Review

Near-infrared light-responsive inorganic nanomaterials for photothermal therapy

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ABSTRACT

Novel nanomaterials and advanced nanotechnologies prompt the fast development of new protocols for biomedical application. The unique light-to-heat conversion property of nanoscale materials can be utilized to produce novel and effective therapeutics for cancer treatment. In particular, near-infrared (NIR) photothermal therapy (PTT) has gained popularity and very quickly developed in recent years due to minimally invasive treatments for patients. This review summarizes the current state-of-the-art in the development of inorganic nanocomposites for photothermal cancer therapy. The current states of the design, synthesis, the cellular uptake behavior, the cellular cytotoxicity and both in vivo and in vitro nanoparticle assisted photothermal treatments of inorganic photothermal therapy agents (PTA) are described. Finally, the perspective and challenges of PTT development are presented and some proposals are suggested for its further development and exploration. This summary should provide improved understanding of cancer treatment with photothermal nanomaterials and push nanoscience and nanotechnology one step at a time toward clinical applications.

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1. Introduction

Directly or indirectly, cancer affects most people’s lives. Cancer is one of the leading causes of death and accounted for 8.2 million deaths worldwide in 2012 [1] and the incidence rate is increasing year by year. The main reason for this dismal picture is that even with the current state of the art of cancer diagnosis, usually this disease is detected in an advanced stage, when the primary tumor has metastasized and invaded other organs, which is beyond surgical intervention. In addition, current chemo- and radiation therapies have many well-known disadvantages, including relatively poor specificity toward malignant tissues, systemic side effects, low efficacy and drug resistance [2]. Therefore, to advance cancer therapy, therapeutic methods that should selectively eliminate only diseased cells/tissues without causing collateral damage will be expected.

As a promising alternative or supplement to conventional cancer treating approaches, photothermal therapy (PTT) has

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caused considerable attention because of its advantages including minimal invasion, few complications, and rapid recovery. PTT, also known as photothermal ablation or optical hyperthermia, employing photo-absorbers and near-infrared light energy sources, provides a precise and minimally invasive alternative for cancer treatment [3,4]. It is a procedure based on localized heating due to light absorption for selective destruction of abnormal cells. To enhance anti-cancer efficacy and optimize therapy, integration of multiple treatment strategies with synergistic effects is highly expected [5]. Among these treatment strategies, the combination of PTT with chemotherapy, termed chemo-photothermal therapy, as a minimally invasive, controllable, and highly efficient treatment method, has drawn widespread attention [6,7]. In PTT, near-infrared (NIR) light (650–900 nm) is preferred for such an application because of its easy operation, its ability to be locally focused on a specific region, and its minimal absorbance by skin and tissues to allow for noninvasive penetration of reasonably deep tissues [8]. The key component of this technique is a photothermal transducer that can efficiently absorb and convert NIR light into heat through a non-radiative mechanism.

Over the past decade, many different types of photothermal therapy agents (PTA) have been reported, including organic compounds or materials (e.g., indocyanine green [9,10] and polyaniline [11]) and inorganic nanomaterials (e.g., noble metal nanoparticles [12,13], metal chalcogenide [14,15], and carbon-based materials [16–19]). When combined with NIR light, all of them are able to generate sufficient heat to raise the local temperature and thus kill cancer cells. Organic photothermal therapy has good biocompatibility and biodegradability, and therefore can be used for nanobiotechnology. However, the low photothermal conversion efficiency, poor photothermal stability and complicated synthesis process of these materials limit their application for PTT. As an alternative, inorganic PTA have received great interest in recent years, because of their high photothermal conversion efficiency and the ease of synthesis and modification; for example, the inorganic nanoparticle size, shape and surface properties can be facilely controlled.

In the past decade, near-infrared light-responsive inorganic nanomaterials, such as gold nanoparticles, carbon nanotubes, and copper sulfide nanoparticles (Fig. 1) efficiently convert optical energy into thermal energy and enhance the efficacy of photothermal ablation therapy. Some applications are under clinical trials. In this review, we summarize the recent advances in the structural and functional evolution of inorganic nanomaterials employed in PTT. These recent progresses in materials design will lead to deeper insight of the chemistry and photonic as well as to promote the development of PTT into practical applications. The aim of this review is to arouse more attention toward inorganic photothermal nanomaterials used in cancer therapy and to encourage future work to push forward the advancement of this biomedical area.

2. Various inorganic nanomaterials for PTT

For biomedical applications, inorganic nanomaterials, including Au-based nanomaterials, Pd nanoparticles, CuS nanoparticles, graphene, and carbon nanotubes etc., have attracted much attention in PTT. This article summarizes recent progress on various inorganic photothermal nanomaterials, including the background, synthesis, modification, cytotoxicity as well as their applications in biomedicine.

2.1. Colloidal noble metal nanoparticles

Noble metal nanoparticles, especially for Au and Pd nanoparticles, have been proven to show strong scattering and absorption of light in visible and near-infrared region owing to their localized surface plasmon resonances. The absorbed light is then turned into thermal energy. With pulsed light irradiation, transient thermal power generated in nanoparticles introduces abundant thermodynamic effects, such as ablation, ultrafast heating, thermal expansion, surface melting, and reshaping.

2.1.1. Gold nanoparticles

Localized surface plasmon resonance of gold nanocrystals endows them the capability to strongly absorb and/or scatter light at synthetically controllable resonance wavelengths, which is the underlying reason for their many applications [20–23]. A wide variety of Au nanostructures, including aggregates of colloidal particles, nanoshells, nanocages, nanorods and nanocrosses have been demonstrated for cancer photothermal therapy with NIR light. In the case of spherical gold nanoparticles, the absorption maximum exists between 400 and 600 nm. Therefore, in in vivo applications, very low light penetration and thus inefficient photothermal heating is generated [24]. In contrast, gold nanorods (GNRs) have attracted much interest because the absorption range of light can be finely tuned by adjusting the aspect ratio, so the heating efficiency can be maximized by using ~800 nm absorption maximum. Also, they
have the advantages of efficient large-scale synthesis, easy functionalization, and colloidal stability [25,26]. However the clinical application of GNRs was limited due to a slight cytotoxicity caused by the remaining excess cetyltrimethylammonium bromide (CTAB), which is used as a template during synthesis and envelops the surfaces of the GNRs [27,28]. To distinguish between the toxicity of the GNRs core and the exterior ligands, Zhu et al. [29] systematically evaluated the cellular uptake behavior and cytotoxicity of Au nanorods with various surface coatings, including organic cetyltrimethylammonium bromide (CTAB), poly(sodium 4-styrenesulfonate) (PSS), and poly(ethylene glycol) (PEG), and inorganic mesoporous silica (m-SiO₂), dense silica (d-SiO₂), and titanium dioxide (TiO₂). The cellular behavior of Au nanorods was found to be highly dependent on the surface coating. CTAB-, PSS-, and m-SiO₂-coated Au nanorods exhibit notable cytotoxicity, while PEG-, d-SiO₂-, and TiO₂-coated Au nanorods do not induce cell injury. Thus, the surface modifications of GNRs shall reduce the cytotoxic effect. For example, phosphatidylcholine (PC)-modified nanorods [30], poly(4-styrenesulfonic acid) (PSS)-coated nanorods [31], GNR-embedded polymeric nanoparticles [32], and PEG-modified nanorods [33] have shown cytotoxicities lower than that of the CTAB-capped nanorods themselves and good photothermal effects.

Although ligand-conjugated GNRs are effective for photothermal killing of cancer cells in vitro, desirable photothermal therapeutic effects in an in vivo animal model are limited due to a high liver uptake during circulation [34]. A high-level localization in the liver of CTAB-stabilized GNRs at 0.5 h after intravenous injection, which might be associated with the hard and rigid characteristics of GNRs, was reported [35]. To overcome the limited effect of GNRs on in vivo cancer photothermal therapy, PEGylation modified GNRs attempted to lower the cytotoxicity and the liver accumulation of GNRs. However, complete suppression of tumor growth when using a hyperthermia-based treatment was not achieved, probably due to the very fast excretion of the PEGylated GNRs from the body (half-life of ~1 h). Thus, a new biocompatible vehicle for the efficient delivery of GNRs into tumor sites is still an unmet need for safe and effective cancer therapy based on GNRs. In addition, specific targeting therapy of GNRs also is another key issue for efficient cancer photothermal therapy. The biological modification of Au nanoparticles can be achieved on their surfaces, which is beneficial to improve biological activity and provide targeting property. For example, Aptamer-conjugated nanorods [36], folate-conjugated nanorods [37], and RGD-conjugated dendrimer modified nanorods [38] have demonstrated selective and efficient photothermal killing of targeted tumor cells. Choi et al. [39] developed photo-cross-linked, Pluronic-based, temperature-sensitive nanocarriers that possessed excellent reservoir characteristics and a simple loading method with high loading capacity for large molecules (e.g., proteins and gold nanoparticles). Importantly, these nanocarriers showed a long circulation time, a good tumor accumulation, and low liver uptake, which were associated with the flexible and soft characteristics as well as the hydrophilic surface from the PEG part of Pluronic. Furthermore, the tumor targeting and prolonged circulation (up to 72 h) were significantly improved and could be optimized by chitosan conjugation. The GNR-loaded, Pluronic-based nanocarriers as a hyperthermia agent were applied for enhanced cancer photothermal therapy. The GNR-loaded nanocarriers showed serum stability and photothermolysis of cancer cells in vitro. The GNR concentration and the laser power density required for photodestruction of cancer cells were also significantly reduced, compared to other formulations, by using the nanocarrier system. Most of all, the optimized GNR-loaded nanocarriers resulted in a very impressive therapeutic effect in vivo in nude mice bearing tumors, and complete resorption of the tumor was achieved (Fig. 2). As a theranostic platform, GNRs bear two disadvantages: (1) a relatively low specific surface area limits the loading amount of drugs, and due to the often-observed clustering and aggregating of the GNRs within cells; (2) when GNRs were exposed to NIR laser, the desirable NIR window shifts to the visible spectral region and the advantage of deep tissue penetration is lost. To overcome these two drawbacks of GNRs, Zhang et al. [40] developed mesoporous silica-coated gold nanorods (Au@SiO₂) as a novel cancer theranostic platform. The large specific surface area of mesoporous silica guarantees a high drug payload. More interestingly, Au@SiO₂ as a drug carrier, under laser irradiation the drug

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**Fig. 2** – (a) Schematic illustration of the preparation of the pluronic-based nanocarriers and GNR loading into the nanocarriers. (b) Change in tumor volumes (an enlarged graph at initial time) and (c) the tumor images after NIR laser irradiations at 24 and 48 h after single i.v. injection of the nanomaterials. Reproduced with permission from Reference [39].
release rate becomes much faster for any pH because the laser-
converted heat dissociates the strong interactions between
doxorubicin (DOX) and silica, thus more DOX molecules are re-
leased. The incorporation of the two nanomaterials created a
new functionality: NIR laser-controlled drug release. For the goal
of on-demand therapy and personalized medicine, the ther-
apeutic modes of Au@SiO\textsubscript{2}-DOX, either chemotherapy or
hyperthermia, can be manipulated simply by changing laser
power density.

PTT has been demonstrated with certain types of Au
nanostructures in early clinical trials. As an example, pilot clin-
cal studies with Au nanoshell (Au nanoshells about 150 nm in
diameter with a coating of polyethylene glycol 5000) have been
approved by the Food and Drug Administration (FDA), wherein
the nanoshells are given intravenously to patients for the treat-
ment of head and neck cancer, as well as primary and/or
metastatic lung tumors [41,42]. For practical application, lo-
cation and size of cancer shall be confirmed first before therapy.
Second, the treatment procedure needs to be monitored in real
time during therapy. Finally, the effectiveness has to be as-
essed after therapy. Based on aforementioned claims, the
design and synthesis of new PTT agents with imaging are of
great importance. Ke et al. [43] developed a novel multifunc-
tional theranostic agent for ultrasound contrast imaging and
PTT. The gold-nanoshell microcapsules (GNS-MCs) were ob-
tained by electrostatic adsorption of gold nanoparticles as seeds
onto the polymeric microcapsule surfaces, followed by the for-
formation of gold nanoshells by using a surface seeding method.
Poly(lactic acid) (PLA), which is biodegradable and possesses
an ultrasound signaling capability, was used as polymeric mi-
crocapsule in this study. NH\textsubscript{2}O\textsubscript{H}-HCl was used to reduce the
gold precursor (HAuCl\textsubscript{4}) to bulk metal without the nucleation
of new particles. Subsequently, all the added gold precursor
was reduced and incorporated into larger particles that were
deposited on the surface of the capsules. Finally, the micro-
capsules were freeze-dried. The encapsulated water in the inner
aqueous phase of the microcapsule was sublimated to leave
a small hollow space that could provide a basis for the ultrasoun-d-responsive properties. In the vivo ultrasound imaging process, GNS-MCs were intravenously injected into
rabbits, pulse inversion harmonic imaging (PIHI) contrast mode
(with mechanical index, MI = 0.42) and conventional B-mode
sonograms before and after administration of GNS-MCs, as
shown in Fig. 3a and b. Excellent enhancements of rabbit kidney
images suggested that GNS-MCs were able to traverse pulmo-

nary capillaries to achieve systemic enhancement. The contrast
enhancement can last more than 5 minutes, which is long
enough to satisfy the clinical requirements. To evaluate the
localized tumor photothermischergetic effect of GNS-MCs, HeLa cells
(human cervical carcinoma cell lines) cultured in six-well plates
were incubated with the GNS-MCs for 1 h, followed by illumi-
nation with an NIR laser (808 nm and 8 W/cm\textsuperscript{2} for 10 min).
Under an inverted fluorescence microscope (Fig. 3c–f), a dark
region was observed in the presence of both the agent and the
laser (Fig. 3f) that arises from the NIR laser-induced hyper-
thermic effect on cancer cells. In contrast, exposure of cancer
cells to either GNS-MCs or a high-intensity NIR laser alone did
not compromise cell viability (Fig. 3c–e), thus indicating that
GNS-MCs will cause cancer cells to die through photother-
mal effect only under NIR laser irradiation.

At the next step in the development of Au nanoshells, a
novel multifunctional drug-delivery platform is developed based
on cholesteryl succinyl silane (CSS) nanomicelles loaded with
doxorubicin, Fe\textsubscript{3}O\textsubscript{4} magnetic nanoparticles, and gold nanoshells
(CSS-DOX-Fe\textsubscript{3}O\textsubscript{4}-Au-shell nanomicelles), which can combine
magnetic resonance (MR) imaging, magnetic-targeted drug
delivery, light-triggered drug release, and PTT into one
nanomaterial [44]. The synthesis of the CSS-DOX-Fe\textsubscript{3}O\textsubscript{4}-Au-
shell nanomicelles was a multistep procedure. Especially, an
enhancement for T\textsubscript{2}-weighted MR imaging is observed for the
CSS-DOX-Fe\textsubscript{3}O\textsubscript{4}-Au-shell nanomicelles compared with that of
sodium citrate modified Fe\textsubscript{3}O\textsubscript{4} nanoparticles. In addition, the
samples were irradiated repeatedly over a period of 10 min, fol-
lowed by 1 h intervals with the laser turned off. A rapid release
was observed upon NIR irradiation and the DOX release rate
slowed down when the NIR irradiation was switched off. After
the first NIR exposure for 10 min, the percentage of released
DOX increased from 7.1% to 18.4%. The percentage increased to
19.7% over the whole period without NIR laser irradiation,
significantly lower than that with NIR laser irradiation (39.5%).
This research achieved a synergetic effect in killing cancer cells
by combined photothermal therapy and the magnetic-field-
guided drug delivery.

Recently, the branched or star-shaped Au nanostructures
consisting of a core and protruding arms have received par-
ticular interest due to their unique morphology and optical
properties [45,46]. Owing to the presence of sharp tips as well
as their high surface to volume ratios, branched Au nano-
structures could be more effective in photothermal conver-
sion and drug loading relative to those with smooth surfaces
[47]. Wang et al. [48] prepared the Au nanohexapods, consist-
ing of an octahedral core and six arms grown on its six vertices,
by reducing HAuCl\textsubscript{4} with DMS in an aqueous solution contain-
ing Au octahedral seeds (Fig. 4a). By controlling the length of
the arms, their localized surface plasmon resonance (SPR) peaks
could be tuned from the visible to the near-infrared region for
dep deep penetration of light into soft tissues. When compared with the PEGylated nanorods (53.0 ± 0.5 °C) and nanocages
(48.7 ± 3.5 °C), PEGylated nanohexapods showed the highest
(55.7 ± 2.4 °C) photothermal conversion efficiency in vivo, owing
to their highest tumor uptake and photothermal conversion
efficiency per Au atom. The different result using Au
nanohexapods, nanorods, and nanocages indicates that all these
Au nanostructures could absorb and convert NIR light into heat
(Fig. 4b). Au nanohexapods exhibited the highest cellular uptake
and the lowest cytotoxicity in vitro for both the as-prepared and
the PEGylated samples. Combined together, Au nanohexapods
are promising candidates for cancer theranostics in terms of
both photothermal destruction and contrast-enhanced
diagnosis.

2.1.2. Palladium nanoparticles
A wide variety of anisotropic gold nanostructures, including
aggregates of colloidal particles, nanorods, nanoshells,
nanocrosses, have been demonstrated for cancer photother-
mal therapy with NIR light. However, studies have shown that
many anisotropic gold nanostructures exhibiting NIR SPR lack
good photothermal stability upon irradiation with high-
power NIR lasers. The heat generated by NIR irradiation can
melt the anisotropic gold nanostructures into solid particles,
leading to the loss of their NIR SPR \[49,50\]. To overcome this limit, other noble nanoparticles with tunable localized surface plasmon resonance in NIR region have been designed and synthesized. Especially, palladium, because of its significantly higher bulk melting point (MP\(_{\text{Pd}}\) = 1,828 K versus MP\(_{\text{Au}}\) = 1,337 K), should show enhanced photothermal stability.

Using this point, Huang et al. \[13\] prepared ultrathin hexagonal palladium nanosheets with tunable (826–1068 nm) and strong SPR absorption (extinction coefficient, \(4.1 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}\)) in the NIR region using a general CO-confined growth method. The nanosheet edge length is synthetically controllable from 20 to 160 nm, leading to tunable NIR SPR. Unlike anisotropic gold nanorods, the two-dimensional structure of the palladium nanosheets appears to be highly stable upon NIR irradiation. Upon irradiation for 30 min by a NIR laser (808 nm, 2 W), the sheet-like structure of the palladium nanosheets was retained well leading to a good SPR response in the NIR region, whereas gold nanorods were severely distorted under a similar irradiation power. Furthermore, the as-prepared palladium nanosheets appear to be...
largely biocompatible. The viable cell count for healthy liver cells was reduced by only 20% after 48 h of exposure to a 600 mg/mL solution of palladium nanosheets by incubating liver cancer cells with polyethyleneimine-exchanged palladium nanosheets. After 5 min of irradiation of an 808 nm laser with a power of 1.4 W/cm$^2$, ~100% of the cells were killed. This work first suggests that Pd nanosheets have great potential in cancer photothermal therapy.

In addition, the sizes of mentioned Au nanorods and nanoshells are considerably large. For example, the size of Au nanorods is typically of ~10 nm in diameter and ~50 nm in length and Au nanoshells are more than 100 nm in diameter [27,51,52]. A lot of investigations have demonstrated that the optimum intravenously administered nanoparticles should be between 10 and 50 nm in diameter because larger nanoparticles are removed by the reticuloendothelial system (expressed as RES; e.g. liver, spleen), and smaller particles are removed by the renal system [55,56]. To overcome slow renal clearance and high, non-specific accumulation in the reticuloendothelial system after systematic administration in the applications of those nanomaterials. Tang et al. [57] further successfully synthesized the ultrasmall Pd nanosheets (SPNS) with an average diameter of ~4.4 nm (Fig. 5), which is below the glomerular filtration-size threshold (10 nm) and thus particularly interesting for renal clearance studies. In addition, these SPNS were surface functionalized with reduced glutathione (SPNS-GSH). GSH (a tripeptide) can not only serve as capping agent to render the nanoparticles with relatively low affinities to serum proteins and lead to the desired stealthiness to the RES organs, but also contribute to efficient renal clearance of small-sized nanoparticles out of the body [58]. In Fig. 5d, the smaller sized Pd nanosheets modified by GSH were both helpful in prolonging their circulation and half-life in the blood. The circulation half-lives was remarkably increased from 0.08 h for large Pd nanosheets (LPNS) to 1.25 h for SPNS-GSH. Additionally, a higher tumor accumulation was observed. Importantly, the total amount of Pd in SPNS-GSH formulation was significantly low in major organs, indicating that plenty of the SPNS-GSH were rapidly excreted from the body within the first 24 h (Fig. 5e).

To minimize toxicity risks, an ideal nanomaterial-based therapy agent should be effectively cleared out of the body after treatment. The renal excretion has been recognized as a desirable pathway for nanoparticle clearance. It was found that more than 6.6% of the SPNS-GSH were excreted out of the body within 1 day p.i. and up to 30.9% after 15 day p.i. (Fig. 5f). These observations confirm that SPNS-GSH could be cleared out from the body through the renal excretion route into the urine. Subsequently, they also developed a versatile system combining chemotherapy with PTT for cancer therapy [59]. The system is based on ultrasmall Pd nanosheets (SPNS) functionalized with the anticancer drug doxorubicin hydrochloride (DOX) mainly through Pd–N coordination bonding. SPNS have an average diameter of ~4.4 nm. After the SPNS-DOX, hybrid nanoparticles are surface-functionalized with reduced glutathione (GSH), the obtained SPNS-DOX-GSH composite exhibits the following synergistic properties for cancer therapy: (1) The coordinative loading of DOX on SPNS enhances its accumulation in tumor tissue, which significantly reduces the laser power required to achieve effective tumor ablation; (2) The DOX was released from
compared degradability and toxicity between two types developed hollow copper sulfide (13 nm) plate-like nanocrystals (70 nm size, shape, surface charge and functional groups) cytotoxicity is observed up to 100 mg/mL for non-modified metal chalcogenide nanoparticles (MCNPs) have to be before PTT application, cellular uptake and cytotoxicity properties of metal chalcogenide nanoparticles (MCNPs) have to be investigated. Cellular uptake of CuS and WS and their good biocompatibility were confirmed with both healthy and cancer cell lines. Several research groups have demonstrated that cellular uptake and cellular toxicity of MCNPs depend on the particle size, shape, surface charge and functional groups. No cytotoxicity is observed up to 100 mg/mL for non-modified 100 nm MCNPs, which is far beyond the concentration required for most therapeutic treatments.

In contrast to exogenous gold, copper is essential for human health. In adults, the highest safe intake level of Cu is 10 mg daily, indicating that CuS nanoparticles (CuS NPs) may be metabolized by humans. CuS NPs with particle sizes of 35 and 11 nm, flower-like CuS superstructures (1 μm in diameter) [73], and CuS, plate-like nanocrystals (70 nm × 13 nm) [74] have intense optical absorption at NIR region. However, critical pharmacological parameters such as body disposition and long-term metabolism of these CuS nanostructures remain unknown. Moreover, data regarding the cytotoxicity profile of the CuS nanostructures are lacking. This knowledge is essential for clinical applications of CuS nanomaterials. Recently, Guo et al. [75] compared degradability and toxicity between two types of photothermal nanoparticles, i.e., hollow gold nanospheres (HAuNSs) and hollow CuS nanoparticles (HCuSNPs), in mice following systemic administration. The two nanoparticles were formulated with similar particle size and morphology. They were both surface-modified with polyethylene glycol (PEG) to evade uptake by monophagocytic systems. The injected PEGylated HCuSNPs (PEG-HCuSNPs) are eliminated through both hepatobiliary (67 percentage of injected dose, %ID) and renal (23%ID) excretion within one month post injection. Comparatively, PEG-HAuNSs are almost nonmetabolizable, while PEG-HCuSNPs are considered biodegradable nanoparticles. PEG-HCuSNPs do not show significant toxicity by histological or blood chemistry analysis. However, with further studies, the researchers found that nanoparticle-mediated photothermal ablation is employed primarily as a local cancer treatment at the primary site. Thus, it is less effective in controlling metastatic cancer. An ideal cancer PTT should not only eradicate the treated primary tumors, but also induce a systemic antitumor immunity, control metastatic tumors and achieve the goal of long-term tumor resistance. For this reason, one promising strategy is to combine photothermal therapy with immunotherapy [76,77]. Laser-induced tumor cell death, on the other hand, can release tumor antigens into the surrounding milieu. Concomitantly, immunoadjuvants for cancer immunotherapy promote antigen uptake and presentation by professional antigen-presenting cells, thus triggering specific antitumor immunity. Therefore, PTT may act synergistically with immunotherapy to enhance immune responses, rendering the tumor residues and metastases more susceptible to immune-mediated killing. Recently, Lu et al. [78] developed hollow copper sulfide nanoparticles with photothermal immunotherapy. They...
synthesized immunoadjuvants, oligodeoxynucleotides containing cytosine-quinine (CpG) coated hollow copper sulfide nanoparticles (HCuSNPs-CpG) for “photothermal immunotherapy” in a mouse breast cancer model. Success of this technique relies on photothermally triggered disintegration of HCuSNPs, allowing the HCuSNPs-CpG conjugates to reassemble and transform into chitosan–CpG nanocomplexes. The chitosan–CpG nanocomplexes increase their tumor retention and promote CpG uptake by plasmacytoid dendritic cells. The HCuSNPs-CpG-mediated photothermal immunotherapy elicits more effective systemic immune responses than immunotherapy or PTT alone, resulting in combined anticancer effects against primary treated as well as distant untreated tumors. Strong antitumor effectiveness, combined with quick elimination, would seem to justify further development of this HCuSNPs conjugate-based photothermal immunotherapy.

Besides aforementioned CuS nanostructures, novel class of metal chalcogenide as photothermal therapy agent is two-dimensional (2D) transition-metal dichalcogenides (TMDCs). For example, MoS₂, MoSe₂, WSe₂, and WS₂ all consist of a hexagonal layer of metal atoms (M) sandwiched between two layers of chalcogen atoms (X) within stoichiometry MX₂. The common feature of these materials is the layered structure with strong covalent bonding within each layer and weak van der Waals forces between different MX₂ sheets. For their special characteristics, TMDCs have become the rising star in recent years, offering great opportunities in physics, chemistry and materials science. However, the exploration of this new class of TMDCs nanomaterials in the area of biomedicine is still at its infant stage. Currently, Chou et al. demonstrated the possibility of using as-made MoS₂ nanosheets as a new NIR absorbing agent for in vitro-photothermal-killing of cancer cells for the first time [79]. Chen and co-workers presented the fabrication of a two-dimensional MoS₂/Bi₂S₃ composite theranostic nanosystem for multimodality tumor CT and PA imaging and photothermal therapy [80]. Li et al. demonstrated in vivo-photothermal-ablation of tumors by local injection of Bi₂Se₃ nanosheets directly into tumors [81]. In recent years, Cheng et al. [82] used the Morrison method to fabricate single-layered WS₂ nanosheets with high-yield. Subsequently, using the thiol chemistry method, the surface of WS₂ nanosheets is coated with polyethylene glycol (PEG), which greatly improves the physiological stability and biocompatibility of those nanosheets (Fig. 7a). It is well-known that X-ray computed tomography (CT) imaging is one of the most commonly used imaging tools for clinic diagnosis and medical research. Based on a lot of studies, CT contrast agents often absorb and weaken the incident X-rays to produce tissue contrasts in the diagnostic

Fig. 7 – (a) A scheme showing the exfoliation and PEGylation of WS₂ nanosheets. (b) CT images of WS₂–PEG solutions with different concentrations. In vivo dual-modal imaging in 4T1-tumor bearing mice. (c) CT images of mice before and after i.t. injection with WS₂–PEG (5 mg/ml, 20 μl). (d) CT images of mice before and after i.v. injection with WS₂–PEG (5 mg/ml, 200 μl). The CT contrast was obviously enhanced in the mouse liver (green dashed circle) and tumor (red dashed circle). (f) Survival curves of mice after various treatments as indicated in (e). Reproduced with permission from Reference [82].
regime. Thus the attenuation of CT contrast depends on the interaction between X-ray and the inner shell electrons of atoms with high atomic numbers. Fig. 7b–d presents the CT images and Hounsfield unit (HU) values of different concentrations of WS$_2$–PEG in water, which show a sharp signal enhancement as the increase of WS$_2$–PEG concentrations. The slope of the HU value for WS$_2$–PEG is about 22.01 HU L/g, which appeared to be much higher than that of iopromide (15.9 HU L/g), a commercial iodine-based CT contrast agent used in the clinic. Utilizing the strong absorbance in the NIR region and strong X-ray attenuation ability of WS$_2$, Liu et al. successfully demonstrated the in vivo enhanced X-ray CT and photoacoustic tomography (PAT) bimodal imaging of tumors, respectively. In animal experiments, after either intratumoral injection with a low dose of WS$_2$–PEG or intravenous injection with a moderate dose of this nanoagent, realizing 100% of tumor elimination after NIR laser irradiation at a relatively low power density (Fig. 7e and f). These works encourage further in-depth investigations of this novel type of nanomaterials for biomedical applications.

2.3. Carbon-based nanomaterials (e.g., graphene oxide and carbon nanotubes)

2.3.1. Graphene oxide

Graphene oxide (GO) is a two-dimensional material obtained from the oxidative exfoliation of graphite. Graphene and GO have become one of the most attractive materials for the following reasons: (1) large surface area; (2) lightweight; (3) high strength and electrical conductivity; (4) the capacity of optical property-expressing plasmon, fluorescence, and nonlinear emission. The absorbance of GO extends from the ultraviolet (UV) wavelength to the NIR region. Thus, the absorbance at 808 nm was used to express the PTT. This photothermal property of GO was applied in in vivo photothermal ablation of tumors [83]. However, this GO dispersion was not easily achieved in bio-applications because of the aggregation that is caused by the high degree of the binding between GO and proteins or with other salts in serum. Therefore, the carbonyl groups in the as-prepared GO were functionalized covalently using amine-terminated PEG (PEG–GO) to increase the level of dispersion and decrease cytotoxicity. Robinson et al. [84] developed nanosized, reduced GO sheets (nano-rGO) (~20 nm in average lateral dimension) with noncovalent PEGylation (PEG–rGO). The nano-rGO was aggregated in the solution after reduction due to the removal of functional groups from the GO sheets. The increased hydrophobicity of the nano-rGO sheets caused aggregation even with the remaining PEG chains attached to GO through the reduction. To restore the dispersion, the PEG–rGO was PEGylated functioned again using sonication with a polymer (two methoxy-terminated PEG and one C17 chain attached to the poly maleic anhydride) to form polymer coated PEG–rGO (expressed as polymer-2PEG–rGO). The polymer-2PEG–rGO regained stability as a homogeneous suspension in buffers and other biological solutions without aggregation even under harsh centrifugation conditions. In addition, it is worth noting that the polymer-2PEG–rGO resulted in a significant ~6.8 fold increase in the NIR absorption at 808 nm than non-reduced nano-GO and covalently PEGylated nano-GO. This enhance was ascribed to the increase of the degree of the π conjugation in GO after chemical reduction. Subsequently, the high NIR absorbance of polymer-2PEG–rGO allowed for effective photothermal heating of solutions at a low concentration of polymer-2PEG–rGO. At a concentration of ~20 mg/L, rapid photothermal heating occurred upon irradiation of a low power 808 nm laser at 0.6 W/cm$^2$. Temperatures above the photobleaching limit of 50 °C were readily reached within 5 min of irradiation. This work shall lead to systematic in vivo investigations of nano-rGO for photothermal treatment of tumor models in mice using low doses of nano-rGO at low laser powers.

To further enhance the photothermal effect of nanomaterials, the plasmon-rich Au nanoparticles [85] and quantum dots (QDs) [86] were combined with GO. For example, Lim et al. [85] synthesized reduced GO-coated gold nanoparticles (gold nanoshells and nanorods) by electrostatic interaction in situ chemical reduction. The new hybrid material generated well-defined r-GO-AuNS and r-GO-AuNR. The r-GO as shell and Au nanoshell/Au nanorod as core existed in the hybrid nanostructures. The r-GO-AuNS and r-GO-AuNR colloidal solutions exhibit good stability at room temperature, because the carboxylic acid and hydroxyl groups still exist in incomplete reduced r-GO. The photothermal performance of r-GO-AuNS or r-GO-AuNR was studied in dry and solution state under NIR illumination (808 nm, continuous wave, power density: 3.0 W/cm$^2$). For the dry state, r-GO-AuNS/r-GO-AuNR led to a 2.9 fold increase in ΔT upon irradiation compared with Au nanoparticles and non-reduced GO-AuNS/GO-AuNR. For the solution state, solutions with the same optical density and sample volume were illuminated for 5 min at 3.0 W/cm$^2$, continuous wave (CW): 808 nm. The heating rates of r-GO-AuNS/r-GO-AuNR solution were greater than Au nanoparticles and non-reduced GO-AuNS/GO-AuNR. These independent measurements demonstrate the greater photothermal effect of particles coated with r-GO, which could be ascribed to the interactions between the r-GO and the gold plasmons. The therapeutic effect of the photothermal rGO-AuNS/r-GO-AuNR was further demonstrated on human umbilical vein endothelial cells (HUVECs). HUVECs were incubated with non-reduced GO- or r-GO-coated and uncoated Au nanoparticles for 24 h followed by irradiation (3.0 W/cm$^2$, CW: 808 nm) for 1 min. The cell viability in r-GO-AuNS and r-GO-AuNR were 23% and 33%, respectively, whereas 41–43% for Au NS or GO-Au NS, and 53–57% for Au NR and GO-Au NR, respectively. These results showed that r-GO coating on plasmonic nanoparticles accelerated cell killing. The main reason for increased killing of cells is that r-GO-Au nanoparticles showed very powerful phototoxicity for cancer cells. Showed excellent photothermal properties, which may be useful in improving biomedical applications based on the photothermal effect, by increasing their efficacy and/or decreasing the duration of therapy.

As we all know, when early studies on GO-assisted cancer therapeutics, GO was limited to serving as the drug delivery vehicle, as GO-assisted chemotherapy. Generally, the hexagonal arrangement of carbon in GO favors the noncovalent loading of anticancer drug cargo using π–π stacking. The GO exhibits a radical increase in drug loading of approximately 200% by weight, and this is the first drug carrier to achieve over 100% loading consistently. In addition, the GO was able to unload the cargo under highly acidic and basic conditions because of the compromise in the hydrogen bonds between the —COOH
and the —OH groups of GO, and between the —OH and the —NH₂ groups of DOX [87, 88]. Following the studies on the aforementioned spontaneous release, the photothermal characteristics of GO were subsequently introduced to determine the combination of PTT and chemotherapy. Wang et al. [89] used mesoporous silica-coated GO (expressed as GS) to administer chemotherapy and PTT. In this study, the GO was coated with mesoporous silica to form a sandwich structure. The GS was then coated with PEG (expressed as GSP) to achieve solubility and IL31 peptides for glioma cell targeting (expressed as GSPI). Finally, GSPI loaded the chemotherapeutic drug DOX, yielding GSPID (Fig. 8a), the photothermal effect of GSPI could promote the release of DOX. The NIR irradiation apparently enhanced the cumulative release of DOX at different time and pH values due to heat stimulative dissociation of the strong interactions between DOX and GSPI including π–π stacking and pore adsorption (Fig. 8b). The result means the photothermal effect of GSPI could significantly increase the sensitivity of chemotherapy (Fig. 8c). Regarding the targeting property of IL31 peptides, we confirmed that GSPI could not only target glioma cells, but also achieve high cellular uptake and cytoselectivity in glioma cells, and no apparent effect on normal cells, compared to GSPD. These findings provided an excellent drug delivery system for combined therapy of glioma due to the advanced chemo-photothermal synergistic targeted therapy and good drug release properties of GSPI, which could effectively avoid frequent and invasive dosing and improve patient compliance.

2.3.2. Carbon nanotubes

Carbon nanotubes (CNTs) are carbon nanomaterials, including both single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). The strong optical absorption and high photon-to-thermal energy conversion efficiency of CNTs in the NIR region combined with a high-absorption cross-section make CNTs a suitable candidate for PTT [90, 91]. The proper surface functionalization of CNTs renders them biocompatible and enables them to serve as efficient cancer drug delivery vehicles. Based on a lot of studies, the loading of aromatic drugs (e.g. DOX) using CNTs by employing noncovalent π–π stacking is a simple process. In particular, the surface of the CNTs can be occupied approximately 70–80% by DOX molecules [92]. Based on this, Liu et al. [93, 94] demonstrated DOX loading, delivery and chemotherapy using the composite. The SWNTs were coated with mesoporous silica (MS) to load drug and then
affected by laser irradiation. Photothermal heating induced by SWNT@MS-PEG without chemotherapy, on the other hand, appeared to be much less effective compared to the combination therapy, especially under lower laser powers (Fig. 9b). Therefore, it is concluded that NIR-light triggered intracellular drug release in such combined photothermal and chemotherapy could offer an obvious synergistic effect to destroy cancer cells.

In vivo Balb/c mice were developed by cancer 4T1 cells and i.v. injected with SWNT@MS-PEG/DOX, SWNT@MS-PEG, DOX, and PBS, respectively. After 24 h, the tumors were irradiated by the 808 nm laser at a moderate power density of 0.5 W/cm² for 20 min. It was found that the tumor surface temperatures of mice treated with SWNT@MS-PEG/DOX and SWNT@MS-PEG were increased and maintained at ~48 °C during laser irradiation. In contrast, the mice treated with PBS and DOX showed no apparent temperature increase in the tumor region after being irradiated by the laser. Remarkably, the tumor growths on mice with injection of SWNT@MS-PEG/DOX were effectively inhibited after NIR laser irradiation as a result of the combined chemo-photothermal therapy. In addition, SWNT was loaded with docetaxel (DTX) using π–π accumulation, and was subsequently subjected to surface modification conducted using poly-N-vinylpyrrolidone (PVP) and 1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]-maleimide [95]. Maleimide was further conjugated with the targeting NGR peptide (Asn-Gly-Arg) to form NGR-SWNT/DTX. The NGR-SWNT/DTX was administered intravenously to the mice bearing S180 tumor xenografts followed by irradiation with an 808 nm laser at a power density of 1.4 W/cm² (NGR-SWNT/DTX with laser) for 13 days. The tumor volume was inhibited at the early stages (7th day) of combined chemotherapy and PTT. The other groups that were treated by chemotherapy only (NGR-SWNT/DTX) or PTT only (NGR-SWNT with laser) exhibited a continual increase in tumor volume. Further surface engineering of those nanostructures may allow active tumor targeting and more precisely controlled drug release under other stimuli in addition to NIR light, to achieve cancer therapy with even better specificity.

The combination of PTT nanomedicine-treatment together with antibody-based immunotherapy may be a novel cancer therapeutic strategy, which not only is able to destroy the primary tumor, but also able to inhibit cancer metastasis at distant organs in the body [96]. However, whether and how CNT-based photothermal therapy would trigger any immunological response and play any effect in inhibiting tumor metastasis remain largely unknown. Recently, Wang et al. [97] reported that photothermal ablation of primary tumors with single-walled carbon nanotubes (SWNTs) in combination with anti-CTLA-4 antibody therapy is able to prevent the development of tumor metastasis in mice. It is found that polymer-coated SWNTs could not only be used for photothermal tumor destruction, which releases tumor-associated antigens, but also can act as an immunological adjuvant to greatly promote maturation of dendritic cells (DCs) and production of anti-tumor cytokines. The mice bearing subcutaneous 4T1 murine breast tumors were intratumorally injected with SWNTs (dose = 0.33 mg/kg). After irradiation with an 808 nm NIR laser at 0.5 W/cm² for 10 min, the tumor temperature jumped to 53 °C, which is high enough to effectively ablate tumor cells. After SWNT-induced photothermal treatment, all tumors on mice
were completely eliminated, without showing a single case of tumor relapse at their original sites. In addition, both SWNTs alone and SWNT-based PTT were able to increase the secretion of pro-inflammatory cytokines IL-1β, IL-12p70, IL-6 and TNF-α. Particularly, the serum level of TNF-α, which plays an important role in anti-tumor immune responses, was dramatically enhanced after SWNT-treated PTT. It is likely that PTT with CNTs is not just burning of tumors, but also to inhibit cancer metastasis. Thus, immunological responses triggered by PTT may offer clinically valuable therapeutic advantages over surgery in cancer treatment.

In addition, SWNTs decorated with noble metals were used to conduct efficient PTT and surface-enhanced Raman spectroscopy (SERS) imaging. The DNA-functionalized SWNTs are modified with noble metal (Ag or Au) nanoparticles via an in situ solution phase synthesis method comprised of seed attachment, seeded growth, and surface modification with PEG, yielding SWNT-Ag-PEG and SWNT-Au-PEG nanocomposites stable in physiological environments [98]. Subsequently, utilizing folic acid (FA) conjugated SWNT-Au-PEG-FA, selective cancer cell labeling and Raman imaging is realized. Owing to the strongly enhanced Raman signals of SWNT-Au-PEG-FA, the cancer cells showed remarkably shortened imaging time compared to that when using a non-enhanced SWNT-nanoprobe (Fig. 10). Moreover, the SWNT-Au-PEG-FA nanocomposite also exhibits dramatically improved photothermal cancer cell killing efficacy. The enhancement is attributable to the strong surface plasmon resonance absorption by the gold shell grown on the nanotube surface. The photostability of SWNT-Au-PEG-FA was compared with that of Au NR by exposing them for 1 h to an 808 nm laser with a power density of 1 W/cm². Au NR exhibited a complete loss of NIR absorbance, whereas SWNT-Au-PEG-FA retained nearly 87% of the absorbance intensity. Taking the intrinsic properties of both SWNTs and gold nanoparticles together, the SWNT-Au nanocomposite developed here may be an interesting and promising nano-platform in biosensing, optical imaging, and phototherapy.

Carbon-based nanomaterials, i.e. graphene oxide and carbon nanotubes, are fabricated and utilized as a multifunctional platform for chemotherapy and photothermal therapy. Carbon-based nanomaterials have demonstrated large heating efficiency and high drug loading amount. However potential clinical implementations of carbon-based nanomaterials are still hampered by distinctive barriers such as poor bioavailability and intrinsic toxicity, which cause difficulties in tumor targeting and penetration as well as an improving therapeutic outcome. For sure, this will be one of the main working areas in the field of carbon-based nanomaterials during the next years.

3. Conclusions and perspectives

In summary, we have presented a detailed review of the inorganic nanocomposite materials for PTT. Inorganic nanocomposites such as gold nanoparticles, palladium nanoparticles, metal chalcogenide nanoparticles, carbon nanotubes and graphene oxide have been extensively explored as photothermal therapy agents (PTA) for cancer therapy. We summarized for each kind of inorganic PTA, fundamental light-to-heat conversion property, synthesis method, the efficiency of in vitro and in vivo thermal therapies, and multifunctional synergy therapies (already possible by the combination of many different techniques such as PTT, CT, and PAT). Based on studies reviewed, inorganic PTA-assisted cancer therapy can effectively induce site-specific cell death in both in vitro and in vivo treatments. The tremendous development of nanotechnology brings us closer to the dream of clinical application of nanoparticles in photothermal therapies of tumors. However, the following disadvantages of inorganic PTA are required to note for the clinical execution of these cancer therapies in the future:

(1) **Photothermal conversion efficiency and stability**: According to clinical requirement, the inorganic nanoparticles with high energy conversion efficiency and good stability should be synthesized. The studies show that high photothermal conversion efficiency requires large absorption cross sections of nanoparticles for optical wavelengths. This would ensure an efficient absorption of optical radiation, thus achieving the PTT with low-power laser sources. For example, the optical-response band and the photothermal efficiency of Au nanoparticles can be tuned and improved by exploring plasmon hybridization by the introduction of the dielectric gap in the form of core–shell structures [99]. In addition, owing to the presence of sharp tips as well as their high surface-to-volume ratios, the absorption of branched Au nanostructures could be more effective in photothermal conversion.

Besides high photothermal conversion, good photothermal stability also is necessary in PTT application. From the shapes of the molecules, the anisotropic nanostructures lack good thermal stability. For example, gold nanorods (GNRs) have the tendency to transform into nanospheres when exposed to NIR laser, accompanied with the disappearance of the NIR absorption band [50]. The photothermal stability of GNRs also can be improved by design of core–shell structures. Thus, it is believed that the performance of PTA can be further improved with the reasonable design and synthesis.

(2) **Toxicity**: Generally, the clearance of PTA and their acute and long-term toxicity need to be thoroughly examined.
before use. In addition, toxicity of PTA should only be activated in the presence of optical radiation. PTA should be non-toxic to both healthy cells and cancer cells without NIR radiation. This is required to achieve a selective treatment with minimum side effects. Thus, these nanoparticles should meet the requirement of the safety, effectiveness, and quality control standards of new drug development. For GNPs, the cetyltrimethylammonium bromide (CTAB) used as a surfactant stabilizer during the synthesis process could cause cytotoxicity and thus needs to be replaced prior to any in vitro or in vivo application. To overcome the limitation of GNPs in in vivo cancer PTT, PEGylation of GNPs was attempted to lower the cytotoxicity and the liver accumulation of GNPs. Additionally, the CNTs were reported to show genotoxicity, as they can pierce the cells and enter the nucleus easily. CNTs with appropriate surface coatings have been found to be not obviously toxic to animals and could be gradually excreted from mice over time [100,101]. Based on aforementioned studies, to reduce the toxicity of PTA, the surface of the PTA is often modified by biological molecules (PEG, FA, GSH, etc.). Thus, it is required to have charge, or functional groups, or hydrogen bonds, etc. on the surface of the photothermal materials.

(3) Visual-guided therapies: The unique physicochemical properties of nanomaterials have offered an opportunity to integrate different theranostic modalities into a single nanoplatform for combined cancer treatments with real-time diagnosis. Before these new nanomaterials are tested in cancer patients, detailed preclinical studies should be conducted, especially to investigate the pharmacokinetics, in vivo tumor targeting, and therapeutic effects of these nanoparticles. The PEGylated PTA of gold nanoparticles and carbon-based materials have demonstrated large heating efficiency and outstanding biocompatibility. However, they have the main drawback of showing a weak fluorescence, which makes it hard to track them in real in vivo treatments. This implies the incorporation of luminescent nanothermometers in the volume to be treated simultaneously with the photothermal agents. Therefore, fluorescence imaging and real-time control can be realized and adjusted using various dopants such as quantum dots, organic dye, etc. We believe that multifunctional nanoparticles will be developed offering heating, tracking and sensing in a single structure in the near future.

On the basis of the current research, we think that the ultimate challenge for cancer treatment is to be able to diagnose and cure cancer without surgical intervention and avoiding the occurrence of side effects. New technology should aim to develop nanomaterials that allow for efficient, specific in vivo delivery of therapeutic agents without systemic toxicity, and the dose delivered as well as the therapeutic efficacy can be accurately monitored non-invasively over time. Therefore, the research on new inorganic nanomaterials as PTA still is an attractive field that should be highly improved. This will be one of the main working areas in the field of nanotechnology and nanomedication in the next years.

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