al. Targetable gold nanorods for epithelial cancer therapy guided by near-IR absorption imaging. *Small* 2012; 8(5): 746–753.
68. El-Sayed IH, Huang XH, El-Sayed MA. Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. *Cancer Lett* 2006; 239(1): 129–135.
69. Ray PC, Khan SA, Singh AK, Senapati D, Fan Z. Nanomaterials for targeted detection and photothermal killing of bacteria. *Chem Soc Rev* 2012; 41(8): 3193–3209.
70. Pissuwan D, Cortie CH, Valenzuela SM, Cortie MB. Functionalised gold nanoparticles for controlling pathogenic bacteria. *Trends Biotechnol* 2010; 28(4): 207–213.
71. Qian W, Murakami M, Ichikawa Y, Che Y. Highly efficient and controllable PEGylation of gold nanoparticles prepared by femtosecond laser ablation in water. *J Phys Chem C* 2011; 115(47): 23293–23298.
72. Curry TY. Nanoparticles for Biomedical Applications: Photothermal Therapy and Nuclear Delivery. PhD. Thesis, University of Michigan, Physics: Ann Arbor, MI, 2012; 154.
73. Yuan H, Khoury CG, Wilson CM, Grant GA, Bennett AJ, Vo-Dinh T. In vivo particle tracking and photothermal ablation using plasmon-resonant gold nanostars. *Nanomed Nanotechnol Biol Med* 2012; 8(8): 1355–1363.
74. Zharov VP, Galitovskaya EN, Johnson C, Kelly T. Synergistic enhancement of selective nanophotothermolysis with gold nanoclusters: potential for cancer therapy. *Lasers Surg Med* 2005; 37(4): 329–329.
75. Nam J, Won N, Jin H, Chung H, Kim S. pH-Induced aggregation of gold nanoparticles for photothermal cancer therapy. *J Am Chem Soc* 2009; 131(38): 13639–13645.
76. Huhn D, Govorov A, Gil PR, Parak WJ. Photostimulated Au nanoheaters in polymer and biological media: characterization of mechanical destruction and boiling. *Adv Funct Mater* 2012; 22(2): 294–303.
77. Murthy AK, Stover RJ, Borwankar AU, et al. Equilibrium gold nanoclusters quenched with biodegradable polymers. *ACS Nano* 2013; 7(1): 239–251.
78. Coronado EA, Encina ER, Stefani FD. Optical properties of metallic nanoparticles: manipulating light, heat and forces at the nanoscale. *Nanoscale* 2011; 3(10): 4042–4059.
79. Orringer DA, Chen T, Huang DL, et al. The brain tumor window model: a combined cranial window and implanted glioma model for evaluating intraoperative contrast agents. *Neurosurgery* 2010; 66(4): 736–743.
80. Koo YEL, Reddy GR, Bhojani M, et al. Brain cancer diagnosis and therapy with nanoplatfoms. *Adv Drug Deliv Rev* 2006; 58(14): 1556–1577.
81. Ross B, Rehemtulla A, Koo YEL, et al. Photonic and magnetic nanoexplorers for biomedical use: from subcellular imaging to cancer diagnostics and therapy. Conference on Nanobiophotonics and Biomedical Applications, San Jose, CA, 2004; 76–83.