Gold-Nanoparticle-Enhanced Cancer Photothermal Therapy

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(Invited Paper)

***Abstract*—In this paper, progress on the gold-nanoparticle-enhanced photothermal therapy is reviewed. Size- and shape-dependent optical absorption of gold nanoparticles, the effects of various parameters on the therapeutic efficiency, and the mechanisms of gold-nanoparticle-assisted cancer therapy are discussed. Future research directions of gold-nanoparticle-assisted cancer photothermal therapy are also suggested.**

***Index Terms*—Cancer, gold nanoparticle, laser, therapy.**

I. Introduction

HYPERTHERMIA is a noninvasive approach to cancer treatment, in which tissues are exposed to higher than normal temperatures to promote selective destruction of abnormal cells. Cancer cells are more susceptible to hyperthermia effects than normal cells because of their higher metabolic rates. A marked reduction in tumor size after treatment by localized hyperthermia has been demonstrated by numerous clinical studies [1]. Several methods, including microwave irradiation [2], RF pulses [3], and ultrasound [4], have been used for the delivery of thermal energy. Although they can penetrate deep into tissue, high fluences are required because of their diffusive nature, which produces undesirable hyperthermic effects on surrounding tissues. Near-infrared (NIR) laser beams can penetrate tissues with sufficient intensity and high spatial precision. However, the low absorption of NIR light by tumors requires high levels of energy input to produce enough hyperthermic effects. The safety concerns associated with laser at such high energy levels discourage its clinical considerations. To make this treatment clinically safe and viable, the hyperthermic effect has to be intensified and highly localized, which makes it necessary to enhance the light absorption and energy conversion in the tumors. In this sense, localized hyperthermia with gold nanoparticles is being developed as an alternative to the conventional hyperthermia methods [5]–[7]. Gold nanoparticles are able to quickly convert the absorbed energy into heat energy in the picosecond time domain, making them excellent agents for hyperthermic cancer treatment. Apart from the light–heat conversion capability, gold nanoparticles are also good contrast agents for cancer detection. Due to their scattering and photoluminescence properties, various techniques suitable for 3-D *in vivo* imaging can be applied for imaging at a cellular level. These techniques include two-photon fluorescence, second/third harmonic generation (SHG/THG) [8], optical coherence tomography (OCT) [9], and reflectance confocal microscopy (RCM) [10]. This feature also makes gold nanoparticles advantageous over other nanomaterials such as carbon nanotubes [11], another photothermal agent used to destruct cancer cells. Gold nanoparticles have been extensively used in other biological aspects such as intracellular imaging [12], [13], biosensing [14], [15], etc., attributable to their good biocompatibility [16], and size- and shape-dependent optical properties [17], [18]. Their excellent affinity to biomolecules makes it possible to functionalize them with various specific targeting molecules [10], [16], [19].

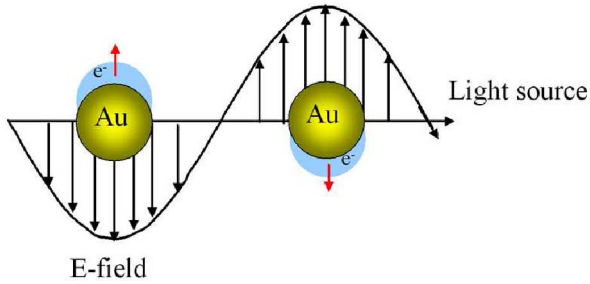

Fig. 1. Schematic description of plasmon oscillation of a gold nanoparticle under light irradiation. The electromagnetic field of the light causes the resonance of electrons across the nanoparticle.

II. Surface Plasmon Resonance of Gold Nanoparticles

For a metal nanoparticle such as a gold nanoparticle with size much smaller than the wavelength of incident light, the presence of an electromagnetic field at a certain wavelength can induce a resonance of the free electrons across the particle, known as surface plasmon resonance (SPR; Fig. 1). As a result of SPR, the particle absorbs and scatters the electromagnetic radiation intensely. The resonance wavelength of a gold nanoparticle depends strongly on the size, shape, and surface composition of the particle, and the dielectric properties of the surrounding medium. This characteristic of gold nanoparticles has been explored for enhancing the hyperthermic cancer treatment [20]–[22]. Gold-nanoparticle-enhanced photothermal therapy has been an active research area for the last few years. Recently, gold nanoparticles have also been used to enhance X-ray radiation and RF treatment of cancers [23]–[26].

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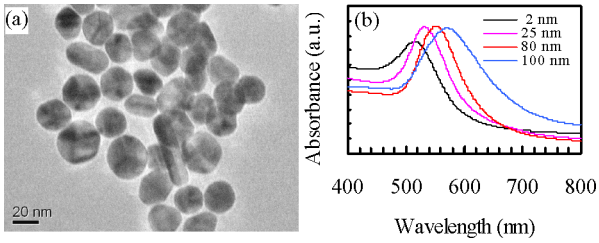

Fig. 2. (a) TEM picture of gold nanospheres with an average diameter of 25 nm. (b) Optical absorption of gold nanospheres of different sizes.

III. Size- and Shape-Dependent Optical Absorption of Gold Nanoparticles

Gold nanoparticles can be produced in a variety of shapes, including sphere [27], rod [28], cube [29], triangle/prism [30], hexagon, star [31], [32], etc. Depending on the shape and size, gold nanoparticles can absorb and scatter light in the visible and NIR wavelength regions. So far, the commonly utilized gold nanoparticles for cancer therapy include gold nanospheres, nanoshells, nanorods, and nanocages. Therefore, in the following sections, the optical absorption of these four types of gold nanoparticles and their applications in photothermal therapy will be discussed.

A. Gold Nanospheres

Gold nanospheres are the simplest form of gold nanoparticles. They are easy to fabricate, which is a factor that contribute to their extensive applications. The most commonly used gold nanospheres have sizes ranging from a few nanometers to around a hundred nanometers [Fig. 2(a)], with absorption wavelength ranging from 500 to 600 nm. The maximum absorption wavelength of a gold nanosphere moves to the longer wavelength as its diameter increases [33].

B. Gold Nanoshells

Gold nanoshells are composite particles of gold and a dielectric material. A gold nanoshell is consisted of a dielectric core, usually of silica, and a gold shell has optical resonance wavelength from visible to NIR region.

The preparation of gold nanoshells involves coating silica nanoparticles having amine groups on the surface with a layer of small gold nanospheres, usually a few nanometers in diameter, and nucleating gold with the gold nanospheres as nucleating centers to form a gold shell. The absorption wavelength of gold nanoshells depends strongly on the ratio of the shell thickness to the diameter of silica core (Fig. 3) [34]. With decrease in this ratio, the absorption shifts to longer wavelengths in the NIR region, making gold nanoshells attractive for *in vivo* study.

C. Gold Nanorods

Among the gold nanoparticles of various shapes, gold nanorods have been attracting special interest. This is attributable to their larger cross section for light absorption, convenience in controlling their absorption in the NIR region, and the recent success in their size-controlled large-scale synthesis [28], [35].

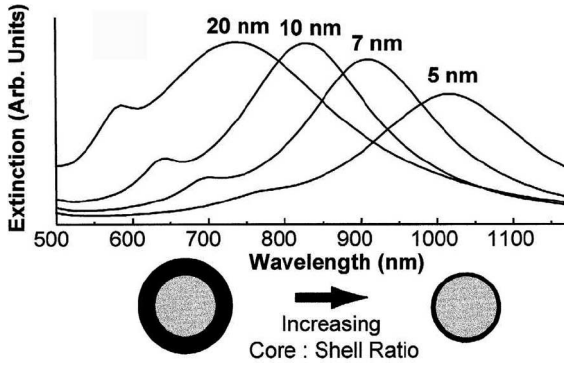

Fig. 3. Simulated optical absorption of gold nanoshells as a function of the thickness of gold shell. The diameter of the silica core was fixed at 60 nm. The absorption wavelength is tunable in the NIR region. With a decrease in shell thickness, the absorption shifts to a longer wavelength. (Reproduced with permission from [34], © 1998 Elsevier.)

Another factor contributing to the popularity of gold nanorod is its strong two-photon-excited photoluminescence (TPL), which is suitable for 3-D *in vivo* imaging [36], [37]. Typically, a gold nanorod has two absorption bands, which correspond to the transverse (short axis) plasmon resonance and longitudinal (long axis) plasmon resonance. The crucial factor determining the longitudinal absorption wavelength of a gold nanorod is its aspect ratio, which is defined as the ratio between the length of the long axis and that of the short-axis direction. By varying the aspect ratio, the longitudinal absorption can be made tunable in the NIR region [Fig. 4(b)], making gold nanorods suitable for *in vivo* applications.

D. Gold Nanocages

Xia and his colleagues developed gold nanocages by etching silver nanocubes with gold chloride [40]–[42]. Therefore, gold nanocages are hollow structures with thin, porous, and robust gold wall [Fig. 5(a)]. By using different amounts of gold chloride, gold nanocages with controllable wall thickness and absorption at wavelengths located from visible to NIR regions are obtained [Fig. 5(b)]. The thicker the gold wall, the longer the maximum absorption wavelength.

IV. Gold-Nanoparticle-Enhanced Photothermal Therapy

Compared with the conventional cancer therapeutic techniques such as chemotherapy, the advantage of gold-nanoparticle-assisted photothermal therapy is highly localized cell damage (Fig. 6), which minimizes the side effects on healthy cells. This technique provides a noninvasive approach to treatment of cancers, particularly those at early stages.

A. Gold Nanospheres

Pitsillides *et al*. used light absorbing micro- and nanoparticles, including iron oxide microparticles and gold nanospheres, to treat cancer cells [43]. It was observed that irradiating lymphocytes cells with a short pulsed laser (20 ns) in the presence of gold nanospheres could increase the plasma membrane permeability, which led to cell death. In a later work, Zharov

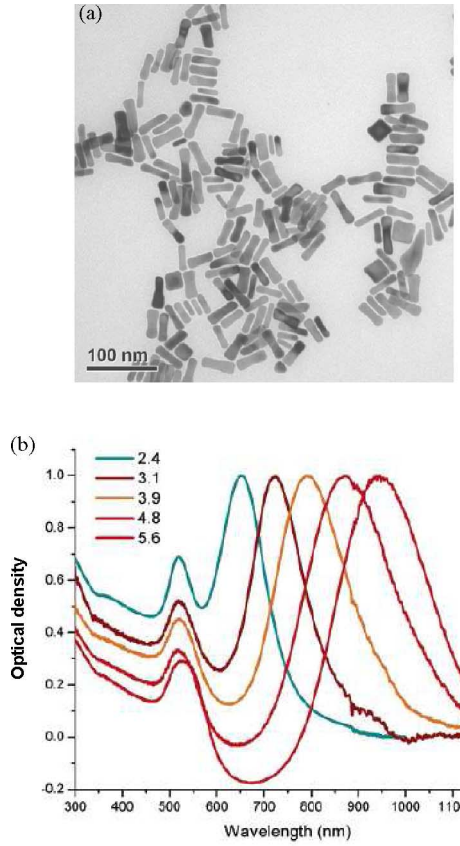

Fig. 4. (a) TEM picture of gold nanorods with an aspect ratio of 4.0 (reprinted with permission from [38], © 2008 Wiley–VCH Verlag GmbH & Company KGaA). (b) Absorption spectra of gold nanorods with aspect ratios ranging from 2.4 to 5.6. (Reprinted with permission from [39], © 2006 American Chemical Society.)

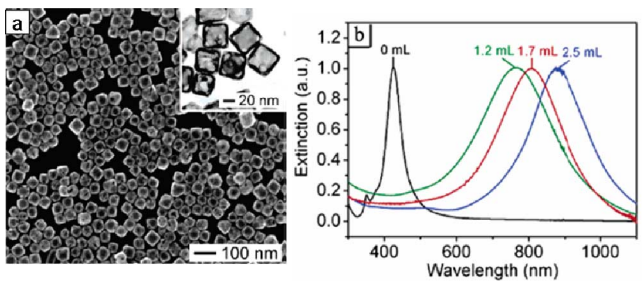

Fig. 5. (a) TEM image of gold nanocages. (b) Dependence of absorption on the thickness of gold wall (the spectra from left to right corresponds to walls with increasing thickness). (Reprinted with permission from [40], © 2007 American Chemical Society.)

et al. reported enhanced cancer cell therapy using a nanosecond laser [44]. It was found in both of the aforementioned two works that particle size strongly influences the photothermal effects of the particles on cells. Particles with size from 10 to 30 nm have the strongest effect. Reduced effects were observed for particles with size outside this range. Although the photothermal effect of larger particles is more significant, as indicated by theoretical calculations, their lower cellular uptake reduced overall efficiency. In addition, the particle size of 10–30 nm was found to be very well correlated to microbubble formation, a cause of cell damage, induced by the particles.

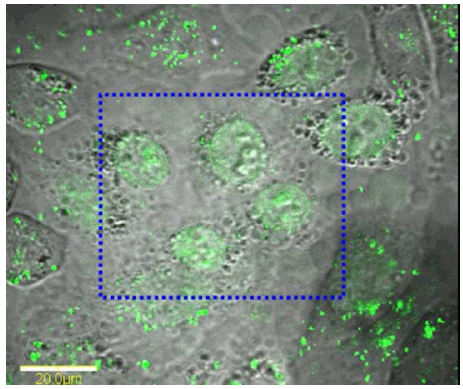

Fig. 6. Picture showing the localized photothermal damage of cancer cells (HeLa) labeled with gold nanorods and irradiated with a femtosecond laser. Ethidium bromide (EB) was used to label dead cells. EB is membrane impermeable to live cells and is generally used for the examination of cell membrane permeability, and thus, cell viability. The rectangular region corresponds to laser irradiated area. It shows that all the cells in the area irradiated with laser were killed and cells outside this region were not affected. The result indicates that the photothermal effect is highly localized. Scale bar: 20 μm. Experimental details are available in [38].

Research on cancer therapy using gold nanoparticles has also been carried out by the group of El-Sayed *et al.* [45]. A continuous-wave (CW) argon ion laser operating at 514 nm was used to treat two lines of oral squamous carcinoma cells (HSC 3 and HOC 313 Clone 8) and one benign epithelial cell line (HaCaT). To achieve effective targeting, gold nanospheres were conjugated with antiepithelial growth factor (anti-EGRF). It was found that the effective powers for malignant cells (25 and 19 W/cm^2 for HSC and HOC cells, respectively) are less than half of that for the benign cells (57 W/cm^2) due to the greater uptake of the antibody-conjugated gold nanoparticles by the cancer cells. In the absence of gold nanoparticles, all the cells were not killed at a much higher power of 76 W/cm^2.

In a recent work, Li *et al*. used gold nanospheres to treat a breast cancer cell line (HS578T) with a nanosecond laser operating at a wavelength of 530 nm [46]. The gold nanospheres with an average diameter of 25 nm were conjugated with transferrin to enhance the cellular uptake of the nanoparticles by the cells. Cell damage was observed at a laser power at 7 W/cm^2. In comparison, cell damage was observed at laser powers as high as 1600 W/cm^2, two orders of magnitude higher, in the absence of gold nanoparticles.

Despite the success in using gold nanospheres for *in vitro* cancer cell treatment, their *in vivo* applicability is limited due to their optical absorption in the visible region, which is a factor that contributes to the less interest in their application for cancer treatment. Huang *et al.* extended the application of gold nanospheres to the NIR region by employing the nonlinear properties of gold nanospheres [20]. Gold nanospheres were conjugated with anti-EGFR antibody for targeting and photothermal therapy of HSC oral cancer cells using a femtosecond laser at 800 nm. A quadratic dependence of the photothermal efficiency on the laser power was obtained, indicating an SHG or a two-photon absorption process. Despite the success in NIR excitation of gold nanospheres, higher incident laser power has

to be applied, leading to higher energy fluence inputs due to the nonlinearity.

B. Gold Nanoshells

West and coworkers did pioneering work on both *in vitro* and *in vivo* photothermal therapy of cancers using gold nanoshells. In one of their works, gold nanoshells were conjugated with antibody for both dark field imaging and therapy of HER-2-positive breast cancer cells SKBr3 [47]. Cells targeted with the functional nanoshells were killed by an NIR laser operating at 80 mW/cm^2 for 7 min, while cells without nanoshells were not affected. In addition, cell damage was confined in the laser exposure area, indicating the highly localized thermal effect. Detailed *in vivo* work on the effects of laser irradiation on tumor size and mice surviving rate were carried out in a recent work [48]. Tumors treated with nanoshells were substantially regressed 12 days after therapy, compared with the substantial growing of tumors untreated and treated with phosphate-buffered saline (PBS). The surviving rate of mice treated with nanoshells was monitored within seven weeks after therapy and a rate of more than 80% was obtained, compared with the much lower rate ($<$20%) of the mice not treated with nanoshells. This study indicates that nanoshells could be potentially used to enhance the photothermal therapy of cancers. In a recent publication, gold nanoshells were used by the same research group to treat central nervous system tumors [49]. Enhanced therapy in the presence of antibody-conjugated gold nanoshells was reported.

Increasing interest is being shown on the applications of magnetic gold nanoshells for cancer cell imaging and therapy [50]–[53]. Magnetic gold nanoshells were formed by embedding magnetic nanoparticles inside the gold shell. The magnetic property of the nanoshells makes it feasible to use another imaging-mode MRI for observing the distribution of the particles *in vitro* or *in vivo*. *In vivo* study of tumor treatment under magnetic resonance guidance showed that tumors treated with magnetic gold nanoshells reached an average maximum temperature increase of 37.4 $\pm$ 6.6 °C, which was capable of inducing irreversible tissue damage. However, without nanoshell injection, the temperature increase was less than 10 °C, below the threshold for tissue damage [54]. Kim *et al.* studied the photothermal therapy of two cancer cell lines using multifunctional magnetic gold nanoshells [50]. It was observed that cells targeted with the nanoshells could be damaged after a short 10 s exposure of a femtosecond laser. Without nanoshells, the cells could not be killed after being exposed to a power of 80 mW (laser beam diameter 1 mm). The power threshold for the nanoshell-labeled cells was found to be 20 mW. At this power, cells only at the center of the laser beam exposure were damaged, and with increasing laser power, the area of the dead cell spread due to the Gaussian energy distribution. Gold nanoshells with magnetic cores were also used for the effective photothermal therapy of other cancer cells [52].

C. Gold Nanorods

El-Sayed and his colleagues used gold nanorods for photothermal treatment of cancer cells [39]. Gold nanorods with NIR absorption at 800 nm were conjugated with anti-EGFR antibody for targeted therapy of oral cancer cells. An NIR CW laser was used. The normal cells were damaged at a laser power of 20 W/cm^2, while the value for the malignant cells was 10 W/cm^2. In addition, cell damage was observed to be confined within the laser spot area, indicating highly localized thermal effect. The strong dark field scattering of the nanorods made it possible for the cancer diagnostics to be carried out. *In vivo* cancer treatment using gold nanorods was also carried out by the authors [55]. The nanorods were administrated into mice with squamous cell carcinoma xenografts by both direct injection into the tumors and intravenous injection. Inhibition of average tumor growth for both delivery methods was observed over a 13-day period, with resorption of $>$57% of the directly injected tumors and 25% of the intravenously injected tumors.

In vitro cancer therapy using gold nanorods has also been performed by Li *et al.* [38] and Tong *et al.* [56], which will be discussed in the following sections.

The advantages of gold nanorods over nanospheres and nanoshells are the narrower absorption band and stronger two-photon luminescence. The strong two-photon luminescence offers a labeling-free method for 3-D cancer diagnostics.

D. Gold Nanocages

Xia and his colleagues used gold nanocages for cancer photothermal therapy [40], [42]. A quantitative study on the photothermal effect of gold nanocages targeted to breast cancer cells was reported recently [57]. To achieve targeting purpose, gold nanocages with an average edge length of 65 $\pm$ 7 nm and a maximal absorption at 800 nm were conjugated with monoclonal antibodies (anti-HER2). The number of gold nanocages immobilized per cell and the photothermal effect were quantified using flow cytometry. The optimal dosage of nanocages and the parameters of the laser irradiation (pulsed NIR laser), including laser power density and irradiation time, were determined.

Despite the success in using gold nanocages for cancer cell treatment, its low popularity is in contrast to those of other types of gold nanoparticles, due to the current complicated procedure to produce the nanocages. The crucial step of the gold nanocage fabrication is the preparation of silver nanocubes, which needs to be processed in nonaqueous solvents at elevated temperatures. With the development of simpler synthetic method, more research on this type of gold nanoparticles can be expected.

V. Mechanisms of Photothermal Therapy

In general, it is believed that the damage of cells labeled with gold nanoparticles upon laser irradiation is a result of the rapid conversion of absorbed light by the gold nanoparticles to thermal energy, which heats and kills the cells. The process involves the transfer of absorbed light from gold nanoparticles to cells by rapid electron–phonon relaxation in the nanoparticles followed by phonon–phonon relaxation, resulting in an increase in the temperature of the nanoparticles and cells. However, in many cases, cell therapy by laser in the presence of gold nanoparticles has been observed to be the results of effects other than the direct temperature increase. Nevertheless, these effects can be

amplified by the temperature increase of the gold nanoparticles, leading to mechanical damage of the cells.

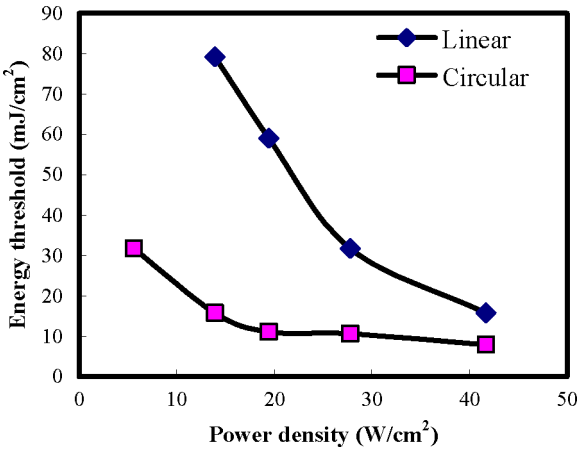

Fig. 7. Energy thresholds for cancer cell (HeLa) therapy under linearly and circularly polarized light illumination. The results show that depending on the incident power density, the adoption of a circularly polarized beam can reduce the threshold of energy fluence to values from one-fifth to half of those obtained under the irradiation of a linearly polarized beam. Experimental details are available in [38].

safety standard of 100 mJ/cm^2, for cell damage were reported in the case of nanorods when the cell samples were scanned by lasers instead of being irradiated with lasers focused on one spot [38], [56]. The low energy fluences are attributable to the very short interaction of light with nanorods, which is only 40 [38] and 126 μs [56] during one scan. The ultralow energy fluences obtained in these cases indicate that a continuous laser irradiation might not ensure higher energy efficiency. Continuous exposure of nanoparticles to laser will cause a severe change of the nanoparticles both in shape and size [61], [62]. This change causes shift in absorption wavelength, and hence, reduces the light absorption efficiency. Nanocages have cross sections comparable to those of gold nanorods. The high energy fluence required for this type of gold nanoparticles might be due to the use of a pulsed laser with a larger pulsewidth (picoseconds) [40].

It is worth mentioning that a comparison of results from different works cannot provide conclusive definition of the role of laser properties in deciding the energy input, due to the nonconformity of other parameters, such as the nanoparticle concentration, the location of the nanoparticles in cells, the aggregation state of the nanoparticles, etc. The comparison made between CW and femtosecond pulsed laser by Tong *et al.* in the same work led to a more concrete conclusion that short pulsed laser was more effective than a CW laser [56].

VII. Summary and Future Direction

The recent research summarized before indicates that gold nanoparticles can be promisingly utilized to enhance hyperthermic cancer treatment, making this approach to cancer therapy less invasive. Despite the increasing number of publications on gold-nanoparticle-assisted cancer detection and therapy over the last few years, there is still a lot of work to be done before this technique is clinically applicable, due to safety concerns. It is predictable that future research in area will be focused on the development of a medically safe and clinically viable photothermal therapeutic technique. To this end, the following considerations or combinations of them should be taken.

1) *Selecting suitable gold nanoparticles:* Gold nanoparticles with absorption in the NIR region and with larger cross sections should be used in order to maximize the light absorption by malignant tissues and minimize energy input. Gold nanorods, nanoshells, and nanocages are advantageous over nanospheres.
2) *Selecting ultrafast pulsed lasers, particularly femtosecond laser, instead of CW laser:* Femtosecond laser microsurgery (FLMS) has emerged as a superior technique for ablation of cells and subcellular structures [63], [64]. In FLMS, laser absorption is confined to the focal volume as a result of nonlinear interaction. This technique has been used clinically for surgery since 2003 [65]. A combination of gold nanoparticles with FLMS can lead to the development of less invasive hyperthermia treatments.
3) *Enhancing light absorption efficiency:* In order to reduce the energy input, the polarization dependence of anisotropic gold nanoparticles such as gold nanorods can be harnessed. More efficient absorption can be realized by using a circular polarized beam to excite as many nanorods in the cells as possible.
4) *Combining photothermal therapy with other therapeutic techniques:* Hyperthermia has been conventionally combined with other therapeutic techniques, such as chemotherapy and radiation therapy, to achieve efficient cancer treatment [66]–[68]. In a recent work, gold-nanorods-enhanced hyperthermia was combined with chemotherapy to treat cancer cells. In this paper, the laser power was tuned to effectively halt cell proliferation, instead of killing the cells directly with a laser, as done in previous works [69]. The combination of these two modes led to increased cytotoxicity, greater than sum of the independent treatments. The speculated mechanism for the superadditive or synergistic effect is that upon optical excitation, gold nanorods can break down the cell membrane so that the permeability of cell membrane and cellular uptake of the drug are enhanced. In an earlier work, gold nanoparticles were also linked with a viral vector, which can be potentially used in combined photothermal and gene cancer therapy [70].
5) *Combining the gold-nanoparticle-assisted photothermal therapy with two-photon fluorescence microscopy:* To realize the potential of gold-nanoparticle-assisted FLMS in clinically applications, it is essential that the application of this technique can be performed in an observable and guided manner. Two-photon fluorescence microscopy utilizes NIR femtosecond laser pulses, similar to those for FLMS. Therefore, by integrating two-photon fluorescence microscopy with FLMS, cell surgery can be done in an observable and controlled manner [71]. In addition, laser beams can be directed to tumors located inside body through optical fibers, which minimizes the possible damage of healthy tissues by laser and also contributes to a reduced energy input. The strong two-photon-induced photoluminescence from gold nanoparticles will also make

this hybrid technique feasible to cancer diagnosis without the use of additional molecular markers.

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