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Gold cluster-labeled thermosensitive liposmes enhance triggered drug release in the tumor microenvironment by a photothermal effect

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ABSTRACT

Stimulus-triggered drug release based on the liposomal drug delivery platform has been studied vigorously to increase drug release at the target site. Although the delivery system has been developed, an effective carrier system is needed to achieve effective therapeutic efficacy. Therefore, we focused on the development of gold cluster bound thermosensitive liposomes (G-TSL), which are capable of triggered drug release when stimulated by external near-infrared (NIR) irradiation in the tumor microenvironment. The size of doxorubicin (DOX)-loaded G-TSL (DOX/G-TSL) was 171.5 ± 8.3 nm, and the efficiency of DOX encapsulation was up to 90%. The release of DOX from DOX/G-TSL was increased 70% by NIR irradiation (1.50 W/cm^2 for 0.5 min) compared to non-gold-coated TSL. Consequentially, the gold cluster on the TSL enabled the light-controlled DOX release through the photothermal conversion of the energy of NIR-absorbed light, leading to membrane destabilization. Cell cytotoxicity of DOX/G-TSL was also increased by their NIR irradiation-triggered DOX release compared to non-NIR-irradiated DOX/G-TSL. In addition, we demonstrated the therapeutic efficacy of DOX/G-TSL against the MDA-MB-231 tumor model. The NIR-irradiated DOX/G-TSL treatment showed greater therapeutic efficacy than that of the non-NIR-irradiated DOX/G-TSL and control ($p < 0.05$). Taken together, DOX/G-TSL has the potential for remote-triggered drug release upon stimulation with NIR irradiation in the tumor microenvironment, and may be applied to a broad range of photothermal-based disease therapies.

1. Introduction

Liposome based drug delivery systems hold great potential for cancer therapy and are being used widely because of their particular capacity and biocompatibility characteristics [1–4]. Although PEGylated formulations increase the circulation time of liposomes [5,6], further improvement of their therapeutic efficacy is needed because of the slow and decreased drug release from liposomes in the tumor microenvironment [7]. Therefore, many of the current strategies focus on liposomes sensitive to stimuli, such as pH [8,9], temperature [10,11], high intensity focused ultrasound (HIFU) [12,13], and microwaves (MWs) [14,15], to enhance drug release. In particular, thermosensitive liposomes (TSL) have received considerable attention to increase drug release by external hyperthermia. However, it is still difficult to achieve effective drug release at the desired site, and the clinical applications of TSL often are limited because of weak drug release from liposomes, which may affect drug resistance and tumor cell malignancy [2,16].

Recently, a photothermal approach using gold nanoshells [17], gold nanorods [18], and gold nanocages [19] was used to achieve effective therapeutic efficacy of cancer treatment. These gold nanoparticles absorb light in the near-infrared (NIR) region (700–900 nm) and thus can present NIR irradiation-induced photothermal effects [20]. However, these approaches showed limited drug encapsulation into the inner area of the gold nanoparticles. Therefore, the in vivo performance of photothermal therapy (PTT) was not effective because of the low drug accumulation in the gold nanoparticles.

To overcome these limitations, we sought to combine TSL with a photothermal approach using a gold cluster and NIR irradiation to achieve a potential synergistic drug release effect at the target site. NIR irradiation-triggered drug release strategies have been applied to carrier systems using polymer nanoparticles [21–23] but have been insufficiently reported for liposomal systems [24]. To improve the NIR-based photothermal approach, we developed a NIR-sensitive TSL system by binding gold clusters, which absorb light in the NIR region and expose heat energy from the absorbed light, to the liposomal

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surface. The TSL membrane can be destabilized over the phase transition temperature of the lipid composition. Through the photothermal effect it can act synergistically with the gold cluster on the liposomal surface to trigger drug release from the TSL (Fig. 1).

Here, we demonstrated that the gold cluster-bound liposomal system (G-TSL) achieved an effective drug release in the tumor microenvironment by external NIR irradiation with synergistic therapeutic efficacy. In this study, DOX/G-TSL showed potential for remote-triggered drug release by NIR irradiation, which could be used for a broad range of therapeutic applications for various diseases.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (MPPC), and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-mPEG-2000) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Doxorubicin hydrochloride (DOX), citric acid, Sephadex G-50, gold (III) chloride solution, and L-ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), penicillin–streptomycin, and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco BRL/Life Technologies (New York, NY, USA). All other materials were of analytical grade and used without further purification.

2.2. Preparation of TSL

TSL were prepared by the thin film hydration method [10,25]. Briefly, the composition of lipids (DPPC:MPPC:DSPE-mPEG2000 = 79.5:6.5:14 by weight ratio) was dispersed in chloroform and dried onto a round bottom flask using a rotary evaporator under vacuum (Buchi Rotavapor R-210, Flawil, Switzerland). The lipid film was hydrated at 43 °C with 300 mM citric acid buffer (pH 4). The liposomal solution was sonicated to reduce the size of the liposomes and was passed through a Sephadex G-50 column to remove the free lipid and the drug. The liposomal preparations were stored at 4 °C.

Gold coating on the liposomal surface was carried out by plasmon resonance coating methods [26,27]. The gold (III) chloride solution (65 μL of 100 mM) was added to 1 mL of the liposomal solution and was gently stirred. Then, the ascorbic acid solution (97.5 μL of 500 mM) was subsequently added to the liposomal solution. Upon an abrupt color change from white to dark blue, the absorbance spectra were measured using a UV–visible spectrophotometer (Optizen 2120UV, Mecasys, Deajeon, Korea).

After gold coating the TSL surface, DOX was loaded into the G-TSL by the remote loading method using a pH gradient [28]. Briefly, DOX was added to the G-TSL at a 10:0.3 lipid:drug weight ratio, and the mixtures were incubated at 29 °C overnight. The mixtures were subsequently passed through a Sephadex G-50 column to remove free DOX. The final DOX concentration was determined by lysis of the liposome with a chloroform–methanol mixture (8:2, v/v) and by fluorescence spectrophotometry (Ex: 490 nm, Em: 590 nm) (F4500, HITACHI Co., Tokyo, Japan) [16,29]. The size and zeta potential of the G-TSL were measured by light scattering with a particle size analyzer and Zeta Plus (Brookhaven Instrument Co., CA, USA), respectively. The morphology of the G-TSL was observed by cryogenic scanning electron microscopy (cryo-SEM). In addition, the phase transition temperature of the TSL and G-TSL was measured by differential scanning calorimetry (DSC, Q1000 V9.9 Build 303, TA Instruments, New Castle, DE 19720).

2.3. Au content assay

Gold binding to the liposomal surface was confirmed using energy dispersive X-ray spectroscopy (EDS, Quantax 200 Energy Dispersive X-ray Spectrometer, Bruker, Berlin, Germany). The liposomes were freeze-dried as a solid form required for EDS analysis, and the samples were bombarded with a focused beam of electrons for localized chemical analysis. In addition, the amount of Au on the liposomal surface was measured by ionizing the sample with inductively coupled plasma (ICP) spectroscopy (iCAP 6500 duo Inductively Coupled Plasma-Atomic Emission Spectrometer, Thermo Scientific, Cambridge, United Kingdom) followed by mass spectrometry (MS) to separate and quantify the generated ions.

2.4. Release of DOX from G-TSL

Prior to measuring the drug release from G-TSL with or without NIR irradiation, we first examined the morphology of TSL and G-TSL before and after NIR irradiation by cryo-TEM. We next measured the triggered release of DOX from G-TSL at the physiological body temperature

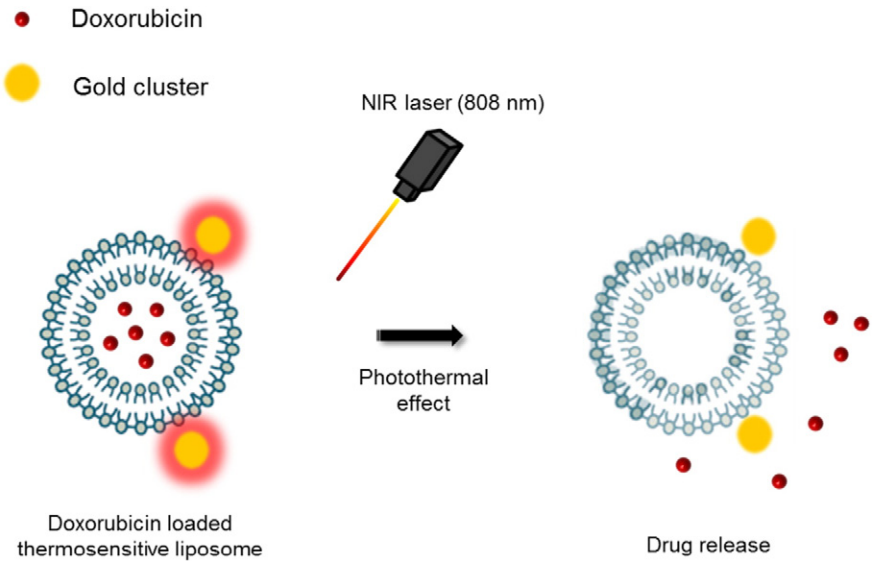

Fig. 1. Schematic illustration of the reaction of gold cluster-bound thermosensitive liposomes (G-TSL) with near infrared (NIR) irradiation.