Gold nanoparticle based photothermal therapy: development and application for effective cancer treatment

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Abstract

Recent advances in nanomedicine make it auspicious for cancer diagnosis and treatment. A possible non-invasive photothermal therapy (PTT) for cancer treatment could be constructed by combining the nanomedicine and laser. PTT employs photothermal agents (PTAs) with high photothermal conversion efficacy for converting light into heat to selectively kill cancer cells under the help of lasers. Because of the unique Surface Plasmon Resonance (SPR) phenomenon and the tunable near-infrared (NIR) region absorption, noble metal nanoparticles like gold nanoparticles can be applied as a PTA for PTT. Gold nanoparticles (AuNPs) offer the enhanced absorption and scattering properties, the optical properties tunability, and specific tumor targeting capability, therefore, AuNPs based PTT turns to furthermore promising. However, drawbacks such as long retention time, cytotoxicity, and insufficient cancer cells targeting restrict the application of AuNPs as PTTs. This review overviews a systematic research of the PTT applications of various modified AuNPs in previous publications. With the advancement of chemical synthesis technology, AuNPs of various shapes and sizes can be synthesized with desired properties, which can achieve multimodal cancer treatment with enhanced anti- tumor effect. In this review, we summarized the major features of five principal types of AuNPs: gold nanorods, gold nanoshells, gold nanospheres, gold nanocages, and gold nanostars with different size, discussed their advantages and disadvantages in PTT. We also detailed the surface modification of AuNPs which could be beneficial for the performance of AuNPs based PTT. In addition, depending on properties of AuNPs and lasers, the underlying mechanism of cell death triggered by NIR laser can be different, which also affect the anti-cancer effects and outcomes of PTT. However, controlling cell death through a desired cell death mechanism to achieve desired PTT outcome is still a challenge.

Keywords: Photothermal therapy, gold nanoparticles, near-infrared absorption, cancer

Contents

Introduction

Photothermal therapy (PTT) is a promising biomedical application in cancer treatment due to its minimally invasive character as well as its high selectivity and accuracy. Nanoparticle normally used for PTT as photothermal agents (PTA) due to their unique properties such as high photothermal efficiency and small diameters which allow tumor penetration. The structure, shape, size, and composite of nanoparticles could be adjusted to tune their optical, electronic and mechanic properties in order to get the desired PTA properties [1]. Most recently, noble metal nanoparticles attract attention to their application in PTT because they possess unique properties that make them excellent PTAs. Noble metal nanoparticles have a unique Surface Plasmon Resonance (SPR) phenomenon [2] which enhances their radioactive absorption and scattering properties. Noble metal nanoparticles are promising for PTT because of their SPR effect to generate heat through absorbing laser energy. Apart from this, their excellent radioactive scattering properties make multiple modalities such as the imaging-guided PTT is achievable [3, 4].

Gold nanoparticles (AuNPs) are promising and typical noble metal PTAs. As a PTT mediator, the AuNPs offer (1) high biocompatibility, (2) high yield and smooth production of surface modification controllable colloidal gold [5], (3) The ability to conjugate with other drugs through gold-thiol bioconjugation chemistry [6], (4) no photobleaching. Different shapes of AuNPs can be synthesized with various sizes, such as Gold Nanorods [7-9], Gold Nanoshells [10, 11], Gold Nanospheres [12, 13], Gold Nanocages [14, 15] and Gold Nanostars [16-18]. All of them offer SPR effect and enhanced radioactive properties. AuNPs with different shapes also possess unique properties which could be used for different modalities of PTT to treat cancer. In this review, the optical properties of AuNPs, the influence of different structures, composites, shapes and sizes of AuNPs on PTT, the application of AuNPs in PTT and the mechanism of cell death triggered by PTT will be discussed.

Optical properties of gold nanoparticles

Optical properties such as scattering and absorption are determinative in photothermal therapy. The conduction electrons of noble nanoparticles show strong resonant oscillation within visible light frequency region. Thus, the AuNPs have strong visible light absorption. [19] However, visible light absorption is not suitable for PTT and cancer imaging if the cancer is not on the body surface due to poor penetration of visible light. To increase penetration, the maximal absorption wavelength of nanoparticles must fall into the near-infrared (NIR) light range, which means the wavelength of around 600 to 1400nm. NIR light offers deep body penetration because the minimal absorption coefficient of water molecules, melanin and hemoglobin are achieved within the NIR range. Therefore, NIR light could shine deeper in the body than light in the visible range. Because the SPR band of noble nanoparticles is tunable and which could alter the absorption range, the unique property Surface Plasmon Resonance of noble nanoparticles enhances optical absorption and scattering properties. The maximum absorption wavelength of

AuNPs can be tuned to the NIR range [20]. The material, structure, size, shape, etc. affect the SPR band of AuNPs. Hence absorbance of AuNPs can be adjusted by various modifications. The optical NIR region can be divided into the first NIR window (λ =650-850nm) and the second NIR window (λ=950-1350nm). For deeply embedded tumors, AuNPs with the second NIR window absorption can be used for PTT because they penetrate deeper with a maximal penetration distance of around 10cm of healthy tissues. The first NIR window is beneficial for superficial solid tumors, NIR light with these wavelengths can reach tumors which are 2 to 3cm under the skin [6, 21, 22]. Depending on the location of the solid tumors, the maximal absorbance of AuNPs should be tuned for precise tumor positioning.

Light scattering property of the AuNPs determines the quality of cancer imaging. Cancer imaging systems work as a guide system for PTT. Thus, the light scattering property should be evaluated when designing AuNPs. With the help of the imaging system, the local environment of the region of interest can be sensitively imaged or even 3D visualized. The location and size of a tumor can be identified and traced, which guarantees the completion of precise photothermal tumor ablation. The imaging system can also be used as a real-time monitoring system for PTT. According to the full Mie theory and works by El-Sayed et al. [23], the scattering and absorption of the AuNPs are highly dependent on their shapes and sizes. Generally, for a particular type of AuNPs, increasing the size of the AuNPs would increase the maximum extinction wavelength (λ_{max}) and the extinction cross-section area, and the scattering efficiency would contribute more to the total extinction efficiency. AuNPs with smaller sizes usually offers a better absorption ability and a lower scattering to absorption efficacy ratio (Table 1). Therefore, for a specific type of AuNPs, higher scattering efficiency means a larger size of AuNPs. Larger AuNPs are preferred for high resolution and sensitive cancer imaging-guided PTT, and smaller AuNPs having higher absorption efficiency that is preferred for higher photothermal efficiency. However, there is no clear relationship between AuNPs with different shapes or surface modifications and their optical property change trend. Individual experiments must be done in the laboratory to determine the optical properties of various AuNPs. The effects of the AuNPs structure, shape, and size on the optical properties will be further discussed in this review.

Table 1 Calculated maximum extinction wavelength (λ_{max}) and the ratio of scattering efficacy to absorption efficacy of the extinction (μ_s/μ_a) for AuNPs with different shape and size [23]

AuNPs type	Dimensions	λ_{max} (nm)	μ_s/μ_a
Silica @ Gold nanoshells	R1=40nm, R2=70nm	843	0.09
Silica @ Gold nanoshells	R1=90nm,R2=105nm	984	2.33
Silica @ Gold nanoshells	R1=120nm,R2=140nm	1120	2.44
gold nanospheres	D=20nm	521	0.01
gold nanospheres	D=40nm	528	0.06

gold nanospheres	D=80nm	549	0.68
gold nanorods	Aspect ratio=3.9 effective radius=8.74nm	788	0.03
gold nanorods	Aspect ratio=3.9 effective radius=11.43nm	797	0.08
gold nanorods	Aspect ratio=3.9 effective radius=21.86nm	842	0.54

Surface modification of AuNPs

Although AuNPs alone have many above mentioned advantages compared with other photothermal agents, the potential long-term toxicity due to low tissue clearance is one of the critical side effects that should be eliminated before they can be used *in vivo*. As stated previously, it is difficult to identify the location of cancer cells or tissues and to eliminate the tumor simultaneously by AuNPs alone in PTT. Low specificity could result in damage to healthy tissues, which leads to some side effects. To achieve better and desired therapeutic outcome, surface modification strategies like coating and conjugation are used to reduce or even eliminate the side effects of AuNPs while keeping their efficacy.

One example is coating AuNPs with amorphous SiO_2 [24]. According to Jabeen et al., the nanoaggregates, formed by AuNPs and SiO_2, have a size greater than 1.4nm. They have a similar photothermal efficiency to raw AuNPs with an Au concentration of 30mg/L [25]. Besides, due to the isolation property of these nanoaggregates, they also can act as dielectric spacers for phototherapy. Their biocompatibility has improved compared with that of AuNPs alone [24, 26]. Another strategy is coating AuNPs with Prussian Blue (PB) [27]. PB is a clinical drug approved by FDA for treatment of radioactive exposure. The Au@PB NPs have a gold core of 9.1±0.64 nm in the experiment of Jiang et al. And this small-size surface modified AuNPs shows excellent scattering property and work well as a CT imaging agent. However, this finding is conflicting with the general trend that AuNPs with a small size have poor scattering property [23]. Therefore, the general optical property change trend may not be used for surface modification of AuNPs. Moreover, the Au@PB NPs can be used not only for a highly efficient and safe radioactive exposure treatment, due to their strong photostability, high NIR region light absorption and absence of toxicity (without laser), but also as a desired PA for PTT. The Au@PB NPs can kill cancer cells precisely and efficiently when used for CT imaging-guided PTT (Fig. 2).

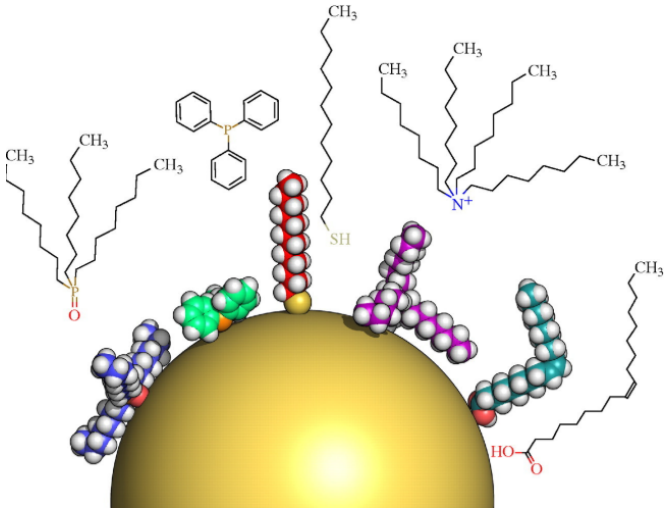


Figure 1 Schematic representation of surface modification of gold nanoparticles. (The particle used for this scheme was idealized as a sphere)

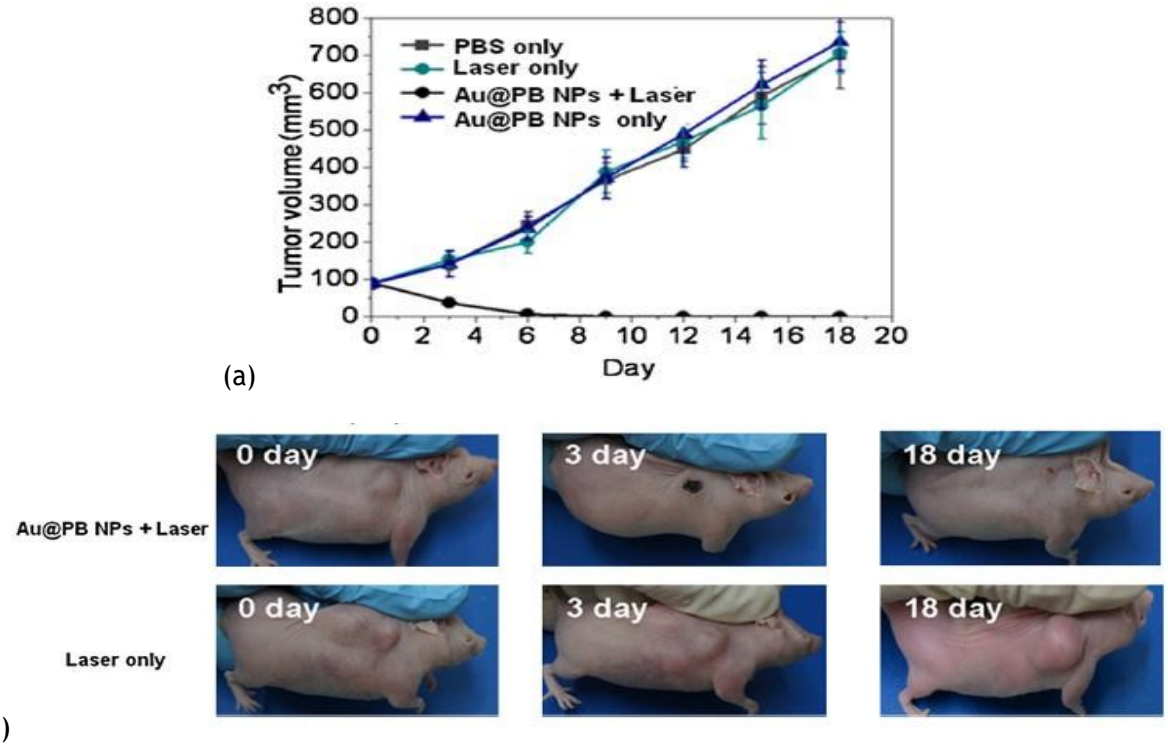


Figure 2 Tumor change under different conditions (a) the tumor volume change curves of different groups of nude mice after different treatments. (b) Representative photographs of nude mice tumor changes after 0 to 18days of two different types of treatments. [27]

Cetyltrimethyl ammonium bromide (CTAB) is widely used in the synthesis of AuNPs. It works as a surfactant to bind to the membrane of AuNPs and to facilitate and stabilize the formation of different structures [28]. CTAB-stabilized AuNPs are generally cytotoxic, so they must be

modified to avoid cytotoxicity and including inflammation. In order to eliminate the impact of cytotoxicity, Metal-Organic Frameworks (MOFs) could be applied to coat AuNPs. MOFs are a novel group of compounds produced from metal ions or clusters, and they can coordinate with organic ligands. Thus, it possesses both inorganic and organic characteristics. Coating MOFs shell with AuNPs can significantly reduce their cytotoxicity and enhance their photothermal efficiency. Another appealing example is coating AuNPs with the zeolitic imidazole framework (ZIF). According to Fang et al., the AuNPs@ZIF has an excellent performance in biocompatibility, photothermal efficiency, and nano-interaction, and it can also promote the secondary PTT: the combination of chemotherapy and PTT by loading the chemotherapeutic drug into the ZIF mesopores [29].

In addition to surface modifications mentioned above, AuNPs can combine with various types of materials to improve the performance of AuNPs. Based on these modifications, the multifunctional AuNPs can be manufactured, and they have great theranostic potential. *Table 2* demonstrates some modified AuNPs with different features used in PTT.

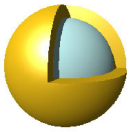
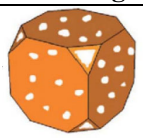
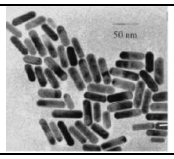
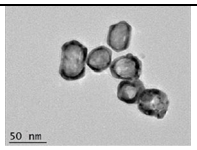
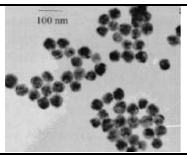
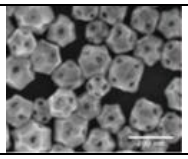
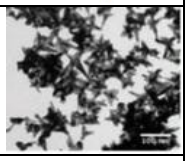
Table 2 various types of modified AuNPs used in PTT

Modification Type	**Therapy**	**Feature**	**Ref.**
ICG-Au@BSA-Gd	PTT/Photodynamic therapy (PDT)	Suitable for NIRF/PA/CT/MR image-guided therapy	[30]
PheoA-HA@AuNPs	PTT/PDT	Switchable photoactivity, prolonged blood circulation, enhanced tumor specificity	[30]
Polydopamine coated AuNPs	PTT	Available for MRI/CT image-guided PTT	[24]
polyethylene glycol coated AuNPs	PTT	Prolonged circulation time, effective light absorption, suitable for X-ray/CT image-guided PTT	[31]
DOX-loaded PLGA-Au H-S NPs	PTT	Drug loading ability, short treatment time, high therapeutic efficacy	[32]
GNS-PEG-Ce6	PTT/PDT	Enhanced cellular uptake, high anti-cancer efficiency, and water dispersibility	[33]
5-FU@Au	PTT	Anti-cancer activity, low dose requirement, high photothermal conversion efficiency	[34]
Au–Fe_2C JNPs	PTT	Excellent magnetic properties, available for MRI/MSOT/CT image-guided therapy, high photothermal efficiency	[35]
DOX@GNR/PSS/HSA NPs	PTT & Chemotherapy	Strong synergistic anti-cancer effect, high photothermal efficacy, excellent stability and biocompatibility	[36]

Feature adjusting by changing shapes and sizes of AuNPs

Advanced synthetic chemistry provides the possibility to construct AuNPs of various sizes and shapes. The optical and physiochemical properties of AuNPs vary among different modifications, and these properties could be precisely controlled by synthetic chemistry with the purpose of meeting various biological, physical and chemical requirements of the multifunctional photothermal therapy. Five major types of AuNPs have entered the preclinical development stage or the clinical trial stage, and they are promising for PTT: gold nanorods, gold nanoshells, gold nanospheres, gold nanocages and gold nanostars (Table 3). Each of these AuNPs of different shapes possesses some unique characteristics, and their feature change barely follows any rule. Since critical properties for PTT, such as the NIR absorbance and clearance rate, are highly dependent on the shapes and sizes of these AuNPs. Researchers must conduct separate experiments to test the properties of these AuNPs in order to find those AuNPs with desired properties for PTT. This section describes some methods to synthesize these AuNPs and highlights some unique qualities and key features of AuNPs with different shapes and sizes.

Table 3 the most common types of the AuNPs for potential photothermal therapy application (Nanospheres, Nanoshell, Nanorods, Nanocages, Nanostars) [6],[37],[85]

	Nanorods	**Nanoshells**	**Nanospheres**	**Nanocages**	**Nanostars**
Structure					
SEM					
Size for PTT	~40-100nm in length with aspect ratio~2-4	~100-150nm diameter	~5nm-100nm	~40-60nm length	~30-100nm
Unique features	two surface Plasmon resonance bands	Core & Shell structure	Easy synthesis and bioconjugation	Target agent loading ability	Large surface area

Au Nanorods for PTT

Yu et al. developed an electrochemical synthesis method to prepare gold nanorods (AuNRs) with a different average aspect ratio in 1997 [7]. In 2002, Kim et al. [9] developed a photochemical synthesis method which uses photochemically reducing gold ions and micellar solution to generate AuNRs. Both of the methods can produce AuNRs with various aspect ratios. Gold nanorods generally have relatively smaller sizes (~10×40nm) compared with other gold nanostructures, which contributes to better cell uptake efficacy. Because for NPs with small sizes, fewer receptors can wrap many NPs and even each receptor can initiate the cellular uptake of many NPs; while for large sizes NPs, many receptors and larger motile forces are needed to start the uptake of the NPs. However, cellular uptake is also affected by shape. Compared with their spherical or cage shape counterpart, the cellular uptake of AuNRs is lower. Because the aspect ratio of nanospheres and nanocages (~1) are much lower than that of nanorods (~2-4), for cellular uptake of nanorods, more receptors are needed to wrap the AuNRs in the longitudinal direction and initiate the cellular uptake of one nanorod. Gold nanorods usually synthesized based on seed growth method, and CTAB is typically applied as a surfactant to stabilize the NPs and prevent aggregation. The residual CTA^+ coated on the AuNRs contributes a positive Zeta potential. Because cell membranes have a negative surface potential, CTA^+ promotes cellular uptake due to ionic interaction. In 2006, Huang et al. demonstrated the *in vitro* PTT using AuNRs for the first time. Their experiment showed that the AuNRs have the ability to locate cancer cells and tissues, and PTT can be conducted with a high photothermal efficacy when used with a NIR laser [39]. AuNRs have two surface Plasmon resonance bands. One band has small extinction in the visible region with a wavelength of around 500 to 550nm. This band also referred to the transverse band, the wavelength of it shows independence with aspect ratio of the nanoparticles. Another band belonging to the NIR light region is stronger and called the longitudinal band. This band dominates the absorption spectra of these AuNRs [5]. The red shift, the wavelength of the longitudinal band increases with the increase of aspect ratio of the AuNRs and the relationship between these parameters was defined by Link et al. as [40]:

$$\lambda_{max} = 95 * (\text{Aspect Ratio}) + 420$$

Therefore, the aspect ratio can be used to precisely control the longitudinal resonance and the maximum SPR of AuNPs to meet the PTT optical absorption requirement. Recently, the PTT ability of the AuNRs with different sizes was studied by Morales-Dalmau et al. [41], They found that the cytotoxicity of AuNRs has no correlation with the size of the particles, but increases with the absolute quantity of AuNRs. The smaller AuNRs show better potential for photothermal ablation because of their higher cell uptake. Although theoretically, AuNRs have a strong optical absorption, AuNRs are randomly oriented in human cells after injection. The high absorption efficiency is hard to achieve and maintain under linear polarized NIR laser light. Thus, NIR laser with high energy is necessary to be applied to photothermal ablation. However, laser with high power density could damage or even 'fire' healthy cells and tissues during light-induced hyperthermia. Therefore, circular laser light should be used with the purpose of producing a laser

beam from all different directions to enhance absorption ability of AuNRs. Circular NIR light-induced hyperthermia results in ultra-low energy threshold and minimum damage to healthy cells and tissues [42].

Au Nanoshells for PTT

Compared to AuNRs, Au Nanoshells (AuNSs) used for PTT have a much larger size, which is usually great than 100nm. The AuNSs usually compose an approximately 100nm silica core and a gold shell with a thickness of around 5 to 20nm. AuNSs have tunable resonance mode. The plasmons response, the strength of the coupling between the inner and outer shell surface plasmons and the hybridization of the plasmons are dependent on the distance between the plasmons on different shell layers. By changing the thickness of the shell, the plasmon resonance will change due to change of free electrons in the surface, phase delay and hybrid plasmon, the plasmon resonance will change [86]. In the theory of hybridization of plasmon, shown as Fig 3, the gold nanoshell plasmon resonance results from the hybridization of a solid sphere (with a radius of r_2 and vibration energy of $\omega_{s)}$ and a cavity (with a radius of r_1 and vibration energy of ω_c). The degree of hybridization is related to the thickness of the gold shell. The thinner of the gold shell, the stronger the plasmon hybridization, and which leads to two new resonance modes. The high energy mode ω_+ corresponds to the anti-symmetric coupling of ω_s and ω_c, ω_c plays a dominant role in this mode; the low energy mode ω_- corresponds to the symmetric coupling of ω_s and ω_c, and ω_s plays a dominant role in this particular model [87]. Thus, the thickness of the gold shell determines the SPR wavelength of the AuNSs [43], and SPR red shifting increases when the shell thickness decreases. Owing to their tunable NIR region resonances and enhanced scattering and absorption ability, they can be used as sensitive cancer cell imaging agents and effective PTAs. Usually, the maximum NIR absorption wavelength can be tuned between 800nm and 1200nm. AuNSs are good candidates for multifunctional PTT, not only because they can work as an imaging and hyperthermia agent due to their excellent scattering and absorption properties, but also because they can be used for drug loading and drug delivery. In 2000, Sershen et al. developed a composite composed of a thermal sensitive hydrogel and AuNSs and used this composite as a drug-loaded PTA. Under the irradiation light at 1064nm, the AuNSs in the composite generate heat efficiently, and the subsequent hyperthermia improves the release of the loaded drug from the hydrogel [44]. In 2003, Hirsch et al. completed both *in vivo* and *in vitro* NIR laser-induced photothermal therapy for the first time using AuNSs [45]. In the *in vitro* PTT trial, photothermal destruction of breast carcinoma cells was observed in all AuNSs treated samples under the NIR light condition (coherent, 820 nm, 35 W^2cm) for 7 minutes, which was evidenced by reduction of Calcein-AM stained cells (Fig4.A), while the laser only treatment does not trigger cancer cell death (Fig4.B) . In the *in vivo* PTT, Hirsch et al. used MRTI to monitor temperature increase after delivering treatments. It showed that the tumor tissue had an average temperature increase by 37.4±6.6°C after being treated with AuNSs and NIR light laser for 4 to 6 minutes. This temperature increase is high enough to induce irreversible damage to tumors cells and permanently kill them. In contrast, the NIR light laser only treatment results in an average of 9.1±4.7°C temperature increase and this change is below the threshold of permanent damage for

tumor cells.

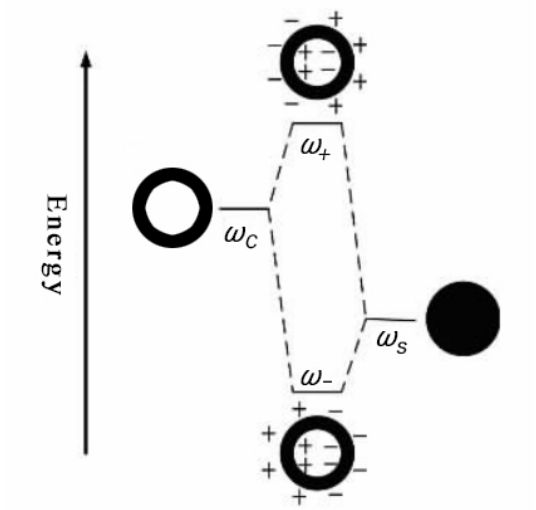


Figure 3 Energy level diagram of gold nanoshell plasmon hybridization

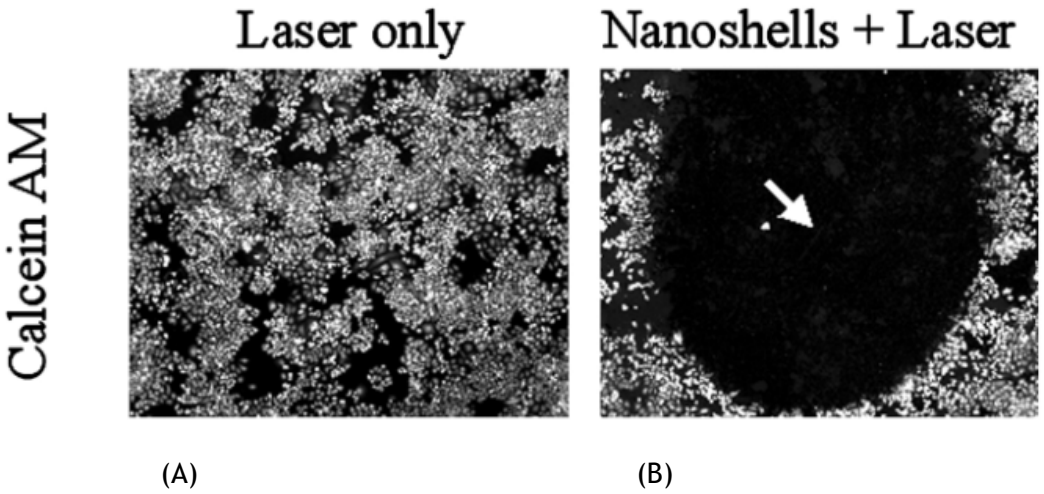


Figure 4 Calcein AM staining test after 7mins of NIR light 820 nm, 35 W^2cm laser treatment (A) NIR light 820 nm, 35 W^2cm laser + Nanoshells treatment (B) [46]

Hollow gold nanoshells are another important structure of AuNSs morphology. The hollow AuNSs can be synthesized by oxidizing the template of cobalt or silver with the chloroauric acid [47, 48]. The hollow core of the AuNSs offers space for drugs or enzymes to be loaded, so this type of AuNSs can be used as delivery vehicles [49]. Drug loading ability along with good photothermal ability is promising for secondary PTT so that, PTT and chemotherapy can be combined. Compared with monotherapy, the secondary PTT possesses excellent synergistic effect for cancer treatment, requiring lower doses of PTA and the chemical drug, minimizes the toxicity of the therapies, decreases off-target effect and increases the possibility of wiping out all of the malignant cells. The hollow AuNSs have higher tumor specificity than normal AuNSs owing to their feasibility of simple bioconjugation. Epidermal Growth Factor Receptor (EGFR)

overexpressing cells can be specifically targeted by hollow AuNSs if anti-Epidermal Growth Factor Rector (EGFR) receptor is conjugated with hollow AuNSs. With the specific targeting and drug delivery strategies as well as the enhanced optical properties, precise tumor imaging and photothermal tumor ablation could be safely and effectively implemented. Therefore, highly specific secondary PTT with an imaging guide system is feasible using hollow AuNSs.

Au Nanospheres for PTT

Compared with AuNRs and AuNSs, Au Nanospheres (AuNSPs) have some unique features which could be beneficial for PTT research. (1) AuNSPs have simpler synthesis processes. (2) Bioconjugation can be easily achieved using simple methods, and the easy bioconjugation ability can improve the specificity of PTT. (3) Aggregates of AuNSPs have non-linear optical properties. Synthesis of AuNSPs only needs several minutes to be completed using the citrate reduction method [50]. By changing the concentration of the sodium citrate solution, the nanoparticle diameter can be tuned from 5 to >100nm [51]. The latter can be used to control the optical properties of AuNSPs. The methods of synthesizing hollow AuNSPs with tunable inside and outside diameters were developed by Adam M et al. [13] Using cobalt sacrificial galvanic replacement. AuNSPs show lower cytotoxicity and better biocompatibility compared with CATB surfactant derived AuNRs which might remain cytotoxic. AuNSPs also shows better cellular uptake rate than the AuNPs with other shapes, their counterparts. However, AuNSPs do not have surface plasmon resonance peak within the NIR region; it has an absorption peak around 500-600nm. Gold nanosphere is a kind of nonlinear material. Benefiting from the nonlinear optical properties of AuNSPs, AuNSPs is a sound system for harmonic generations [88]. Thus, although AuNSPs do not have broad tunable light adsorption peak in NIR region, it can still be used for PTT application. In the nonlinear optical process of second harmonic generation, two NIR region photos with the frequency of ω interact with the AuNSPs. The interaction causes them to convert into a new photon with a wavelength of 2ω in the visible light region. This particular visible light region is the region where the AuNSPs have strong surface plasmon adsorption (~550nm). Thus, through the second harmonic generation, AuNSPs can also be used for NIR laser-induced PTT by non-radioactively absorbing NIR light energy and generating heat. The *in vivo* photothermal effect of AuNSPs with bioconjugation of anti-EGFR antibodies was tested by Huang et al. [52]. With 800nm NIR laser at laser energy greater than 120mJ, HSC cancer cells were irreversibly destroyed, and this energy requirement is 20 times lower than PTT without using gold nanospheres. The difference in laser power requirement shows that AuNSPs have high photothermal efficacy. Au@Ag Nanospheres are another novel AuNSPs structure which is developed by Ye et al. [53]. The Au@Ag Nanospheres with approximately 40nm diameter shows board absorption cross-section and high absorption in the NIR region till wavelength of 1100nm. PTT was performed using this novel structure under 980nm irradiation. The *in vitro* study showed that Au@Ag nanospheres have a stronger hyperthermic effect (5.7times stronger) than normal AuNRs with lower cytotoxicity. Thus, this novel AuNSPs require a lower dose of PTAs and less laser energy to conduct a tumor ablation using laser- induced hyperthermia.

Au Nanocages for PTT

Au Nanocages (AuNCs) can be synthesized by galvanic replacement reaction, replacing silver nanoparticles with gold salts [14]. According to Chen et al. the optical property can be controlled by changing the amount of auric acid during the synthesis process or by modifying the thickness of the AuNCs wall [54]. The light absorption wavelength of AuNCs varies from 400nm to 1200nm, from the visible light region to the NIR region. Sara et al. [14], reported that the AuNCs with 30nm inner edge length and 5nm wall thickness provide absorption cross-section around $20\times10^{-15}m^2$ when tuned to 710nm. Compared to conventional organic dyes absorption cross-section (normally 5-10 times smaller), AuNCs have huge absorption cross-sections. The large areas are beneficial for high photothermal efficiency PTT. However, the magnitude of the absorption cross-section is one factor which researchers must consider when they design the AuNCs for application. When AuNCs have small absorption cross-section (edge length<45nm), more energy of the incident light is absorbed, while when AuNCs have broad absorption cross-section, light scattering predominant.

Similar to hollow AuNSs, AuNCs have hollow interiors which can work as a delivery system. For certain drugs, the controlled release of drug can be accomplished by AuNCs. During the processes of encapsulation, release and laser treatment, AuNCs do not melt or react with drugs. Therefore, the bioactivity of the encapsulated drugs does not alter [55]. An *in vitro* study on breast cancer cells treated with doxorubicin (DOX) and AuNCs found that the cell death rate increased with the increasing exposure time of irradiation (laser at a power density of $20mWcm^{-2}$) due to the increased release of DOX from the nanocages. In comparison, AuNCs only treatment showed little effect on cancer cell viability due to their low toxicity and the absence of the photothermal effect [40]. Structures of nanocages provide a special platform for PTT application. The drug loading ability improves the specificity and efficacy of killing malignant cells [55, 56]. The photothermal effect of the nanocages has also been tested by Leslie Au et al. in 2008. They conjugated 65nm AuNCs with monoclonal antibodies to target and kill SK-BR-3 breast cancer cells. They found out that the irreversible cell damage would happen when the laser at a power density greater than $1.6Wcm^{-2}$, and the efficacy of the photothermal effect increases with the increase of laser exposure time up to 5 minutes. Cancer cells treated with the same condition but in the absence of the AuNCs maintained their viability [57]. This indicates that AuNCs can work as both a drug delivery agent and a photothermal agent.

In 2017, Huiping sun et al. reported the first biomimetic drug delivery system consisted of Doxorubicin (DOX) loaded AuNCs and 4T1 breast cancer cell membrane, shown as Fig 5 [90]. This system shows the ability to against breast cancer by the combination of photothermal therapy and chemotherapy. The outer shells which derived from cancer cell membrane can homotypic targeting the breast cancer cells with superior efficiency. Under NIR irradiation, not only the generated heat result from plasmon resonance of AuNCs causes the tumor cell inhibition effect, but also the hyperthermia leads to the release of the anticancer agent DOX. As a result, this biomimetic CDAuNCs system act as a promising PTT and chemotherapy nano-platform with high tumor targeting ability and anti-breast-tumor growth effect.

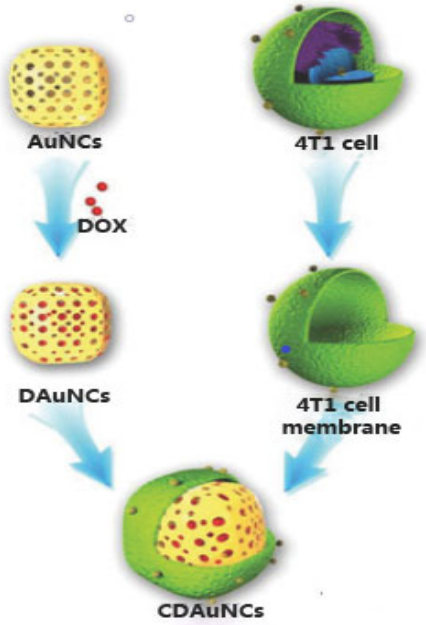


Figure 5 Schematic demonstration of the 4T1 cell membrane coated AuNCs biomimetic drug delivery system [90].

Au Nanostars for PTT

Au Nanostars (AuNSTs) are a type of AuNPs with sharp multidimensional tips. Pandian et al. developed a high yield synthesis method in 2007 using poly(vinylpyrrolidone) (PVP) to reduce $HAuCl_4$ in N,N-dimethylformamide (DMF) to Au seeds with which the AuNSTs could be synthesized by stirring the PVP coated Au seeds in the ethanol solution [58]. AuNSTs have strong crystallinity, and each tip of the AuNSTs possesses a single crystalline nature. A large number of tips in AuNSTs increase the surface-enhanced Raman scattering (SERS), while it has little influence on the position of the main longitudinal resonance wavelength. AuNSTs can also be prepared using a surfactant-free synthesis method. This method could produce AuNSTs with little cytotoxicity due to the absence of toxic surfactant. However, control of the size and the optical properties of surfactant-free AuNSTs are still inefficient. Khlebtsov et al. provided a novel surfactant-free synthesis method to construct AuNSTs-1,4-aminothiophenol(ATP) complex with excellent SERS imaging ability[59]. They obtained AuNSTs with plasmon resonances from 630nm to 900nm by varying the Au seed size from 3nm to 35nm, and the AuNSTs product size is within the range of 40nm to 200nm. Bioconjugation is more accessible to perform in producing AuNSTs than producing other AuNPs because the AuNSTs have relatively larger surface areas due to the existence of the multi-dimensional tips. In 2017, Liu et al. proposed a gold nanostars application related to synergistic immune photothermal nanotherapy (SYMPHONY) concept (Fig 6) [89]. In this nanotherapy, they combined gold nanostars and anti-programmed death-ligand 1 antibodies (Anti-PD-L1 Antibodies) to conduct photothermal therapy and immunotherapy simultaneously to kill both primary tumor cells and metastases. Benefiting from the tip-enhanced plasmonics properties, gold nanostars trigger a photothermal therapy with high efficiency under the irradiation of NIR laser to increase the temperature, which causes the death of the primary tumor cells. Activated by the dead primary

tumor cells, the immune system shows the ability to kill the distant tumors. The Anti-PD-L1 antibodies in this system are selected to block the interaction of PD-L1/PD-1, which inhibits the cytotoxic T-cell function. The immunotherapy resulted in PD-L1 blockade disable the immune system resistance of the tumor cells. Thus, without defending system, the distant tumor cells can be more easily killed by the action of the immune system.

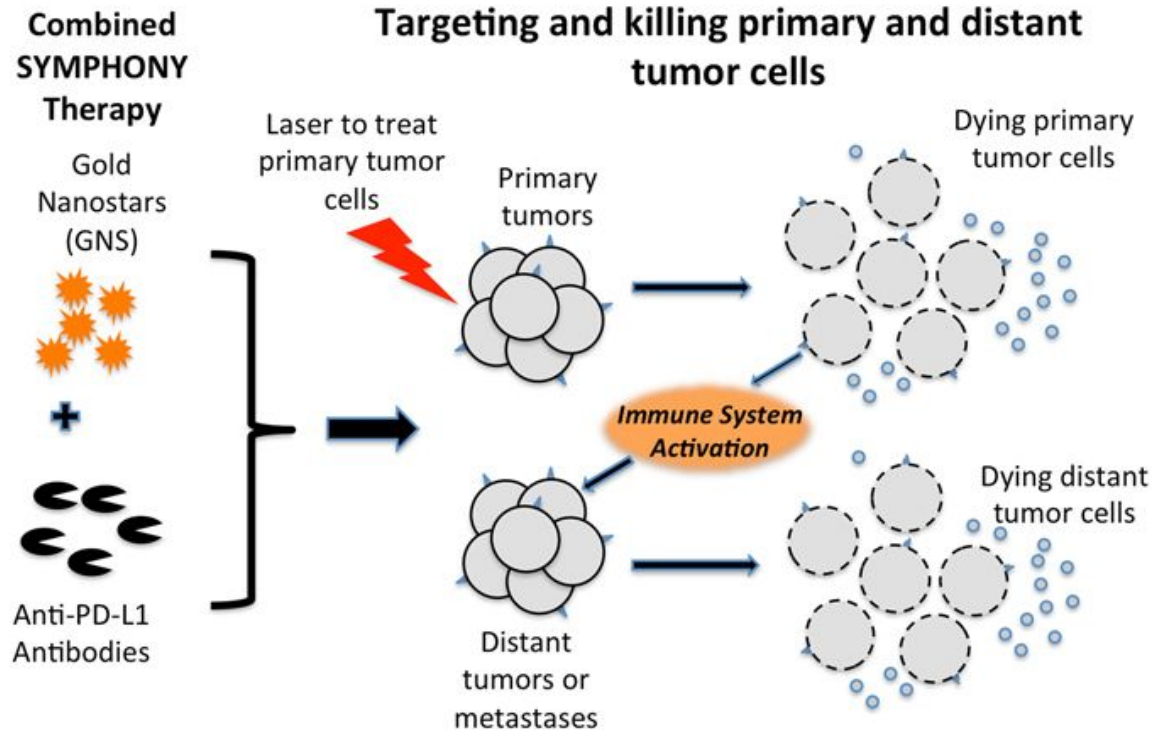


Figure 6 Killing primary and distant tumor cells by SYMPHONY triggered by GNS and Anti-PD-L1 Antibodies [89].

Regarding cytotoxicity, an *in vivo* experiment showed that the specific AuNSTs do not have acute toxicity for rats even at a high dosage (48mg/kg) [60]. AuNSTs with relatively large diameters are beneficial for bioconjugation, but they are not as specific as PEG conjugated AuNSTs in targeting tumors. Also, the nanoparticles with sizes larger than 100nm are relatively difficult to be delivered to the tumor site, and the clearance of them from the human body is another critical issue. The long retention time of gold particles within the human body could result in cytotoxicity. Regarding AuNSTs based PTT, an in vivo experiment found that AuNSTs have higher absorption to scattering ratio than other AuNPs, and the photothermal efficacy (around 90%) of the AuNSTs is higher than that of 30 nm and 60 nm AuNSs (61%)[18]. High photothermal efficacy is essential for a low dose agent and low off-target PTT.

All of these *in vitro* and *in vivo* studies of AuNPs based PTT provide useful information for selecting suitable nanoconstructs for specific therapeutic applications.

Size effect on PTT

For PTT clinical trials, the size of nanoparticles should be precisely controlled because the optical properties such as scattering and absorption, the clearance ability, extravasation from vasculatures and solid tumor penetration are highly dependent on the size of nanoparticles. Generally, like mentioned above, larger AuNPs have better scattering properties which entitle them to imaging-guided PTT because they can provide a better image resolution than smaller AuNPs. Smaller AuNPs have better absorption ability and higher photothermal efficacy because they require much less laser energy than larger AuNPs of the same type to kill the individual tumor. The clearance ability of AuNPs is vital because gold alone is relatively bio-inert and slightly cytotoxic. Retention of gold could result in some health-related problems. AuNPs smaller than 5nm can be successfully eliminated by renal clearance, and AuNPs (D<5nm) retention in the spleen, liver, and the kidney is low [61]. However, if the nanoparticle size is too small, it would be cleared rapidly, even from the extracellular milieu before the therapy. Sadauskas et al. reported that the daily elimination rate of AuNPs with a diameter of 40nm is approximately 0.05% for the first six months after Intravenous nanoparticle injection. They claimed that AuNPs with a diameter more than 40nm would remain in the organs permanently. The *in vivo* animal experiment observed no consequences of the retention of these AuNPs [62]. It might suggest these AuNPs have no acute cytotoxicity, but it may potentially have long-term toxicity. Another limitation for large size NPs is that they can only be used to treat superficial tumors. Due to size limitation, these large NPs can hardly travel the long distance from superficial blood vessels to deep tumor sites. Apart from these, the AuNPs size also affects the tumor targeting efficacy, and this will be explained in the section of targeting properties of AuNPs.

Targeting properties of gold nanoparticles

Although AuNPs show low cytotoxicity in most of the *in vivo* experiments, retention of AuNPs in vital organs is still one of the potential threats for AuNPs based on PTT. They might still poison or damage organ cells and cause some health problems to patients. Therefore, precise tumor targeting should be effectively achieved with minimal threat to patient health. As mentioned in the surface coating section, the specific and precise targeting of cancer cells can be achieved after modifying the AuNPs. To increase the specificity and selectively deliver the AuNPs to tumor tissues, the AuNPs can be used with various targeting agents apart from the surface coating. Depending on the nature of modifications and structures of the AuNPs, selectively delivery to solid tumors can be accomplished by the passive targeting or the active targeting method (Fig 7).

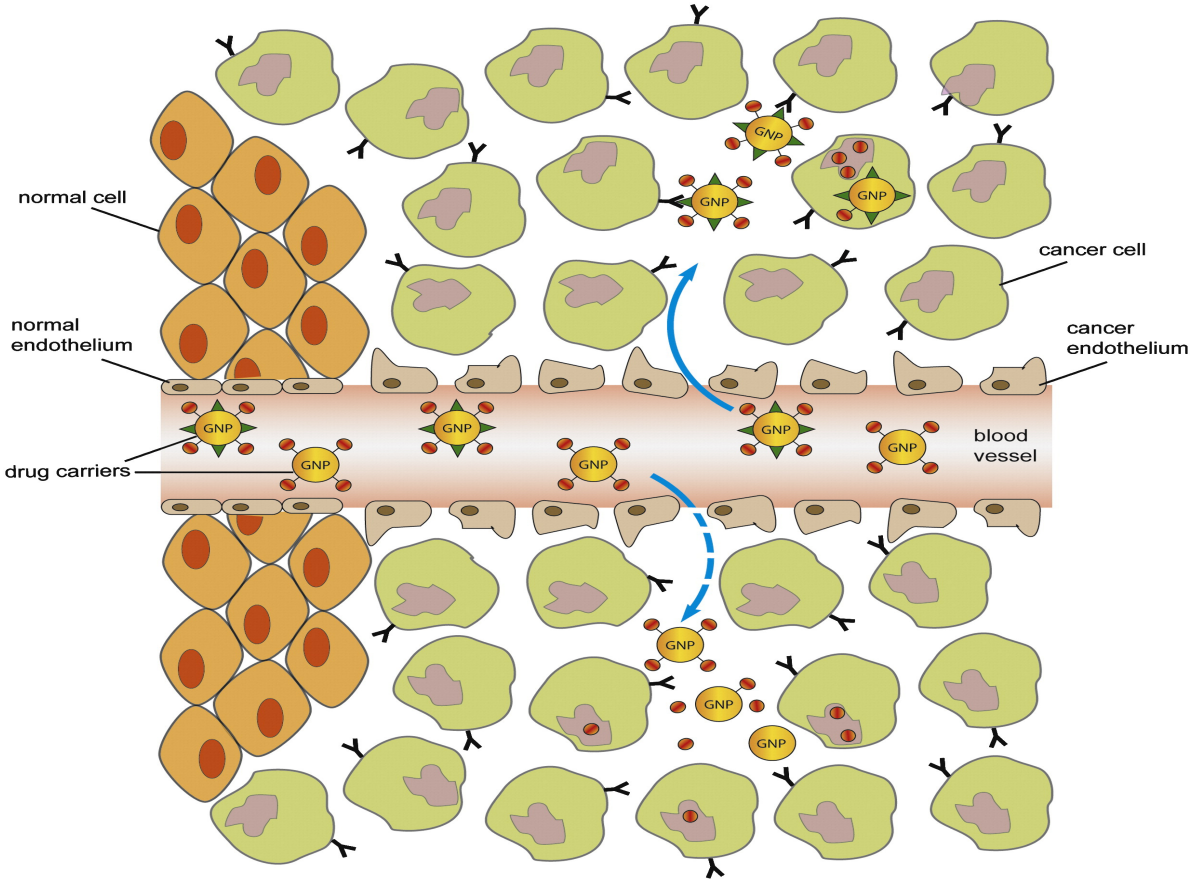


Figure 7 Illustration of passive and active targeting method [63]

The passive targeting method

AuNPs can target certain tumors in a passive pattern due to the increased permeability and retention (EPR) effect. This unique feature is attributed to the unique anatomical and pathophysiological nature of solid tumors compared with normal cells and tissues: the majority of solid tumors have leaky blood vessels and poor lymphatic drainage [64]. Blood vessels of solid tumors can proliferate rapidly in order to provide large amounts of nutrients and oxygen to tumor tissues for their non-stop growth. Compared with healthy blood vessels, blood vessels with hyperplasia have wider inter-epithelial space. Consequently, the AuNPs in the body extravasate from the leaky vessels and accumulate in the tumor interstitium. Because of poor lymphatic drainage of solid tumors, AuNPs can be retained for a long time in the body and cannot be eliminated as waste [65-67]. The accumulation efficiency of AuNPs in the tumor tissue is important for optimal PTT, and the passive targeting efficiency is dependent on the shape, size and aspect ratio of the AuNPs. It has been reported that the citrate-capped spherical AuNPs with a diameter of 50nm have the highest accumulation efficiency for HeLa cells, compared to 14,30,74 and 100nm citrate AuNPs [68]. In contrast, another study showed that smaller AuNPs have better tumor accumulation, but the smaller AuNPs (D<50nm) may be cleared faster from the tumor tissue due to the high interstitial matrix pressure [69]. 74 and 14nm AuNPs with

different shapes (Spherical citrate–AuNPs & citrate/CTAB-AuNPs) show a considerable difference in per particle uptake in HeLa cells, the two sizes of spherical AuNPs shows 5times and 3.75times higher uptake in HeLa cells, respectively. The greater uptake of

40×14nm AuNPs than 74×14nm AuNPs in He La cells shows the aspect ratio effect on the passive targeting of PTA, which should be considered when designing AuNPs for PTT.

The passive targeting method has some critical limitations for PTT. Firstly, the passive targeting AuNPs are not directly delivered to cancer cells, the position of PTA is relatively dispersed in the body, and the PTA may not target all of the solid tumor sites due to the non-ubiquitous distribution of cells in the solid tumor tissue. This situation may result in damage to healthy cells and tissues due to the relatively random distribution as well as multiple drug resistance (MDR). Secondly, the EPR effect does not exist in all types of tumors. For solid tumors without leaky blood vessel, AuNPs cannot pass through the bloodstream easily and accumulate in the tumor interstitium [70]. Therefore, other targeting methods like the active targeting method and the cellular internalization method should be applied in order to overcome these limitations.

The active targeting method

Compared with the passive targeting scheme, the active targeting method takes advantage of the EPR effect and targets cancer cells and tissues more specifically by antibody-antigen or ligand-receptor interaction. It has better tumor delivery specificity and longer retention. Typically, Active targeting can be achieved by functionalizing AuNPs by some targeting agents (TAs) such as proteins (antibodies and their fragments), nucleic acid (aptamers), and receptor ligands which include peptides, vitamins, and carbohydrates. Similar to the passive targeting scheme, the active targeting efficiency is also dependent on the size and structure of the AuNPs. It has been reported that 60nm transferrin-decorated AuNPs can actively target orthotropic melanoma cells within the range of 15-100nm and the tumor accumulation is 1.9times higher than that of the passive targeting method [71]. If the size was too small (D=15nm), the AuNPs lack multi-valent receptor binding capability and they may be rapidly eliminated from the tumor interstitium. If the AuNPs have a large diameter (D=100nm), they may be rapidly eliminated from blood at the tumor site. Low intracellular penetration is another issue for the large size. In order to design a highly specific and efficient active tumor targeting AuNPs, the functionalized AuNPs should be designed with a diameter in the range of 40-70nm and the specifically conjugated target agent as well as the shape of the nanoparticles should be precisely and specifically designed for different imaging-guided or drug-loaded PTT applications. *Table 2* lists some promising examples of functionalized AuNPs that can be used to target different cancer cells and tissues.

Table 4 Functionalized AuNPs used in PTT

TA Types	Targets	Features	Ref.
Anti-Her2 antibodies(Herceptin)	Human breast tumors	Enhanced CT image quality, higher efficient targeting than passive tumor targeting.	[72]
Bombesin (BBN) peptides	Gastrin-releasing peptide receptor overexpressing cells.	High binding affinity and selectivity. Potential in X-ray/CT imaging-guided PTT.	[73]
Anti-epidermal growth factor receptor(EGFR)	Head and Neck cancer cells and other EGFR overexpressing cells.	Efficient and specific, enhanced CT molecular cancer imaging.	[74]
folic acid (folate receptor ligand)	KB cancer cells	High specificity and strong photothermal effect.	[75]
prostate-specific membrane antigen (PSMA) RNA aptamer	Prostate cancer	Strong CT intensity. Drug loading ability.	[76]
Nuclear localization signal(NLS) peptides and linear Arginine-glycine-aspartate (RGD) peptides	HSC-3 (cancer) cells	Specific delivery of AuNPs to cytoplasm or nucleus, different cell death mechanisms can be achieved.	[77]

Mechanisms of Cell Death triggered by AuNPs based PTT

Understanding mechanisms of cell death in the process of PTT is important for increasing the treatment efficacy and decreasing the undesired side effects. Cell death has two basic mechanisms: apoptosis and necrosis. Apoptosis, also known as programmed cell death, is more like a well-planned sequence of events: Gene regulated cell membrane collapse leads to an influx of Ca^{2+}, then Ca^{2+} elicits blebbing of the cytoplasmic membrane and disrupting of actin filaments [78]. This mechanism results in eliminating cells without producing any harmful chemicals. Necrosis is cell death related to the low blood supply, disease, and injury. It is a type of abnormal breakdown of cells. The integrity of the cell membrane is severally destroyed, and some substances are released, which leads to inflammatory and immune responses because the dead cells and the released substances cannot be timely removed by phagocytes in the body [79]. During apoptosis, apoptosis-related proteins could be inactivated by cancer cells, and this leads to drug resistance and immune escape of the cancer cells. However, cancer cells cannot evolve

resistance due to the immediate death of the cells during necrosis. In contrast to chemotherapy which could only trigger necrosis of cancer, these two mechanisms could both be triggered by AuNPs based PTT, which has been reported by a few studies [77, 80-82]. Depending on AuNPs concentrations, AuNPs properties, targeting methods, and irradiation conditions, the different mechanisms of cell death could be triggered. Nanosecond pulsed laser PTT can only trigger necrosis, while continuous wave (CW) laser PTT can trigger either necrosis or apoptosis depending on the intensity of laser and the position of the AuNPs [77]. Generally, high energy irradiation leads to protein and lipid structure changes, which cause necrosis of cells. Solid tumor elimination typically uses this effective method. Low energy irradiation normally causes apoptosis, which does not contribute to immunogenic or inflammatory responses. Studies have reported cells underwent necrosis under $30W/cm^2$ irradiation for only 2mins. In contrast, increased apoptosis-related gene expression was observed under $1.5W/cm^2$ for 3 minutes and $5W/cm^2$ for 10 minutes in the irradiated cells [[81],[83],[84]]. The duration of irradiation is another factor which could affect the mechanism of cell death. Long irradiation time triggers a larger percentage of cell death, and cell death mechanism can change from apoptosis to the secondary necrosis as irradiation time increases. Because PTT is more likely to activate apoptosis intrinsically by damaging cells through heat generation rather than extrinsically by providing specific ligands binding to death receptors, researchers concluded that the low energy irradiation PTT can trigger intrinsic apoptosis [81]. Besides, a study in 2016 shows targeting AuNPs by different targeting methods leads to different cell death mechanism [91]. For passively targeted AuNPs, most of them retain in the extracellular matrix instead of cells or nucleus based on the EPR effect of tumor cells. Most of the nanoparticles generate heat through PTT in the extracellular matrix, heating all the surrounding microenvironment rather than generating heat within cells. While actively targeted AuNPs would retain inside the cells by receptor-mediated endocytosis, which generates heat within the target cell, they can even heat up localized the nucleus of cells through ionic interaction or covalent interaction. Thus, different targeting methods lead to different heat accumulation, either within the tumor microenvironment or inside the cell or even inside the nucleus of cell. In another word, heating from inside to outside environment of the cell would most likely trigger different cell death mechanism as heating from extracellular matrix to inside. Both of the cell death mechanisms have advantages and undesired outcomes for PTT, important factors like the severity of inflammatory responses and the anti-apoptotic capability should be weighted in order to design a robust PTA which could trigger the desired mechanism of cell death with the help of NIR laser.

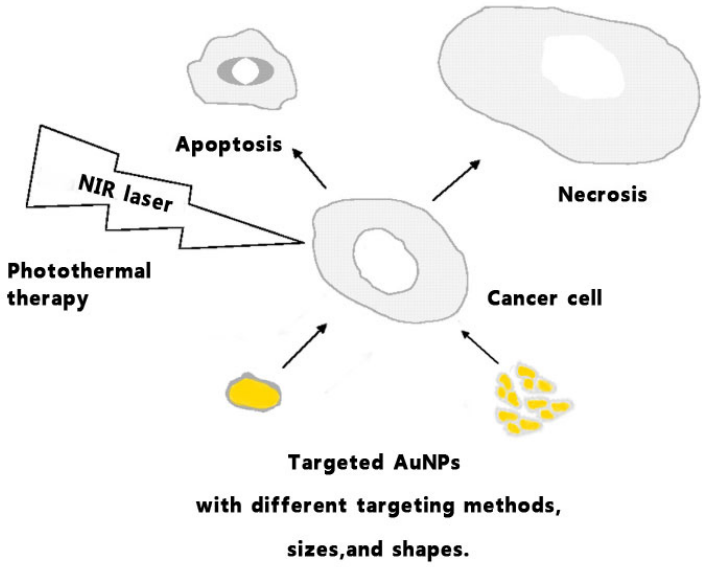


Figure 8 the schematic illumination of different cell death mechanism triggered by PTT with different parameters.

Conclusion

Innovative cancer treatments have emerged with the development of medical imaging techniques. Modern research is now concentrated on designing biocompatible AuNPs with high photothermal efficacy, enhanced scattering property for imaging, low cytotoxicity and reasonable clearance rate from the body. Overall, there are some modified AuNPs having acceptable PTT performances in pre-clinical research or clinical trial, especially gold nanoshells which are the best developed gold PTA so far. The Future development of photothermal therapy should focus more on the combination of different treatments. Limitations like precise control of biodistribution and clearance of PTA for mono-photothermal therapy could be overcome by taking advantages of other treatments such as chemotherapy, photoacoustic therapy, and gene therapy. Researchers should also pay attention to cell death mechanisms triggered by PTT in the future in order to promote a better PTT outcome. Currently, there are still many mysteries that have not been solved regarding cell death mechanisms, and most of PTAs could not trigger cell apoptosis but necrosis. It is still impossible to control cell death through a desired mechanism. For AuNPs themselves, with the improvement of synthetic technology, accurate and precise nanostructure construction techniques have become mature and stable. Various desired shapes and sizes of AuNPs with desired properties can be successfully synthesized, which will improve the photothermal efficiency and cancer imaging quality of future multimodal PTT. However, problems like potential long-term toxicity still need to be handled appropriately. Based on the fact that all different nano-forms of gold possess unique advantages, a large number of comparative experiments about the properties of the different gold forms should be carefully tested in order to select the most suitable AuNPs for certain tumor photothermal ablation.

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