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Published in:
Chemistry of Materials

DOI:
10.1021/acs.chemmater.8b00934

Publication date:
2019

Document version
Accepted manuscript

Citation for published version (APA):

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Chem. Mater., Just Accepted Manuscript • DOI: 10.1021/acs.chemmater.8b00934 • Publication Date (Web): 11 Jun 2018

Downloaded from http://pubs.acs.org on June 12, 2018

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Multifunctional Nanocomposites for Targeted, Photothermal, and Chemotherapy

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ABSTRACT: In the past decades, the advance in nanoparticles (NPs) synthesis and engineering has greatly propelled the application of nanoscale agents for therapeutic and diagnostic functions, promoting an emerging field of “nanotheranostics”. In particular, they are being increasingly exploited for the cancer management, in which diagnosis and therapy are combined to address clinical challenges. In this work, we demonstrated a new approach using histidine (His) to mediate the hydrothermal growth of amorphous zinc oxide (a-ZnO) shells on gold NPs (Au-His@a-ZnO). Au-His@a-ZnO NPs was integrated onto the planar structure of PEGylated graphene oxide (PEG-GO) via carbodiimide crosslinker chemistry. More importantly, the strong absorption and near-infrared (NIR) emission within the range from 700 to 900 nm were observed with preferential uptake at tumors and high photothermal conversion efficiency ($\eta = 38\%$). Both in vitro and in vivo studies showed that the GO@Au-His@a-ZnO NCs was biocompatible materials with low toxicity. Moreover,
GO@Au-His@a-ZnO NCs was further conjugated with antibody of epidermal growth factor receptor aptamer (anti-EGFR Apt) and doxorubicin (DOX) into Apt@GO@Au-His@a-ZnO@DOX NCs, applying for the synergetic treatment of lung cancer. The prepared Apt@GO@Au-His@a-ZnO@DOX NCs showed a high loading capacity of DOX as well as NIR/pH-sensitive drug release in which the metal-drug complex dissociated to release antitumor Zn\(^{2+}\) ions in the acidic endosome/lysosome of cancer environment. In addition, they also showed good biostability and aptamer-promoted binding specificity for lung cancer cells. The specific binding facilitated the cellular uptake into EGFR-mutated cancer site, as compared with non-targeted controls. In particular, human pulmonary adenocarcinoma cells (A549) tumor-bearing mice were selected as the animal model and demonstrated the efficient targeted drug delivery and the high anti-cancer efficacy of Apt@GO@Au-His@a-ZnO NCs in vivo. Taken together, our multifunctional NCs, Apt@GO@Au-His@a-ZnO@DOX NCs, have shown the high efficacy in targeted, photothermal, and chemotherapy to lung cancer. This proof-of-principle example suggests the fascinating perspectives of these functional NPs for the future clinical use.

INTRODUCTION

The use of theragnostic nanoparticles (TNPs) for malignancy treatment is one of the most leading-edge biotechnology research topics.\(^1\)\(^-\)\(^3\) The resulting-nanoplatform is optimized in the precise diagnosis of disease, individualized treatment and real-time monitoring of therapeutic outcomes.\(^4\) For clinically relevant applications in oncology, TNPs with absorption in the near-infrared (NIR) (700-900 nm) is particularly attractive, because the transparency window for biological tissues allows for deeper light penetration and relatively low absorption/scattering, and thereby can efficiently transfer the absorbed NIR optical energy into heat.\(^5,\)\(^6\) A series of nanomaterials including zinc oxide nanoparticles (ZnO NPs),\(^7,\)\(^8\) graphene oxide (GO),\(^9,\)\(^10\) carbon
nanotubes (CNTs),\(^{11}\) carbon dots (CDs),\(^{12}\) and gold nanoparticles (AuNPs)\(^{13,14}\) have been applied for photothermal therapy (PTT) as their strong absorbance in the NIR region. Recently, many researchers have explored the role of GO sheets as the theranostic agent in PTT \emph{in vitro} and \emph{in vivo}.\(^{15}\) The delocalized electron arrangement makes GO capable of absorbing NIR radiation. The absorbed radiation can derive from various GO vibrational modes that are further transferred into thermal energy, leading to the high temperature around cancerous tissue, structural changes in cellular, as well as protein configurations.\(^{9,10}\) Owing to the excellent thermal conductivity (59.2\%) and high effective surface area (90.6 m\(^2\)/g), GO sheets have served as one of the most effective nanomaterials for PTT applications.\(^{9,10,15,16}\) In fact, biocompatibility has always been regarding as one of the most important parameters for graphene-based nanomaterials applications in (especially \emph{in vivo}) photothermal therapy of cancer cells. Cytotoxicity of GO nanosheets exhibits dose-dependent property,\(^{17,18}\) which suggests that biocompatible GO nanosheets should be constructed to reduce cytotoxicity. This reduction can be obtained by preparing a hierarchical GO nanocomposites (NCs).\(^{15}\) To date, thanks to distinct physical and chemical properties, AuNPs have been used as biological safe materials for a variety of biomedical applications such as drug delivery, imaging, biosensing and therapy.\(^{19,20}\) AuNPs have strong absorption in the NIR region because they exhibit a localized surface plasmon resonance band and throughout the NIR region.\(^{21,22}\) Therefore, AuNPs have been applied as promising photothermal agents for cancer treatments. Furthermore, AuNPs can be easily synthesized and chemically modified. These allow the further development of a NCs by the assembly of AuNPs on GO nanosheets in the pursuit of a broad light absorption band and excellent photothermal effect.

Chemotherapy, surgery and radiotherapy treatments of cancer have strong side effects for living beings.\(^{23-25}\) Therefore, in the process of later clinical treatment, improving the drug targeting, enhancing the drug curative effect while minimizing adverse drug side effects are needed.\(^{26,27}\) ZnO NPs have been widely used in biocidal applications, sensor designing, catalysis and gas sensing; however, these
nanostructures in drug delivery system are still in a nascent stage.28-30 Most recently, ZnO NPs have been explored as nano-vectors for targeted drug delivery systems, behaving as (1) biological modification,31 or (2) ZnO NPs based drug delivery system.32 For example, ZnO NPs had been demonstrated for multimodal cancer treatment through loading antitumor drug (DOX).33 The combination of ZnO NPs and DOX gave rise to a synergistic therapy, which was presumed due to the controlled-release of Zn$^{2+}$ and DOX in the tumor microenvironment where the acidic condition (pH < 5.5) triggered the decomposition of ZnO from the NCs.34 Such dissolution behavior of ZnO NPs has been well investigated. ZnO NPs were found to possess significant cytotoxic effects, if and only if post dissolving preferentially killed cancer cells. These excellent properties have propelled the recent development of the pH-sensitive ZnO NPs for drug delivery.35,36 Latest reports have shown that ZnO nanosheet was used as the bifunctional vector for chemo-photothermal therapy.35 However, only few ZnO structures like that has been found in cancer therapy.7,37 For example, ZnO nanodots/nanosheet structures easily aggregated and ZnO nanotubes/pyramids/hexagon/cages hasn’t been reported. It is important for effective cancer therapy to select a ZnO nano-structure for targeting tumor site with high drug loading efficiency and releasing payload to the cancer cells.38 Taken together, we present a one-step process that uses free-base L-histidine (His) to mediate the growth of continuous and uniform shells of either amorphous ZnO on AuNPs in aqueous solutions (denoted as Au-His@a-ZnO). The use of L-histidine can benefit to not only construct monodisperse and unaggregated core-shell colloids, but also precisely tune the shell thickness to 2.5 nm, thereby meeting the requirements of plasmonic particles with either ultrathin or customizable coatings for biological applications.39 Besides, zinc chalcogen materials can promote shell growth.36 L-histidine have been previously identified as possessing affinity for both zinc chalcogenides and gold. Its presence can, therefore, modify the growth and morphology of ZnO.39 Considering the stability and biodegradability of ZnO in the different pH, we used modified Au-His@a-ZnO NPs for the efficient drug delivery in tumors environment where acidic pH promotes the controlled release of drugs.
Herein, we aim at the development of multifunctional therapeutic, doxorubicin-loaded, anti-EGFR aptamer-conjugated, Au-His@a-ZnO-loaded and PEGylated GO nanocomposites (Apt@GO@Au-His@a-ZnO@DOX NCs) which release drug or Zn\(^{2+}\) induced by external stimuli (photothermal or pH) to selectively damage cancer cells. In this system, GO@Au-His@a-ZnO NCs emit a strong surface plasmon resonance band in the NIR region for photothermal drug-releasing, and EGFR serves as the marker for specific targeting cancer cell.\(^2,19\) Doxorubicin (DOX) is the chemotherapeutic agent.\(^2,3,27,40\) The drug delivery ability, targeting ability, cellular uptake, and cytotoxicity were investigated. Furthermore, the anticancer effects of Apt@GO@Au-His@a-ZnO@DOX NCs have been exploited \textit{in vitro} and \textit{in vivo}.

\textbf{EXPERIMENTAL SECTION}

\textbf{Chemicals.} All chemicals were purchased from Aladdin Chemical Co., Ltd. (Shanghai, China) and used as received. Biological reagents were obtained from KeyGEN Biological Technology Co. Ltd. (Beijing, China). Fresh blood from healthy consenting volunteers was collected into sodium citrate tubes. Female Balb/c mice and Wistar rats were purchased from Shanghai BK Lab. All procedures for animal experiments were handled under the guidelines approved and supervised by the ethics committee of Nanjing University.

\textbf{Instruments and Characterization.} The photoluminescent (PL) spectra were recorded using molecular fluorescence spectrometer (Cary Eclipse, Varian, USA). Nexus 670 FTIR type (Nicolet) infrared spectrometer was used to analyze the infrared spectrum. The X-ray diffraction (XRD) analysis was performed using a D/Max 2500V/PC diffractometer (Rigaku Corporation, Japan). Ultraviolet-Visible (UV-Vis) absorption spectra were recorded using UV spectra (Cary-50, Varian, USA). The surface composition and element analysis were recorded using X-ray photoelectron spectroscopy (XPS, EscaLab-250, Thermo, USA). The morphologies of the samples
and elemental analyses were characterized using the transmission electron microscope (TEM, H-7650, Hitachi, Japan) and energy dispersive X-ray spectroscopy (EDS, H-7650, Hitachi, Japan). In vitro bright field and fluorescence images were performed with a laser scanning confocal microscope (LSCM, LTI-EA1R, Nikon, Japan). The specific surface area was carried out using a Brunauer-Emmett-Teller (BET, Belsorp mini II, Japan). To monitor the temperature changes at the tumor site during irradiation, infrared thermal images were recorded with a PTT monitoring system MG33 (Shanghai Magnity Electronics Co. Ltd). The methods used for material characterization were displayed in the “Experimental Section” (Supplementary File).

RESULTS AND DISCUSSION

Synthesis and Characteristics of the Au-His@a-ZnO NPs. An aqueous solution of His was mixed with a replaceable colloid of citrate-capped AuNPs (AuNPs-Cit), followed by adding NH₄OH and ZnCl₂. The added His caused no change to the color of the aqueous solution (red) (Figure 1A), indicating that well dispersed AuNPs colloids were stabilized by the His that had strong affinity to displace Cit from the gold surface as shell precursor ions (Figure S1). The observations are in line with the previous study, which reported His possessed the strongest affinities for the gold surface. The synthesis process is illustrated in Figure 1A to show the formation of Au-His@a-ZnO NPs. Specifically, His first displaced the Cit capping agent from AuNPs and bound to the gold surface. The particles were then stabilized by the deprotonated charge (i.e., -COO⁻ from His) on the outer shell after adding NH₄OH. Upon the further addition of aqueous ZnCl₂, Zn²⁺ ions were coordinated by ammonia to form [Zn(NH₃)₄]²⁺ complexes. These complexes could form the visible white precipitates, or soluble solution, depending on the amount of ZnCl₂ in the reaction solution. The positively charged complexes are attracted to the negatively charged AuNPs; however, the relatively large size of the complex could not densely pack around the NPs, thus unable to completely neutralize the surface charge. Once being heated, the complexes decomposed and hydrothermally nucleated into a-ZnO on the
AuNPs. As long as the new materials were formed, the excess amount of His continued to stabilize the particles. This is due to that His has sufficient affinity to displace Cit from the surface of AuNPs and stabilize the particles in the presence of the shell precursor ions.  

Figure 1. (A) Proposed synthetic pathway for the hydrothermal growth of ZnO shells on AuNPs in aqueous solution through His mediation. (B) XRD spectra for Au-His@a-ZnO NPs with several shell thicknesses. (C) XPS survey scan of
Au-His@a-ZnO NPs confirms the shell of AuNPs-Cit is displaced by His. (D) EDS characterization of (a) AuNPs and (b) Au-His@a-ZnO NPs through a survey spectrum. The inset is the dark-field TEM image. (E) TEM, HRTEM and elemental maps image of His-mediated particles confirm the shell is amorphous for (a) AuNPs and (b) Au-His@a-ZnO NPs.

A number of techniques were taken to characterize each core-shell material. The XRD spectra (Figure 1B) revealed that the crystalline component in His-mediated shells is the gold core. Elemental analyses by XPS (Figure 1C) and EDS (Figure 1D), shows the presence of Au, Zn, and O in the core-shell particles (note that the EDS signals of Cu arise from the TEM grid). In particular, XPS data shows that Au-His@a-ZnO has composition of C, O, N, Zn, and Au with 38.49, 23.66, 12.80, 9.56 wt %, and 15.49 wt %, respectively, revealing its gold and zinc dual-doped NCs. The lattice fringes in AuNPs core without crystallinity are observed by the high-resolution TEM (HRTEM) in Figure 1E, a, indicating the amorphous structure of the shell. TEM elemental mapping of His-mediated particles in Figure 1E, b clearly shows that the shell is composed of O and Zn elements. The TEM images in Figure 1E, b demonstrate that uniform and continuous shells with thicknesses of 2.5 nm can be grown on individual AuNPs. The XPS scans of the O 1s and Zn 2p3/2 peaks shown in Figure S2 reveal that the binding energies correspond with ZnO rather than Zn(OH)2. All these characterization data indicate that His mediates the growth of a-ZnO shells on AuNPs. The nitrogen peak in XPS spectra (Figure 1C) confirm that the His molecules exist on the AuNPs shell surface.

**Synthesis and Characterization of the GO@Au-His@a-ZnO NCs.** GO nanosheets were synthesized from graphite powders by a modified Hummers method. Functional groups, such as -OH and -COOH on GO nanosheets, are beneficial for their dispersion in aqueous solution (Figure 2A). The structures and morphologies of GO nanosheets are fully characterized by atomic force microscope (AFM) and TEM images in Figure S3 and Figure 2B. The thickness of GO nanosheets is about 0.8 nm, indicating the majority of GO nanosheets exists as monolayer
structure (Figure S3). TEM images in Figure 2B shows that the most GO nanosheets are ca 100 nm in lateral width. In order to obtain multimodal therapeutic functions, Au-His@a-ZnO NPs, Au-His@a-ZnO NPs as both photothermal agent and carriers for photothermal agent, were covalently grafted with PEGylated GO (GO-PEG) via amide coupling, as illustrated in Figure 2A. GO-PEG was obtained by grafting amino carboxylic PEG with GO sheet, and the left -COOH groups at the PEG terminals were available for loading Au-His@a-ZnO NPs. Such PEG functionalization was proved by FI-IR (Figure S4). The morphology of GO-PEG was presented by TEM (Figure 2C) and AFM (Figure S5). GO-PEG was almost single layered sheet with average topographic height of 1.6 nm, as shown in Figure S5. The formation of GO@Au-His@a-ZnO NCs was first verified by TEM measurements. Figure 2D showed a typical TEM image of the as-prepared GO@Au-His@a-ZnO NCs, in which Au-His@a-ZnO NPs with a narrow size distribution were deposited uniformly on the GO nanosheets surface while no aggregated Au-His@a-ZnO nanoclusters or bigger Au-His@a-ZnO NPs could be observed. Furthermore, the modification of Au-His@a-ZnO NPs to the GO surface was also confirmed by XPS. Figure S6 demonstrates that Au 4f peaks clearly occurred at 83.2 and 87.9 eV, which was not obtained for bare GO (Figure 2E), thus verifying the success of Au-His@a-ZnO modification on the GO surface. Figure S7A shows the N\textsubscript{2} adsorption-desorption isotherms of the GO@Au-His@a-ZnO NCs. The BET surface areas of GO@Au-His@a-ZnO NCs are estimated as 30.56 m\textsuperscript{2}/g.

**Photothermal Performance of the GO@Au-His@a-ZnO NCs.** The GO@Au-His@a-ZnO NCs can be easily dispersed in water with negligible morphological change. They have good water stability, typically showing as khaki color (Figure 2A). Moreover, GO@Au-His@a-ZnO NCs exhibits higher absorption in the NIR region within the biological-window (650-950 nm), which is attributed to the extended \pi-conjugation. Such NIR adsorption can be proved by UV-Vis-NIR analysis (Figure S7B). The broad range of NIR absorption of GO@Au-His@a-ZnO NCs suggests their potential for single light induced PTT by transforming photothermal
agent excitation wavelengths from visible to NIR. To verify this potential, we exposed GO@Au-His@a-ZnO NCs solution (100 μg/mL) under an 808 nm NIR laser with different power density (Figure 2F) to detect their temperature. As shown in the inset of Figures S8, by 5 min irradiation at the power densities of 1.5 W/cm², the GO@Au-His@a-ZnO NCs solution temperature increased from 25 to 58 °C, which is high enough to cause cancer cells damage.12,15,27 These results clearly confirm that pronounced heat can be generated by NIR irradiation of GO@Au-His@a-ZnO NCs. Second, in order to measure the photothermal conversion efficiency (η) of GO@Au-His@a-ZnO NCs, the temperature changes of the solution (50 μg/mL) as a function of time were monitored under continuous irradiation of 808 nm laser (1.5 W/cm²). The η value of GO@Au-His@a-ZnO NCs was calculated according to the reported model,6,43 obtaining a value of 38% (Figure S8), which is higher than that of the previously reported Au nanostars,44 CDs,45 AuNPs,6 and Cu2-xSe NPs.46 This improved η value makes GO@Au-His@a-ZnO as a promising photothermal coupling agent. In addition, the η value of GO nanosheets and Au-His@a-ZnO NPs was determined to be 41% and 29% (Table S1), suggesting that the compositions can be finely-tuned for the optimal photothermal effect.
Figure 2. (A) Schematic illustration for the preparation of anti-EGFR aptamer-conjugated and doxorubicin-loaded GO@Au-His@a-ZnO NCs (Apt@GO@Au-His@a-ZnO@DOX NCs). TEM images of (B) GO nanosheets, (C) PEG-GO nanosheets, and (D) GO@Au-His@a-ZnO NCs. (E) XPS survey scan of GO nanosheets and GO@Au-His@a-ZnO NCs. (F) Photothermal heating curves of GO@Au-His@a-ZnO NCs at various power intensities with GO@Au-His@a-ZnO NCs concentration at 50 µg/mL.

Cytotoxicity Assay of the GO@Au-His@a-ZnO NCs. We also investigated the biocompatibility of GO@Au-His@a-ZnO NCs through MTT assay, in vitro blood coagulation and hemolysis assay. It was found that GO@Au-His@a-ZnO NCs caused no cytotoxicity to both human liver cells (HL-7702) and mouse embryonic fibroblasts cells (NIH3T3) even when their concentrations were as high as 200 µg/mL (Figure 3A). However, their cytotoxicity effects became significant using cancer cell lines like
human cervical cancer (HeLa) and human pulmonary adenocarcinoma (A549) cell lines. This pronounced cytotoxicity to cancer cells is due to the accelerated dissolution of free Zn\(^{2+}\) ions in the tumor environment, which is consistent with the previous findings in other ZnO NPs.\(^{31,32}\) As shown in Figure 3B, there is no significant hemolysis (<5%) when GO@Au-His@a-ZnO NCs were cocultured with red blood cells.

**Figure 3.** Cytotoxicity of GO@Au-His@a-ZnO NCs incubated with HL-7702, NIH3T3 cells, HeLa cells, and A549 cells for 24 h. (B) Hemolysis percentage of RBCs at various concentrations of GO@Au-His@a-ZnO NCs. Effect of the intravenously administered GO@Au-His@a-ZnO NCs on (C) APTT and (D) PT. (E) RBCs photomicrographs of the rat after injection of PBS and GO@Au-His@a-ZnO NCs (0.1, 1, and 10 mg/mL).

*In vivo* biocompatibility evaluation is a key index for clinical application of nanomaterials. We measured the activated partial thromboplastin time (APTT) and prothrombin time (PT) of blood collected from rats, 24 h after intravenously injecting
GO@Au-His@a-ZnO NCs. The APTT and PT data for the rats injected with GO@Au-His@a-ZnO NCs were not significantly different from the control group, as shown in Figure 3C and 3D. Therefore, intravenous administration of GO@Au-His@a-ZnO NCs at doses up to 10 mg/kg appears to be safe for the blood coagulation function of rats. Morphologically aberrant forms of red blood cells (RBCs) always can sever as a distinct explanation for diagnosis of various medical conditions, including hemolytic anemia. The photomicrographs from Figure 3E indicate that the treatment of GO@Au-His@a-ZnO NCs does not alter the number and shape of RBCs in the test rat. The in vitro and in vivo biocompatibility study provides more practical data for clinical applications of our particles.

Synthesis of the Apt@GO@Au-His@a-ZnO@DOX NCs. EGFR antibody is selected as an ideal agent for targeting cancer cells because it has strong correlation with survival of cancer cells (e.g., lung cancers) and disease progression. We prepared the Apt@GO@Au-His@a-ZnO@DOX NCs to target specific cells with PTT and NIR-triggered drug release. DOX was applied as a model drug to check the feasibility of our drug delivery system due to its effective cytotoxic to cancers. For the preparation, the Au-His@a-ZnO NPs was first conjugated with the anti-EGFR Apt using the EDC/NHS coupling method (Figure 2A). Then, Apt@GO@Au-His@a-ZnO@DOX NCs was obtained by simple mixing Apt@GO@Au-His@a-ZnO NCs with DOX for 24 h at room temperature, followed by a washing step to remove unloaded doxorubicin. After the DOX loading, the color of the Apt@GO@Au-His@a-ZnO solution did not change significantly (Figure S9). Apt@GO@Au-His@a-ZnO NCs are designed for three functions: (i) combining with EGFR antibody and targeted specific cancer cell, (ii) PTT by NIR irradiation, and (iii) the controlled-release of dual drugs (Zn$^{2+}$ ions released in acidic endosome of cancer cells and DOX). As such, Apt@GO@Au-His@a-ZnO@DOX NCs are used to target for lung cancer cells where the high level of EGFR receptors are often expressed, and the loaded drugs are released in the acidic microenvironment (Figure 4A).

For the DOX-loaded NPs, we speculated that DOX was loaded onto
Apt@GO@Au-His@a-ZnO NCs by three methods: (i) hydrophobic interaction and π-π stacking between GO with the surface of sp²-bonded carbon nanostructures and DOX with aromatic structures, (ii) the complexation of DOX and Zn²⁺ on NCs, and (iii) electrostatic interaction between the negative charge (COO⁻) of the Apt@GO@Au-His@a-ZnO NCs and the positive charge (-NH₂) on DOX.³² The UV-Vis and PL spectra of DOX and Apt@Au-His@a-ZnO@GO@DOX NCs were displayed in Figure 4B and Figure 4C. Notably, the UV absorption peak of Apt@GO@Au-His@a-ZnO@DOX NCs was closed to 490 nm that responds to the absorption wavelength of DOX. The fluorescence of the free DOX and after Apt@GO@Au-His@a-ZnO@DOX showed the same excitation peaks in independent tests (Figure 4C), indicating the successful DOX loading onto the Apt@GO@Au-His@a-ZnO NCs surface. The UV-Vis spectra of the resulting products were measured to determine the loading efficiency of DOX on the Apt@GO@Au-His@a-ZnO NCs. The drug loading efficiency was evaluated by incubating the Apt@GO@Au-His@a-ZnO NCs with the different DOX concentrations in PBS (pH 7.4). The loading amount of DOX increased progressively with the increasing drug concentrations and could reach a saturation value (250 mg/g) using an initial DOX concentration above 1000 mg/mL (Figure 4D), suggesting the controlled loading profile of DOX on the Apt@GO@Au-His@a-ZnO NCs surfaces. This maximum loading capacity, 250 mg/g, is close to that of aptamer-conjugated carbon hollow nanoshells,⁴⁷ but higher than that of FA-conjugated ZnO or GO nanosheets.⁷,⁴⁸ Moreover, the resulting Apt@GO@Au-His@a-ZnO@DOX NCs exhibited excellent stability in PBS (pH 7.4) for more than 21 days (Figure S9).
Figure 4. (A) The DOX-loaded Apt@Au-His@α-ZnO@GO NCs effectively lead to specific cancer cell death through the synergetic effect of pH/NIR light-triggered DOX release, pH-triggered Zn\(^{2+}\) ions release and heat generation. (B) UV-vis absorption spectra of DOX and Apt@Au-His@α-ZnO@GO@DOX NCs. (C) Fluorescence spectra of DOX and Apt@Au-His@α-ZnO@GO@DOX NCs. (E) The plot of DOX loading amount on Apt@Au-His@α-ZnO@GO NCs versus the drug concentration. (F) Drug release profile of Apt@Au-His@α-ZnO@GO@DOX NCs at different conditions (pH 7.5, pH 5.5, and pH 5.5+NIR) in 24 h.

Cumulative Release Profiles of DOX. In order to evaluate the chemotherapy efficacy, drug release behavior from Apt@GO@Au-His@α-ZnO@DOX NCs was first explored. PBS solutions were chosen to mimic microenvironments of the normal body (pH 7.4) and cancers (pH 5.5), respectively. An 808 nm laser (1.5 W/cm\(^2\)) was
used to mimic PTT conditions. As shown in Figure 4E, the accumulative DOX release rate is 11.6% in PBS pH 7.4, while a release rate 30.8% was observed in PBS solution (pH 5.5). This upsurge release rate at pH 5.5 can be explained by the acidic environment that disrupts the binding forces between DOX and NCs. The application of 808-nm laser caused a fast drug release rate up to 75.4%, and the sharp increases occurred at time points of 0.5, 4, and 16 h (Figure 4E), with the release rate 20.2%, 13.4%, and 12.7%, respectively. This irradiation-accelerated drug release should be due to the rising temperature that speeded up the molecular movement from NCs nucleus during incubation, indicating the ultimate fate of the DOX released from Apt@GO@Au-His@a-ZnO@DOX.23

**Targeting Efficiencies of Apt@GO@Au-His@a-ZnO@DOX NCs.** In addition to release profile, the targeting efficiency of Apt@GO@Au-His@a-ZnO@DOX NCs was next investigated in lung cancer cells, as shown in Figure 5A. To find an optimal cell line for the targeted drug delivery of Apt@GO@Au-His@a-ZnO@DOX NCs, we evaluated the expression of EGFR in both A549 (adenocarcinomic human alveolar basal epithelial) and H522 cells (non-small lung cancer) cell lines. As shown in Figure 5B, a, the expression of EGFR in A549 cell line was higher as compared to H522 cell line. In contrast, EGFR levels in the scrambled control aptamer group (Figure 5B, b) were much lowered in both A549 and H522 cell lines, presumably resulted from non-specific binding. This result agrees with previous studies about the quantity of EGFR in lung cancer cells.19 Furthermore, LSCM was utilized to confirm the EGFR antibody targeted delivery of DOX as shown in Figure 5C. Interestingly, DOX fluorescence in the co-incubated A549 cells was obviously higher for Apt@GO@Au-His@a-ZnO@DOX NCs than GO@Au-His@a-ZnO@DOX NCs, suggesting that GO@Au-His@a-ZnO@DOX NCs was up-taken by A549 cells more inefficiently than Apt@GO@Au-His@a-ZnO@DOX NCs. In addition, the fluorescence intensity of the DOX was quantitatively analyzed by ImageJ software. The fluorescence intensity of the Apt@Au-His@a-ZnO@GO@DOX NCs was much stronger than GO@Au-His@a-ZnO@DOX NCs (Figure 5D), indicating that their
high targeting efficacy to A549 cells. The western blot and LSCM images results provide strong evidence that anti-EGFR aptamer in Apt@GO@Au-His@a-ZnO@DOX NCs account for the high targeting efficacy in cancer cells that contains overexpressing EGFR.

Figure 5. (A) The drug delivery scheme for cellular uptake by EGFR receptor. (B) Comparisons of EGFR levels in A549 and H522 cells (a: anti-EGFR DNA aptamer; b: scrambled control aptamer). Bands are representative of three individuals from each group. Densitometric analysis of western blots (lower location). (C) LSCM images of (a) PBS, (b) GO@Au-His@a-ZnO@DOX NCs, and (c) Apt@GO@Au-His@a-ZnO@DOX NCs at the same concentration of DOX incubated with A549 cells for 1 h. The scale bar: 50 μm. (D) The intracellular fluorescence was analyzed by ImageJ software.

NIR Trigger Drug Release Behavior in A549 Cells. Next, also investigated the in vitro drug release profile by NIR light was investigated. To this end, A549 cells were incubated Apt@Au-His@a-ZnO@GO@DOX NCs for 1 h, followed by removing
nano-carriers and drugs from the incubation fresh cell culture. Subsequently, cell images were taken immediately after irradiation under 808 nm laser (0.5 W/cm², 10 min) by a Nikon LSCM. The released free DOX could be identified by the reoccurred fluorescence, while DOX bound to Apt@GO@Au-His@a-ZnO NCs didn’t show fluorescence due to the quenching by the coordination effect. The LSCM images showed that free DOX molecules could distribute uniformly into the cell cytoplasm regardless of laser irradiation, indicating the irradiation had no impact to free-DOX delivery (Figure 6A). In contrast, DOX release from Apt@GO@Au-His@a-ZnO@DOX NCs could be remarkably enhanced after exposure to the laser irradiation (Figure 6A). This finding encouraged us to further explore the correlation between DOX release (from nanocarriers) and irradiation. Analysis by ImageJ software on LSCM images disclosed the quantitative evidence that NIR laser irradiation promoted drug release from Apt@GO@Au-His@a-ZnO@DOX NCs in cells.

**In Vitro Combination Therapy.** To gain the new insights in therapeutic effect of our nanocarriers, a live/dead assay was carried out by incubating Apt@GO@Au-His@a-ZnO@DOX NCs with cancer cells for 4 h under laser irradiation (808 nm, 1.0 W/cm², 10 min). After proper staining, a control experiment, where cancer cells were incubated without nanocarriers but exposed to the laser irradiation, showed the high cell viability (Figure 6B, a), indicating the irradiation alone had no adverse impact to cell survival. However, adding free DOX into the incubation culture gave rise to more cell death (Figure 6B, b, c). An even lower cell viability was found when Apt@GO@Au-His@a-ZnO@DOX NCs were used under no laser irradiation (Figure 6B, d), suggesting that nanocarriers effectively enhanced the DOX delivery. Interestingly, the cell viability further decreased when the DOX-free nanocarriers, Apt@GO@Au-His@a-ZnO NCs, were applied along with laser irradiation (Figure 6B, e), indicative that the cell death is not only DOX-dependent, but also a synergetic effect from the whole nanocarriers. Surprisingly, this synergetic effect could be clearly testified using only 100 μg/mL.
Apt@GO@Au-His@a-ZnO@DOX NCs under laser irradiation, resulting in almost 100% cell death (Figure 6B, f).

**Figure 6.** (A) LSCM was performed in A549 cells incubated with Apt@GO@Au-His@a-ZnO@DOX NCs (or free DOX) ([DOX] = 25 μM) for 1 h, washed with PBS to remove extracellular nanoparticles, then treated with laser irradiation (808 nm, 0.5 W/cm², 10 min) (L+). Controls (L−) refers to un-irradiated cells. DOX and cell nuclei were represented by red and blue fluorescence, respectively. The scale bar: 50 μm. (B) Fluorescence images of calcein AM/PI-stained A549 cells incubated with various media: (a) laser only, (b) DOX; (c) DOX (L+), (d) Apt@GO@Au-His@a-ZnO@DOX NCs, (e) Apt@GO@Au-His@a-ZnO NCs (L+), and (f) Apt@GO@Au-His@a-ZnO@DOX NCs. (C) Relative viabilities of A549 cells treated with laser only, DOX, DOX (L+), Apt@GO@Au-His@a-ZnO@DOX NCs, Apt@GO@Au-His@a-ZnO NCs (L+), and Apt@GO@Au-His@a-ZnO@DOX NCs. (***p < 0.001, **p < 0.01, or *p < 0.05). The scale bar: 100 μm.

In order to fully explore the potential of photothermal therapy,
Apt@GO@Au-His@a-ZnO@DOX NCs were again incubated with A549 cells for 1 h, followed by irradiation with the 808 nm laser at different power densities for 5 min. After that, MTT assay was performed to incubate the cells for additional 24 h (Figure 6C). It was discovered that Apt@GO@Au-His@a-ZnO@DOX NCs treated cells exhibited remarkably reduced viabilities with increasing laser power intensities, whereas the DOX-treated cells (control) was not significantly affected by laser irradiation in causing cancer cell damage. Simple photothermal heating induced by Apt@GO@Au-His@a-ZnO NCs without chemotherapy, on the other hand, appeared to be much less effective compared to the combination therapy, especially under lower laser powers. Therefore, the synergistic effect caused by photothermal and chemotherapy in the form of NIR-light triggered intracellular drug release make obvious achievements in destructing cancer cells.

**In Vivo Photothermal and Fluorescence Imaging.** In addition to in vitro study, in vivo explorations were carried out on six groups of A549-bearing nude tumor mice (n=5) that were respectively injected with Apt@GO@Au-His@a-ZnO NCs and Apt@GO@Au-His@a-ZnO@DOX NCs via the tail vein irradiated with an 808 nm laser (1.5 W/cm², 5 min) (Figure 7A, B). The tumor temperature under irradiation was monitored using the thermal camera. Five min after the irradiation, tumor temperature was increased to higher than 50 °C in both GO@Au-His@a-ZnO and Apt@GO@Au-His@a-ZnO@DOX treated groups, which is sufficient to cause tumor cells damage in vivo. Interestingly, the use of Apt@GO@Au-His@a-ZnO@DOX NCs resulted in obviously higher temperature than that of GO@Au-His@a-ZnO NCs. It was speculated that anti-EGFR aptamer conjugated GO@Au-His@a-ZnO@DOX NCs prolonged retention time by the tumor passive targeting of the enhanced permeability and retention (EPR) effects and EGFR receptor, which increased the efficiency of Apt@GO@Au-His@a-ZnO@DOX NCs recognized and accumulated into the tumor side. In a stark contrast, the tumor temperature in the control group was only increased by about 2.1 °C, which agrees with the results of water measurements in Figure 2F.
Figure 7. (A) Thermographs of tumor-bearing mice with photothermal treatment for different periods of time (0-5 min). Mice were injected intratumorally with control, Apt@GO@Au-His@a-ZnO NCs, and Apt@GO@Au-His@a-ZnO@DOX NCs. The laser power density was 1.5 W/cm$^2$. (B) The heating curve of the three laser-irradiated groups. (C) The fluorescence images of mice and tissues after injected with DOX, GO@Au-His@a-ZnO@DOX NCs, and Apt@GO@Au-His@a-ZnO@DOX NCs. (D) Major organs were excised 24 h after post-intravenous injection to detect the biodistributions of DOX, GO@Au-His@a-ZnO@DOX NCs, and Apt@GO@Au-His@a-ZnO@DOX NCs.

The long circulation time of drugs can be realized by both EPR effect and increased EGFR receptors, resulting in the improved antitumor efficacy.$^{49,50}$ Owing to the fluorescence nature of DOX, it is feasible to investigate the EPR effect of our nanocarriers in mice using the agile intrinsic laser fluorescence, which can avoid external radio or fluorescence. DOX biodistribution study in A549 tumor-bearing mice was subsequently explored by the whole-body fluorescence imaging technique, and images were taken at interval time after intravenous injection of free DOX, Au-His@a-ZnO@DOX NCs, and Apt@GO@Au-His@a-ZnO@GO@DOX NCs (5 mg/kg equivalent DOX). For free DOX, 24 h after injection, its major fraction was
found in liver, and only a negligible amount of drug was identified in the tumor site. This implies that free DOX was quickly cleared out by kupffer cell (Figure 7C). However, 24 h after injection of GO@Au-His@a-ZnO@DOX NCs, there was clear fluorescence intensity observed in tumor site of mice. More interestingly, using Apt@GO@Au-His@a-ZnO@DOX NCs caused a much pronounced DOX accumulation in the diseased region. A quantitative assessment of DOX concentration in the normal tissue and tumor region supported the conclusion that Apt@GO@Au-His@a-ZnO@DOX NCs presented the best drug delivery system (Figure 7D). For example, the drug accumulation in the tumors using Apt@GO@Au-His@a-ZnO@DOX NCs and GO@Au-His@a-ZnO@DOX NCs was increased by 5.8-fold and 2.7-fold, respectively, compared to free DOX. These results demonstrated that DOX could be delivered effectively to the tumor by the combined EPR effects and EGFR receptor.

**In Vivo Combination Therapy.** The above-mentioned imaging study suggested the high delivery capacity of our nanocarriers, which thus encouraged us to further explore their therapy potential in vivo. For this, A549 tumor-bearing nude mice was investigated using PBS, DOX (L+), DOX, Apt@GO@Au-His@a-ZnO NCs (L+), Apt@GO@Au-His@a-ZnO@DOX NCs, and Apt@GO@Au-His@a-ZnO NCs (L+), respectively. Tumor volumes decreased progressively with treatments of drugs or NIR irradiation while increased in control groups, e.g., using PBS (Figure 8A, B). A pronounced synergetic therapy was found using Apt@GO@Au-His@a-ZnO@DOX NCs (L+), contributing to the completely disappear of tumors while causing no harm to mice lives till the end (Figure S10). This is a clear proof that Apt@GO@Au-His@a-ZnO@DOX NCs (L+) effectively suppress the tumor growth in vivo. Accordingly, experiments of tumor weight (14 days) showed that Apt@GO@Au-His@a-ZnO@DOX NCs (L+) significantly inhibited the proliferation of A549 transplanted cancer (Figure 8C). Besides, the tumor tissues were timely collected and analyzed through hematoxylin and eosin (H&E) staining (Figure 8D). It turned out that Apt@GO@Au-His@a-ZnO@DOX NCs (L+) treatment caused
damage in the cancer cell, such as nuclear condensation, cell shrinkage, and even the corruption of tumor extracellular matrix. Moreover, the tumors treated with DOX (L+), DOX, Apt@GO@Au-His@a-ZnO NCs (L+), Apt@GO@Au-His@a-ZnO@DOX NCs, and Apt@Au-His@a-ZnO@GO NCs (L+) showed the characteristic features like thermal or chemical damage. In particular, abundant pyknosis, coagulative necrosis, and considerable regions of karyolysis inside tumors were found, which was caused by the Zn\textsuperscript{2+} release.\textsuperscript{34} To further determine the combined therapy in terms of Zn\textsuperscript{2+} ions and chemo-photothermal effects in Apt@GO@Au-His@a-ZnO@DOX NCs (L+), TUNEL assay were applied for A549 tumor tissues where apoptosis cells were labeled green fluorescent (Figure 8E). DOX (L+), DOX, Apt@GO@Au-His@a-ZnO NCs (L+), Apt@GO@Au-His@a-ZnO@DOX NCs, and Apt@GO@Au-His@a-ZnO NCs (L+) all enhanced apoptosis. However, Apt@GO@Au-His@a-ZnO@DOX NCs (L+) resulted in mostly destructive apoptosis to A549 tumor tissues.

![Figure 8](image-url)

**Figure 8.** (A) Photos of the A549 tumor-bearing mouse after different treatments: (a) PBS, (b) Apt@GO@Au-His@a-ZnO NCs, (c) DOX, (d) DOX (L+), (e)
Apt@GO@Au-His@a-ZnO@DOX NCs, and (f) Apt@GO@Au-His@a-ZnO NCs (L+), and (g) Apt@GO@Au-His@a-ZnO NCs (L+). The tumor is marked with the red arrows. (B) Relative tumor volume for mice treated with different treatments. (C) The weight of excised tumor. (D) Hematoxylin and eosin (H&E) staining photomicrographs of tumor tissue after different treatments: (a) PBS, (b) Apt@GO@Au-His@a-ZnO NCs, (c) DOX, (d) DOX (L+), (e) Apt@GO@Au-His@a-ZnO@DOX NCs, (f) Apt@GO@Au-His@a-ZnO NCs (L+), and (g) Apt@GO@Au-His@a-ZnO NCs (L+). The scale bar: 100 μm. (E) Apoptosis of A549 cells in tumor tissues after different treatments. The apoptotic cells labeled green fluorescent were evidently identified by TUNEL assay. The scale bar: 100 μm.

**Toxicity Evaluation of Combination Therapy.** Nanotoxicity has drawn a broad spectrum of concern in clinic therapy. Weight loss is considered as the effective index to evaluate the toxicity. As such, we measured the body weight of mice in all groups. As shown in Figure 9B, mice weight between the treatment and negative groups show no significant difference. In addition, no abnormality was identified in the normal tissues and no obvious toxicity of our nanosystem was detected in the organs according to the result of H&E staining (Figure 9A). Besides, organ indices were measured to assess the general toxicity. It is worth mentioning that the increasing organ index is strongly correlated with its strengthened function, congestion, proliferation, or swelling while the decrease presented the opposite changes. The comparison between control and Apt@GO@Au-His@a-ZnO@DOX NCs (L+) groups (Figure 9C) showed that applying Apt@GO@Au-His@a-ZnO@DOX NCs (L+) caused neither toxicity nor any side effects. In vivo long-term tissue biodistribution of Apt@GO@Au-His@a-ZnO@DOX NCs in mice was detected using ICP-MS, as shown in Figure S11. By monitoring the presence of Au element in the tissue and organs, it was found that the NCs could be generally expelled from the body as time prolonged.

Furthermore, the toxicity of Apt@GO@Au-His@a-ZnO@DOX NCs (L+) was determined by biochemical analysis of mice blood. As shown in Figure 9D, E, the
Apt@GO@Au-His@a-ZnO@DOX NCs (L+) group caused no obvious increase in levels of lactate dehydrogenase (LDH) and creatine kinase (CK), which are important parameters of heart function, suggesting that the nanosystem could reduce the side effect of DOX. In addition, comparing with free DOX, the use of Apt@GO@Au-His@a-ZnO@DOX NCs (L+) caused no damage to liver function (aspartate aminotransferase (AST), alanine aminotransferase (ALT)) (Figure 9F, G), and kidney function (creatinine, uric acid (UA)) (Figure 9H, I). Therefore, all toxicity study proved that Apt@GO@Au-His@a-ZnO@DOX NCs (L+) has good biocompatibility, suggesting them as effective and safe nanomedicine to treat lung cancer.

Figure 9. (A) Histopathological analysis of major organs after different treatments: (a) PBS, (b) Apt@GO@Au-His@a-ZnO NCs, (c) DOX, (d) DOX (L+), (e)
Apt@GO@Au-His@a-ZnO@DOX NCs, (f) Apt@GO@Au-His@a-ZnO NCs (L+), and (g) Apt@GO@Au-His@a-ZnO NCs (L+). (B) Mouse weights after irradiation. (C) Comparison of the organ indices of PBS, Apt@GO@Au-His@a-ZnO NCs, DOX, DOX (L+), Apt@GO@Au-His@a-ZnO@DOX NCs, Apt@GO@Au-His@a-ZnO NCs (L+), and Apt@GO@Au-His@a-ZnO NCs (L+), injected after 14 days of inoculation. Blood biochemistry analysis of the indices of (D) LDH, (E) CK, (F) AST, (G) ALT, (H) Creatinine, and (I) UA in mice treated with PBS, Apt@GO@Au-His@a-ZnO NCs, DOX, DOX (L+), Apt@GO@Au-His@a-ZnO@DOX NCs, Apt@GO@Au-His@a-ZnO NCs (L+), and Apt@GO@Au-His@a-ZnO NCs (L+) at 14th days of treatment, respectively.

CONCLUSION

In summary, we have successfully prepared multifunctional NCs, Apt@GO@Au-His@a-ZnO@DOX NCs that have combined functions as enhanced targeting delivery and chemo-photothermal therapy. In the designed nanocomposites, anti-EGFR-aptamer was responsive for the targeting delivery, whereas AuNPs, and GO nanosheets generated heat upon NIR irradiation. By loading the chemical drugs of doxorubicin, the NPs enabled to function as the dual drug system where toxic Zn$^{2+}$ ions released in the acidic tumor sites. All these features contribute the Apt@GO@Au-His@a-ZnO@DOX NCs with synergetic therapeutic effects including targeted-tumor accumulation, NIR absorbance, photothermal performance, as well as biocompatibility for the normal cells. Furthermore, in vivo studies show that our NCs could significantly inhibit tumor growth without apparent toxicity to in nude mice model. Therefore, on a broader perspective, these successful demonstrations open new avenues in the field of nanomedicine where a plateau of other hieratical nanostructured materials can be developed using functional building blocks and drug sources for the combined therapies in cancer treatment.

ASSOCIATED CONTENT
The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxxx. A detailed description of experimental methods and Figures S1–S11 (PDF)

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ACKNOWLEDGEMENT

The authors gratefully acknowledge the support of this work by Jiangsu province science and technology support plan (BE2015367), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and the Jiangsu Collaborative Innovation Center of Biomedical Functional Materials.

REFERENCES


(11) Liang, X.; Shang, W.; Chi, C.; Zeng, C.; Wang, K.; Fang, C.; Tian, J. Dye-Conjugated Single-Walled Carbon Nanotubes Induce Photothermal Therapy


Biomaterials 2015, 45, 81-92.


(27) Zhang, M.; Wang, W.; Cui, Y.; Zhou, N.; Shen, J. Magnetofluorescent Carbon


(31) Zhou, Y.; Fang, X.; Gong, Y.; Xiao, A.; Xie, Y.; Liu, L.; Cao, Y. The Interactions Between ZnO Nanoparticles (NPs) and α-Linolenic Acid (LNA) Complexed to BSA Did not Influence the Toxicity of ZnO NPs on HepG2 cells. *Nanomaterials* **2017**, *7*, 91.


(49) Liu, B.; Zhang, X.; Li, C.; He, F.; Chen, Y.; Huang, S.; Lin, J. Magnetically Targeted Delivery of DOX Loaded Cu$_9$S$_5@mSiO$_2@Fe$_3$O$_4$-PEG Nanocomposites for Combined MR Imaging and Chemo/Photothermal Synergistic Therapy. *Nanoscale*
Multifunctional Nanocomposites for Targeted, Photothermal, and Chemotherapy

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The multifunctional Apt@GO@Au-His@a-ZnO@DOX NCs were prepared successfully for tumor enhanced targeting delivery and chemo-photothermal therapy.