Chapter 13

Applications of Gold Nanoparticles in Cancer Imaging and Treatment

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Additional information is available at the end of the chapter

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Abstract

Cancer is one of the leading causes of death worldwide. In the last two decades, the development of nanotechnology has facilitated our ability to design new nanoparticles for the diagnosis and treatment of cancer. In this chapter, we reviewed the applications of gold nanoparticles as contrast agents for cancer imaging, including optical imaging, photoacoustic imaging, and X-ray–based imaging. We also reviewed their applications as delivery carriers for small molecule drugs, therapeutic genes, vaccines, and adjuvants and as therapeutic agents by themselves in cancer treatment, including photothermal therapy, photodynamic therapy, and radiation therapy.

Keywords: gold nanoparticles, cancer, cancer imaging, cancer treatment, localized surface plasmon resonance

1. Introduction

With over 7 million new deaths per year, cancer remains one of the leading causes of death worldwide. The mortality of cancer is estimated to reach 13.1 million in the next two decades. Surgery, radiation therapy, and chemotherapy are key players in treatment of cancer. These treatments slow the progression of disease and prolong the survival of patients. Nonetheless, new treatments are in urgent need due to the greater understanding of the complexity of genetic and environmental factors. Recently, the development of nanotechnology has facilitated our ability to design new nanoparticles for the diagnosis and treatment of cancer [1].

Due to their unique physical and chemical properties, gold nanoparticles (ranging from 1 to 100 nm in one dimension at least) have attracted remarkable attention in recent years [2]. The gold nanoparticles are suitable for drug delivery given their large surface area to

volume ratio. Owing to localized surface plasmon resonance (LSPR), gold nanoparticles, such as gold nanorods, nanocages, nanoshells, and nanostars, have strong light scattering and/or absorbance and have been extensively explored for bioimaging, cancer treatment, and both. In addition, given the strong binding affinity of gold to thiol and amine groups, the surface of gold nanoparticles can be easily functionalized with biomolecules such as DNA, siRNA, peptides, antibodies, and receptors. Gold nanoparticles also have a long history for clinical applications (for the treatment of rheumatoid arthritis) [3].

2. Gold nanoparticles in cancer imaging

2.1. Light scattering–based imaging

The scattering cross section of gold nanoparticles increases with the growth of their size. In general, gold nanoparticles can scatter light with the cross section more than 1 million times stronger than that of the emission from a fluorescent dye. Gold nanoparticles with a diameter greater than 10 nm can be visualized by dark-field scattering microscopy. Compared to a fluorescent dye, the gold nanoparticles are photostable and the scattering light does not blink. These features make gold nanoparticles attractive imaging probes for optical imaging.

Different shapes of gold nanoparticles, such as gold nanorods, nanocages, and nanostars, have been tested by dark-field scattering microscopy. In 2003, Sokolov *et al.* [4] conjugated antibodies against epidermal growth factor receptor (EGFR), a glycoprotein that is overexpressed in epithelial malignant cells, for molecular-specific optical imaging. The gold nanoparticles they used were ~12 nm in diameter, which is approximately the same size as that of antibodies. The EGFR-overexpressed cells can be visualized individually by the strong scattering light reflected from a laser pointer with power output less than 5 mW. Later, El-Sayed *et al.* [5] demonstrated that the anti-EGFR antibodies–conjugated gold nanoparticles can differentiate EGFR-overexpressed malignant cells from healthy cells by simple dark-field optical microscopy. Other gold nanoparticles with different size and shapes were also explored. As demonstrated by Huang *et al.* [6], gold nanorods could scatter light in near-infrared (NIR) region and thus enable detection of head and neck cancer cells in biological tissues. In this work, they found that the extinction spectra of the gold nanorods on the cell surface were greatly red-shifted compared with that of free nanorods of the same size. This red shift is believed to rise from the change in the local refractive index after binding between gold nanoparticles and cells or the interparticle interaction between the gold nanorods on the cell surface. By conjugation, gold nanostars with hyaluronic acid, our group [7] demonstrated that CD44-overexpressed malignant cells could be observed under dark-field optical microscopy through the binding between cell surface receptor and functionalized gold nanostars.

Another imaging modality based on light scattering is optical coherence tomography (OCT). OCT captures the changes in phase and intensity from the scattered light to provide optical cross-sectional images of tissues. It employs NIR light to produce a three-dimensional image of tissues with micrometer resolution. Gobin *et al*. [8] demonstrated dramatic contrast

enhancement of tumors on OCT images after systematically injected gold nanoshells. The quantification of OCT images showed a 56% increase in tumor intensity 20 h after the injection of gold nanoshells. In contrast, conventional optimal contrast agents for OCT, such as microbubbles and microspheres, typically enhance contrast less than 5% *in vivo*. Cang *et al.* [9] described that gold nanocages with a 35 nm edge length can provide enhanced contrast for OCT in tissue phantoms. Similarly, gold nanorods with LSPR wavelengths overlapping the OCT light source have been demonstrated for OCT contrast enhancement [10]. One interesting observation in that report is that the gold nanorods generate little signal when the wavelength of OCT source is outside their spectral bandwidth. This result indicates the possibility of spectral multiplexing for contrast-enhanced OCT.

2.2. Photoacoustic imaging

The photoacoustic imaging (PAI) is based on the acoustic waves generated by the thermal expansion of materials induced by optical excitation. When a pulsed laser irradiates materials, the temperature rise of the materials produces ultrasonic waves by periodic thermal expansion. The images of PAI are then constructed by detection of the ultrasonic waves. Since the ultrasonic wave penetrates deeper than light in tissues, the imaging volume and depth of PAI are significantly higher than those of optical imaging.

Gold nanoparticles have been highly attractive for PAI due to their excellent photothermal conversion ability and tunable optical properties. Jokerst *et al*. [11] visualized photoacoustic signal from gold nanorods accumulated in ovarian cancer tumors *in vivo* by PAI. The increase fold of photoacoustic signal was in good linear relationship with the concentration of intravenously injected gold nanorods ($R^2 = 0.97$). *Song et al*. demonstrated the use of gold nanocages as lymph node tracers for PAI. They identified gold nanocages labeled sentinel lymph nodes as deep as 33 mm below the skin surface in an animal model. Our group worked with Lin and coworkers [12] demonstrated that gold vesicles composed of assembled gold nanoparticles have strong photoacoustic signal due to the plasmonic coupling between the adjacent gold nanoparticles in the vesicular membranes. To specifically target tumor neovasculature, our group worked with Nie and coworkers [13] to functionalize gold nanostars with cyclic arginine-glycine-aspartic acid (cRGD) peptides, which possess high affinity to integrin $\alpha_v\beta_3$ overexpressed by neovessels of tumors. As shown in **Figure 1**, benefited from targeting ability of cRGD-conjugated gold nanostars, three-dimensional photoacoustic images of tumor neovessels could be obtained with high spatial resolution and deep imaging depth (up to centimeters under skin) using a laser fluence of 6 mJ/cm^2, which is far less than the safety limit for laser skin exposure (20 mJ/cm^2). Another work from our group designed pH (low) insertion peptides (pHLIPs)-conjugated gold nanostars for tumor targeting PAI [14]. pHLIPs are 35 amino acid peptides with pH-dependent transmembrane activity. After conjugation, the functionalized gold nanostars could be activated in the mild acidic microenvironment of tumors and internalized by cells rapidly. The photoacoustic images showed that at 24 h after systematic injection, the pHLIPs-conjugated nanostars had a 50% higher tumor signal than the gold nanostars without functionalization.

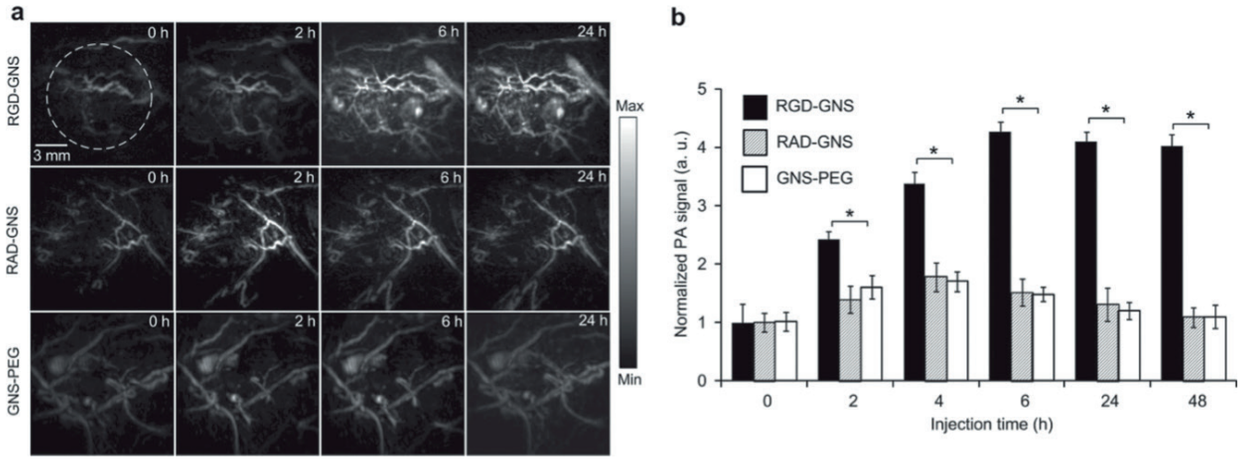

Figure 1. cRGD-conjugated gold nanostars for PAI. (a) Sequential photoacoustic images captured before, 2, 6, and 24 h post injection of RGD-GNS, control peptide–conjugated gold nanostars (RAD-GNS), and PEGylated gold nanostars (GNS-PEG). (b) Quantification of the signal intensity in region of interest. Reproduced from Ref. [13].

2.3. X-ray–based imaging

Computed tomography (CT) is one of the most widely used diagnostic imaging modalities for cancer. It can obtain whole volume 3D anatomical images with high spatial resolution in a cost-effective fashion. However, the contrast between different types of soft tissues is poor on CT images; thus, contrast agents, like iodine-based compounds, need to be injected to differentiate tumors from healthy tissues.

Since the attenuation of X-rays depends greatly on the atomic numbers of elements, gold has a stronger X-ray attenuation coefficient than other elements naturally existed in human bodies. Therefore, the accumulation of gold nanoparticles in tumors can significantly increase X-ray attenuation, resulting in high contrast between tumor and healthy tissues on CT images. Deoxyglucose-labeled gold nanoparticles are described as potential CT contrast agents [15]. The cancer cell samples showed significant contrast enhancement after incubation with these gold nanoparticles on multiple CT slices. The use of acetylated dendrimer-entrapped gold nanoparticles to image cancer *in vivo* is also reported [16]. The gold nanoparticles with a mean diameter of 2.6 nm were able to increase the attenuation of tumors after intratumoral and intraperitoneal administration in a mice model of human lung adenocarcinoma. Our group demonstrated that PEGylated gold nanostars have higher CT values when compared to iodixanol at equivalent concentration. The CT images of tumor-bearing mice exhibited 10-fold enhancement in tumors after injection of PEGylated gold nanostars (**Figure 2**) [17]. We also reported the use of Gd chelates–conjugated gold nanoprisms as a new class of contrast agents for CT/MRI dual-modality imaging [18]. The CT values of Gd chelates–conjugated gold nanoprisms had a linear relationship with the concentration of gold at an attenuation coefficient calculated to be 959.3 HU L g^{-1}. The conjugated Gd chelates exhibited high T1 relaxivity for MR imaging.

The surface of gold nanoparticles can be easily functionalized with tumor-targeting moieties, such as ligands, antibodies, and aptamers, to selectively image tumors via active targeting strategy. For example, Hainfeld *et al.* [19] conjugated gold nanoparticles with polyethylene

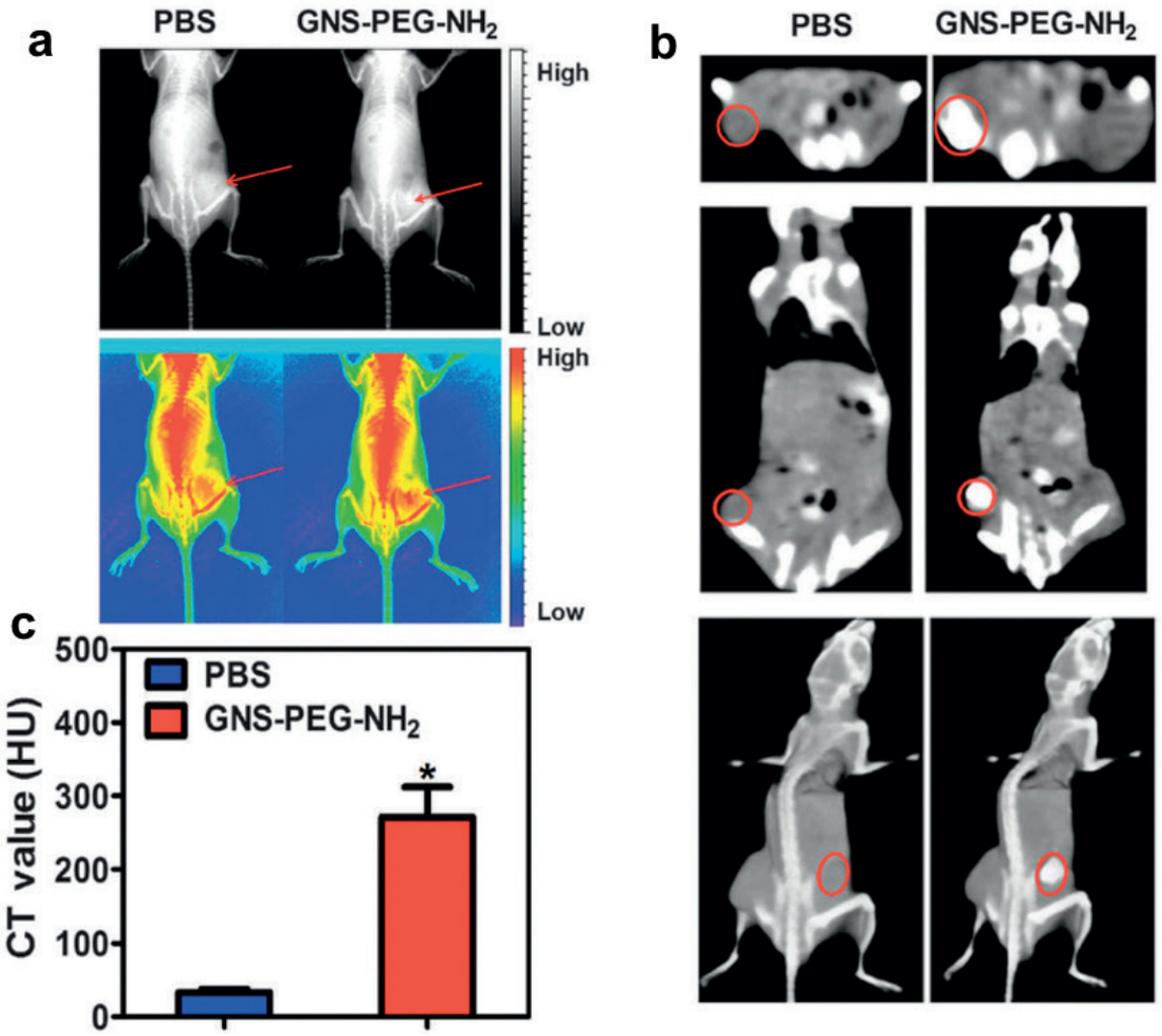

Figure 2. Amino-PEG functionalized gold nanostars (GNS-PEG-NH_2) for X-ray and CT imaging. (a) and (b) X-ray and CT images of MCF-7 tumor-bearing mice after intratumoral of PBS and GNS-PEG-NH_2. (c) Corresponding CT values of tumors in (b). Reproduced from Ref. [17].

glycol (PEG) and anti-Her2 antibodies (Herceptin) as Her2-targeting diagnostic tools. An *in vitro* study showed the nanoconjugates specifically targeting Her2-positive breast cancer BT-474 cells *vs.* Her2-negative breast cancer MCF7 cells with a gold mass ratio of 39.4 ± 2.7:1. In the *in vivo* studies, the BT-474 tumors showed 1.6 times higher CT attenuation than MCF7 tumors after intravenous injection of anti-HER2–conjugated gold nanoparticles. The Housefield Unit (HU) of BT-474 tumors was 22-fold higher than that of surrounding muscles, enabling the detection of small tumors on micro-CT. In another report, Kim *et al.* [20] functionalized gold nanoparticles with a prostate-specific membrane antigen (PSMA) RNA aptamer to establish prostate cancer targeting CT probes. The aptamer-conjugated gold nanoparticles exhibited fourfold higher CT values in PSMA-overexpressed LNCaP cells than that in PSMA negative PC3 cells. Furthermore, the aptamers on gold nanoparticles could be used to load doxorubicin to kill targeted cells more efficiently.

3. Gold nanoparticles in drug delivery

3.1. Small molecule drug delivery

Gold nanoparticles have been explored as drug carriers due to the following advantages: (1) the large surface area provides high loading capacity for drug loading and improves the hydrophilicity and stability of drugs; (2) the ability to modify surface with targeting ligands to enhance the tumor selective accumulation compared to free drugs; (3) the passive targeting ability to tumor site due to their leaky neovessels, which is called enhanced permeability and retention(EPR) effect; and (4) the controlled release of loaded drugs in response to internal or external stimulus.

The enhanced tumor accumulation of gold nanoparticles can be utilized for drug delivery to increase therapeutic potent and reduce side effects. Xiao *et al.* [21] demonstrated that cRGD-conjugated gold nanorods selectively delivered doxorubicin to tumors with a tumor/muscle ratio as high as 16.6 ± 1.2 at 5 h post injection. Given that the nanorods were labeled with radioactive 64Cu, the biodistribution of nanorods could be easily observed and quantified by microPET/CT *in vivo*. In another work, You *et al.* [22] connected TNYL-RAW, a 14-mer peptide with high binding affinity to EphB4, with doxorubicin-loaded gold nanoshells. Since EphB4 is a receptor overexpressed by numerous types of tumor, such nanoshells selectively delivered doxorubicin to Hey tumors in nude mice model at 24 h after intravenous injection.

Gold nanoparticles can release loaded drugs in response to external stimulus like light. You *et al.* [23] reported successful loading of doxorubicin on gold nanoparticles and nanoshells after 24 h of mixing at room temperature. Interestingly, they found that the payload of doxorubicin is exceptionally high (up to 70%) in gold nanoshells than that in gold nanoparticles (~20%). They believed the hollow interior of the nanoparticles increased the effective surface area for the high payload loading of doxorubicin. Furthermore, the release of doxorubicin can be triggered by NIR laser irradiation to selectively increase the drug amount in tumors. Similarly, Yavuz *et al.* [24] used the hollow interiors of gold cages to load doxorubicin and covered the surface of gold nanocages with thermoresponsive polymers to prevent the preloading doxorubicin leaked from the porous walls. Such design enables the gold nanoparticles to release drugs in an NIR laser controlled fashion.

Some gold nanoparticles are designed to behave dramatically to various internal stimuli in the microenvironment of tumors. For instance, Wang *et al.* [25] tethered doxorubicin onto gold nanoparticles with a PEG spacer *via* an acid-labile linkage. They found that this smart drug carrier could release doxorubicin in response to the low pH of endosome and enhance therapeutic efficacy by overcoming the drug resistance of MCF-7/ADR cancer cells.

3.2. Gene delivery

Gene therapy attempts to treat cancer by altering the dysregulated gene expression of tumor cells. The successful regulation of gene expression requires compaction of DNA/RNA, rapid cellular uptake and endosomal escape, protection from degradation in blood stream and cytoplasm, and effective delivery of DNA/RNA to the nucleus. Therefore, carriers for efficient and safe DNA/RNA delivery are critical for the development of gene therapy.

Gold nanoparticles provide a potent platform for therapeutic gene delivery. Braun *et al.* [26] coated 40 nm gold nanoshells with siRNA and Tat peptide-lipid cell internalizing agents to silence gene expression in a particular subset of cells. They showed that the gene silencing can be temporally and spatially controlled using a pulsed NIR laser *via* light-induced siRNA release. Jensen *et al.* [27] complexed a high-density monolayer of siRNA on the surface of gold nanoparticles, a nanostructure so-called spherical nucleic acid (SNA), to knockdown the expression of oncoprotein Bcl2Like12 (Bcl2L12) of glioma cells. The *in vitro* studies showed SNAs accumulated in human glioma U87MG cells efficiently and neutralized Bcl2L12, a potent caspase and p53 inhibitor specifically overexpressed by the majority of human primary gliomas, without any chemical modifications and auxiliary transfection methods. After intravenous injection, the SNAs penetrated the blood-brain barrier, disseminated throughout glioma explants, inhibited Bcl2L12 expression in glioma and reduced tumor volume in mice model, without adverse side effects. To specifically deliver siRNA to tumors, our group constructed a CD44-targeting heat shock protein 72 (Hsp72) depletion nanosystem by assembling gold nanostars with siRNA against Hsp72 and hyaluronic acid, a targeting moiety binds to CD44, in a layer-by-layer manner [7]. We showed that the obtained nanosystem could selectively inhibit the expression of Hsp72 in triple negative breast cancer (TNBC) cells, sensitize TNBC cells to hyperthermia and enhance the therapeutic efficacy of photothermal therapy with minimal side effect both *in vitro* and *in vivo*.

3.3. Adjuvant and vaccine delivery

Immunotherapy is a burgeoning therapeutic modality for cancer treatment. The goal of immunotherapy is to harness the host immune system to attack and eradicate cancer cells [28]. There are several ways to enhance the innate power of immune system to fight cancer. Using cancer vaccine is one of the most extensively studied approaches to boost the immune system's response [29].

Gold nanoparticles are promising for delivery of cancer vaccine, because they preferentially accumulate within tissues and cells of the immune system, and have a large surface area for vaccine loading. Lin *et al.* [30] conjugated large quantities of self-assembled tumor-associated peptides on the surface of gold nanoparticles as a cancer vaccine platform. The so-called gold nanovaccines were able to effectively deliver antigens to dendritic cells (DCs) and stimulated cytotoxic T lymphocytes (CTL) better than free peptides.

Since the tumor cells can secrete immunosuppressive cytokines and/or attract immune suppressive cells to evade the attack from immune system, co-delivery of adjuvants with vaccine has been explored to elicit a stronger CTL response to existing tumors. In one study, Lee *et al.* connected red fluorescent protein (RFP, as a model cancer antigen) and CpG oligodeoxynucleotide (CpG ODN, a short single-stranded synthetic DNA used as vaccine adjuvant) on 7 nm gold nanoparticles [31]. The nanoparticles were injected *via* footpad to RFP-expressing B16F10 melanoma tumor-bearing mice. After interacted with DCs, remarkable antitumor activity was induced through a Th1-mediated cell response. In another report, gold nanoparticles were conjugated with both tumor-associated glycopeptides antigens and a peptide from

complement derived protein C3d to act as an adjuvant to activate B-cells [32]. The immunized mice represented robust immune response with production of immunoglobulins, containing both IgM and IgG isotypes.

4. Gold nanoparticles in cancer treatment

4.1. Photothermal therapy

Photothermal therapy (PTT) is a central application of gold nanoparticles in cancer treatment. Gold nanoparticles absorb incident photons and convert them to heat to destroy cancer cells. Due to their unique optical properties as a result of LSPR, gold nanoparticles absorb light with extremely high efficiency (cross section at ~10^{9} M^{-1} cm^{-1}), which ensures effective PTT at relatively low radiation energy. The abnormal vascular structure of tumor is inefficient in dissipating heat, thus the tumors are more sensitive to hyperthermia than healthy tissues. When irradiated by light, the heat generated by gold nanoparticles causes biomolecule denaturation and cellular membrane disruption and kills tumor cells [33].

The wavelength of incident light is important for PTT. The NIR light has maximal penetration in tissues because most components of biotissues, including water, hemoglobin, skin, and other pigments, show minimal absorption and scattering of light in this region. Typically, the NIR light can reach ~1 cm deep in human body. Various nanoparticles that can absorb NIR light have been synthesized and tested for PPT, including nanorods, nanoshells, nanocages, nanostars, nanovesicles, and so on. Hirsch *et al.* [34] demonstrated that PEGylated silica@Au gold nanoshells induced irreversible thermal damage to tumor under exposure to low dose of NIR light (4 W/cm^2) for 4–6 min after systematic injection. Without nanoshells, the exposure of NIR light increased temperature less than 10°C. In the *in vivo* studies, the temperature of tumor was monitored by MRI in real time, and the maximal depths of thermal damage spanning were about 4–6 mm after 6 min of irradiation.

Selective PTT has been demonstrated by using tumor-targeting gold nanoparticles. One strategy for selective PTT is to design immune-targeted gold nanoparticles against receptors overexpressed by cancer cells. For instance, in an experiment designed by Huang *et al.*, [6] gold nanorods were conjugated to anti-EGFR antibodies, incubated with malignant oral epithelial cells (HOC 313 clone 8 and HSC 3) and normal epithelial cells (HaCat), followed by exposure to NIR laser at 800 nm. It is found that HOC 313 clone 8 and HSC 3 destroyed at about half the laser energy required to destroy HaCat cells. Another strategy is using engineered gold nanoparticles responsible to the microenvironment of tumors. Nam *et al.* [35] synthesized a hydrolysis-susceptible citraconic amide moiety and introduced to the surface of 10-nm sized gold nanoparticles. After internalization by cancer cells, the obtained "smart" gold nanoparticles exhibited both positive and negative charges in response to the low pH of endosomes. Then, the electrostatic between the nanoparticles induced rapid aggregation and shifted the absorption to NIR region. In this way, the gold nanoparticles can selectively destroy cancer upon NIR laser irradiation. In another report from our group [36], gold nanostars were decorated

with long-chain amine/carboxyl-terminated PEG. Such gold nanostars were designed to change cell affinity reversibly in response to the change of extracellular pH by utilizing the reversible protonation of amine and carboxyl groups. After intravenous injection, these gold nanostars showed enhanced tumor accumulation and photothermal therapeutic efficacy than that of pH-insensitive PEGylated gold nanostars.

One strategy to increase the therapeutic index of PTT is to increase the photothermal conversion efficacy of gold nanoparticles. Higher photothermal conversion efficacy means lower laser power density required for tumor ablation and less damage to skin and other healthy tissues. Chen *et al.* [37] found that the photothermal conversion efficacy decreases as the effective radius of gold nanorods increases. The highest conversion efficacy (~95%) was determined in gold nanorods with size of 10 × 38 nm. In another report [38], the conversion efficacy is 21% for gold nanorods with size of 7 × 23 nm. Therefore, the conversion efficacy of gold nanoparticles is highly dependent on their size and shape. Huang *et al.* [39] reported the synthesis of gold bellflowers by a liquid-liquid-gas triphase interface system. The gold bellflowers showed photothermal conversion efficiency at 74%, which is higher than most reported gold nanoparticles. Ma and coworkers working with our group [40] demonstrated similar photothermal conversion efficiency (~70%) in triangular gold nanoprisms (**Figure 3**).

Reversing the thermoresistance of malignant cells is another pathway to enhance the therapeutic effect of PTT. Recently, Wang *et al.* [41] complexed gold nanorods with siRNA against BAG3, a cytoprotective protein preventing cell death from hyperthermia, to sensitize cancer cells to PTT treatment. They demonstrated that the gold nanorods-siRNA complex enhanced apoptosis and antitumor effect of PTT both *in vitro* and *in vivo* by knocking down the expression of BAG3 in tumor cells. Our group entrapped siRNA against Hsp72, a key chaperone for the thermoresistance of cells, onto the surface of gold nanostars [7]. We showed that the siRNA functionalized gold nanostars sensitized breast cancer cells to hyperthermia and significantly enhanced the therapeutic efficacy. Similarly, Chen *et al.* [42] loaded a small molecule Glut1 inhibitor of diclofenac (DC) on the surface of gold nanorods. The DC depleted the Glut1 level in tumor cells, blocked glycolysis, decreased ATP level, and finally inhibited the expression of heat shock proteins (Hsp). Without the protection from Hsp, the tumor cells were more easily to be destroyed by PTT.

Due to the effect of surface melting, gold nanoparticles may undergo laser-induced shape transformation far below the bulk melting point and lose the photothermal conversion ability. Gold nanoparticles are easier to reshape under irradiation of a pulse laser than under that of a continuous wave laser because the light energy is harder to release from the lattice of gold nanoparticles to surrounding tissues upon pulse laser irradiation [43]. Takahashi et al. [44] demonstrated that gold nanorods were reshaped into spherical nanoparticles under pulsed NIR laser irradiation and did not kill cells upon successive laser irradiation. Wang et al. compared the photothermal stability of gold nanorods, nanohexapods, and nanocages under pulsed laser irradiation. They showed that gold nanorods started to melt at 15 mW/cm^2, whereas nanohexapods and nanocages started to melt at 25 mW/cm^2. Chen *et al.* [45] found that gold nanocages were stable under continuous wave laser but melt to nanoparticles under

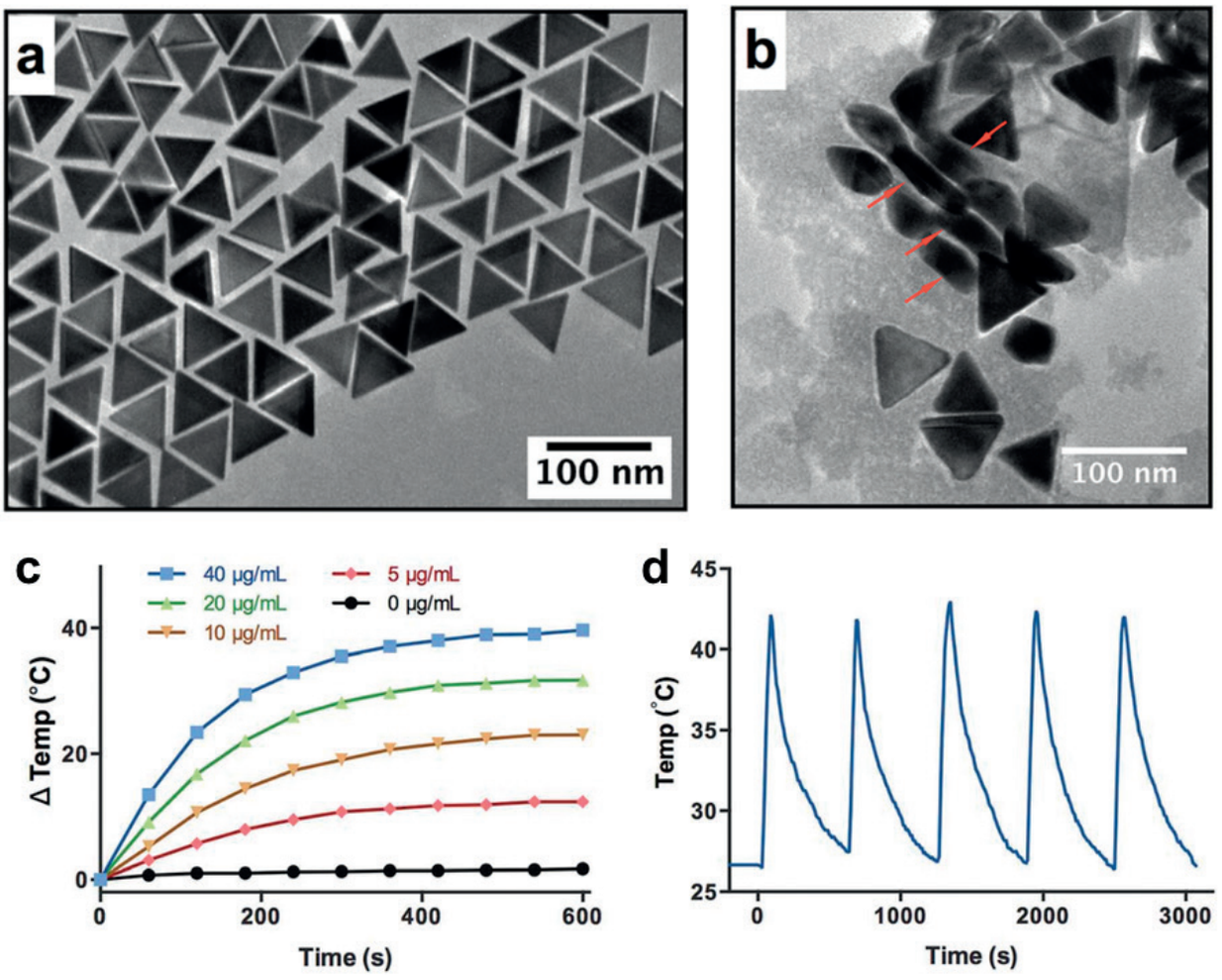

Figure 3. PEGylated gold nanoprism for PTT. (a) and (b) Representative TEM images of PEGylated gold nanoprism. (c) Heating curves of various concentration of PEGylated gold nanoprism under laser irradiation. (d) Photostability of gold nanoprism under five cycles of laser irradiation. Reproduced from Ref. [40].

pulse laser. Thus, the type of laser and the photothermal stability of gold nanoparticles should be considered in practical applications of PTT.

4.2. Photodynamic therapy

The principle of photodynamic therapy (PDT) has been known for over 100 years. PDT involves three nontoxic components, photosensitizer, light, and oxygen, that are needed to generate singlet oxygen ($^1O_2$) or/and reactive oxygen species (ROS) to kill cells. Generally, the photosensitizer is administrated to localize in tumor first, activated by light of a specific wavelength to transfer energy from light to molecular oxygen, and damage cells by the generated $^1O_2$ and ROS. Since $^1O_2$ and ROS quench after a very short time, PDT is a localized treatment similar to PTT. However, due to the poor solubility of most photosensitizers, it is challenging to deliver them to tumor with high specificity for treatment.

To address this challenge, gold nanoparticles are engineered as carriers of photosensitizer. Camerin et al. [46] bound Zn(II)-phthalocyanine disulfide (C11Pc), a photosensitizer bearing

hexyl chains and a sulfur terminated C11 chain, with gold nanoparticles and investigated the photodynamic therapeutic effect in a melanoma mice model. Compared with free C11Pc, the ratio between the amount of photosensitizer recovered from melanoma and skin increased from 2.3 to 5.5 for gold nanoparticles bound C11Pc at 24 h after injection. The nanoparticle-bound C11Pc also showed a better antitumor effect than that of free C11Pc at the same concentration.

With rational design, the PDT and PTT effect of photosensitizer-loaded gold nanoparticles can be combined to further enhance the therapeutic efficacy via synergistic effect. Jang *et al.* [47] developed a gold nanorods-photosensitizer complex (GNR-AlPcS4) for PDT/PTT dual-modality treatment. In *in vitro* studies, GNR-AlPcS4–treated cells showed fourfold greater intracellular uptake and enhanced therapeutic efficacy than that of free AlPcS4-treated cells. After intravenous injection of GNR-AlPcS4, PDT and PTT effect can be induced by irradiation of 670 and 810 nm laser separately. Compared with PDT alone, the tumor growth inhibition increased from 79 to 95% for PDT/PTT treatment. Our group covalently conjugated Chlorin e6, a commonly used photosensitizer, with gold nanostars [48]. The peak of LSPR of gold nanostars was tuned to match the absorbance of Chlorin e6. Such design enables the simultaneous activation of PDT and PTT effect upon irradiation of single wavelength NIR laser (**Figure 4**). Our results demonstrated that the photostability of photosensitizers and gold nanostars is different, and thus the ratio between PDT and PTT effect can be modulated by adjusting irradiation time. The synergistic PDT/PTT effect significantly enhanced the therapeutic efficacy and inhibited the growth of breast cancer in animal models.

Certain gold nanoparticles have the ability to generate $^1O_2$ and ROS upon light irradiation, allowing them to act as photosensitizers by themselves rather than as delivery vehicles. Krpetić *et al.* [49] showed endosomal gold nanoparticles disrupted endosome and distributed into cytosol of cancer cells upon low-intensity laser irradiation. They ruled out the possibility of photothermal effect as the underlying mechanism due to the low intensity of laser used in their experiments and observed higher concentration of ROS in the cells incubated with gold nanoparticles than in the control cells incubated without nanoparticles after laser irradiation. Pasparakis [50] found that the gold nanoparticles, with a mean diameter of 40 nm, could generate $^1O_2$ upon either pulse or continuous wave laser irradiation. Cancer cell death was observed after incubation with gold nanoparticles, followed by laser irradiation. Although these reports demonstrated the potential of using gold nanoparticles as photosensitizers in the future, the wavelengths of light used in these experiments are 514 and 532 nm. The poor penetration ability of light in this wavelength region limits their potential for *in vivo* applications.

4.3. Photon-based radiation therapy

Radiation therapy (RT) is a major component of the modern therapeutic modalities of cancer. Photon-based radiation therapy uses high-energy gamma rays, typically 8–18 MeV for deep tumors, to control the growth of tumor by causing DNA damage *via* direct ionization or through production of free radicals and secondary electrons [51]. Since gamma rays have a low linear energy transfer (LET, energy deposited per unit distance traversed), a large fraction of radiation dose deposits in healthy tissue before and behind the planned target volume [52].

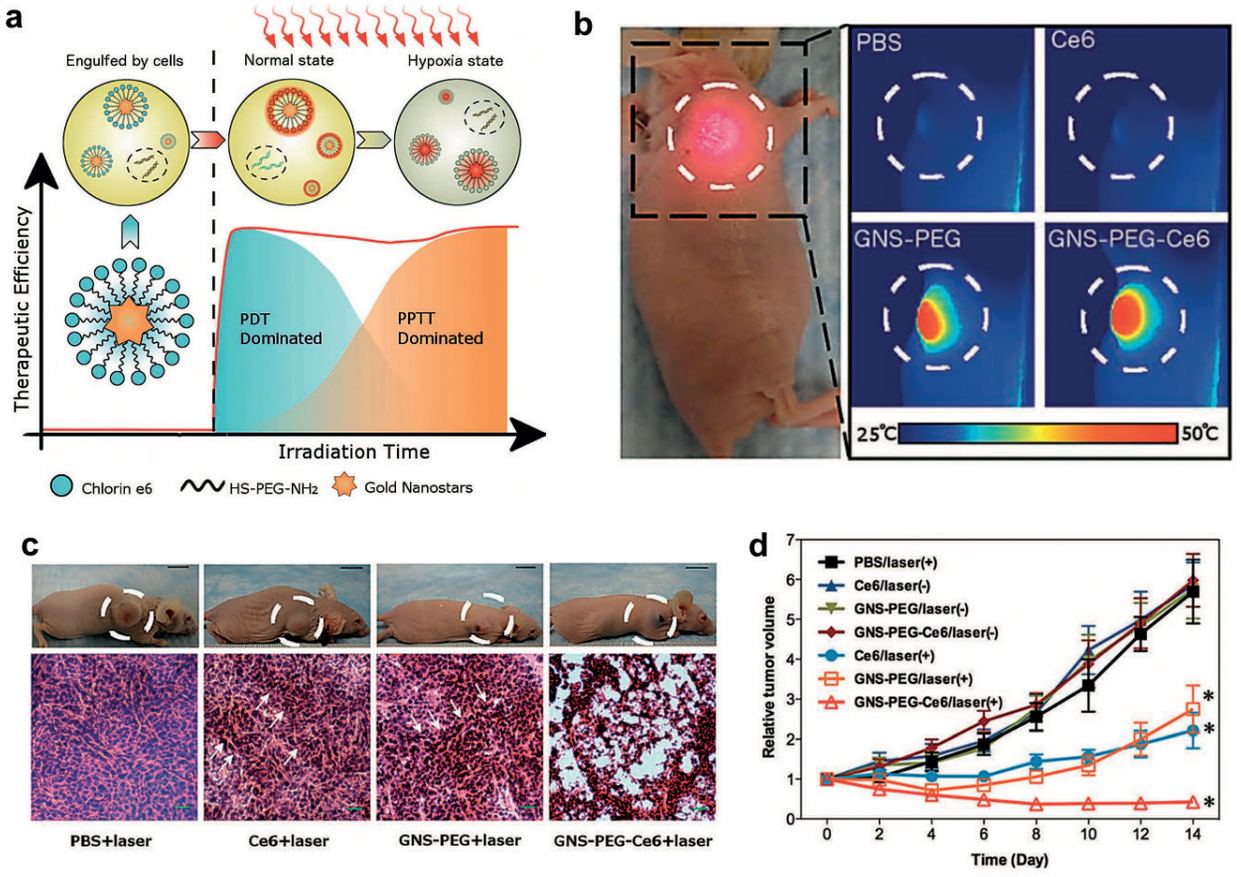

Figure 4. Chlorin e6-conjugated gold nanostars (GNS-PEG-Ce6) for PDT/PTT. (a) Schematic representation of PDT/PTT upon single laser irradiation. (b) Thermal images of MDA-MB-435 tumor-bearing mice under laser irradiation. (c) Digital photos and H&E stained tumor sections collected from each group of mice at the 8th day post treatment. (d) Tumor growth curves of each group after treatment. Reproduced from Ref. [48].

When gamma rays excited core electrons near the atomic nucleus of elements, low energy electrons may be released by a so-called Auger de-excitation processes. Gold nanoparticles have high atomic number and are more likely to generate Auger electrons when compared to light elements of biological tissues. The Auger electrons are effective in breaking DNA and only damage cells in a short distance less than the size of a single cell. This short-range therapeutic effect makes gold nanoparticles potent to selectively sensitize tumor cells to photon-based radiation therapy. Hainfeld *et al.* [53] demonstrated the use of gold nanoparticles with a mean diameter of 1.9 nm for enhancement of radiation therapy. After a single intravenous injection of gold nanoparticles, the tumor-to-normal tissue gold concentration ratios reached about 8:1. The 1-year survival of mice bearing EMT-6 breast cancer increased from 20% (with X-rays alone) to 86% (sensitized by gold nanoparticles) after several minutes of 250 kV gamma ray irradiation. The nanoparticles treated mice presented no overt clinical signs for more than 1 year.

One problem of using ultrasmall nanoparticles is the relatively short half-life due to the rapid excretion from kidney. To reduce the dosage of gold, larger nanoparticles with tumor-targeting moieties have been exploited. In one study, thio-glucose bound gold nanoparticles were synthesized as a sensitizer to enhance radiation therapy for ovarian cancer cells [54]. Since

malignant cells metabolize faster than healthy cells, the glucose coating of gold nanoparticles resulted in an ~31% increase of cell uptake compared to that of naked nanoparticles. As a result, the inhibition of cell proliferation increased 30.48% for 90 kV and 26.88% for 6 MV gamma ray irradiation. In another report, 30 nm gold nanoparticles were conjugated to Herceptin, a monoclonal antibody against Her2, to target MDA-MB-361 in a subcutaneous mice model [55]. After gamma ray irradiation, the tumors treated with gold nanoparticles resulted in 46% tumor regression, whereas the tumors treated by gamma ray alone increased 16% in tumor volume.

Although larger gold nanoparticles achieve improved accumulation in specific tumors due to the EPR (enhanced penetration and retention) effect, some authors argue that as gold nanoparticles become larger, more of the secondary electrons occur in the core of the nanoparticles, thus reducing the dose delivered to the cytoplasm around the nanoparticles. Therefore, the best size range of gold nanoparticles for photon-based radiation therapy is still under debate.

4.4. Ion-based radiation therapy

Ion-based radiation therapy is another type of RT. Instead of utilizing high-energy gamma rays, ion-based radiation therapy uses ion beams as the radiation source, such as the ions of hydrogen (protons), helium, carbon, or oxygen. The ion radiation is attractive because it has a strong LET near the end of the track, which is called the Bragg peak. The location of Bragg peak can be extended by increasing the energy of the ion so that the volume of irradiation can be better defined in ion irradiation than in photon irradiation.

One of the proposed mechanisms for radiosensitization of gold nanoparticles is that the ion beams excite surface plasmons and thus increase the yield of secondary electrons. Li *et al*. [56] demonstrated the radiosensitization effect of gold nanoparticles for proton therapy *in vitro*. They compared the difference between effect of gold nanoparticles with different size (5 and 10 nm) and proton beams with different LET (10 and 25 keV μm^{-1}). A pronounced radiosensitization effect of gold nanoparticles was observed after irradiation of proton beam with high LET, but not with low LET. The effect was more remarkable for large gold nanoparticles than for small gold nanoparticles. Recently, Lin *et al*. established a biological model of gold nanoparticles–enhanced proton therapy [57]. They suggested that the radiosensitization effect would only exist when the gold nanoparticles were internalized into cells, especially into cell nucleus. Kim *et al*. [58] intravenously injected 14 nm gold nanoparticles into CT26 tumor bearing mice and utilized proton beam to irradiate CT26 tumor at 24 h post injection. They observed 100% complete tumor regression (CTR) in mice treated with a proton dose of 31 Gy and tumoral gold concentration of 41 Au/g tissues. In contrast, the CTR was 37–62% for mice treated by proton alone.

5. Future perspectives

As discussed above, the extensive research on gold nanoparticles over the past decade has indicated their potential for a rich variety of applications in cancer imaging and treatment.

Many advances were enabled by the understanding of the chemical synthesis and biobehavior of gold nanoparticles. These understandings will certainly lead to more practical clinical applications. The use of gold nanoparticles for drug delivery and photothermal therapy was proved for phase I and phase II clinical trials [59].

Although gold nanoparticles offer an attractive platform for new novel modalities for cancer imaging and treatment, it is very important to carefully and precisely study their toxicity in potential applications for human. Although the gold is relatively inert for biotissues, these nanoparticles tend to remain in the body, especially in liver and spleen for a long time. Thus, the long-term toxicity of nanoparticles is an issue for their use in humans. Since the reported biodistribution and toxicity vary greatly with the size, shape, and coating of gold nanoparticles, it seems that the safety of gold nanoparticles is dependent on many factors, and so, this needs to be examined for every single synthetic formula and application [60].

One unique property of gold nanoparticles is their LSPR effect, which enables a variety of imaging and treatment modalities, such as light scattering imaging, photothermal therapy, photodynamic therapy, and so on. However, the penetration depth of light in biotissues is no more than several centimeters and cannot reach most tumors in human body. Combining other imaging and therapeutic modalities may provide a strategy to overcome this limitation [61].

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