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Target-specific near-IR induced drug release and photothermal therapy with accumulated Au/Ag hollow nanoshells on pulmonary cancer cell membranes

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ABSTRACT

Au/Ag hollow nanoshells (AuHNSs) were developed as multifunctional therapeutic agents for effective, targeted, photothermally induced drug delivery under near-infrared (NIR) light. AuHNSs were synthesized by galvanic replacement reaction. We further conjugated antibodies against the epidermal growth factor receptor (EGFR) to the PEGylated AuHNS, followed by loading with the antitumor drug doxorubicin (AuHNS-EGFR-DOX) for lung cancer treatment. AuHNSs showed similar photothermal efficiency to gold nanorods under optimized NIR laser power. The targeting of AuHNS-EGFR-DOX was confirmed by light-scattering images of A549 cells, and doxorubicin release from the AuHNSs was evaluated under low pH and NIR-irradiated conditions. Multifunctional AuHNS-EGFR-DOX induced photothermal ablation of the targeted lung cancer cells and rapid doxorubicin release following irradiation with NIR laser. Furthermore, we evaluated the effectiveness of AuHNS-EGFR-DOX drug delivery by comparing two drug delivery methods: receptor-mediated endocytosis and cell-surface targeting. Accumulation of the AuHNS-EGFR-DOX on the cell surfaces by targeting EGFR turned out to be more effective for lung cancer treatments than uptake of AuHNS-EGFR-DOX. Taken together, our data suggest a new and optimal method of NIR-induced drug release via the accumulation of targeted AuHNS-EGFR-DOX on cancer cell membranes.

1. Introduction

Effective drug delivery by nanocarriers has been intensively investigated using dendrimers [1,2], polymeric micelles [3], liposomes [4,5], carbon-based materials [6–8], magnetic particles [9,10], and noble metal nanoparticles (NPs) [7,11]. Nanocarriers were developed to protect drugs from premature degradation and to enhance drug absorption into specific tissues. Compared to organic carriers, which only serve as a drug reservoir, novel metal

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NPs have many advantages and multifunctionality, due to their unique optical properties and the various surface functional groups that can be introduced.

Near-infrared (NIR) resonant nanomaterials such as Au nanorods (GNRs) [7,12,13], multi-branched particles [14,15], nanoshells [16], and hollow-shells (HSs) [11,17] are attractive therapeutic agents because they can deliver drugs via non-invasive hyperthermia. NIR light in the range of 700–900 nm has been widely used to locally raise cell temperature, thereby optimizing the photothermal effect [18].

Triggered drug release by an external stimulus such as NIR irradiation could significantly advance cancer treatment by overcoming the indiscriminate drug distribution observed in conventional drug delivery. The combination of photothermal therapy (PTT) and chemotherapy, termed chemo-thermotherapy [17], can achieve enhanced anti-cancer efficacy via synergistic effects.

Previous reports have shown that nanocarriers are internalized by cells through endocytosis. However, these nanocarriers were not specifically targeted to cancer cells [17,19,20]. Drug carriers that specifically target cancer cells are advantageous, because they selectively kill cancer cells with minimal side effects, particularly when combined with controlled drug release. Thus, we sought to develop novel drug-loaded nanocarriers that could reach the desired cancer cells or tissues via selective tumor cell targeting [21,22].

We chose Au/Ag hollow nanoshell (AuHNS) to develop a novel, multifunctional nanocarrier for selective chemo-thermotherapy. The hollow nanoshells (HNSs) are advantageous for chemo-thermotherapy, because they are simply synthesized by galvanic replacement reactions from Ag nanoshells (NSs) [17,23]. Furthermore, the hollow vacancy allows for high drug loading, due to the large effective surface areas for drug attachment [24]. In addition, in contrast to GNR, cytotoxic surfactants such as cetyltrimethyl ammonium bromide (CTAB) are not used during the preparation of HNS structures [17,23,25].

In this study, we report a novel, multifunctional therapeutic agent-doxorubicin-loaded, anti-EGFR antibody-conjugated and PEGylated Au/Ag nanoshells (AuHNS-EGFR-DOX) which can selectively target cancer cells and kill them via photothermal-induced drug release. In this system, AuHNS emits a strong surface plasmon resonance (SPR) band in the NIR region for photothermal drug releasing, EGFR is a marker allowing for specific cancer cell targeting [26–28], and doxorubicin is a chemotherapeutic agent [4,17,24,29]. Targeting was confirmed by light-scattering images of lung cancer cells, and drug release was evaluated under low pH and NIR irradiation conditions.

Additionally, we developed a novel approach to maximize the ability of multifunctional NPs to kill lung cancer cells by evaluating two drug delivery mechanisms: receptor-mediated endocytosis and the accumulation of targeted AuHNS-EGFR-DOX on the cell membranes. In previous reports, NIR irradiation-induced drug release was shown to be dependent on endocytosis [17,19,20,30]. Our data suggest a new and optimal method of NIR-induced drug release, via the accumulation of targeted AuHNS-EGFR-DOX on cancer cell membranes.

2. Experimental section

2.1. Materials

Tetraethylorthosilicate (TEOS), 3-mercaptopropyl trimethoxysilane (MPTS), ethylene glycol (EG), poly(vinyl pyrrolidone) (PVP, Mw ~40,000), silver nitrate ($AgNO_3$, 99.99 + %), octylamine (OA), tetrachloroaurate trihydrate ($HAuCl_4$, 99.9 + %), doxorubicin, *N*-hydroxysuccinimide (NHS), and *N*-(3-dimethyl aminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. PEG coated GNR (GR750-PG-1 mL) were purchased from Nanocos (New York, USA). Absolute ethanol (99.8%) was purchased from Carlo Erba (Milano, Italy). Methoxypoly(ethylene glycol)sulfhydryl (m-PEG-SH, Mw 5000) was purchased from Sunbio (Anyang, Korea). Poly(ethylene glycol)-2-mercaptoethyl ether acetic acid (HOOC-PEG-SH, Mw 5000) was purchased from Creative PEG-Works (Winston Salem, NC, USA). Ammonium hydroxide (NH_4OH, 27%), sodium chloride, and ethanol (98%) were purchased from Daejung (Busan, Korea). Deionized (DI) water was used for all experiments.

2.2. Cell culture

A549 (adenocarcinomic human alveolar basal epithelial cells) and H522 cell lines (non-small lung cancer cells) were obtained from Korean cell line bank (Seoul, Korea). A549 cell line was grown in F-12 medium and H522 cell line was grown in RPMI-1640 medium. The media for A549 and H522 cell lines were supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (GibcoBRL, Grand Island, NY, USA). The cells were kept at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Preparation of Au/Ag hollow nanoshells (AuHNSs)

Tetraethylorthosilicate (TEOS, 1.6 mL) was dissolved in 40 mL of absolute ethanol, followed by addition of a 3 mL portion of ammonium hydroxide (~27%). The resulting mixture was vigorously stirred using a magnetic bar for 20 h at room temperature. The resulting silica NPs were centrifuged and washed with ethanol several times to remove the excess reagents. The resulting silica NPs were then functionalized with the thiol group. Silica NPs (300 mg) were dispersed in 6 mL of ethanol containing 300 μL of MPTS and 60 μL of ammonium hydroxide (27%). The mixture was stirred for 12 h at room temperature, after which the MPTS-treated silica NPs were centrifuged and washed with ethanol several times. In order to synthesize Ag nanoshells (AgNSs), 2 mg of MPTS-treated silica NPs were dispersed in 25 mL of ethylene glycol containing PVP (5 mg), followed by the addition of a 25 mL of $AgNO_3$ solution in ethylene glycol (final concentration of $AgNO_3$ was 3.5 mM). Next, 41.3 μL of octylamine (5 mM) was rapidly added to the NP solution, which was stirred for 1 h at room temperature. The resulting AgNSs were centrifuged and washed with ethanol several times for purification. To form hollow structure via galvanic replacement reaction, 100 μL aliquot of AgNS (10 mg/mL) was dispersed in 5 mL of PVP (8 wt%) aqueous solution. Next, 0.1 mM of $HAuCl_4$ solution was loaded into a plastic syringe with PVC tubing, and was placed on a syringe pump, which was used to add $HAuCl_4$ solution (4.5 mL) to the AgNS dispersion at a rate of 0.75 mL/min at 110 °C. After adding the $HAuCl_4$ solution, the reaction was allowed to stabilize the Au/Ag hollow nanoshells (AuHNSs) for 10 min, after which the dispersion was cooled to room temperature with vigorous magnetic stirring. The resulting mixture was washed with a saturated solution of NaCl to remove residual AgCl, centrifuged and washed with DI water (×3) and ethanol (×3). In order to confer biocompatibility and functionality, the AuHNS surface was grafted with two types of PEG derivative depending on the further modification. For conjugation with EGFR antibody, the AuHNS surface was modified with HOOC-PEG-SH solution (2 mM in ethanol). The PEG grafted AuHNS (AuHNS-COOH) dispersions were washed with ethanol several times by centrifugation and then resuspensed in 0.1 M of phosphate buffered saline (PBS pH 7.0).

2.4. Preparation of AuHNS-EGFR antibody

After the activation of carboxylic acid group with 2 mM of EDC and 5 mM of NHS in 0.1 M of PBS (pH 6.0), 20 μg portion of the antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to the pre-activated PEGylated AuHNS dispersion. The mixture was shaken for 2 h at room temperature. The EGFR antibody-conjugated AuHNSs were rinsed with 0.1 M of PBS (pH 7.0) several times.

2.5. Loading of doxorubicin on AuHNS

Doxorubicin was loaded into AuHNS or AuHNS-EGFR (1 mg/mL) by mixing the NPs with a doxorubicin solution (1 mg/mL) in PBS (pH 7.0, 0.1 M, 1 mL). The mixture was gently shaken at room temperature for 24 h in the dark. Free doxorubicin was removed by washing with PBS (pH 7.0) several times by centrifugation.

To determine the amount of doxorubicin loading, the calibration curve was obtained based on the absorbance at 480 nm using various concentrations of doxorubicin dissolved in 0.1 M of PBS (pH 7.0). The amount of doxorubicin loading in NPs was then estimated by the absorbance of doxorubicin-loaded AuHNS or AuHNS-EGFR.

2.6. Characterization of AgNS and AuHNS

Energy-Filtering Transmission Electron Microscope (EF-TEM) images of AgNS and AuHNS were obtained using a LIBRA 120 (Carl Zeiss, Germany). UV-Vis-NIR spectra were collected with a UV-Vis-NIR spectrophotometer (Optizen 2120 UV, Mecasys Co. Ltd., Daejeon, South Korea). Cross-sectional scanning electron microscope (SEM) images of AgNS and AuHNS were obtained with dual beam focused ion beam (FIB) microscope (Helios 550 NanoLab DualBeam, FEI Corporate, Oregon, USA). Prior to obtain the imaging with SEM, a sample stage was tillted and the AgNS and AuHNS particles were milled with a Ga^+ ion beam.