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Gold nanoshells-mediated bimodal photodynamic and photothermal cancer treatment using ultra-low doses of near infra-red light

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ABSTRACT

Previously, gold nanoshells were shown to be able to effectively convert photon energy to heat, leading to hyperthermia and suppression of tumor growths in mice. Herein, we show that in addition to the nanomaterial-mediated photothermal effects (NmPTT), gold nanoshells (including, nanocages, nanorod-in-shell and nanoparticle-in-shell) not only are able to absorb NIR light, but can also emit fluorescence, sensitize formation of singlet oxygen and exert nanomaterial-mediated photodynamic therapeutic (NmPDT) complete destruction of solid tumors in mice. The modes of NmPDT and NmPTT can be controlled and switched from one to the other by changing the excitation wavelength. In the *in vitro* experiments, gold nanocages and nanorod-in-shell show larger percentage of cellular deaths originating from NmPDT along with the minor fraction of NmPTT effects. In contrast, nanoparticle-in-shell exhibits larger fraction of NmPTT-induced cellular deaths together with minor fraction of NmPDT-induced apoptosis. Fluorescence emission spectra and DPBF quenching studies confirm the generation of singlet O_2 upon NIR photoirradiation. Both NmPDT and NmPTT effects were confirmed by measurements of reactive oxygen species (ROS) and subsequent sodium azide quenching, heat shock protein expression (HSP 70), singlet oxygen sensor green (SOSG) sensing, changes in mitochondria membrane potential and apoptosis in the cellular experiments. *In vivo* experiments further demonstrate that upon irradiation at 980 nm under ultra-low doses ($\sim$150 mW/cm^2), gold nanocages mostly exert NmPDT effect to effectively suppress the B16F0 melanoma tumor growth. The combination of NmPDT and NmPTT effects on destruction of solid tumors is far better than pure NmPTT effect by 808 nm irradiation and also doxorubicin. Overall, our study demonstrates that gold nanoshells can serve as excellent multi-functional theranostic agents (fluorescence imaging + NmPDT + NmPTT) upon single photon NIR light excitation under ultra-low laser doses.

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1. Introduction

In the recent years, the use of plasmonic noble metal nanostructures for phototherapy has attracted considerable attention as a powerful non-invasive technique for treating various cancers as well as malignant tumors [1,2]. In the literature, it was known that upon photoexcitation of metal nanostructures at their respective localized surface plasmon resonance (LSPR) band can efficiently convert the excited state photon energy into heat and thereby facilitate the so called photothermal therapeutic (PTT) effects in curing cancers [3]. Owing to the poor tissue penetration depths of UV and visible light, it has become mandatory to design metal nanostructures to be able to absorb the near infra-red (NIR) light in the biological window for the treatment of deep-tissue buried tumors [4]. To this end, several metal nanostructures, such as, gold nanoparticles [5], nanorods [6], nanoshells [7], nanohexapods [8] as well as palladium nanosheets [9] were reported as NIR light activatable nanomaterial-mediated PTT (NmPTT) agents for the destruction of malignant tumors. However, the usage of very high laser powers (1–48 W/cm^2) is practically not applicable for clinical treatments of tumors [10], as they are far higher than the skin tolerance threshold values (Maximum Permissible Exposure (MPE) of skin for 808 and 980 nm wavelengths are 330–350 mW/cm^2, with an exposure time of 10–1000 s) set by American National Standards Institute (ANSI) [11]. As a brief overview, the laser

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wavelengths, laser light intensities and irradiation times were summarized for various gold nanomaterials mediated PTT effects reported in the literature (see Table 1) [12–21]. Therefore, it is urgently needed to develop a new modality or new nanomaterials which can utilize photo-energy much more effectively to kill cancer cells upon low laser doses within the tolerance of skin burning thresholds.

Photodynamic therapy (PDT) is an alternative cancer treatment modality, which, in principle, is far more effective than PTT, where every singlet O_2 molecule can facilitate the destruction of cancer cells, and, unlike PTT effect, will not be suppressed by low temperatures [22]. Most of the organic photosensitizers used in clinical PDT treatments are, however, restricted to be activated only by UV and visible light, which has very poor tissue penetration depths and therefore limited to the treatments of surface tumors [23]. Moreover, organic photosensitizers possess very low molar extinction coefficients and can also easily undergo photobleaching as well as enzymatic degradation. Metal nanostructures can overcome these limitations, as they possess 5–6 orders high molar extinction coefficients, better photostabilities and resistant to enzymatic degradation when compared to organic photosensitizers [24]. Metal nanostructures also show unique photophysical and photochemical properties owing to their involvement of LSPRs [25,26]. Upon photoexcitation of metal nanoparticles, the excited state photon energy can undergo energy transfer to molecular oxygen and generate highly reactive oxygen species, such as singlet oxygen ($^1O_2$) [24]. Previously, metal nanoparticles (M = Au, Ag & Pt) were shown to sensitize formation of singlet O_2 upon excitation of its LSPR band in the visible region [24]. In order to tackle the treatment of deep-tissue buried tumors, it is necessary to develop a multifunctional theranostic reagent which can able to exert PDT effects upon NIR light activation. Nanomaterials able to sensitize formation of singlet O_2 and exert PDT effects are still very rare [24,28]. Very recently, it was reported that gold nanorods (Au NRs) and PEGylated $W_{18}O_{49}$ nanowires can act as NIR light activatable nanomaterial-mediated PDT (NmPDT) reagents for the complete destruction of tumors [28,29]. In addition to Au NRs, gold nanoshells is also fascinating, due to their excellent light absorbing capabilities in the NIR biological window. Previously, it was reported that gold nanorod-in shells possess better light absorbing capabilities than nanorods and hence they can become very effective photothermal transducers [17]. Recently, nanohexapods were also reported which consists of an octahedral core and six arms grown on its six vertices. Owing to its sharper tips protruded to outside and superior light absorbing capability in the NIR region, Au nanohexapods were claimed to act as very good photothermal agents [15]. However, none of the previous papers has experimentally determined the magnitudes of extinction coefficients as a function of wavelengths to determine their light absorbing abilities.

To compensate with the poor extinction coefficients and achieve high phototherapeutic efficiencies in a single theranostic strategy, organic photosensitizers were often loaded on gold nanostructures to produce synergistic effects combining both PTT and PDT [30]. Previously, it was reported that photosensitizer-loaded Au nanocages can be used as two-photon induced bioimaging probes as well as PDT [31]. To achieve such type of two photon-excitation induced luminescence and PDT therapeutic efficacy, it is, however, required the use of expensive femto-second lasers. Therefore, it is highly desired to develop a NIR light activatable theranostic strategy to achieve PDT and PTT effects simultaneously under one-photon excitation using ultra-low laser doses. Currently, such type of ideal multi-functional theranostic nanomaterials is still very rare [28].

In this work, we report that gold nanomaterials, including nanocages (Au NCs), nanorod-in shell (NR shell) and nanoparticle-in shell (NP shell), in fact, are ideal multi-functional theranostic nanomaterials and can act as fluorescent cellular markers, as well as exert NmPTT and NmPDT effects on destruction of tumors in mice upon ultra-low doses of NIR light excitation. We believe that ultra-low doses of NIR light excitable gold nanomaterials for bimodal photothermal and photodynamic therapy can open up new avenues in the cancer treatment as well as future biomedical investigations.

2. Materials and methods

2.1. Preparation of lipid-coated Au nanoshells

Gold nanoshells were synthesized from the previously adopted literature procedures [17,33]. The molar extinction coefficients of Au nanoshells at LSPR maximum is $\sim 10^9$ M^{-1} cm^{-1} (see Fig. 1(d)). As-obtained nanoshells were washed thoroughly using DI water to remove the surface-adsorbed PVP via centrifugation, and then re-dispersed in an aqueous solution containing 100 μL of Lipofectamine 2000 reagent (Invitrogen, USA) to facilitate formation of the surface-coated bilayer structures.

2.2. Singlet oxygen phosphorescence measurements

An aliquot of different Au nanoshells were dispersed in D_2O, and then used for singlet oxygen sensitization. Phosphorescence emission of singlet O_2 was recorded using luminescence spectrometer (FLS920, Edinburgh, equipped with a 450 W broadband Xe lamp) with a 1000 nm longpass filter (Isuzu Optics, LP1000) located in-between the sample and the detector to cutoff both the scattering light and stray light having wavelengths shorter than 1000 nm.

2.3. DPBF quenching studies

DPBF was used as a singlet O_2 trapping reagent in ethanol solution. In a typical experiment, 2 mL of an ethanol solution containing 0.08 mM DPBF and 1 mg/mL Au nanoshells were placed in a quartz cuvette. A 300 W tungsten halogen lamp equipped with a bandpass filter of 750–1380 nm was used as the light source. The absorbance of the solution at 410 nm was measured every 1 min for a 12 min period using a UV–visible spectrophotometer. The decrease of the absorbance caused by photobleaching of DPBF was measured and corrected in all experiments.

Table 1
Brief literature overview of the laser wavelengths, power intensities and irradiation time used in the various gold nanomaterials mediated PTT effects.

Nanomaterial	Laser wavelength	Power intensity	Irradiation time	Cell line	Reference
Immuno Au nanocages	810 nm	4.7 W/cm^2	5 min	SK-BR-3 (*In vitro*)	[12]
PEGylated Au nanocages	800 nm	1 W/cm^2	10 min	U87MG with EGFR (*In vivo*)	[13]
Immuno Au nanocages	805 nm	1.6 W/cm^2	5 min	SK-BR-3 (*In vitro*)	[14]
Au nanocages	808 nm	0.8 W/cm^2	10 min	MDA-MB-435 (*In vitro*)	[15]
Au nanocages	750 nm	0.6 W/cm^2	10 min	L929 (*In vitro*)	[16]
Au NR in-shell	808 nm	27 W/cm^2	8 min	A549 (*In vitro*)	[17]
PEGylated Au nanoshells	808 nm	4 W/cm^2	3 min	CT26.WT (*In vitro*)	[18]
Silica @ Au nanoshells	808 nm	30 W/cm^2	7 min	A549 (*In vitro*)	[19]
Au nanoshell	820 nm	35 W/cm^2	7 min	SK-BR-3 (*In vitro*)	[29]
Au NS–micelle	808 nm	8 W/cm^2	10 min	HeLa (*In vitro*)	[21]
Lipid-coated Au nanocages	940 nm	0.056 W/cm^2	40 min	HeLa (*In vitro*)	Present study
Lipid-coated Au nanocages	980 nm	0.15 W/cm^2	10 min	B16F0 (*In vivo*)	Present study

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