Biomed Eng Lett (2013) 3:67-73
DOI 10.1007/s13534-013-0097-8

REVIEW ARTICLE

Nanoparticle Platforms for Combined Photothermal and Photodynamic Therapy

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Received: 20 June 2013 / Accepted: 25 June 2013

Abstract
Phototherapy is a promising strategy for cancer treatment due to its selective and localized therapeutic effect by laser irradiation. Photothermal therapy damages malignant cells by using heat converted from light by an agent. On the other hand, photodynamic therapy uses photosensitizers that become cytotoxic upon irradiation with laser light at excitation wavelength. As singular treatment of each phototherapy showed some limitations, there have been significant efforts to enhance therapeutic effect by combining photothermal and photodynamic therapy. Here we review recent developments of nanoparticle platforms, in which inorganic nanostructures (photothermal therapy) are integrated with photosensitizers (photodynamic therapy) for combined phototherapeutic effect.

Keywords Cancer, Gold nanostructure, Photodynamic therapy, Photosensitizer, Photothermal therapy

INTRODUCTION

Phototherapy uses specific wavelengths of light to induce selective photodamage on the cancer cells. There are two major types of phototherapy: photothermal therapy and photodynamic therapy (Table 1).

Nanomaterials in photothermal therapy

Therapeutic strategy using heat to kill the pathogenic cells with irreversible damage by loosening cell membranes and denaturing proteins from various heating source is called hyperthermia. A laser is a main tool for hyperthermia by conversion of laser energy to heat. Direct irradiation to the target region using a light source generates sufficient amount of heat. However, there have been problems of direct conversion of light energy to heat energy because the laser travels all the tissues in its pathway, causing unintended damage to normal tissues. The needs of agents for selective and efficient photothermal therapy were uttered to solve this problem. Ideal photothermal agent should satisfy several requirements; biocompatibility, large absorption coefficient, near infrared absorption, and photostability. One of the promising photothermal agents is gold nanostructures which are highly light-absorbing and biocompatible [1].

Metals such as gold (Au) or silver (Ag) have different optical properties from standard dielectrics. Metallic materials have free electrons surrounding fixed positive ions, and these electrons interact with light on the interface between metal surface and dielectric environment. When irradiated with a certain external light, free electrons collectively oscillate upon light absorption and propagate along a metal surface, which is called surface plasmon [2]. On the other hand, when particle diameter is even smaller than wavelengths, surface plasmon cannot propagate but stay within the particle, keep oscillating. This state is called 'localized surface plasmon resonance', and thereby most of light at the resonance wavelength is absorbed by or scattered from the nanoparticle. Thus, strong light absorption of gold nanostuctures at the resonant wavelength enables them to generate sufficient amount of heat by converting incident light into heat efficiently. This gold nanostructure-mediated photothermal heating can be further utilized to kill malignant cells, which is called localized photothermal therapy [3].

For gold nanostructures to generate heat effectively in biological systems, light must penetrate deep into target

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Table 1. Photothermal therapy and photodynamic therapy.

	Photothermal therapy (PTT)	Photodynamic therapy (PDT)
Activation source	External light	External light
Role of agent	Heat generation	Reactive singlet oxygen generation
Cell damage	Denaturation of proteins Disruption of cell membrane	Destruction of localized organelles

tissues. Light encounters several obstacles in its pathway to the target region such as scattering from or absorption by various biomolecules in the tissues. In particular, it has been known that hemoglobin molecules absorb most of visible light. On the other hand, near infrared (NIR) light at the wavelengths ranging from 650 nm and 900 nm travels deep though tissues because hemoglobin and water exhibit relatively low light absorption in this region [4]. At this point of view, nanoparticle-based agents that can generate heat efficiently by NIR light are more favorable for photothermal therapy. Various structures of gold nanoparticles have been extensively developed for new generation of photothermal therapy [5-7]. Resonant absorption peak and cross-section of gold nanostructures can vary based on their size and shape. According to the previous reports, gold nanorods, gold nanoshells, gold nanostars and gold nanocages can be suggested as effective photothermal agents because they show their resonant peaks in the NIR region with high absorption cross-section.

One weak feature of photothermal therapy is that hyperthermia leads to thermotolerance in cancer cells, mediated by heat-shock proteins (HSPs) [8]. In previous findings, HSP 27, 72 and 90 have been known to play a significant role in enabling the survival of cancer cells in high temperature conditions [9, 10]. Thus, a synergistic approach in which photothermal therapy is combined with another therapeutic modality is needed to overcome the limitation of the photothermal therapy.

Nanomaterials in photodynamic therapy

Photodynamic therapy is a clinical method to damage disease cells by highly toxic singlet oxygen generated by photosensitizer, which is excited by external light source. Singlet oxygen species have short lifetime (<0.04 ms) and show small active region (0.02 mm), so that localizing photosensitizer inside the cells of our interest to kill is very important. Photodynamic therapy induces apoptosis of the cells, as various intracellular organelles essential for metabolism are destructed by photo-damage. Mitochondrial photo-damage is one of the most important reasons for apoptotic response, followed by release of cytochrome c, which govern later apoptotic reaction [11].

Delivery of photosensitizers to the target region is also important. Most of the photosensitizers with high therapeutic activity are hydrophobic, which hampers direct administration of the photosensitizers into systemic circulation. In order to solubilize the hydrophobic photosensitizer in physiological solutions and improve localization of the photosenstizers in

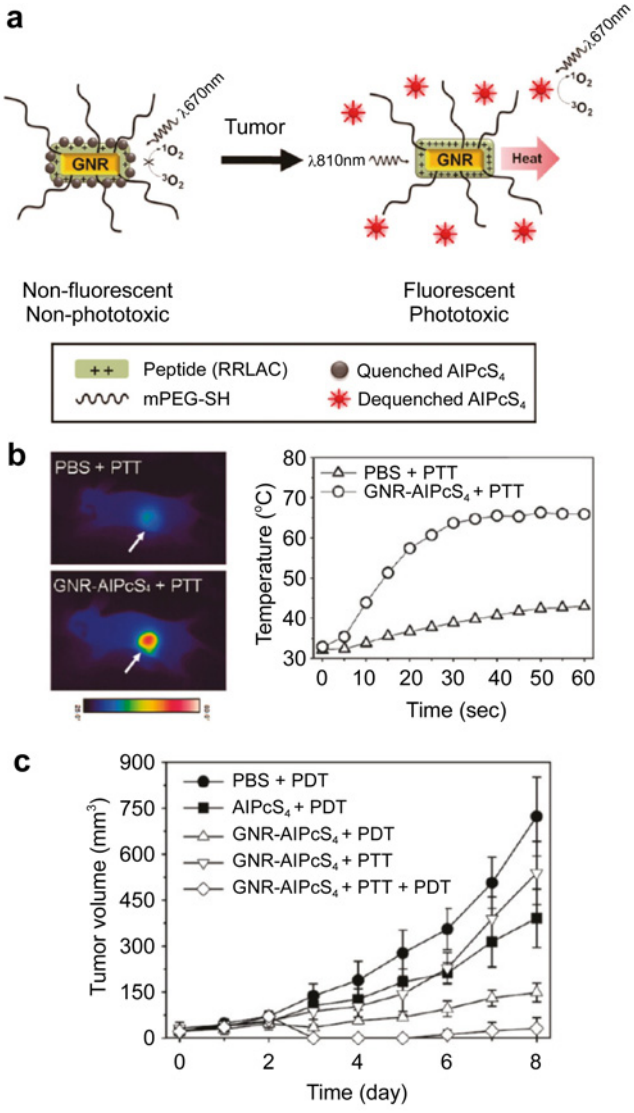

Fig. 1. Gold nanorod-photosentizer complex for NIR-based imaging and combined phototherapy. (a) Scheme of combined photothermal and photodynamic therapy using photosensitizer($AlPcS_4$)-oated gold nanorods. (b) Temperature change of tumors injected with photosensitizer-coated gold nanorods upon laser irradiation. (c) Tumor size change after combined photothermal and photodynamic therapy using $AlPcS_4$-coated gold nanorods. Adapted with permission from reference [23]. Copyright (2011) American Chemical Society.

the target region, a variety of delivery nanosystems have been developed. Polymeric nanoparticles [12], liposomes [13], lipoproteins [14], and micelles [15] with amphiphilic properties have been developed as photosensitizer nanocarriers in the laboratory, and some of them are now undergoing clinical trials. Moreover, in order to improve selective accumulation of photosensitizers at tumor site, active targeting systems with targeting ligands (antibody, peptide, and aptamer) have been also widely developed [16]. In addition, hydrophobic photosensitizers are prone to aggregation in the nanocarrier, which reduces their phtotherapeutic effect [17]. Among various nanocarriers, liposomes have offered more promise in the photodynamic therapy because photosensitizers with a high unimer form can be loaded in the liposomal transmembrane [18-20]. However, combining photodynamic therapy with another therapeutic modality is still encouraged for cancer therapy because injection dose of the photosensitizer can be reduced to avoid potential side effects.

NANOMATERIALS FOR COMBINED PHOTOTHERMAL AND PHOTODYNAMIC THERAPY

Nanomaterials using dual wavelength light sources

As singular treatment of each phototherapy showed such limitations, there have been increasing efforts to enhance therapeutic index by combining photothermal and photodynamic therapy. First generation of nanoparticle platforms for combined photothermal and photodynamic therapy used two different light sources to excite photosensitizers and photothermal nanomaterials separately due to their absorption mismatch. Many research groups used a gold nanorod along with different kinds of photosensitizers for this combined phototherapy.

Yeh and co-workers demonstrated that gold nanorods coated with indocyanine green (ICG) could be used for combined photothermal and photodynamic therapy and biological imaging simultaneously [21, 22]. In these two studies, ICG molecules were served as both photosensitizing and NIR-imaging agents. Choi and co-workers reported a gold nanorod-photosensitizer complex for combined photothermal and photodynamic therapy of cancer (Fig. 1) [23]. In this work, negatively charged $AlPcS_4$ molecules were attached onto the positively charged surface of gold nanorod by electrostatic interaction, and the photodynamic effect of AlPcS4 photosensitizer was temporarily suppressed on the gold surface. Once the photosensitizer was released from the nanocomplex in the intracellular environments, it finally could be optically activated for phototherapeutic effect. Two different light sources were used to separately excite gold nanorods (810 nm laser) and $AlPcS_4$ photosensitizer (675 nm laser).

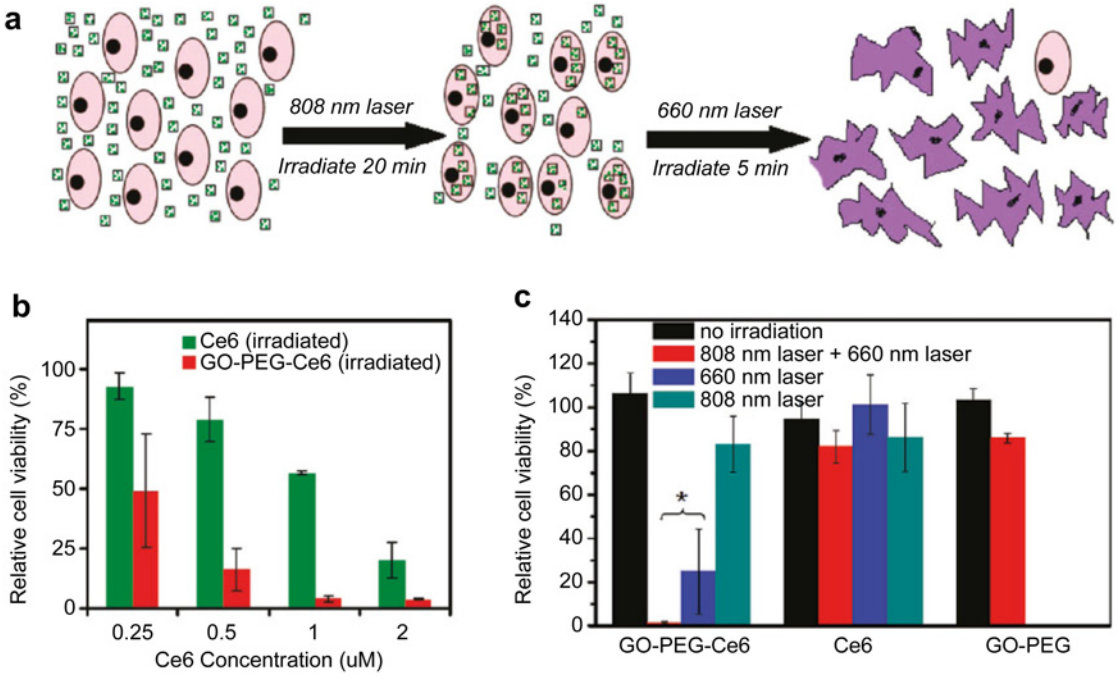

Fig. 2. Ce6-coated nano-graphene oxide formulation for combined phtothermal and photodynamic therapy. (a) Scheme of combined photothermal and photodynamic therapy using photosensitizer(Ce6)-coated PEGyated nano-graphene oxide (GO-PEG-Ce6). (b) Cell viability of KB cells treated either Ce6 or GO-PEG-Ce6 followed by laser irradiation. (c) Cell viability of KB cells incubated with various samples under 808-nm laser irradiation and then re-irradiated with 660-nm laser. Adapted with permission from reference [24]. Copyright (2011) American Chemical Society.

Besides gold nanostructures, Liu and co-workers developed polyethylene glycol (PEG)-functionalized nano-graphene oxide (GO) incorporated with photosensitizer Chlorin e6 (Ce6) for synergistic photothermal and photodynamic therapy (Fig. 2) [24]. Ce6 molecules were loaded on the surface of PEGylated GO via supramolecular π-π stacking. GO-PEG-Ce6 nanocomplex maintained excellent water solubility and efficiently generated singlet oxygen species for photodynamic therapy. Because of enhanced cellular uptake of Ce6-loaded nanocomplex, photodynamic effect was dramatically increased in the tested cancer cells compared to free Ce6 molecules. As previously reported [25], graphene-based nanomaterials displayed mild photothermal effect when exposed to relatively low-power NIR laser. In this work, the photothermal heating of GO was utilized to further improve intracellular delivery of the nanocomplex, thus enhancing overall therapeutic effect. The GO and Ce6 in the nanocomplex were irradiated with 808-nm and 660-nm lasers for combined photothermal and photodynamic therapy, respectively.

More recently, gold nanorod and photosensitizer were linked using aptamer switch probe (ASP) in order to achieve a combined photothermal and photodynamic therapy (Fig. 3) [26]. 812-nm NIR laser and white light were used to activate gold nanorod and Ce6 photosensitizer, respectively. The ASP was composed of leukemia-specific aptamer with a hairpin form and Ce6 molecule conjugated to the end of aptamer. When ASP-modified nanocomplex interacted with the target cancer cells, the ASP allowed the photosensitizer to be released from the surface of gold nanorod, thereby generating both cytotoxic singlet oxygen for photodynamic therapy and heating for photothermal therapy upon dual light irradiation. However, although such combined approach to use two different light sources showed some improvement in the cancer phototherapy, singular laser sources to simultaneously activate two different phototherapies would be more preferred to achieve a synergistic phototherapeutic effect.

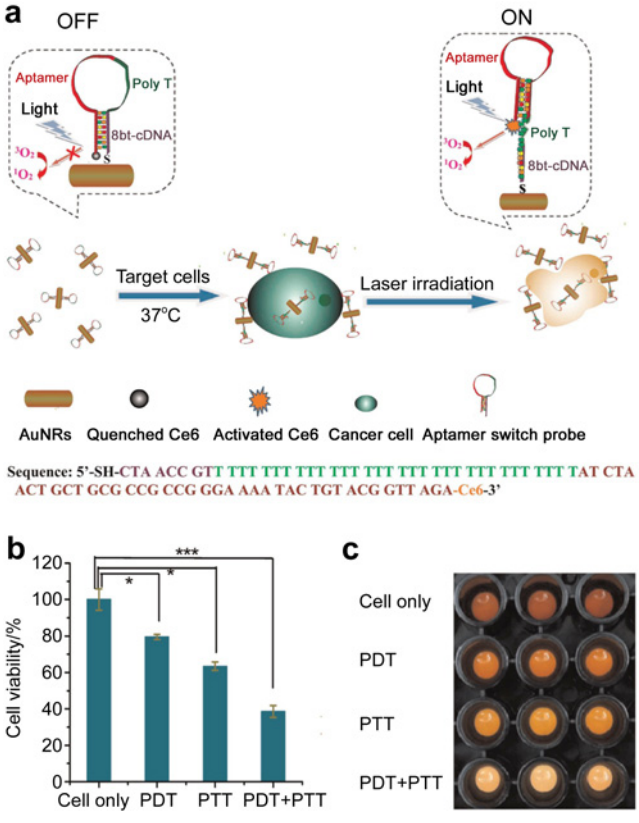

Fig. 3. gold nanorod-photosensitizer complex linked with aptamer switch probes (ASP) for combined photothermal and photodynamic therapy. (a) Schematic representation of photosensitizer-aptamer switch probes-conjugated gold nanorods for photothermal and photodynamic therapy. (b and c) Cell viability results (b) and fluorescence imaging (c) of CCRFCEM cells treated with photosensitizer-ASP-conjugated gold nanorods followed by light irradiation. PDT and PTT indicate white light irradiation and 812-nm laser irradiation, respectively. Adapted with permission from reference [26]. Copyright (2012) American Chemical Society.

Nanomaterials using a single wavelength light source

For single laser-based combined phototherapy, zinc phthalocyanine (ZnPc) photosensitizer was loaded in the holes of bovine serum albumin -coated carbon nanotube derivatives, carbon nanohorns (Fig. 4) [27]. The carbon nanotubes have been utilized as photothermal agents in cancer therapy because they efficiently absorb a NIR light [28]. In this work, carbon nanohorn was served as both photosensitizer carrier and photothermal agent. A single 670-nm laser was used to simultaneously activate ZnPc photosensitizer and carbon nanohorn for synergistic phototherapy. This combined phototherapy exhibited a superior anti-cancer effect both *in vitro* and *in vivo* compared with photothermal or photodynamic therapy alone.

Li and co-workers also prepared hypocrellin-loaded gold nanocages for two-photon photothermal/photodynamic therapy of cancer. The gold nanocages were optimized to have efficient NIR absorption and further coated with lipids containing hydrophobic photosensitizer hypocrellin B (HB) (Fig. 5) [29]. Photodynamic effect of the photosensitizer was quenched in the gold nanostructure, thereby reducing side effect of the photosensitizer in unintended tissues. For *in vitro* experiments, cancer cells were treated with the nanocomplex and irradiated with a 790-nm pulsed NIR laser. The photosensitizer HB was released from the gold nanocage in the intracellular region, which induced dramatic phototoxicity due to synergistic effect of photothermal and photodynamic therapy under two-photon irradiation.

Lastly, Chen and co-workers demonstrated that photosensitizer-coated gold nanostars could induce a synergistic photodynamic/photothermal effect under single-laser irradiation [30]. Gold nanostars was precisely tuned to have an absorption peak around 670 nm to match with absorption of photosensitizer

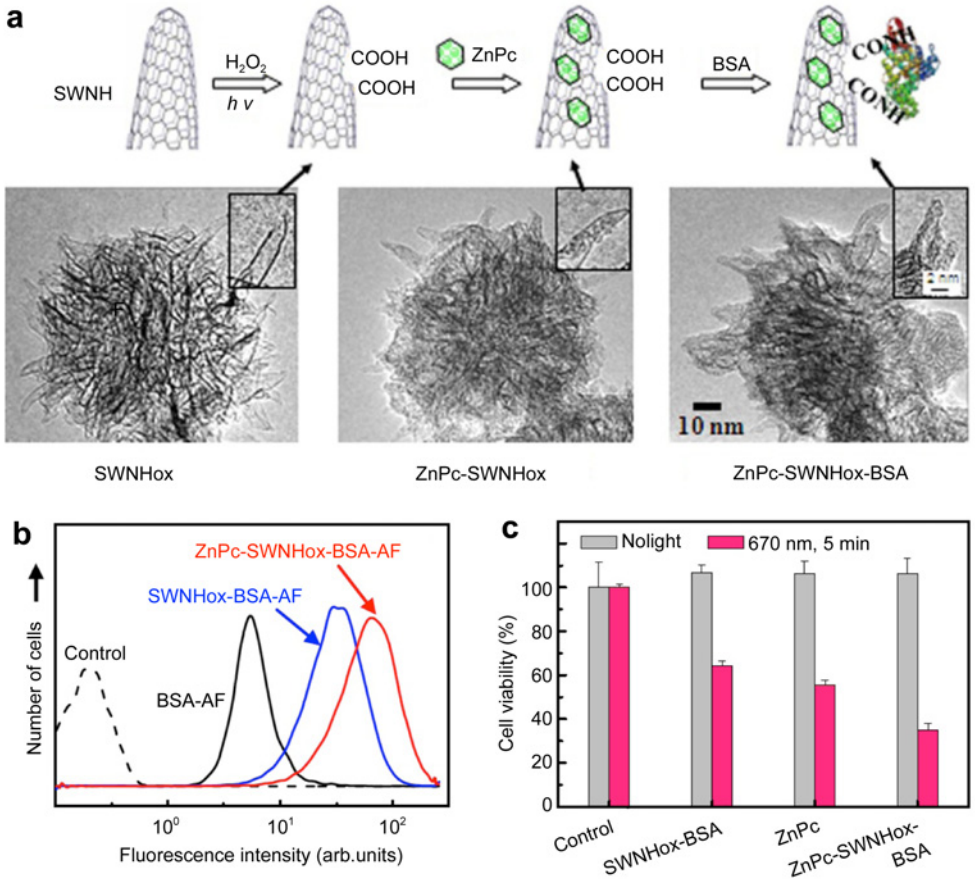

Fig. 4. ZnPc-incorporated carbon nanohorns for combined photothermal and photodynamic therapy of cancer. (a) Transmission electron microscopic images of single-wall carbon nanohorns with hall opened (SWNHox), ZnPc-incorporated (SWNHox), and BSA-coated ZnPc-incorporated (SWNHox). (b) Flow cytometry of the 5RP7 cells treated with Alexa Fluor 488 dye-conjugated BSA (BSA-AF), BSA-AF-conjugated SWNHox (SWN-Hox-BSA-AF), and BSA-AF-conjugated ZnPc-incorporated SWNHox (ZnPc-SWNHox-BSA-AF). (c) Cell viability of the 5RP7 cells treated with Alexa Fluor 488 dye-conjugated BSA (BSA-AF), BSA-AF-conjugated SWNHox (SWNHox-BSA-AF), and BSA-AF-conjugated ZnPc-incorporated SWNHox (ZnPc-SWNHox-BSA-AF) without or with light irradiation. Adapted with permission from reference [27]. Copyright (2008) Proceedings of the National Academy of Sciences.

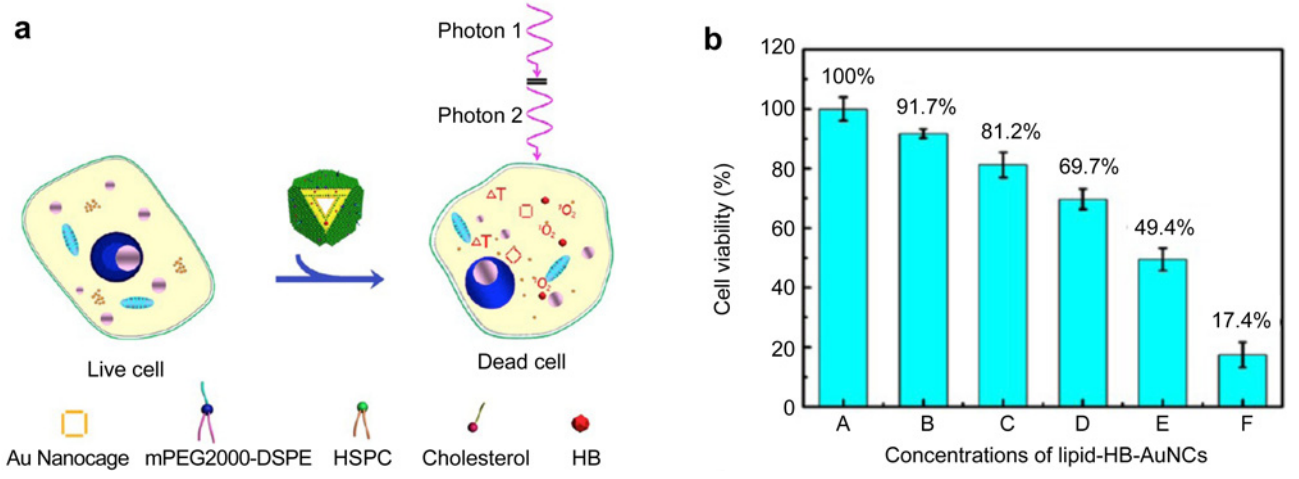

Fig. 5. Hypocrellin-loaded gold nanocages for two-photon combined photodynamic and photothermal therapy. (a) Schematic diagram of combinational treatment with two-photon photodynamic/photothermal therapy using lipid-photosensitizer(hypocrellin, HB)-coated gold nanocages (AuNC). (b) Cell viability of HeLa cells treated with lipid-HB-AuNCs at various concentrations followed by two-photon irradiation. Adapted with permission from reference [29]. Copyright (2012) American Chemical Society.

Ce6, PEGylated and then conjugated with Ce6 molecules. Ce6-incorporated gold nanostars efficiently destructed cancer cells *in vitro* under single-laser irradiation (671 nm) compared to singular phototherapy. For *in vivo* experiments, mice bearing MDA-MB-435 tumors were intratumorally injected with the Ce6-incorporated gold nanostars and irradiated with a single laser at 4 h post-injection. The combined phototherapy with the Ce6-incorporated gold nanostars significantly reduced tumor growth compared to singular phototherapies. It was also demonstrated in this study that synergistic effect of the combined phototherapeutic system could be modulated by adjusting the irradiation times due to photostability difference of gold nanostars and photosensitizers.

SUMMARY AND PERSPECTIVES

Here, we reviewed recent developments of nanoparticle platforms, in which inorganic nanostructures (photothermal therapy) are integrated with photosensitizers (photodynamic therapy) for combined phototherapeutic effect. Although considerable advances have been made in developing nanoparticle platforms for combined photothermal and photodynamic therapy in the past decade, there remain significant barriers to their clinical translation such as non-biodegradability of gold nanostructures and low penetration depth of light source. Therefore, much more effort should be made to improve *in vivo* behavior of the nanoparticle platforms for combined photothermal and photodynamic therapy of cancer.

ACKNOWLEDGEMENTS

This work was supported by the National R&D Program for Cancer Control, Ministry for Health and Welfare, Republic of Korea, through grant no. 1220070.

CONFLICT OF INTEREST STATEMENTS

Oh J declares that s/he has no conflict of interest in relation to the work in this article. Yoon H declares that s/he has no conflict of interest in relation to the work in this article. Park JH declares that s/he has no conflict of interest in relation to the work in this article.

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