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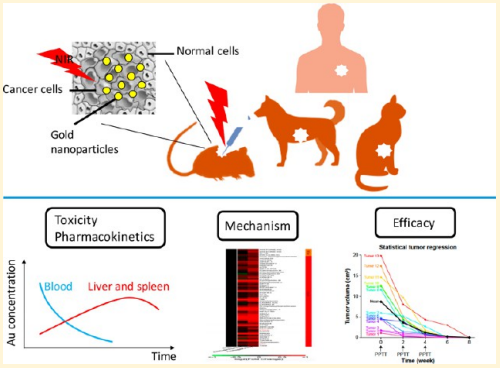
Gold-Nanoparticle-Assisted Plasmonic Photothermal Therapy Advances Toward Clinical Application

Moustafa R. K. Ali,*,# Yue Wu,# and Mostafa A. El-Sayed*

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332-0400, United States

ABSTRACT: Plasmonic photothermal therapy (PPTT), a type of treatment involving the intravenous or intratumoral injection to introduce gold nanoparticles to cancerous cells and the subsequent exposure to heat-generating near-infrared (NIR) light, is a potentially favorable alternative to traditional treatments of localized tumors such as chemotherapy, radiotherapy, and surgery. The current main concern of PPTT, however, is the feasibility of the treatment in clinical settings. Since PPTT's initial use 15 years ago, thousands of studies have been published. In this feature article, we summarize the most recent scientific progress, including the efficacy, molecular mechanism, toxicity, and pharmacokinetics of PPTT *in vitro* with cancer cells and *in vivo* through mouse/rat model testing, animal clinical cases (such as dogs and cats), and human clinical trials. Given the benefits of PPTT, we believe that it will ultimately become a human clinical treatment that can aid in our ultimate goal of beating cancer.



1. INTRODUCTION

Throughout history, gold has been one of the most prized metals on Earth. With its malleable and chemically inert properties, the number of applications for gold has increased over time. The fact that gold is chemically inert, relatively resistant to bacteria, and biocompatible has made it a prime candidate in this quest to improve human condition by serving as a more effective and unique material to treat patients. There is evidence that gold was used in dental repairs over 4000 years ago. Throughout history, gold has been added to medicine to treat diseases, and proof of this practice is demonstrated by the use of gold cordial that was used for treating ailments caused by a decrease in vital spirits (melancholey, fainting, fevers, etc.) and by the application of or a mixture of gold chloride and sodium chloride to treat syphilis.[1] More recently, Robert Koch, a German bacteriologist, discovered that the presence of gold in compounds, specifically gold cyanide, inhibited the growth of tuberculosis-causing bacteria. In 1890, Jacques Forestier, a French scientist, discovered the anti-inflammatory properties of gold compounds, and the manipulation of those properties made them useful drugs that treated rheumatoid arthritis in 1929.[2,3] Also, the qualities of gold have made it a great choice of metal in surgical implants and for wires in pacemakers or stents.[4]

Additionally, the transformation of chemical properties that results from scaling gold down to the nanoscale provides an opportunity. Gold nanoparticles (AuNPs), since the first report by Michael Faraday in the mid-19th century when he prepared gold colloidal solutions,[5] have been widely applied to biology and medicine, extending from genomics and gene therapy[6,7] to the plasmonic photothermal therapy (PPTT) used for cancerous cells and tumors,[8−11] to selective bacterial destruction,[12−14] and to the treatment of HIV.[15−17] These applications are derived from the many unique properties of AuNPs compared to traditional drugs. In the presence of light (which is an oscillating electromagnetic field), the free electrons of the AuNPs will oscillate. This process, termed surface plasmon resonance (SPR), happened when electrons resonate at a particular frequency of light. The surface plasmon oscillation can nonradiatively decay by converting energy to heat. In addition, AuNPs are able to selectively target the AuNP treatment to a specific region within a biological system, leading to high hopes for the potential employment of AuNPs to serve as vessels capable of carrying cargoes such as drugs and genetic materials to where they are needed.[18−20]

Since its ancient usage in 1700 BC, heat has demonstrated its functionality in tumor therapy when the glowing tip of fire was used to treat breast cancer.[21] Photothermal therapy is a minimally invasive therapeutic strategy in which photon energy from light is converted into heat in order to destroy cancer cells. This avoids the severe infection-related complications that are commonly encountered after surgery[22] and circumvents the side effects from using toxic drugs in chemotherapy. Heating sources, including NIR or visible light, the magnetic field, radiofrequency waves, microwaves, and ultrasound waves, are used to induce a moderate temperature rise, clinically termed as hyperthermia, in a specific target region in order to destroy the cancer cells.[11] Due to the low absorption efficiency of natural tissue absorbents, porphyrins and photosensitizers such as the synthetic and organic dye molecule, indocyanine green, that are coordinated with transition metals are externally injected into the tumor sites to enhance their photothermal

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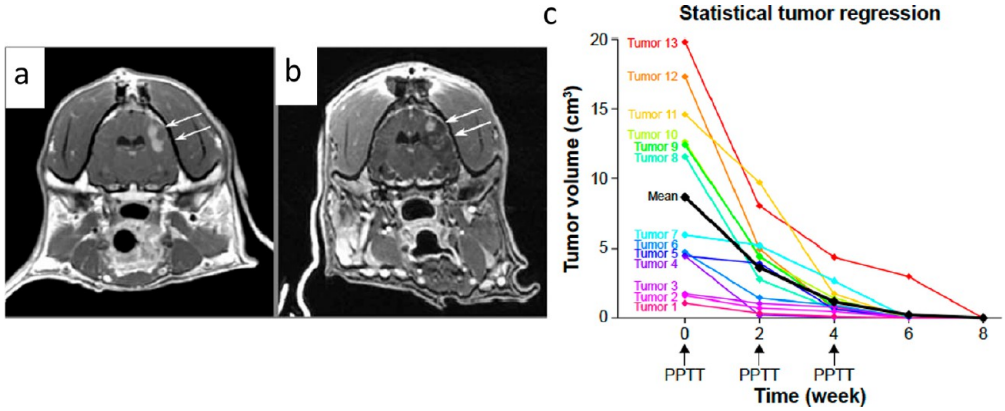

Figure 1. Tumor regression in dogs and cats in different organs using either gold nanoshells (a,b) or nanorods (c). (a,b) AuroLase treatment of a transmissible venereal tumor in a canine brain. MR-DCE axial images of the dog brain showing contrast between (a) enhancement of bilobed tumor before treatment and (b) ablation of tumor after treatment. Reprinted with permission from ref 28. Copyright 2009 American Association for Cancer Research. (c) Our study shows the tumor regression curves from 13 mammary gland tumors in cats and dogs with variable volumes under multiple PPTT treatments using gold nanorods. Reprinted with permission from ref 33. Copyright 2016 Dove Medical Press.

effects when NIR light is applied.[23] However, the dye molecules photo bleach quickly, rendering insufficient therapeutic outcomes.

The nonradiative properties of AuNPs have shown great potential in plasmonic photothermal cancer therapy (PPTT). It is reported that gold nanospheres about 40 nm in size absorb light 10^5 times stronger than do most efficient light-absorbing dye molecules.[24] The light absorbed by nanoparticles with specific shapes and sizes can be converted into nonradiative heat with very high efficiency and speed.[25] A study carried out in our lab regarding the photothermal process has revealed that photoexcitation of metal nanostructures can generate a heated electron gas that can quickly cool within ~1 ps via energy transfer to the nanoparticle lattice, which can then exchange energy with its surroundings on a time scale of ~100 ps.[26,27] SPR greatly enhances the photothermal effect of AuNPs by a few orders of magnitude in a short period of time. Therefore, if AuNPs are attached to or inside of cancer cells and are simultaneously exposed to light, intensive heat can be generated to destroy the cells. Compared to conventional dye absorbers, AuNPs are more effective and more stable without any exposure to photobleaching.

Preclinical studies have shown the high degree of safeness and efficacy of PPTT in treating xenograft mice tumors. Currently, AuroLase therapy that is based on the photothermal effect of silica-gold nanoshells (AuNSs) is undergoing investigation in human clinical trials (ClinicalTrials.gov Identifiers: NCT02680535). In addition, gold nanorods (AuNRs) have been used to treat spontaneously growing tumors in canine and feline patients. The efficacy of PPTT compared to other traditional treatments is considered a very important factor in driving researchers around the world to intensely study the methods of enhancing PPTT efficacy. Changes in the shape, size, surface conjugations, and administration routes of AuNPs play key roles in PPTT applications. Furthermore, the mechanism of PPTT is highly coveted as it would allow PPTT to progress to the clinical stages. In addition, as for any cancer treatment, the toxicity, biodistribution, and pharmacokinetics of PPTT should be studied in a very systematic manner. Thus, it is promising to show the progress in these three points (efficacy, mechanism, and toxicity) in order to move this form of cancer therapy to the clinical stages. Herein we have summarized the progress toward the complete understanding of the efficacy, mechanism, and toxicity of PPTT.

2. EFFICACY OF GOLD NANOPARTICLE-ASSISTED PPTT

2.1. Current Stages of PPTT. Currently, AuroLase therapy, a type of PPTT based on 150 nm silica-gold nanoshells (AuNSs) that absorb NIR light, produce heat, and are coated with polyethylene glycol (PEG), was developed by Nanospectra Biosciences, Inc. and has been under clinical trials (ClinicalTrials.gov Identifiers: NCT00848042 for refractory and/or recurrent tumors of the head and neck (2008−2014), NCT01679470 for metastatic lung tumors (2012−2014), and currently recruiting clinical NCT02680535 for localized prostate cancer (2016 until now)). The clinical trials for AuroLase are based on intravenous (i.v.) injections of AuNSs in the blood, and the accumulation of these nanospheres inside tumors via the enhanced permeability and retention (EPR) effect is due to leaky and poorly organized tumoral blood vessels. Prior to human clinical trials, AuroLase-based PPTT was utilized in the treatment of brain tumors in orthotropic canines, and subsequent tumor ablation was observed after PPTT (Figures 1a and 1b).[28]

Studies of gold nanorods (AuNRs) have been applied in the treatment of spontaneous tumors in canine and feline patients as cancer is very common in cats and dogs. It is estimated that 23% of all dogs[29] die of cancer, and the percentage of feline deaths due to cancer is half that of dogs.[30] Generally, dogs and cats develop similar types of cancer that are analogous to human cancer manifestations,[30] such as mammary gland tumors.[31,32] Ali et al. have performed several studies on the treatment of spontaneous mammary gland tumors in cats and dogs by directly injecting AuNRs into solid tumors (intratumoral injection, i.t.), followed by NIR irradiation.[33] After three sessions of treatments, efficient tumor regression was achieved in all cases with no recurrence or metastasis; additionally, there were not any toxic effects on the blood profile nor a decrease in liver and kidney functioning afterward (Figure 1c).[33] Abdoon et al. also conducted similar studies on dogs and cats with mammary gland tumors.[34] Their results showed that the treated animals had complete remission

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(62.5% (10/16)), partial remission (25% (4/16)), and significantly low remission (12.5% (2/16)), respectively (Figure 1d).[34] The two groups' differences in the conditions as shown in the study by Ali et al. have adopted more gentle conditions to trigger tumor apoptosis, while Abdoon et al. relied on heating the tumor harshly. London and co-workers used AuNR-PPTT to treat spontaneous neoplasia in dogs (carcinoma, sarcoma, or mast cell tumors). The AuNRs were injected intravenously into seven canines 72 h before using the 30 W, 808 nm NIR laser to irradiate the tumor mass. At the end of the study, London and co-workers observed either partial or complete remission of tumors, and the overall response rate was 28.6%.[35]

2.2. Different Types of Gold Nanoparticles Used in Photothermal Therapy. While various nanomaterials, such as noble metals (including Au, Ag, Pt, Pd, etc.), carbon-based materials (carbon nanotubes, graphene, etc.), quantum dots, metal oxides, and organic nanoparticles (such as semiconducting polymers),[23] have been used in photothermal therapy, gold nanoparticles have emerged to be the leading agents that are thoroughly studied as they offer major advantages for the following reasons: (1) high biocompatibility, (2) ease of synthetization and surface modification, and (3) ease of optical and physical property tuning.

The AuNPs that enable the photothermal effect generated with NIR light are more often used in photothermal therapy. NIR light exhibits wavelengths between 750 and 1700 nm (the first window is between 750 and 1000 nm, and the second window is between 1000 and 1700 nm[36]) where the water absorption is minimal, and light can deeply penetrate the tissues in order to reach the tumor area. Spherical AuNPs show a SPR peak at around 520−540 nm. Several Au nanostructures that absorb NIR light have been reported, with structures including Au nanorods,[37] Au nanoshells,[38] Au nanocages,[39] and Au nanostars.[40] It has been recently found that other shapes of AuNPs also exhibit photothermal capability, with shapes including Au bipyramids,[41] Au nanoprisms,[42] Au nanorings,[43] and AuNP assemblies with caterpillar-like structures.[44]

The first demonstration of AuNP-based photothermal therapy was published in 2003 using gold nanoshells (AuNSs) by West, Halas, and co-workers.[9] The AuNSs exhibit a spherical silica core with a gold shell layer. By varying the sizes of the core and shell, the SPR wavelength can be tuned to the NIR region (as shown in Figure 2a).[45] The synthetic protocol for AuNS synthesis (Figure 2b) consists of: (1) growing silica nanoparticles, (2) attaching Au (gold) seeds (1−2 nm) to the silica surface via molecular linkage (such as amino groups),[46] and (3) adding Au nanoparticles to the seeds by reducing Au ions in the growth solution.[47] A TEM image illustrating how the Au layer is formed on the silica surface is shown in Figure 2c.

Later on, our lab performed the first gold nanorod (AuNR)-based photothermal therapy in 2006.[37] Dickerson et al. made gold nanorods (AuNRs) of the right geometry to make them able to absorb infrared light that heats and melts the cancer cells and their surroundings.[37] As shown in the following figure, AuNRs possess two SPR peaks, the transverse peak and the longitudinal peak. The small transverse SPR peak is located around 520 nm. The longitudinal SPR peak of the AuNRs shifts from the visible to the near-infrared (NIR) range due to the change in the aspect ratio (length of a rod divided by its width), as shown in Figures 3a and 3b. AuNR synthesis is one

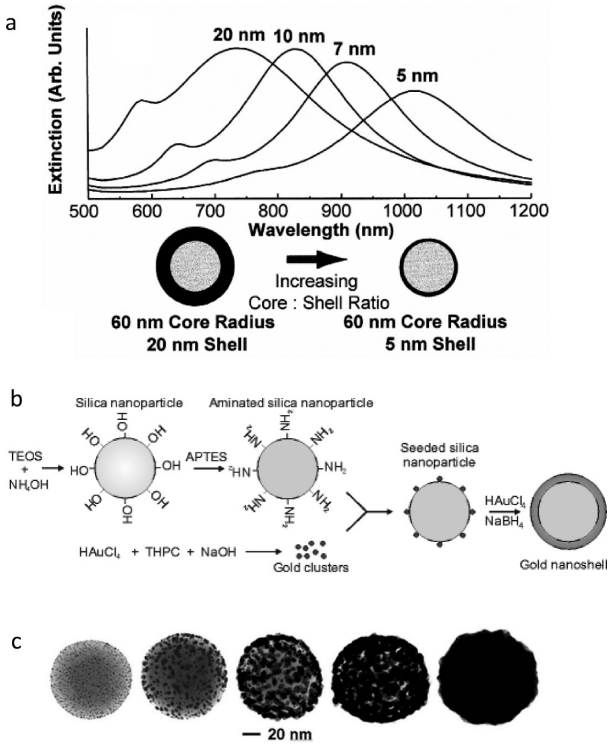

Figure 2. (a) Tuning the surface plasmon resonance by synthetically controlling the shell size of gold nanoshells (AuNSs). Reprinted with permission from ref 45. Copyright 1998 Published by Elsevier. (b) Schematic of silica-core AuNS synthesis. Reprinted with permission from ref 46. Copyright 2016 Springer. (c) Transmission electron microscope images of AuNSs during shell growth. Reprinted with permission from ref 47. Copyright 2004 SAGE Publications.

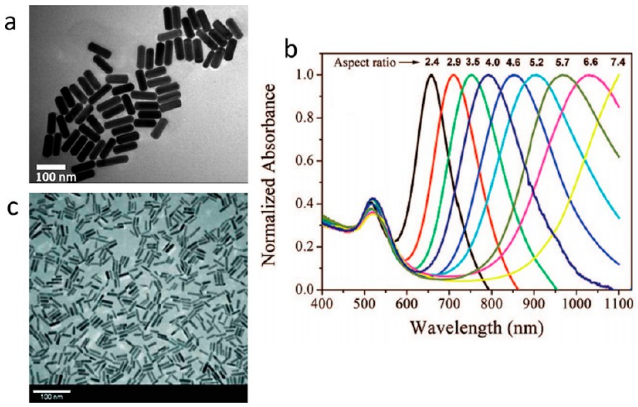

Figure 3. Gold nanorods (AuNRs). (a) TEM images of AuNRs (72 × 16 nm, scale bar 100 nm). Reprinted with permission from ref 56. Copyright 2017 National Academy of Sciences. (b) Tuning of the longitudinal SPR by synthetically controlling the aspect ratio. Reprinted with permission from ref 37. Copyright 2006 American Chemical Society. (C) TEM images of AuNRs (25 × 5.5 nm, scale bar 100 nm). Reprinted with permission from ref 56. Copyright 2017 National Academy of Sciences.

of the most mature and established protocols among all of the anisotropic AuNPs. The most successful strategy is the seed-mediated, wet chemical synthesis method (originally developed in Murphy's lab[48−51]) that utilizes cetyltrimethylammonium bromide (CTAB), which forms the rod-shaped micelles,[52] and Ag^+ that leads to an increase in the AuNR yield and allows for better control of the aspect ratio.[53] Our lab

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has developed one of the most popular synthesis routes to yield an extremely high amount of AuNRs (can reach 99%) by using CTAB-capped Au seeds with added $AgNO_3$.[54] Additionally, we have developed a synthetic route for small AuNRs (Figure 3c) that produces higher efficacy in heat conversion and the photothermal effect.[55]

Xia and co-workers developed Au nanocages (AuNCs) used in the photothermal destruction of cancer cells in 2007.[57] AuNCs were synthesized using the method of galvanic replacement of silver nanocubes with gold ions in water (Figures 4a and 4b). By controlling the amount of $HAuCl_4$ titrated into the reaction, the wavelengths of the SPR peak can be tuned between 600 and 1200 nm (Figure 4c).[58] The increase in the amount of Au is directly correlated with the increase in the SPR wavelength.

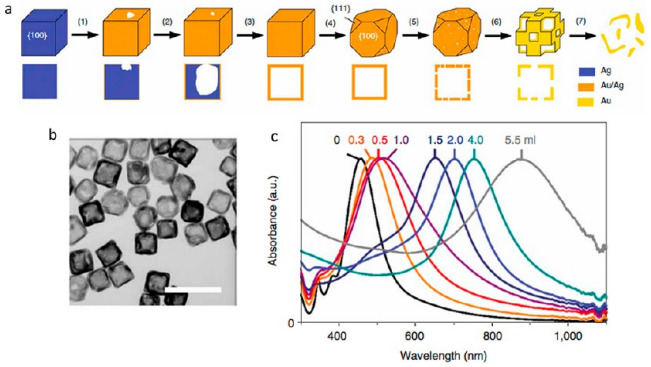

Figure 4. (a) Schematic of the synthesis of AuNCs evolved from Ag nanocubes with increasing concentration of Au by galvanic replacement reaction. The major steps include the deposition of gold on the surface of Ag nanocubes, the oxidation and removal of Ag on the nanocube interior, the alloying and dealloying of Ag/Au, and eventually the production of hollow and porous structures. (b) A TEM image of Au nanocages (scale bar 100 nm). (c) Tuning of SPR by synthetically controlling Au content. Reprinted with permission from ref 58. Copyright 2007 Springer Nature.

Comparison of the Different Amounts of Heat Generation from Different Shapes/Sizes of AuNPs for PPTT. A good agent that displays favorable photothermal efficiency should have a high cross-section of NIR absorption and high photothermal conversion efficiency. In general, small AuNPs show higher absorbance levels than the larger AuNPs (Figure 5a),[24,59] resulting in more efficient heat generation. Using the Mie theory and the discrete dipole approximation (DDA) method, our lab calculated the absorption and scattering efficiencies of Au nanospheres, AuNSs, and AuNRs.[24] In regard to AuNSs, their absorption contributions relative to their extinction can rapidly decrease as the nanoshell size decreases (see the lower panel in Figure 5b).[60] As for AuNRs, we have shown that smaller AuNRs exhibit higher effectiveness in heat generation (see the upper panel in Figure 5b).[60,61]

2.3. Advantages and Disadvantages of PPTT and Types of Cancer with Which PPTT Has Been Used. AuNP-assisted PPTT offers ample advantages in other types of cancer treatments. First, AuNP-assisted PPTT avoids the systematic side effects associated with traditional cancer therapies, such as chemotherapy. The treatment mainly targets localized solid tumors, with almost no damage to healthy tissues. Second, since PPTT is a physical treatment, there is no restriction on the types of tumors to be treated. Usually, different cancer types have their own treatment drugs, and many cancers develop resistance to particular drugs after a certain time period. In this way, PPTT could be a "universal" treatment for many types of cancer.

However, AuNP-assisted PPTT presents its own unique challenges. The first issue concerns the biological fate of AuNPs, especially in the long term. The potential applications of AuNPs are endless, but their practical value in nanomedicine is dependent upon their toxicity level. Despite evidence that AuNPs are biocompatible and chemically inert, there have also been studies with contradictory results.[62] However, for many cases, the toxicity does not come from the AuNPs, but rather from either the nonbiocompatible surface ligands,[63] the high power of the laser,[56] or the high treatment dosage of AuNPs.[64,65] The first issue regarding AuNP-based PPTT is the fact that AuNPs primarily accumulate in the liver

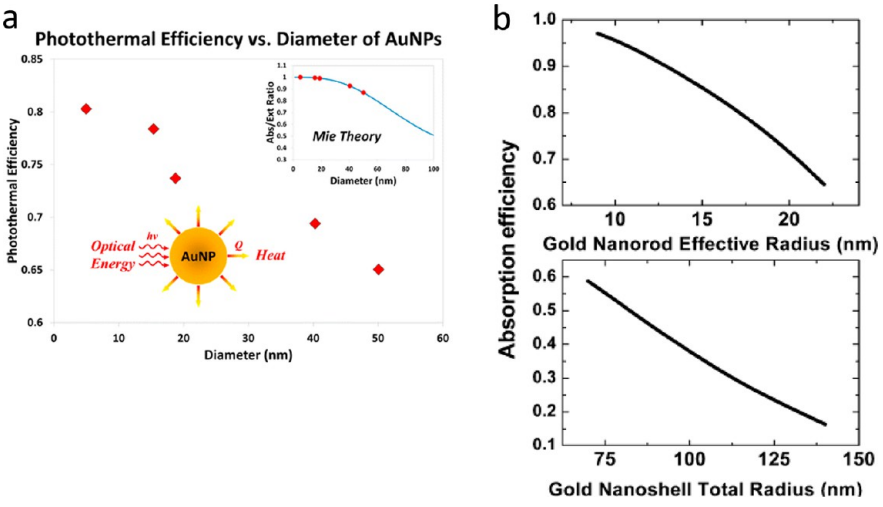

Figure 5. Sizes of gold nanoparticles and photothermal efficiencies. (a) Smaller AuNPs display higher photothermal efficacy. Reprinted with permission from ref 59. Copyright 2013 American Chemical Society. (b) (Top) Dependence of the absorption efficiency of AuNRs with their effective radius at fixed aspect ratio of 3.9. (Bottom) Dependence of the absorption efficiency of AuNSs with sizes at a fixed core−shell ratio of 0.857. (Data extracted from previous work in our lab[24].) Reprinted with permission from ref 60. Copyright 2014 The Royal Society of Chemistry.

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and spleen. Many studies, however, have shown that their negative effect is negligible in the aforementioned organs.[56,66] The second issue deals with the inconsistency in the treatments due to different laboratory variables such as lab personnel, types of AuNPs, surface modifications, laser dosage, different handling, etc. These inconsistencies will result in different outcomes, which sometimes account for high variations in PPTT. The third issue is that PPTT is largely limited to the treatment of localized solid tumors. For delocalized or advanced metastatic cancers, the effectiveness of PPTT might be greatly impaired. Despite this, the recent advances of PPTT show its feasibility in preventing and inhibiting cancer recurrence and metastasis.[67]

Types of Tumors for PPTT Treatment. Due to the nature of PPTT, localized solid tumors are more suitable for treatment. PPTT has been performed in various cancer types, including breast cancer,[41,68] head and neck cancer,[56,69,70] melanoma,[71−73] lung cancer (ClinicalTrials.gov Identifiers NCT01679470), prostate cancer (ClinicalTrials.gov Identifiers NCT02680535), liver cancer,[74] etc. PPTT is more convenient when applied to superficial tumors (such as breast, head and neck, and melanoma tumors) due to its limitation in light penetration depth. However, by using optical fibers for the purpose of delivering light into deep tissues, we are able to achieve photothermal treatment to deeply buried tumors. Here we mentioned conventional PPTT that only involves gold nanoparticles without any other therapeutic supplements such as chemotherapy or immunotherapy; however, the new combined therapies could be more effective.

3. MOLECULAR MECHANISM OF PPTT

3.1. Cancer Cell Death Pathways. Cell death can be induced when cells are exposed to temperatures greater than 42 °C. Generally, cell death follows one of two distinct pathways: apoptosis or necrosis.[75,76] It is reported that cells undergo apoptosis at 44 °C and necrosis at a higher temperature of 46 °C (Figure 6).[77] During necrosis, heat

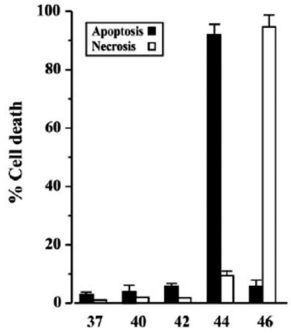

Figure 6. Percentage of cell death after heating cells at different temperatures. Reprinted with permission from ref 77. Copyright 1999 Wiley.

disrupts the plasma membrane, thus causing the cytoplasmic components to leak out and for inflammation to occur. However, apoptosis is a highly regulated cell death pathway that does not cause inflammation[78] and, consequently, serves as a "cleaner" way to eliminate cancer cells.

Though PPTT might share great similarity to traditional heating, it might also activate its unique cell-killing mechanisms associated with the specific properties of gold nanoparticles. Apoptosis and/or necrosis are widely recognized in PPTT.[8] However, as apoptosis discourages an inflammatory response, modulating PPTT to trigger apoptosis would be a more favorable option considering its clinical outcome. Recent studies demonstrate that PPTT can be modulated to trigger apoptosis rather than necrosis through modification of treatment parameters.[79] Specifically, high-dose PPTT (high AuNP concentration, laser power, and/or exposure time) can lead to necrosis, while low-dose PPTT (low AuNP concentration, laser power, and/or exposure time) can promote apoptosis.[33,79] By adjusting the laser exposure time, we developed a mild PPTT strategy to induce cancer cell apoptosis *in vitro* and *in vivo*.[33,56] Figure 7A−D shows that when we irradiate the cancer cells (MCF-7 cells) or tumors (mammary gland tumors in dogs and cats) for 2 min apoptosis predominantly happens (42.7% of the population underwent apoptosis and 2.89% necrosis, respectively, Figure 7C), whereas 5 min laser irradiation (more than 500 times the

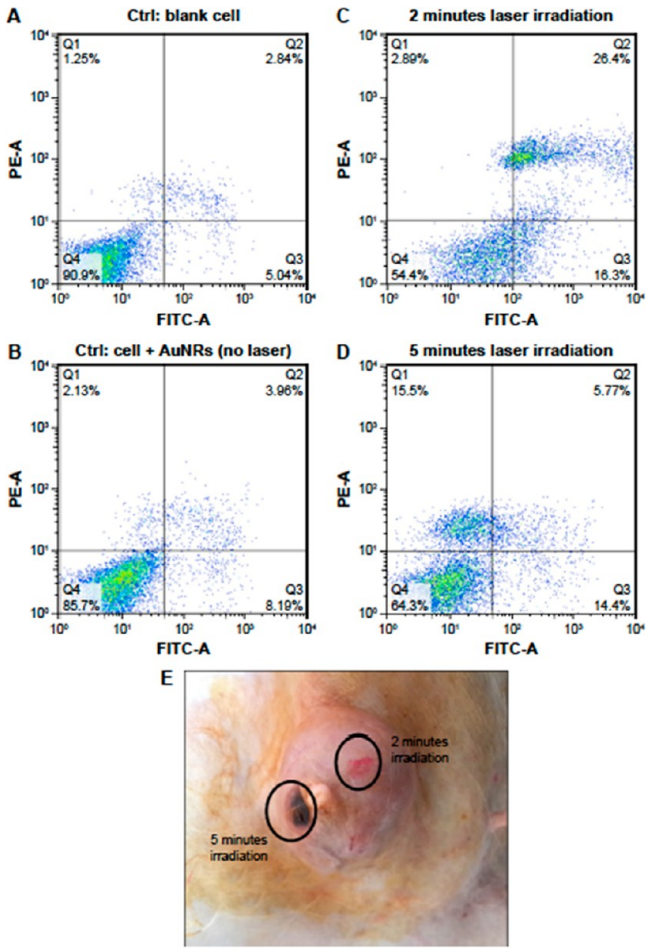

Figure 7. Modulation of PPTT laser exposure time to induce apoptosis in MCF-7 cells (A−D) and mammary gland tumors in a dog. Flow cytometry analysis of normal, apoptotic, and necrotic cells under different conditions: (A) control, (B) incubated with AuNRs (no laser), (C) incubated with AuNRs and 2 min of laser irradiation, and (D) incubated with AuNRs and 5 min of laser irradiation. (E) PPTT on a single mammary gland tumor (5 min irradiation at the left side, 2 min at right side). We observed tumor burning (necrosis) after irradiation for 5 min, whereas there was no obvious change in appearance after 2 min irradiation. Identical conditions were applied for *in vitro* and *in vivo* experiments. Reprinted with permission from ref 33. Copyright 2016 Dove Medical Press.

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dosage of 2 min irradiation) caused 20.17% of the population to undergo apoptosis and 15.5% to undergo necrosis, respectively (Figure 7D). Concerning the tumor response, 2 min of irradiation caused a gentle change within the tumor, whereas 5 min of irradiation caused a very severe burning (Figure 7E). The phenomena in cells (performed *in vitro*) and animal tumors (performed *in vivo*) are quite comparable.

Several mechanisms have been proposed for PPTT *in vitro*. Several reports indicate that it is mediated by the mitochondrial apoptotic pathway.[42,56,79] Pérez-Hernández et al. reported that apoptosis during PPTT with the use of gold nanoprisms is mediated by proteins Bak and Bax through the activation of the Bid protein.[42] A class of proteins termed heat shock proteins can resist heat-induced apoptosis. Ali et al. have reported that AuNR-assisted PPTT has shown varied responses toward different cancer types due to the varying levels of heat shock proteins.[80] Targeting heat shock protein 70 with AuNRs will likely trigger the apoptosis process and magnify the PPTT efficacy.

To better understand the mechanisms involved, systematic analysis such as mass spectrometry (MS)-based proteomics is necessary. In our study, from using AuNR-assisted PPTT for treating head and neck tumor-bearing mice, we found that cytochrome c and p53-related apoptosis mechanisms were contributing to the PPTT mechanism.[56] Proteomics analysis identified multiple apoptosis pathways such as Granzyme B signaling, phosphorylation of the BAD protein (BCL2-associated agonist of cell death), caspase cascade, and others.[56] Additionally, we monitored the SERS spectral signature *in vitro* during apoptosis as a function of PPTT exposure time. Integrative multiomics network analysis revealed specific alterations that explain the underlying changes in the SERS spectral data, demonstrating the power of combining SERS with MS to study cellular processes involved in PPTT.[56]

In conclusion, gentle conditions of PPTT can trigger an apoptotic pathway that is favorable when compared to necrosis since apoptosis is highly regulated and does not cause inflammation. To quantitatively measure apoptosis after PPTT, we can identify several types of markers such as mitochondrial damage, cytochrome c release, plasma membrane permeability, DNA fragmentation, immunological detection, etc.[81] Necrosis can be differentiated from apoptosis by using microscopic techniques or flow cytometry.[82]

Plasmonic Photothermal Therapy Impacts the Tumor Microenvironment and Immune Response. While NPs are traditionally designed to target cancer cells, emerging data suggest that nanomedicine could actually have a larger impact on the tumor microenvironment (TME) (Figure 8). A tumor has now been recognized as an organ whose behavior can only be understood by studying its specialized cellular makeup.[83,84] The TME plays a critical role in cancer development and the metastatic process. Many types of cells exist in the TME, including endothelial cells and immune inflammatory cells (e.g., tumor-associated macrophage (TAM), cancer-associated fibroblasts (CAFs), etc.). The interaction between cancer cells and their TME greatly influences cancer progression.[85,86] In addition to the TME cells, blood vessels also play important roles in promoting tumor progression and metastasis.

AuNPs Innately Exhibit the Ability to Specifically Modulate the TME. It has been reported by Mukherjee et al. that AuNPs could interfere with the crosstalk between cancerous and stromal cells (Figure 9A).[87,88] Furthermore, many immune cells naturally uptake AuNPs, which might help

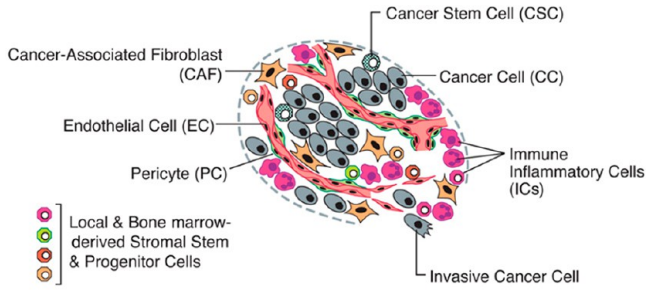

Figure 8. Tumor microenvironment (TME) containing many types of cells. It is worth noting that the cells present in tumors can be tumor-promoting and tumor-suppressing. Reprinted with permission from ref 83. Copyright 2011 Elsevier.

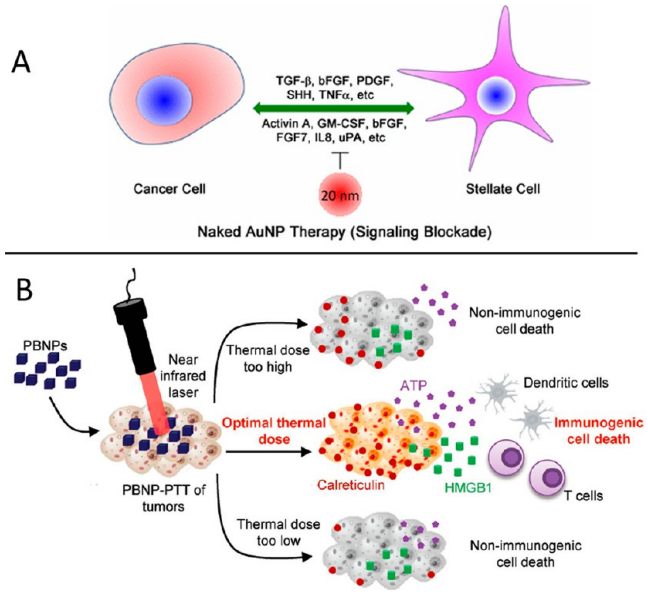

Figure 9. Gold nanoparticles change TME. (A) AuNPs reprogram the pancreatic tumor microenvironment and abrogate the cross-talk between the cancer cell and stellate cell via an alteration of the cell secretome. AuNPs disrupt growth factors/cytokines, resulting in reprogramming of the TME. Reprinted with permission from ref 88. Copyright 2016 American Chemical Society. (B) Photothermal therapy generates immunogenic cell death when an optimal thermal dose is administered to tumors. However, it induces a non-immunogenic response under too high or too low of a thermal dose. Adapted with permission from ref 90. Copyright 2018 Wiley.

boost the immune cells' strength in the TME to eliminate tumors.[89] It is easier to use nanoparticles (NPs) to target immune cells rather than cancer cells, as many immune cells, such as dendritic cells (DCs) and macrophages, naturally uptake more NPs.

Photothermal Therapy Could Effectively Trigger an Immune Response to Eliminate Tumors. An appropriate thermal dose is able to effectively generate an immune response.[90] It has been reported that photothermal therapy could inflame the tumor microenvironment, increase pro-inflammatory cytokines, stimulate local DC maturation,[91] generate a "vaccine-like" immune response, and enhance T cell infiltration.[92] Moreover, several studies showed that PPTT can trigger the immune response for metastasis inhibition. Bear et al. reported that PPTT promotes the maturation of dendritic cells inside tumor-draining lymph nodes, which are regarded as the first organs of metastasis, thereby promoting antitumor T

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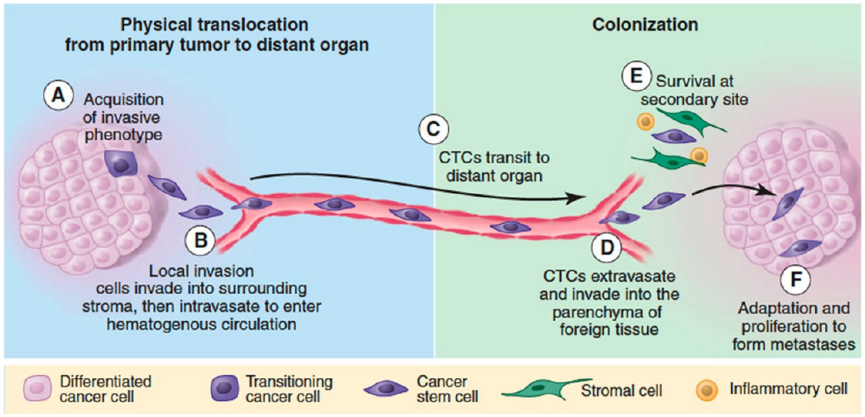

Figure 10. Scheme of the metastatic cascade. Steps A–F describe the process of metastasis. Cancer cells within the primary tumor adopt an invasive phenotype, then locally invade the surrounding stroma to eventually reach blood vessels, enter the blood circulation (cancer cells traveling in this blood circulation are called circulating tumor cells (CTCs)), and finally reach the secondary site from which they begin to colonize. Reprinted from ref 98. Copyright 2011 American Association for the Advancement of Science.

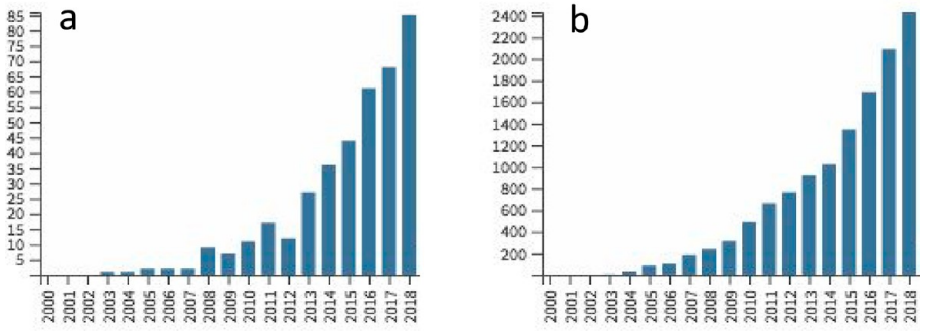

Figure 11. Total publications (left) and citations (right) per year until the end of 2018 regarding the topic "AuNPs inhibit metastasis". The figure is referenced to Web of Science search keywords "gold nanoparticles" and "metastasis".

cell responses.[72] In addition, Mukherjee et al. have reported the antiangiogenic properties of AuNPs, one of which being that they can specifically bind the vascular permeability factor/vascular endothelial growth factor (VPF/VEGF)-165, resulting in the inhibition of angiogenesis *in vivo*.[93] In our recent study involving combination therapy of PPTT and surgery, we observed that applying PPTT before surgery did not cause bleeding during breast tumor removal in dogs and cats. Our histopathology results supported this observation with the presence of altered blood vessels after PPTT.[94] On the other hand, Leong et al. reported that nanomaterials could induce the endothelial leakiness (nanoEL) effect, which leads to the formation of micrometer-sized gaps in blood vessel walls.[95] This phenomenon might need to be taken into consideration when designing future nanomedical treatments to attack cancer.

3.3. Gold Nanoparticles and/or PPTT in Inhibiting Metastasis. Metastasis enables cancer cells to migrate to distant secondary sites, thus making metastasis responsible for 90% of cancer-related deaths.[96−99] Metastasis is a multistep process where the primary cancer cells migrate and locally invade regions close to the main tumor. Afterward, they intravasate and circulate in blood or lymphatic vessels, and the cells finally extravasate and colonize in secondary sites, as shown in steps A–F described in Figure 10.

The migration of cancer cells from one site to another requires a dramatic remodeling of the cellular cytoskeleton.[100−102] The process of cell migration can be described in four steps: protrusion, adhesion, contraction, and retraction. Cell migration is initiated by the polarization and extension of actin protrusions. To stabilize the protrusions, the cells generate adhesions that link the actin cytoskeleton to the extracellular matrix (ECM). The adhesions at the rear of the cell then disassemble and allow for the forward retraction of the cell body. Studies on the changes of cytoskeletal components could provide novel therapeutic approaches to prevent cancer cell migration and metastasis.[100]

Past attempts to develop drugs for preventing or treating metastasis have not been efficacious in clinical trials.[103] Moreover, in many cases, the anticancer drugs that target specific proteins might lose their efficacy after several months of treatment due to mutations in the proteins that engender the rise of drug resistance in cancer cells.[104,105] Recent advances in nanomedicine provide us with great opportunities to avoid the drawbacks of commonly used drugs.[106,107] Nanoparticles are able to selectively target tumors,[108] and a great deal of studies showed that nanoparticles are helpful for cancer diagnosis, therapy,[109,110] and the inhibition of cancer cell migration and/or metastasis.[111−114] AuNPs, especially, have been widely used in these studies due to their unique physical and chemical properties, easy surface modification, and exceptional biocompatibility. Figure 11 displays the numbers of publications and citations related to AuNPs inhibiting metastasis, an area of research that started to emerge in the past decade (reached nearly 85 publications in 2018) and is still in its preliminary stages.

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Several methods have been explored for preventing metastatic cancer using nanotechnology. Generally, there are three strategies for using AuNPs in preventing metastasis: (1) utilizing a AuNP-based drug delivery system that delivers chemo-drugs, antibodies, or siRNA to invasive cancer cells, cancer stem cells, and TMEs (since metastasis only occurs in a "supportive" TME, the perturbation of the TME could be a good strategy for inhibiting metastasis), (2) using AuNPs without any drugs, and (3) combining AuNPs with near-infrared light to generate the photothermal effect. In the first strategy, AuNPs are usually conjugated with drugs used for targeting and killing invasive or metastatic cancer cells. Peptides, such as RGD peptides (target integrin)[115] and tumor metastasis targeting (TMT) peptides, have been employed to target metastatic breast cancer.[116] Besides targeting and killing invasive cancer cells, attacking cancer stem cells,[117,118] inducing angiogenesis,[119,120] and altering the tumor microenvironment,[121] conjugated AuNPs could also prevent the development of metastasis.

In the second strategy, AuNPs have recently shown their ability to prevent metastasis even without drug-loading. AuNPs could inhibit the motility of cancer cells,[114,122,123] induce anticancer immune responses for more effective cancer therapy,[124−126] or modulate the tumor microenvironment (TME) and blood vessel components.[127,128] In 2013, Arvizo et al. reported that nonspecifically targeted AuNPs could inhibit tumor growth and metastasis by abrogating MAPK signaling and reversing the epithelial−mesenchymal transition. Murphy et al.[111] informed the scientific community that AuNPs with different surface charges and sizes can affect cancer cell migration differently. In the same year, Zhou et al. showed that AuNRs coated with bovine serum albumin (BSA) caused reduced cell migration and invasion by way of hindering ATP synthesis, an impairment that subsequently inhibits F-actin cytoskeletal assembly and decreases the metastatic ability of the tumor.[114] However, for most of the previously mentioned works, nonspecifically targeted nanoparticles were used. For instance, Zhou et al.[114] used BSA-coated AuNRs that showed inhibitory effects on cancer cell migration, but the high concentration of AuNRs (50−200 μM) used might have been an obstacle for clinical usage. Our lab recently discovered that nucleus-targeting gold nanoparticles (AuNPs) could stimulate the overexpression of Lamin A/C, thus increasing nuclear stiffness and greatly decreasing cancer cell motility (Figure 12). The nucleus-targeting AuNPs decrease the dosage thousand fold.[129]

Third Strategy Involves Using the AuNP-Assisted Plasmonic Photothermal Effect to Inhibit Metastasis. PPTT has been reported by many studies for its ability to prevent metastasis. In our previous studies, we observed that animals with induced or spontaneous tumors were effectively cured, interestingly, without any metastasis.[33] PPTT can be used to eliminate primary cancer cells and treat local metastasis in lymph nodes.[130] Burke et al. reported that breast cancer stem cells, which are resistant to many chemo treatments as well as drive tumor recurrence and metastasis, are sensitive to AuNP-mediated PPTT and lost their long-term proliferative ability.[131]

Although the phenomena that PPTT could prevent metastasis are starting to be discovered by many animal studies, the mechanism is barely understood. We showed that integrin-targeting AuNP-assisted PPTT can cause cytoskeleton remodeling by affecting Rho GTPase and other pathways,

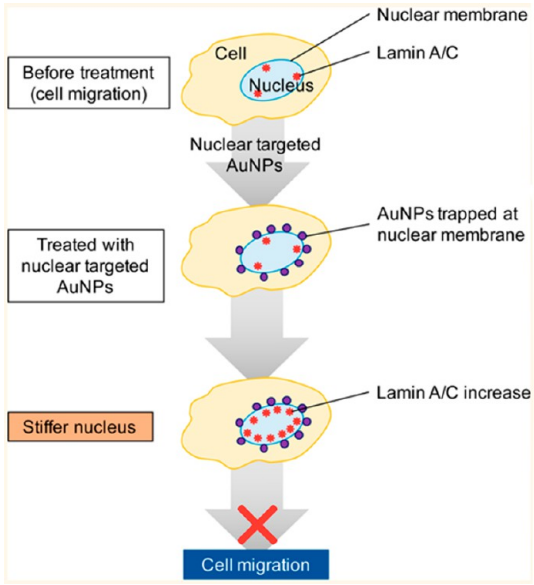

Figure 12. Nucleus-targeting AuNPs increase nuclear stiffness and Lamin A/C formation while also inhibiting ovarian cancer cell migration and invasion. Reprinted with permission from ref 129. Copyright 2017 American Chemical Society.

causing a decrease in cancer cell motility.[132] Additionally, we studied the effect of the same treatment on collective cell migration by phosphoproteomics and high-resolution imaging, both of which reveal impaired cell junctions and actin cytoskeletons.[133]

Besides using this strategy alone, PPTT can also be combined with other therapies such as chemotherapy and radiotherapy in order to prevent metastasis. Wang et al. reported that the combination chemotherapy and photothermal ablation using doxorubicin-loaded DNA-wrapped AuNRs suppresses lung metastasis in an orthotropic 4T1 mammary tumor model.[134] In addition, the same research group developed gold-coated nanocages that loaded DOX and used hyperthermia to trigger drug release for antimetastasis purposes. This method caused an obvious decrease in the number of pulmonary metastatic nodules.[135] In addition, Atkinson et al. reported that gold nanoshells and PPTTs could sensitize breast cancer stem cells (which result in metastasis) to radiation therapy.[136]

3.4. Combination of Photothermal Therapy and Immunotherapy to Combat Cancer and Metastasis. Immunotherapy has recently emerged as a breakthrough for cancer treatment. Although it shows great success in several clinical cases, systematic administration of this type of therapy might lead to many unintended side effects. Nanomedicine, due to the specific properties of nanoparticles (can target specific cells, release of the drug loaded in a controlled manner, etc.), is promising to the future of cancer treatment as it can possibly avoid traditional side effects, decrease the toxicity of conventional immunotherapy,[137] and activate anticancer immunity.[138] The cargo includes tumor antigens that induce responses to the effective cells (such as CD8+ T cells and CD4+ T cells), inhibitors of immunosuppression (i.e., CTLA-4, PD-1, PD-L1), etc. Nanoparticles can recognize specific surface receptors and then enter dendritic cells (DCs).[139] After targeting DCs, Rosalia et al. observed a further boost of tumor-specific CD8+ T cells.[140] Therefore, the resulting cancer cell

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membrane-coated NPs can be used to deliver tumor-associated antigens to antigen-presenting cells,[141] offering a platform for cancer immunotherapy.

Ample evidence suggests that AuNP-assisted PPTT could be well integrated into cancer immunotherapy. Zhou et al. prepared AuNRs conjugated with immunoadjuvant imiquimod (R837).[71] Under NIR irradiation, tumor ablation and immune response triggering were observed in metastatic melanoma in mice (Figure 13). Moreover, lung metastasis and tumor

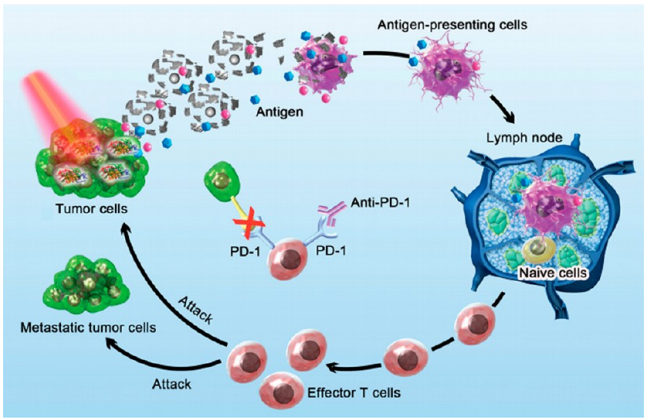

Figure 13. Schematic figure of photothermal therapy using gold nanorods loaded with immune-adjuvant and the mechanism of antitumor immune responses in the treatment of melanoma. Reprinted with permission from ref 71. Copyright 2018 The Royal Society of Chemistry.

recurrence were prevented by the induction of strong, long-term antitumor immunity. Liu and co-workers used photothermal therapy that heated tumors and stimulated DC maturation locally.[91] Their photothermal therapy was synergized with anti-CTLA-4 therapy in order to effectively inhibit cancer metastasis in mice. In addition, the same group adopted a nanocomposite with R837 that showed that photothermal ablation could generate a "vaccine-like" immune response, inflame the tumor microenvironment, increase pro-inflammatory cytokines, and intensify T cell infiltration.[92] This effect can be combined and enhanced with checkpoint blockades for tumor control in order to achieve effective treatment for metastatic cancer (Figure 14).[92] Zhou et al. combined local phototherapy and immunotherapy, and this fusion induced a systemic immune response against primary tumors and metastasis in pancreatic tumor models.[142] Although some of the studies mentioned above used types of nanoparticles other than gold, the concept of photothermal therapy and its ability to effectively promote immunotherapy are shown.

4. BIODISTRIBUTION, TOXICITY, AND PHARMACOKINETICS OF GOLD NANOPARTICLES

For PPTT to be successful, the AuNPs ideally need to accumulate within the tumor. However, the distribution and pharmacokinetics of AuNPs in the body are largely dependent on several factors: size, surface coatings, and administration routes of AuNPs.

In general, for nanoparticles that are i.v.-injected, the main pathway of clearance is through the reticuloendothelial system (RES) via macrophages in the liver and spleen.[143] The diminished interaction between nanoparticles and the RES lengthens the blood circulation time, and this longer period of time is often associated with higher intratumoral penetration.[65] Nanoparticles accumulate in the tumor due to the enhanced permeation and retention (EPR) effect, a phenomenon directly related to immature and leaky tumor blood vessels. In addition, in order for nanoparticles to get inside the tumor, they must first cross a barrier under high interstitial fluid pressure and surrounded by dense stromal tissues. Smaller AuNP sizes might be more beneficial for overcoming these barriers. The effects of AuNP size on toxicity, clearance routes, heat generation efficiency, blood circulation time, and intratumoral penetration ability are summarized in Figure 15. The smaller AuNPs (>20 nm) might be more beneficial for PPTT.[144−146]

4.1. Effects of Size and Shape on Cellular Toxicity, Pharmacokinetics, and Biodistribution. Nanoparticles display distinctive biological behaviors in the body compared to other small molecules. Smaller size enables AuNPs to pass through the blood brain barrier (BBB, <20 nm), and AuNPs less than 5 nm in diameter are able to clear from the kidney.[148] Fraga et al.'s study that utilized 20 nm AuNPs showed a lack of particles in the brain at both assessed time points (30 min and 28 days), indicating that the AuNPs did not cross the BBB.[149] Most of the AuNPs used in PPTT are larger AuNPs (>20 nm), which are usually unable to pass the BBB or achieve renal clearance, consequently showing accumulation mainly in the liver and spleen. There is evidence that smaller nanoparticles are more toxic than larger ones possibly due to their higher probability of interacting with their surroundings and inducing greater immune responses as a result of their high surface area relative to their mass.[144] Pan Y et al. reported that AuNPs with a diameter of 1.4 nm are much more cytotoxic than 15 nm AuNPs of similar chemical composition.[145] In another study, Coradeghini et al. assess the colony-forming efficiency of Balb/3T3 mouse fibroblast cells incubated with 5 and 15 nm AuNPs for 72 h. The results indicated that the 5 nm AuNPs at a concentration higher than 50 μM exhibit cytotoxicity, while there was no cytotoxicity found for the 15 nm AuNPs.[146] Low cytotoxicity was observed in 15−20 nm AuNPs despite the differences in cell lines and in the treatment time. Pattanayak et al. studied AuNPs between 15 and 20 nm in diameter in the L929 mouse cell line for 15−16 h of treatment and reported that they did not observe any toxicity.[152] Murphy et al. show that 18 nm AuNPs do not cause acute cytotoxicity.[153] Khan et al. treated HeLa cells with 18 nm AuNPs before incubation for 3 and 6 h. The AuNPs were not observed to enter the cellular nuclei as they were found to be localized within the cytoplasmic membranes. They generated transcriptional profiles of HeLa cells and found that the expression level of most of the genes remained unaltered.[154]

Li and co-workers studied the size effect on the biodistribution and pharmacokinetics of intravenously injected AuNPs (20, 40, and 80 nm, all coated with PEG) in mice.[151] The 20 nm AuNPs exhibited the slowest clearance from the body and longest blood circulation time (half-life clearance of particles 30−40 h), followed by the 40 nm AuNPs (half-life 10 h). The 80 nm AuNPs exhibited the fastest clearance (half-life <1 h), as shown in Figure 16. In addition, the 20 nm AuNPs had lower accumulation in the liver and spleen than the 80 nm AuNPs, but they exhibited higher accumulation in the tumor due to their longer blood circulation time. Similar observations were reported by Cho et al., who also performed studies regarding the pharmacokinetics and biodistribution of differently sized PEG-coated AuNPs (4, 13, and 100 nm) when injected intravenously into mice.[155] Both of the smaller AuNPs

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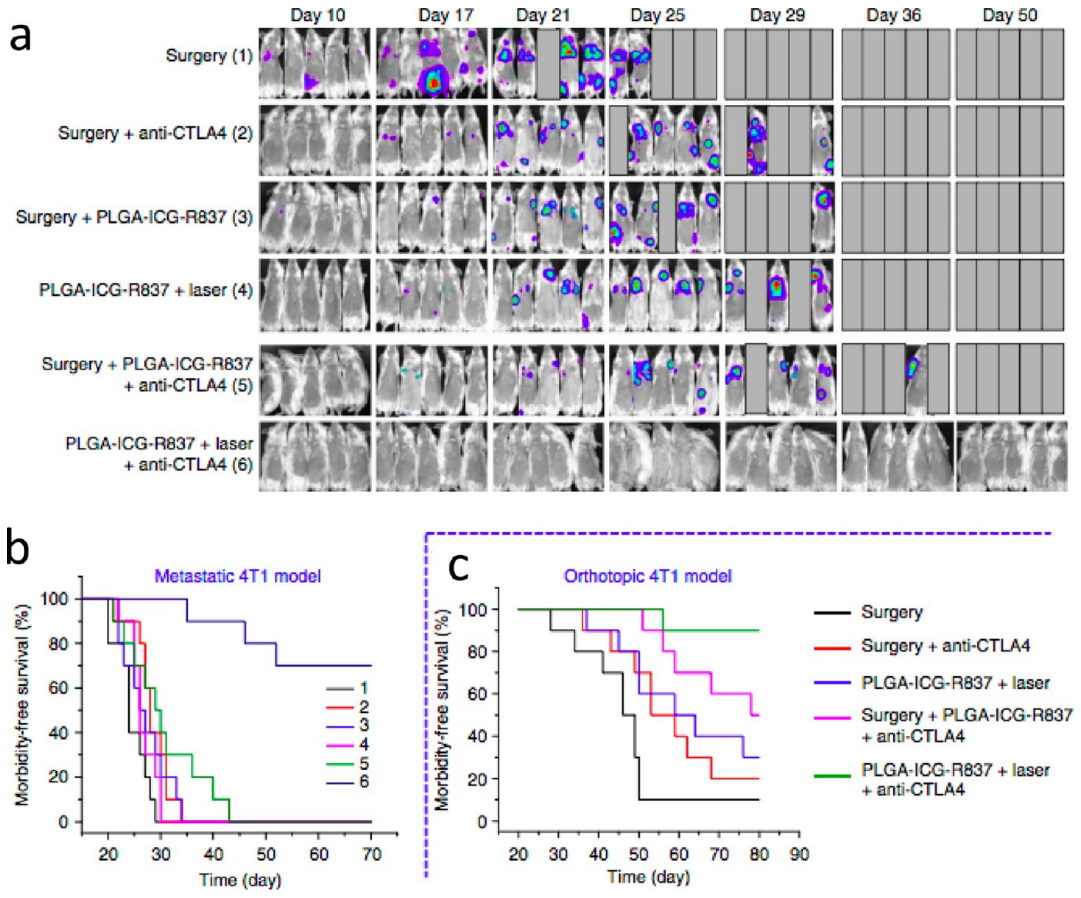

Figure 14. Photothermal ablation could generate a "vaccine-like" immune response, prevent metastasis in 4T1 mice, and extend their life span. (a) Bioluminescence images for tracking breast tumors and metastasis in 4T1 mice after various treatments (conditions # 1−6). (b) Morbidity-free survival of mice with metastatic 4T1 tumors after various treatments. (c) Morbidity-free survival of mice with orthotropic 4T1 tumors with spontaneous metastasis after various treatments (10 mice per group). Reprinted with permission from ref 92. Copyright 2018 Springer Nature.

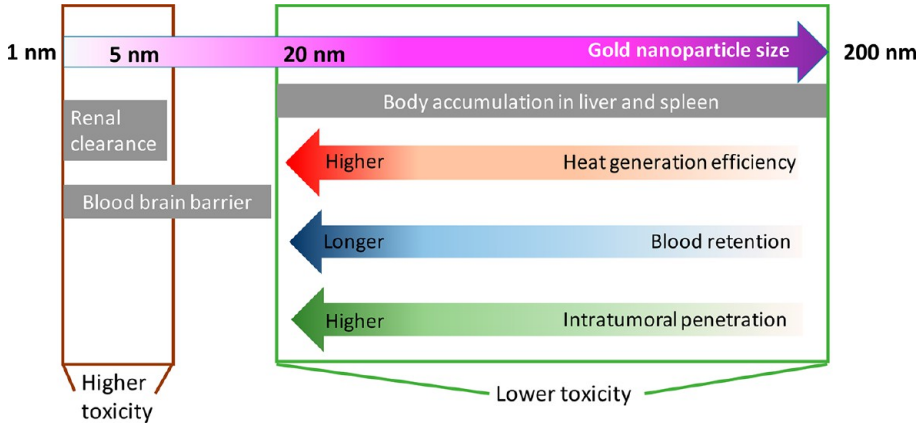

Figure 15. Sizes of the AuNPs in PPTT affect their biological behaviors, concluded from refs 24, 59, and 147−151. Smaller size enables the AuNPs to be cleared from the kidney (with sizes below 5 nm) and pass through the blood brain barrier (BBB, <20 nm). Most of the AuNPs for PPTT are larger AuNPs (>20 nm), which show accumulation mainly in the liver and spleen. For AuNPs used in PPTT, smaller sizes enable higher heat generation efficacy, longer blood retention, and higher intratumoral penetration. Experimental evidence showing that very small nanoparticles are more toxic than larger ones.

(4 and 13 nm) revealed longer blood circulation intervals, peaked at 24 h and cleared by day 7, while the large 100 nm AuNPs were cleared in 24 h. In contrast, 100 nm AuNPs rapidly accumulated (~30 min) in the liver, spleen, and mesenteric lymph nodes, while small AuNPs accumulated more slowly in the organs (peak at 7 days in liver and spleen, 1 month in mesenteric lymph nodes). TEM showed AuNPs existing in cytoplasmic vesicles and lysosomes of Kupffer cells in the liver as well as in macrophages in the spleen and mesenteric lymph nodes.

4.2. Effects of Surface Modifications on Toxicity and Biodistribution. Surface modifications have been observed to have drastic effects on the interactions between AuNPs and biological systems. The structures, functional groups, and

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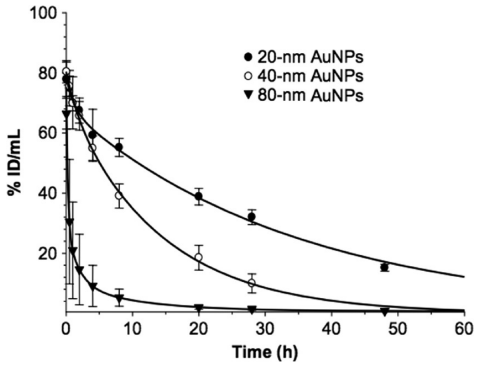

Figure 16. Pharmacokinetics (of 20, 40, and 80 nm AuNPs) expressed as the percentage of the injected dose per gram of tissue in mice (% ID/g). Reprinted with permission from ref 151. Copyright 2009 Elsevier.

charges of specific surface modifications result in different cellular responses to the conjugated AuNPs. It is noteworthy to mention that the aforementioned debate over the perceived toxicity of AuNRs primarily stems from the surfactants of the AuNRs. Cetyltrimethylammonium bromide (CTAB) is one of the surfactants central to this debate, and there have been studies that confirmed that the incomplete purification of AuNRs during synthesis results in free CTAB molecules that induce the observed cytotoxicity.[63,106,156]

In order to assess the toxicity of AuNPs with different surface charges, Goodman et al. synthesized cationic and anionic particles, and they found that the toxicity of the AuNPs was related to their interactions with the cell membrane.[157] The cationic AuNPs were found to be more strongly attracted to the negatively charged membrane than the anionic AuNPs, an observation that is expected given their electrostatic complementarity relative to the negatively charged bilayer of the cell membrane. In another piece of work demonstrating the cellular behavior of positively charged, negatively charged, and neutral AuNPs, Schaeublin et al. found that charged AuNPs displayed toxicity at relatively low dosages (10 mg/mL), while neutral AuNPs displayed significant levels of toxicity at a higher dosage of 25 mg/mL.[158] Their final results illustrated that both the positively and negatively charged AuNPs were toxic, with the negatively charged AuNPs having a magnified response that resulted in necrosis being the primary mechanism of cell death. It is also worth noting that the physiochemical surface properties of AuNRs change after contact with biological media. Therefore, this type of change needs to be considered when examining the biological impact of AuNRs.[159]

The FDA-approved PEG modification was achieved by adding mPEG-SH to the gold nanoparticles to form a nearly neutral surface, which showed little cytotoxicity *in vitro* and is currently becoming one of the most favorable surface modifications of AuNPs for *in vivo* usage. PEG modification creates a nonspecific barrier that reduces unspecific bindings in blood components such as proteins and cells,[160] which could greatly decrease the interaction between AuNPs and RES, leading to extended blood retention and increased uptake in the tumor.[143]

4.3. Gold Nanoparticle Administration Strategies: Intravenous vs Intratumoral. In PPTT treatment, since intravenous injection relies on the EPR effect, it might face challenges regarding the transportation of adequate amounts of AuNSs to the tumor site. Several studies have showed less than 10% ID/g delivered to the tumor when administrated by

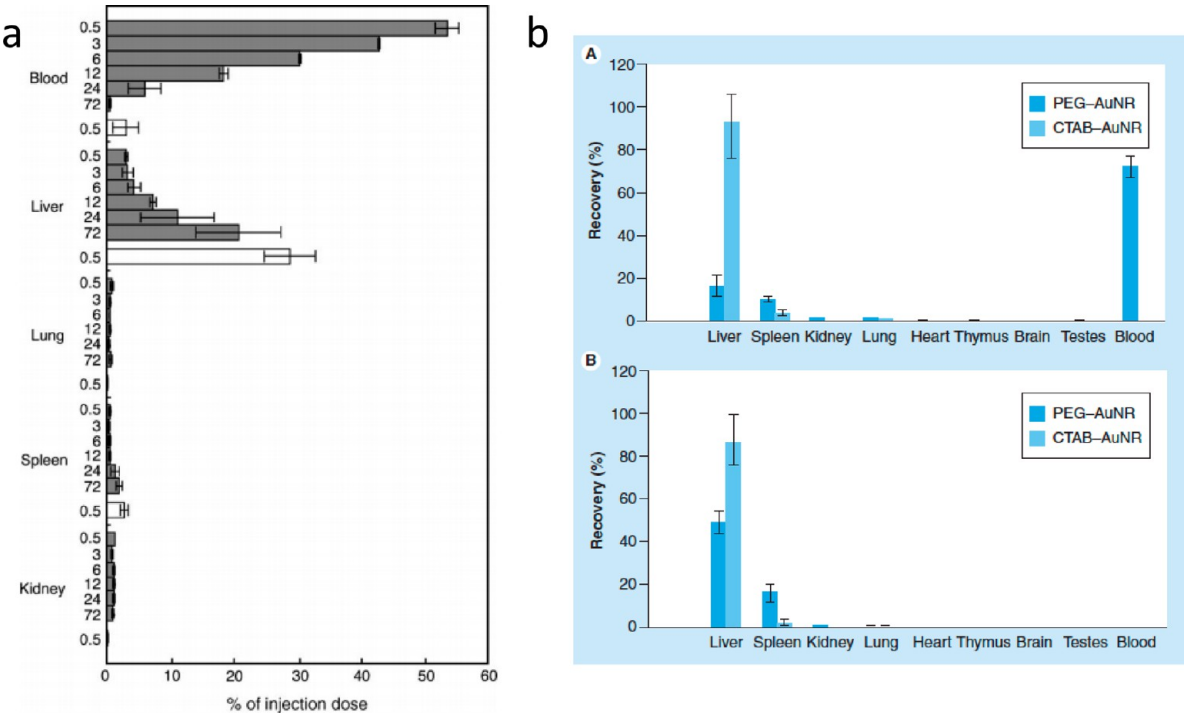

Figure 17. (a) Blood clearance and biodistribution of AuNRs in mice after intravenous injection. Black bars show PEG-coated AuNRs at 0.5, 3, 6, 12, 24, and 72 h after injection, and white bars show CTAB-coated AuNRs at 0.5 h. The CTAB-coated AuNRs were washed once with water to reduce toxicity to mice. Reprinted with permission from ref 160. Copyright 2006 Elsevier. (b) Percentage recovery of gold in different organs at day 1 (top panel) and day 6 (bottom panel). Reprinted with permission from ref 162. Copyright 2011 Future Medicine.

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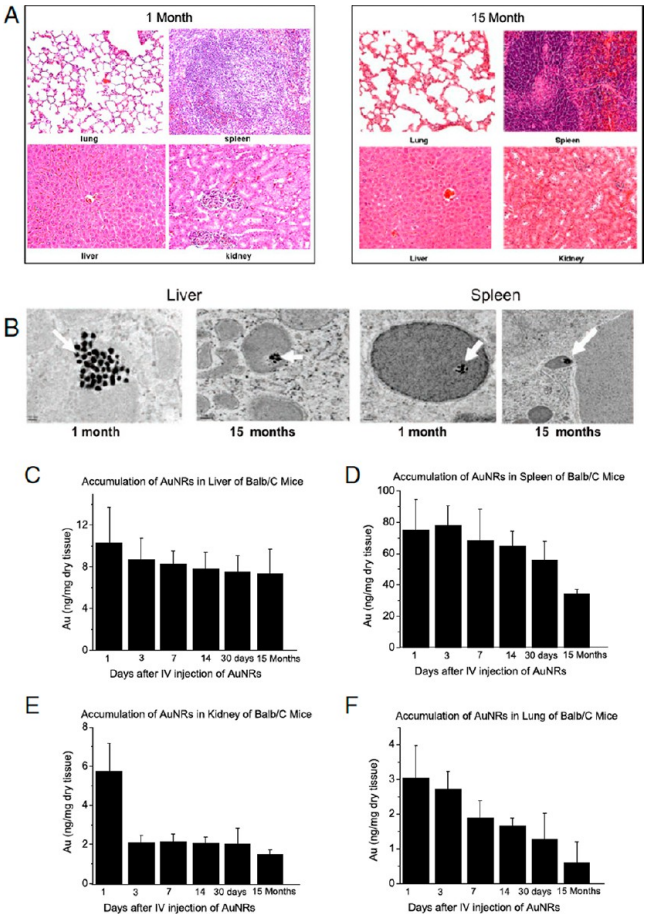

Figure 18. 15-month toxicity study of PEG-coated AuNRs in mice. (A) Histopathological images of the liver, spleen, lung, and kidney of BALB/c mice after 1 and 15 months. (B) TEM images of AuNRs in the liver and spleen without obvious morphology changes after 1 and 15 months. (C–F) Accumulation of AuNRs in different organs (liver (C), spleen (D), kidney (E), and lung (F)) over 15 months. Reprinted with permission from ref 56. Copyright 2017 National Academy of Sciences.

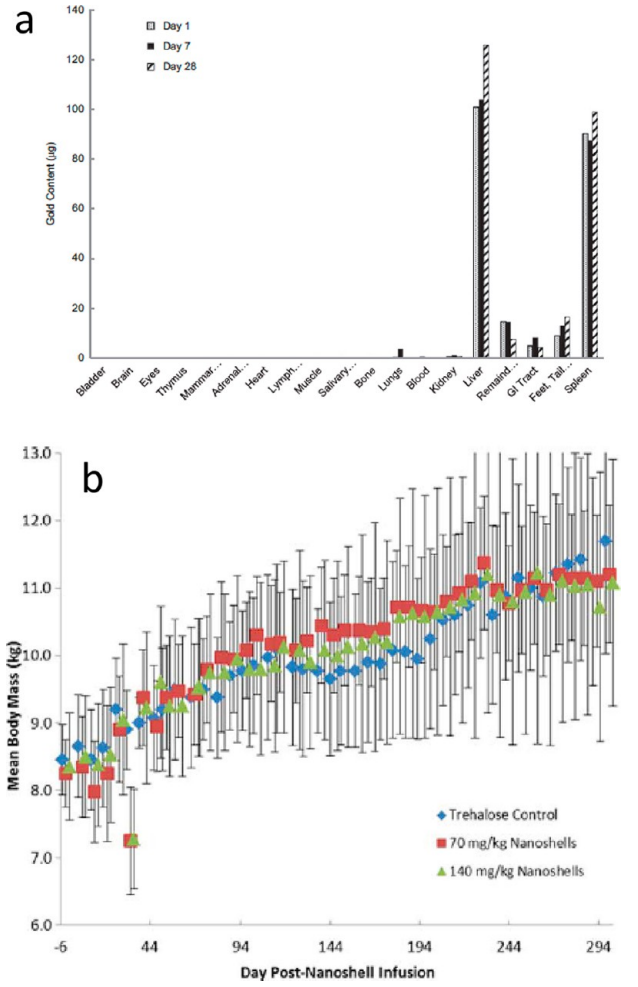

Figure 19. (a) Gold content in major organs after i.v. injection of PEG-coated AuNSs in Balb/c mice. (b) Mean body mass for dogs in a 10-month study; no variations in body mass were observed for the test groups (with i.v. injection of PEG-coated AuNSs) compared to the control. Reprinted with permission from ref 66. Copyright 2012 Sage Journals.

intravenous injection.[143,161] On the other hand, several other groups, including our lab, have been using intratumoral administration of AuNPs.[33,56] The intratumoral administration showed a successful result when a reasonable dose was applied. The intratumoral injection could directly introduce AuNPs into the tumor site and, therefore, provide a more favorable AuNP concentration inside the tumor while also decreasing the injection dosage. However, i.v. injection could be more helpful in some cases, especially for tumors that are not accessible by direct injection of AuNPs.

For PPTT, the AuNRs, AuNSs, and AuNCs are separately discussed in the following contents.

4.4. Pharmacokinetics and Biodistribution of AuNRs. The PEG-coated AuNRs (Length × width, 65 × 11 nm) completely changed the *in vivo* pharmacokinetics of the initially CTAB-coated ones, according to the report from Niidome et al., when using a mouse model.[160] PEG-coated AuNRs exhibited a stable and extended circulation in the blood (half-life of ∼1 h), without any accumulation in major organs (except for the liver) for at least 72 h. As for the CTAB-coated AuNRs, fast clearance in the blood and accumulation at around 0.5 h were observed, as shown in Figure 17. The majority of the AuNR accumulation occurred in the liver. Similar observations in rats comparing CTAB-coated and PEG-coated AuNRs (55.3 × 18.5 nm) were reported by Lankveld by et al., with the conclusion that the PEGylation of AuNRs resulted in a prolongation of blood clearance after intravenous administration[162] and an accumulation of AuNRs mainly inside the liver and spleen.

Although most of the studies involving AuNR biodistribution are performed using ICP-MS to measure the Au content in organs, live animal imaging methods are also used. Su et al. used 3-dimensional optoacoustic tomography imaging to map the biodistributoion of AuNRs coated with PEG in living mice.[163] The optoacoustic imaging system was equipped with two lasers: one sensitive to both the AuNRs and blood (765 nm, close to the SPR of AuNRs) and the other only sensitive to blood (1064 nm). Maximum levels of blood AuNR brightness were observed 24 h postinjection, followed by a slow clearance during the next 6 to 7 days.

Our study has reported a 15 month toxicity study of PEG-conjugated AuNRs (25 × 5 nm) in mice.[56] To assess the toxicity, we examined the histopathology of liver, spleen, lung, and kidney tissues in mice at 1 month and 15 months after the single i.v. injection of AuNRs conjugated to PEG. No

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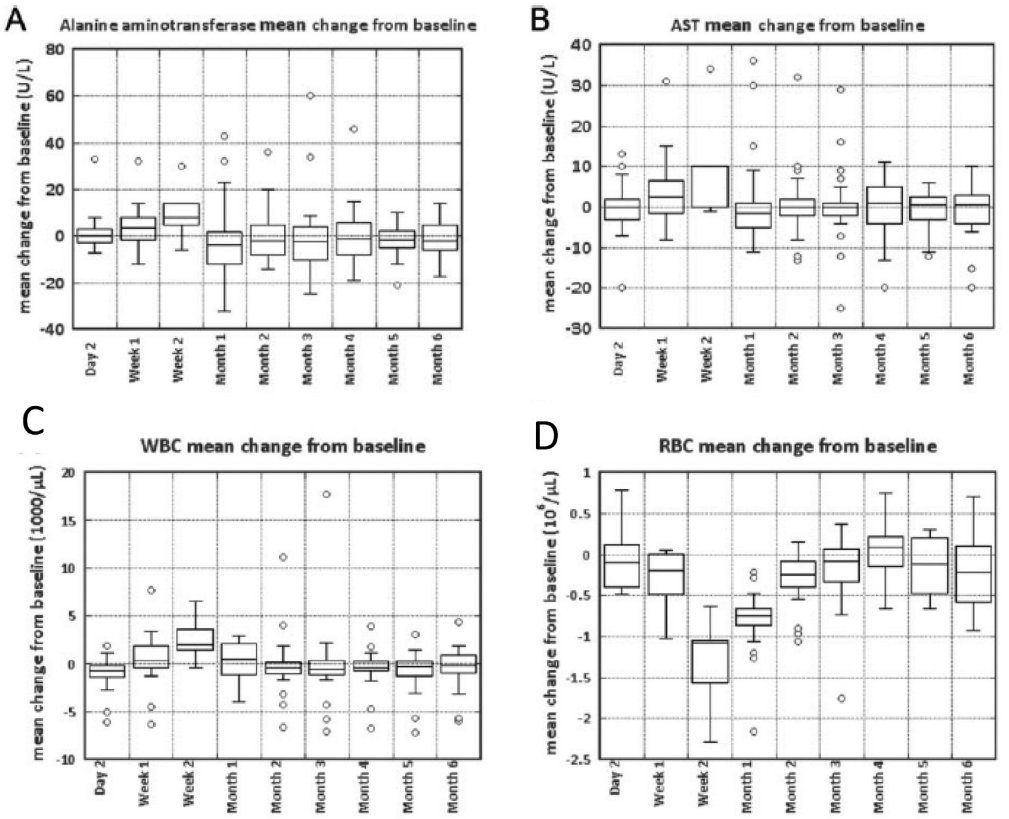

Figure 20. Blood chemistry and hematology. Only ALT, AST, WBC, and RBC are shown here. Reprinted with permission from ref 164. Copyright 2016 Sage Journals.

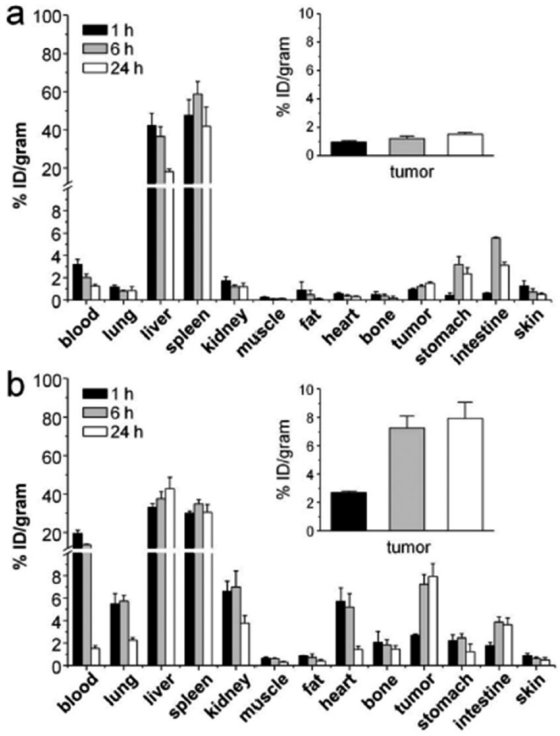

Figure 21. Biodistribution and tumor targeting of (a) large AuNCs (55 nm) and (b) small AuNCs (30 nm) in EMT-6 tumor-bearing mice. Reprinted with permission from ref 150. Copyright 2012 American Chemical Society.

histopathological abnormalities in the preceding organs were observed (Figure 18A). No clinical signs of toxicity, including impeded movement, ruffled fur, signs of abnormal constitution, aberrant behavior, ocular or nasal discharge, loss of weight, respiratory distress, inability to walk, or diarrhea, were observed during the 15 months of testing. The AuNRs were found without morphological changes in the liver and spleen for up to 15 months (Figure 18B). Unlike previous studies by Niidome et al., our results showed a higher AuNR accumulation in spleen than in the liver (Figures 18C and 18D). Slow clearance of AuNRs was observed after 30 days and up to 15 months, but Au content still exists at 15 months (Figure 18C–F).

Furthermore, for the clinical cases of canines and felines (with mammary gland tumors that have been treated with AuNR-assisted PPTT), no evidence of negative impact was observed on their liver and spleen functions.[33]

4.5. Pharmacokinetics and Biodistribution of AuNSs. Gad et al. conducted a series of studies to evaluate the toxicity of PEG-coated nanoshells (AuNSs) when injected intravenously based on the ISO-10993 standard.[66] Additionally, they studied the biodistribution/clearance in mice, acute toxicity in rats, and acute and chronic toxicity in Beagle dogs for time durations up to 404 days. This study provides an extensive description of the biological fate and safety of AuNSs. Results show that the AuNSs were well-tolerated and did not exhibit any toxicities. AuNSs mainly accumulate in the liver and spleen, which account for over 85% of the total gold measured (Figure 19a). Furthermore, no variations in the mean body weights were reported for dogs treated with AuNSs compared to the control in the 10-month long-term study

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(Figure 19b). Stern et al. evaluated the safety of AuNSs in 22 patients with human prostate cancer who were treated with AuNS-based PPTT.[164] Results of this study indicated no toxicity, or lack of tolerance, or lack of immunological effects for 6 months (Figure 20).

4.6. Pharmacokinetics and Biodistribution of AuNCs. There are less data on the pharmacokinetics and biodistribution of gold nanocages. Xia and co-workers evaluated the pharmacokinetics and tumor targeting ability of PEG-coated AuNCs of different sizes (30 vs 55 nm) in an EMT-6 mouse mammary tumor model.[150] Their results suggest that 30 nm AuNCs exhibited more blood retention than 55 nm AuNCs, along with higher uptake in the tumor (Figure 21).

5. CONCLUSIONS AND OUTLOOK

AuNP-assisted PPTT has displayed encouraging therapeutic results and is transitioning from the *in vitro*/*in vivo* studies to the clinical stages. The revolutionary AuNP-assisted PPTT provides an alternative to traditional cancer therapy, and due to its effectiveness in targeting cancer cells and minimizing the side effects on healthy cells as well as its great biocompatibility, it proves to be effective in preliminary clinical trials. In this article, we summarized the most recent progress regarding the efficacy, molecular mechanism, toxicity, and pharmokinetics of PPTT *in vitro*, *in vivo*, and in human trials. Changes in the shape, size, surface conjugations, and administration routes of AuNPs play key roles in PPTT applications. Furthermore, the mechanism of PPTT is highly coveted as it would allow PPTT to progress to the clinical stages. In addition, as for any cancer treatment, the toxicity, biodistribution, and pharmacokinetics of PPTT should be studied in a very systematic manner.

At the current stage, the first challenge for PPTT in clinical use is the AuNPs' long-term biological behavior. Although a large number of publications discussed the AuNPs' toxicity, biodistribution, and pharmacokinetics, there is still no clear answer of what the long-term effects of the AuNPs inside the body are. In spite of many studies showing the deposition of AuNPs in the liver and spleen without short-term toxicity, the question of what is their long-term fate in these organisms upon AuNP exposure is still not answered. In addition, the conflict concerning nanoparticle toxicity studies largely comes from the synthesis of nanoparticles and their surface modifications, as well as the different concentrations that are applied. Are these AuNPs purified enough to remove the toxic surfactant? How good are the AuNPs' surface ligands in the bloodstream and the stability of the AuNPs? The interactions of AuNPs with blood vessels and the impact on blood vessel cells need to be considered too.

The second challenge is the standardization of the treatment. One major barrier blocking the use of AuNPs in clinical application and their commercialization is the lack of nanoparticle model standards. Variant results come from the different experimental conditions in different laboratories. For example, for the dosages of AuNPs and laser power, high-power laser intensities could damage the healthy tissue, cause burning, trigger a toxicity effect, induce inflammation, or cause cancer recurrence and metastasis. In addition, the cost of the treatment is hard to examine at this stage until standardized treatments are used. It is hard to compare the performance of different types of AuNPs due to the big variation in treatment conditions. Therefore, it will be beneficial if the conditions of the treatments such as the specific AuNPs used, the laser intensity, the type of cancer, and the category of animal models are standardized, as this will help accelerate the application of PPTT in clinical settings.

Third, physical restrictions of light penetration depth through tissue (generally <1 cm) and the limited efficiency of delivering AuNPs to the tumor site based on passive EPR effects may slow down the PPTT clinical transition.[165,166] The major administration route for PPTT is via intravenous injection. However, more investigations are needed in order to use different routes such as intratumoral injection, a route that greatly enhances the AuNP concentration in the tumor.

More development of cutting-edge methods of using PPTT or its derived therapies for combating cancer and metastasis is greatly needed. Future work should focus on designing intelligent AuNP-assisted PPTT either alone or in combination with other therapies, such as immunotherapy and surgery, which might greatly improve the efficacy of PPTT in combating cancer and metastasis. In addition, more data are required from clinical trials with more details about the pharmacokinetics and long-term toxicity of AuNPs in humans. More successful clinical trials using AuroLase move us one step closer to the commercialization of PPTT, thus opening the door for other types of AuNPs to follow in the future to treat more types of cancer.

AUTHOR INFORMATION

Corresponding Authors

*Moustafa R. K. Ali. E-mail: mali43@gatech.edu.
*Mostafa A. El-Sayed. E-mail: melsayed@gatech.edu.

ORCID

Moustafa R. K. Ali: 0000-0002-2027-1006
Yue Wu: 0000-0001-7680-9285
Mostafa A. El-Sayed: 0000-0002-7674-8424

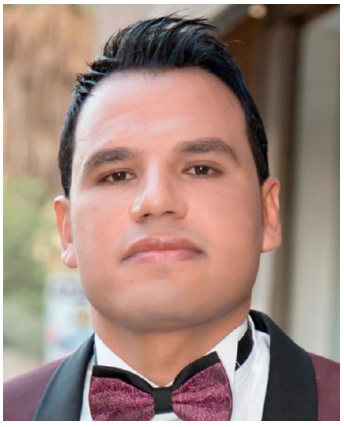
Author Contributions

#M.R.K.A. and Y.W. contributed equally.

Notes

The authors declare no competing financial interest.

Biographies



Moustafa Ali received his B.Sc and M.Sc in chemistry from Cairo University in 2005 and 2012. During his M.Sc period, he was a visiting scholar at Material Science Institute at Connecticut University. He was awarded his Ph.D. from Georgia Institute of Technology in Chemistry in the Group of Prof. El-Sayed in 2017. Currently, he is a postdoctoral Associate at the same group. His research interests include plasmonic nanoparticle synthesis, mechanistic/imaging of bionano interactions using spectroscopy and microscopy techniques, etc. His primary goal is to develop a

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plasmonic nanoparticle treatment for pathogens, cancer progression, and metastasis.

Yue Wu received her B.S. in chemistry from Zhejiang University of Technology, China, in 2007 and M.S. in analytical chemistry from Dalian Institute of Chemical Physics, Chinese Academy of Sciences in 2011. She is currently pursuing a Ph.D. in analytical chemistry at Georgia Institute of Technology under the supervision of Professor Mostafa A. El-Sayed. Her current research focuses on the development of gold nanomaterials for applications related with cancer treatment and biological optical imaging.

Mostafa A. El-Sayed received his B.Sc from Ain Shams University in Egypt and his Ph.D. from Florida State with Michael Kasha. After a research fellowship at Harvard and Cal Tech, he joined the faculty at UCLA in 1961 and Georgia Tech in 1994. He is currently the Julius Brown Chair, Regents Professor of Chemistry and Biochemistry, and the Director of the Laser Dynamics Laboratory. El-Sayed is an elected member of the U.S. National Academy of Sciences (1980), an elected Fellow of the American Academy of Arts and Sciences, an Associate member of the Third World Academy of Science, and a Fellow of the American Association for the Advancement of Science (AAAS) and of the American Physical Society. He is a former editor-in-chief of the Journal of Physical Chemistry and recipient of the US National Medal of Science. His current research includes the optical and electronic properties of nanomaterials and their applications in sensing, nanocatalysis, and nanomedicine.

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