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REVIEW ARTICLE

Application of Plasmonic Gold Nanoparticle for Drug Delivery System

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Abstract: Various kinds of colloidal nanoparticles (NPs) have been widely developed as a promising theranostic probe in biomedical fields. Particularly, colloidal gold nanoparticles (GNP) have been extensively utilized as a theranostic probe for molecular imaging and drug delivery owing to their unique physiochemical properties with excellent biocompatibility. This review will outline the progress along this line and discuss about gold nanoparticle synthesis method for controlled geometry (spherical, rod and cage), surface functionalization (covalent and non-covalent) and associated *in vitro/in vivo* cytotoxicity as well as some of interesting diagnostic (imaging) and therapeutic (photo-thermal, drug delivery) applications.

Keywords: Theranostic probes, diagnostic and therapy, drug delivery, plasmon, gold nanoparticle.

1. INTRODUCTION

Theranostics is aimed to develop platforms, which is capable of combining both diagnostic and therapeutic agents [1, 2]. Ever since, when the conjugation of polymer and drug was firstly suggested by Jatzkewitz in the 1950s, [3] nanotechnology has been continuously applied to the field of medicine to improve the therapeutic outcomes [4-6]. For example, various types of colloidal nanoparticles (*i.e.*, metal, semiconductor, polymer, *etc*.) have received great attention in the diagnosis and treatments of disease as an active imaging agent and drug carrier based on their outstanding properties, such as large surface-to-volume ratios, ease of surface functionalization (*i.e.*, biomolecules, and chemotherapeutic drugs) and tunable optical properties (*i.e.*, absorbance, scattering and luminescence) without photo-bleaching effect [7-11]. Particularly, plasmonic metal nanoparticles (*i.e.*, gold, silver and *etc*.) distinguish themselves from other inorganic nanoparticles based on their unique optical property known as localized surface plasmon resonance (LSPR) [12, 13]. These remarkable optical properties originate from photon confinement to a small particle resulting in the collective coherent oscillation of free conduction band electrons, which affect both radiative and non-radiative properties of the metal nanoparticles. Consequently, radiative decay results to strong visible scattering light and nonradiative decay convert photon energy into thermal energy [14]. According to these multiple modalities, metal nanoparticles have been widely utilized for biological and medical applications [15-17]. Recent advances in the nanotechnology have provided plasmonic metal nanoparticles with tunable optical properties based on controlling parameters such as size, shape, and composition [18, 19]. Additionally, the ease of tailoring functionalities (*i.e.*, tunable optical properties) and inevitable cytotoxicity of gold nanoparticle (GNP) has attracted many interests for biomedical applications, especially for diagnosis (imaging) and therapy (drug delivery) [20]. (Fig. **1**) (Table **1**) As well known, one of the most distinct properties of GNP is tunable optical property (range from visible to near infrared region) based on LSPR mechanism, which is a phenomenon of free electrons oscillation localized at the interface of the metal boundary in resonance with external electromagnetic fields [12]. The surface plasmon absorption and scattering efficiencies of a gold nanoparticle can be theoretically described by Mie theory [21]. El-Sayed and co-workers have demonstrated that the quantitative relationship between the optical absorption and scattering is highly correlated with particle size, when the size of particle becomes larger, the ratio of the scattering to absorption also increases [22]. When the size of GNP is less than ca. 20 nm, the total extinction is nearly all contributed by absorption, at ca. 40 nm the scattering starts to be distinguished and donated by both absorption and scattering in a similar amount at ca. 80 nm. Based on these phenomena, lager nanoparticles are known to be ideal for biological imaging *via* dark-field optical microscopy, [23, 24] surface-enhanced Raman scattering due to higher scattering efficiency [25-27]. In comparison, smaller nanoparticles are preferred for drug delivery and photothermal therapy, since the light is mostly adsorbed and efficiently converted to heat [28, 29]. In addition, GNP can be also used as an imaging contrast agents for photoacoustic (PA) imaging [30, 31] and computed tomography (CT) [32]

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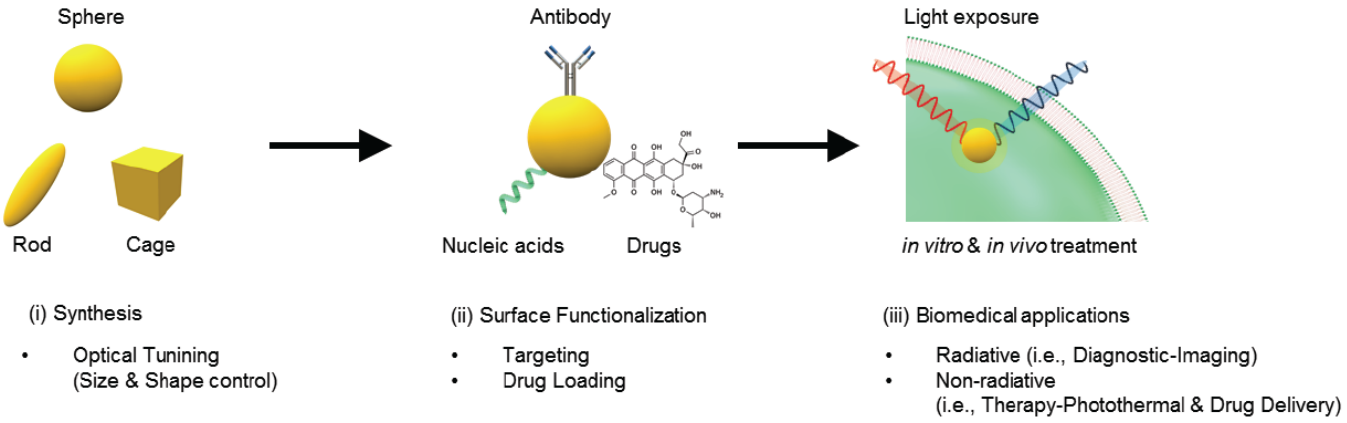

Fig. (1). The gold nanoparticle as a theranostic agent for biomedical applications. (i) Tunable optical property based on size and shape with the different synthetic method. (ii) Targeting & drug loading property by surface functionalization with biomolecules such as proteins (*i.e.*, antibodies), nucleic acids, drugs and *etc*. (iii) apply for both diagnosis and therapy applications based on based on their unique optical properties.

Table 1. GNP as a theranostic agent for biomedical applications.

Process	Feature/Methods		Ref
Synthesis (tunable optical property)	Nanosphere Nanorod Nanocage	Turkevich Brust Seed-mediated growth Galvanic exchange	[34, 35] [36, 37] [33, 38, 39] [40]
Functionalization	Covalent Noncovalent		[41-46] [43]
Biomedical Applications	Diagnosis Therapy	Raman Scattering Scattering Photoacoustic Computed tomography Photo-thermal Drug delivery	[25-27, 48-52] [23, 24, 47] [30, 31] [32] [28, 29, 31, 55] [43, 45, 56]
Cytotoxicity	Non-toxic Toxic		[57, 58, 63, 69] [59, 60-62, 64-68, 70]

and known to be resistant to photo-bleaching [8]. In this review, we will focus on preparation of plasmonic gold nanoparticle, its optical properties, and surface functionalization as well as cytotoxicity and their diagnostic and therapeutic approaches.

2. SYNTHESIS OF PLASMONIC GOLD NANOPARTICLE

Synthesis of Gold nanoparticle for biological applications has been well established by colloidal synthesis method. Utilizing a metal precursor, a reducing agent and a stabilizing agent, different morphology of gold nanoparticles such as spheres, rods, and cages can be synthesized accurately in large quantity [33].

One of the most well-known and widely used gold particles is spherical gold nanoparticles, which is firstly reported by Faraday in 1857 and generally synthesized by Turkevich's citrate reduction method [34, 35]. This method comprises the reduction of chloroauric acid ($HAuCl_4 \cdot 3H_2O$) with sodium citrate (reducing agent) and stabilizes through electrostatic repulsion with citrate-capped surface. In more depth, the size of the nanoparticle is also tunable by varying the ratio between gold and the reducing agent [36]. In a different way, Brust method is generally used for synthesizing smaller nanoparticles less than 10 nm in diameter. This method involves a two-phase system, reduction of chloroauric acid by sodium borohydride and transfer phase by stabilizing agent tetraoctylammonium bromide. In addition, alkanethiol can be used as stabilizing agent to prevent aggregation [37].

Owing to tunable broad absorption band at near infrared (NIR), gold nanorod (GNR) is another most widely used gold nanoparticle. In general, GNR is synthesized by seed-mediated growth method *via* the introduction of a small seed into growth solution comprised of ascorbic acid, silver nitrate and cetyltrimethylammonium bromide (CTAB) [38].

The length and aspect ratio of the nanorod can be controlled by adjusting the concentration of $AgNO_3$ [39].

As another attractive nanostructure, gold nanocage (GNC) can be synthesized by galvanic exchange mechanism with vulnerable optical properties and a hollow interior structure for drug delivery [40]. Formerly synthesized silver nanocube work as the sacrificial template to form gold nanocage. Owing to lower reduction potential energy of silver compare to gold, the oxidization and displacement of silver occur spontaneously as gold reduces on the surface to form cage structure.

2. SURFACE CHEMISTRY FOR FUNCTIONALIZATION OF PLASMONIC GOLD NANOPARTICLE

The addition of ligand such as an oligonucleotide, peptide/protein, and lipids on the surface of the gold nanoparticle can improve the targeting and the endocytosis efficiency to increase therapeutic effect.(41, 42) The surface of the gold nanoparticle can be functionalized with various biological and chemical molecules relative ease *via* non-covalent and covalent bindings. As an example for non-covalent bonding, GNP can be simply modified by electrostatic interaction with using oppositely charged biomolecules such as DNA, peptides, and antibodies.(43) The advantage of this interaction is that the biomolecule does not have to be exposed to the harsh chemical condition that could defect its activity. Alternatively, covalent interaction generally provides better stability and reproducibility with strong interaction between gold and thiol. In this interaction, sulfur atom donates a lone pair of electrons on the surface of gold and resulting an additional functional group such as carboxyl/amine termini for bio/chemical molecules, though molecule can be pre-thiolated and directly bound onto gold surface and utilized as therapy probes.(44-46)

3. RADIATIVE PROPERTY OF PLASMONIC GOLD NANOPARTICLE FOR IMAGING AND DIAGNOSTIC

Upon the exposure of the light, free electrons in gold nanoparticle are excited to a state of collective oscillation and emits photons in the form of scattered light either at the same frequency (Rayleigh scattering) or at a shifted frequency (Raman scattering), which is either lost or gained, translates to a change in the frequency of the scattered photons.(27, 47) This unique shift of frequency corresponds to the energy difference created by molecular motion (molecular bond rotations, stretching or vibrations), hence a Raman spectrum consisting of different signals from molecular vibrations could provide "vibrational fingerprint" for molecule.(27, 48, 49) Based on their optical scattering properties, GNP enables the visualization of region of interest by targeting and accumulation. Through the surface functionalization with specific antibodies, GNP can be directed to the antigens and precise the location in the body. Of particular interest, SERS have been employed for label-free *in situ* monitoring and targeted *in vivo* imaging. Hossain *et al*. presented SERS-based *in situ* monitoring system for time-dependent intracellular anti-cancer drug release by using gold nanoparticle. (Fig. **2**) (50) The anti-HER2 (Human epidermal growth factor receptor 2) antibody was used to target the breast cancer cells (SK-BR-3) and the cysteine modified Tat peptide (Tat-C) was used as a cell penetrating peptide to increase uptake rate. Glutathione-dependent intracellular release rate of doxorubicin (Dox) as an anti-cancer drug was monitored through SERS and cell cytotoxicity assay was conducted to observe the effects of intracellular anti-cancer drug release in

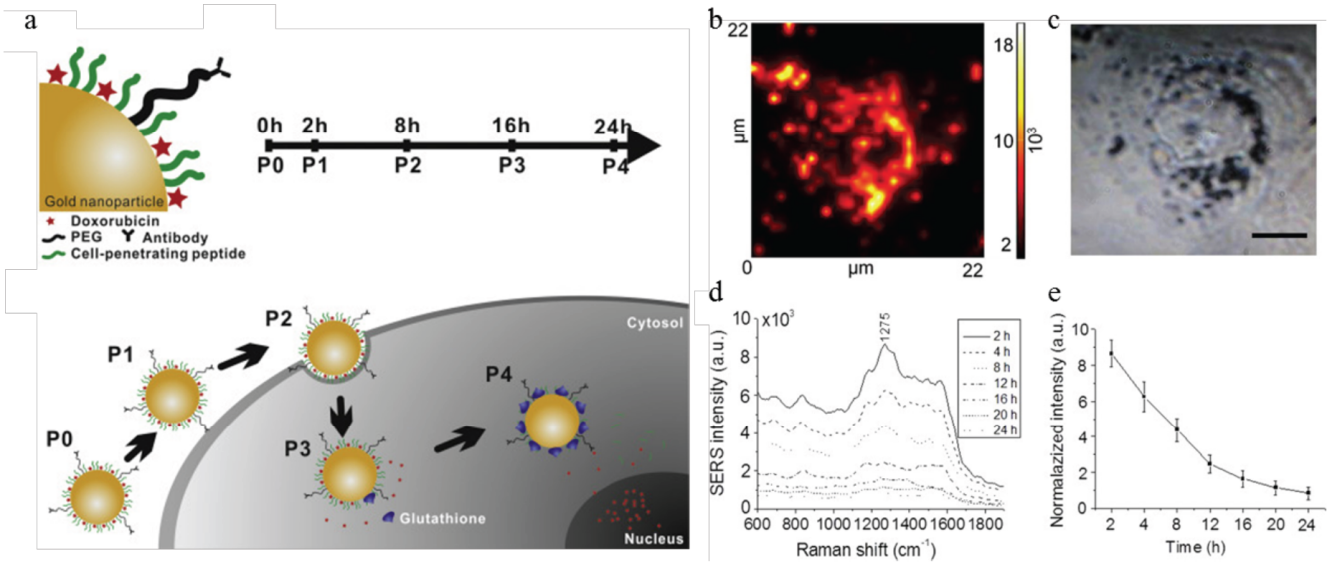

Fig. (2). *in situ* label-free intracellular drug release monitoring system based on gold nanoparticle consist of a cell-penetrating peptide (CPP) and a breast cancer-targeting antibody to facilitate specific cell targeting (**a**) Schematic illustration of biohybrid nanoparticle, time-dependent monitoring of the nanoparticle's specific targeting, uptake of the Dox-loaded biohybrid nanoparticles by the cells and the intracellular release of Dox by GSH. (**b**) SERS mapping image SK-BR-3 (Breast cancer) cells according to 1275 cm^{-1} Raman band and (**c**) bright field image, respectively. (scale bar: 5 μm) (**d**) SERS spectra of the SK-BR-3 cells treated with Dox-loaded biohybrid nanoparticles at different incubation times. (**e**) The relationship between time and release of Dox. (Modified from Ref. [50] with permission, Copyright 2015 Elsevier, Amsterdam, Netherlands).

the target cells. Moreover, Combination with Raman reporters has enabled *in vivo* SERS imaging for the tissues also.(51) Qian *et al.* have reported tumor detection in nude mice with xenograft model consist of human head and neck squamous cell carcinoma (Tu686). GNPs were functionalized with malachite green as a Raman reporter and ScFv EGFR antibodies to localize in the tumor site.(25) These results demonstrate the potential possibility of using GNP as a biomedical probe for *in vivo* imaging and monitoring, however, it should be noted that it cannot be directly employed for clinical use due to the limited tissue penetration of the optical signal.(52) Hence, additional technological improvement is required for *in vivo* Raman imaging for the clinical setting.

4. NON-RADIATIVE PROPERTY OF GOLD NANOPARTICLE FOR PHOTOTHERMAL THERAPY

Comparably, El-Sayed and his group have focused on GNP as energy transducers which convert the light into the heat by ultrafast dynamics.(14, 15) Upon the exposure of the light, the coherently excited electrons by electron-electron collisions lead to hot electrons, which passes the energy to the phonon by electron-phonon interactions resulting in a hot lattice.(53) The heated lattice can be cooled off by passing its heat to the surrounding medium by phonon-phonon relaxation and generate heat to kill the adjacent tumor cells. However, high energy pulse could generate rapid massive heat which could lead to structure changes or ablation of the nanoparticle. For example, Link and his coworker have found that nanorods melted with using a 100-fs laser at 800 nm while fragmented with 7-ns laser or higher energy of fs laser.(53, 54) In order to use the produced heat for photothermic therapy, Xia group demonstrated the successful detection and eradication of breast cells (SK-BR-3) through combination with anti-HER2 and subsequent irradiation of NIR light-induced photothermal effect which leads to cell death resulting from disruption of the cell membrane and denaturation of proteins subjected to high temperatures. (Fig. **3**) (55) Instead of just using direct heat from gold nanoparticles for cancer treatment, shao *et al.* recently reported the synergy effect of photothermally-activated dual treatment (physical and biological) by using tumor necrosis factor-alpha coated gold nanospheres (Au-TNF). Upon light exposure, the heated nanoparticle not only affected the cell directly but also allowed to release anti-cancer drug (TNF) for indirect treatment. As a result, higher therapy efficacy of Au-TNF conjugates could be observed for both *in vitro* and *in vivo*. (Fig. **4**) (56) In consequence, the combination of gold nanoparticles and light exposure has the potential to provide useful therapeutic benefits such as the optically controllable release of drugs and genes with *in situ* monitoring.

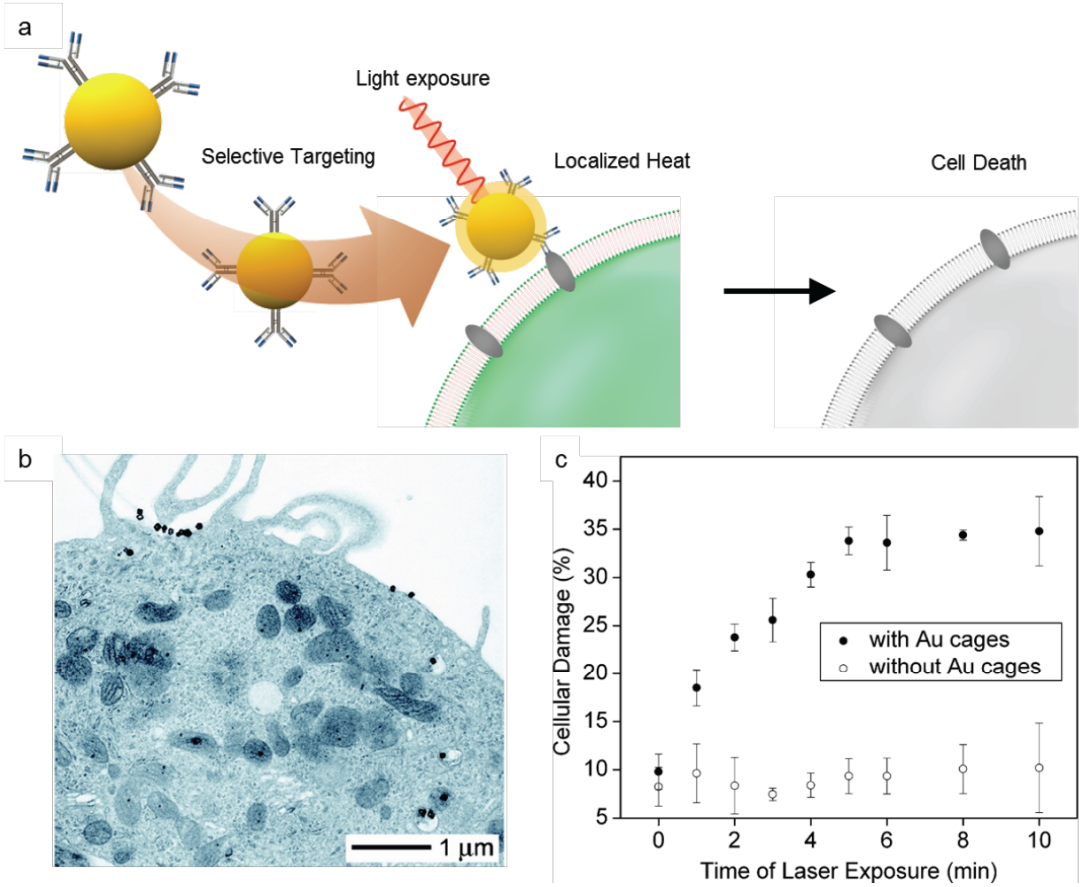

Fig. (3). (**a**) Schematic illustration of selective photothermal treatment based on antibody conjugated gold nanoparticle. (**b**) TEM image demonstrates the targeted SK-BR-3 cell with gold nanocages and (**c**) resulted in cellular damage with laser exposure. (Modified from Ref. [55] with permission, Copyright 2008 American Chemical Society, Washington, D.C., USA).

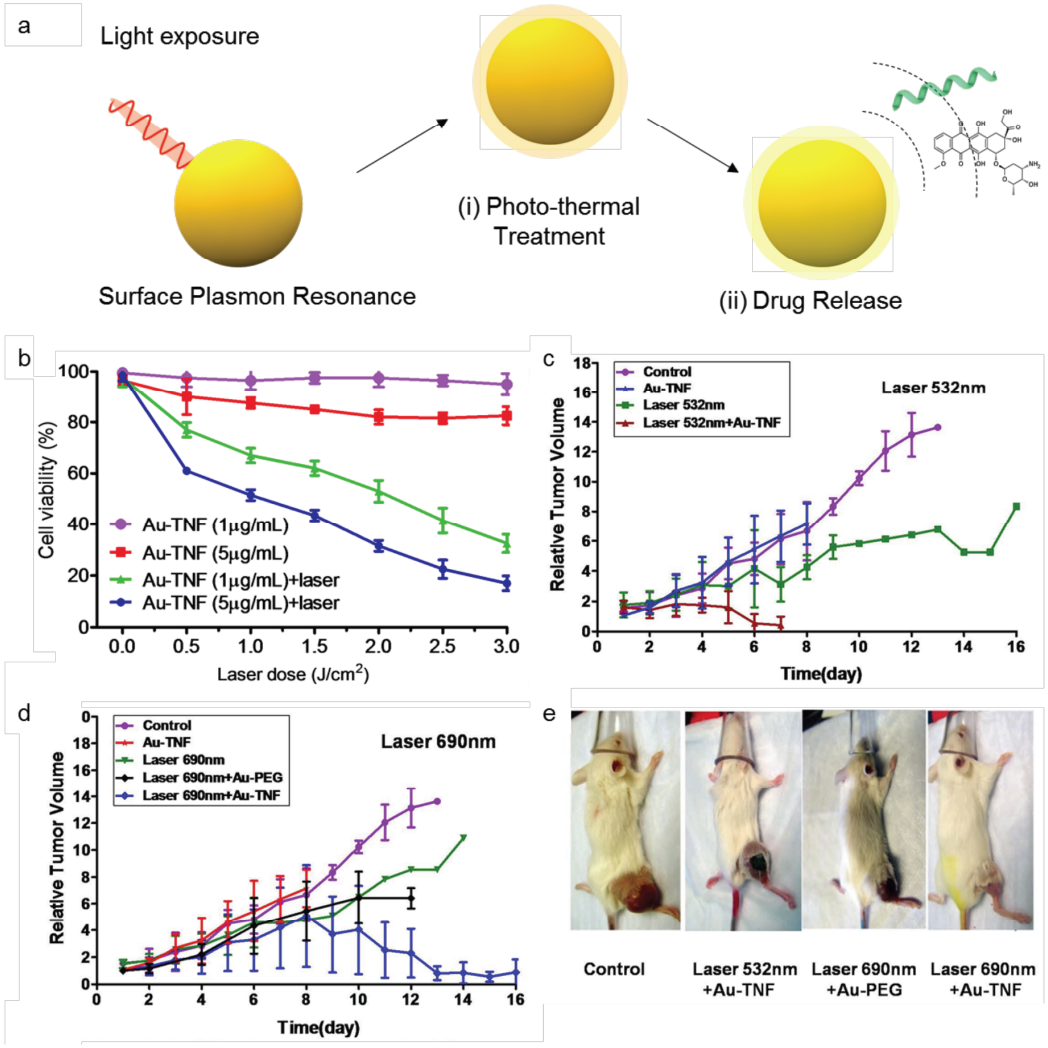

Fig. (4). (**a**) Schematic illustration of the photothermal property of gold nanoparticle for drug delivery. Light induces (i), photo-thermal heat generation and (ii), release drug for treatment. (**b**) *in vitro* cell viability test for synergetic effect based dual modality of Au-TNF. (**c-e**) Relative tumor volume after treatment with laser and Au-TNF at a laser wavelength of 532 nm (**c**) and 690 nm (**d**) at 8 h after an i.v. injection of nanoparticles. (**e**) Relative tumor images of different mouse groups. (Modified from Ref. [56] with permission, Copyright 2013 Nature Publishing Group, London, United Kingdom).

5. BIOCOMPATIBILITY AND CYTOTOXICITY OF PLASMONIC GOLD NANOPARTICLE

While using GNP as a theranostic probe for biological applications, toxicity is also one of the critical issues to be discussed. GNP have been reported to be biocompatible for use in some biomedical applications,(57, 58) however recent studies have shown that the properties of GNP in size, shape and surface chemistry determines the toxicity and stability in blood as well as the permeability and internalization on cells.(59-61) One of the previous study has shown that utilized GNP as embryonic imaging probes only affected 2% of deformities among the 76% of survived zebrafish embryos.(62) However, another contradictory study indicates that N, N, N-tri-methyl-ammonium-ethane-thiol coated GNPs at a size of 1.3 nm could potentially induce systemic cytotoxicity in the zebrafish embryos resulting in a mal pigmented eyes and neuronal damages.(63) The reduced size of GNP could improve the potential for deep penetration of certain tissues and improved internalization by cells, but it could also increase the possibility for cytotoxicity.(64) For example, in the rat model study, 10 nm sized small GNP have shown the widespread distribution in all tissues including blood and other organs such as kidney and brain, while GNP larger than 50 nm were mostly localized at blood and liver.(65) As mentioned, the smaller size of GNPs could be more feasible for a delivery system in the body and therefore it might also possess the higher probability of causing widespread harm. Previous studies indicate that 1.4 nm sized GNPs exhibited cytotoxicity mostly by inducing cell apoptosis on tissue fibroblasts,(66) while relatively larger 50 nm and 200 nm sized GNP showed non-toxicity.(67) As another issue, the surface functionality could also affect the efficiency of cellular uptake and cytotoxicity also.(68) For example, glutathione have improved the targeting efficiency without causing any inflammation in the mouse model.(69) PEGlayted GNP upregulated common genes relate to cell cycle, inflammation, and apoptosis in the liver tissue.(70) Hence, distinct variations such as endoplasm reticular system and genotoxicity should be also considered before shifting from animal models to clinics (Table **2**).

Table 2. Advantages and limitations of using gold nanoparticles as a theranostic agent for clinical applications.

Advantages
Ease to synthesis
Ease of surface modification (Targeting & Drug Loading)
Tunable optical properties based on size and shape
Large surface area & low hydrodynamic mean size
Suitable for multimodal applications (drug delivery, imaging, and therapy)
Limitations
Surface functionalization results to bio-distribution and cytotoxicity
Lack of information on cellular interaction and cytotoxicity
High costs for large-scale production
Non-biodegradability

CONCLUSION

Alongside with an expanding knowledge, GNP have emerged as a promising theranostic probe for biomedical applications. Their unique optical properties, high surface to volume ratios and surface functionalization have provided the tremendous possibility for GNP to be used as not only sensitive optical probes for imaging and diagnosis, but also as a theranostic probe for chemotherapeutic drug delivery and photo-thermal therapy. In addition, the surface functionalization of GNP has provided the selective targeting as well as effective and stable intracellular delivery of various therapeutic molecules. Although many researchers has indicated that GNPs are nontoxic, however still there are many issues to be solved before move on to clinical use such as long-term cytotoxicity, immune response or genotoxicity. Consequently, since it is clear that gold nanoparticle possesses several advantages compared to other traditional theranostic agents, we strongly believe that GNP based clinical approach will gain interest continuously with increasing numbers of innovations such as optically controllable drug delivery with *in situ* monitoring.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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