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Development of light-driven gas-forming liposomes for efficient tumor treatment

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ABSTRACT

In this study, we report a gas-forming liposome containing 1H-1H-2H-perfluoro-1-hexene (PFH) and gold nanoparticles (AuNPs). These liposomes were rapidly destabilized by external near-infrared (NIR) light, as a result of the vaporization of PFH influenced by an AuNPs-mediated photothermal event. In particular, these liposomes showed the triggered release of the encapsulated D-(KLAKLAK)$_2$ peptide drug. The experimental results demonstrated that the light-irradiated liposomes significantly enhanced *in vitro* photothermal and D-(KLAKLAK)$_2$ peptide-mediated cell death of CAOV-3 tumor cells.

1. Introduction

A principal challenge for antitumor drug delivery systems is to suitably deliver drugs and control drug release rates to achieve highly efficient drug therapeutics (Wu et al., 2008). Significant progress in tumor therapy has been made as a result of the use of nano-sized carriers that release therapeutic drugs temporally and spatially in response to external stimuli or biological signals (Kwag et al., 2015; Kwon et al., 2015; Park et al., 2016; Wu et al., 2008; Youn et al., 2016). Among these drug carriers, liposomes have been evaluated as a potent drug vesicle for delivering chemical or biological drug molecules and have been widely used because of their large capacity for drug encapsulation and their biocompatible properties (Kwag et al., 2015). Furthermore, numerous studies have focused on engineering liposomes to exhibit stimulus-responsive properties (*e.g.*, responsiveness to pH, temperature, and enzymes) (Agarwal et al., 2011; Ahn et al., 2009; Garg and Kokkoli, 2011; Nii and Ishii, 2005; Park et al., 2006; Pornpattananangkul et al., 2010; Volodkin et al., 2009). It is also interesting to note that liposomes encapsulating volatile compounds, such as perfluorocarbon (PFC) and 1H-1H-2H-perfluoro-1-hexene (perfluorohexene: PFH), have been designed to satisfy the need for the development of liposomal ultrasound contrast agents that can efficiently generate microbubbles in tumor tissues (Duarte et al., 2014; Duncanson et al., 2014; Fang et al., 2009; Hoang et al., 2016; Kagan et al., 2012; Kopechek et al., 2013). These liposomes generate the microbubbles as a result of the gradual vaporization of volatile compounds in the body (Duncanson et al., 2014; Kagan et al., 2012). Importantly, the ultrasound treatment for these liposomes accelerates the generation of microbbles, resulting in the triggered release of encapsulated drugs (Kopechek et al., 2013; Yan et al., 2013).

In this study, we encapsulated PFH, gold nanoparticles (AuNPs), and D-(KLAKLAK)$_2$ proapoptotic peptide into the liposomes to develop light-driven gas-forming liposomes. It is known that AuNPs have strong light absorbing properties, converting light irradiation to vibrational energy to elevate ambient temperatures (Lee et al., 2014b; Wu et al., 2008). Furthermore, extremely small-sized lipophilic-coated AuNPs have sufficient lipophilicity and can be easily entrapped in the lipid bilayer (Park et al., 2006; Rasch et al., 2010). The D-(KLAKLAK)$_2$ disintegrates mitochondrial membranes and induces cell death by mitochondrial-dependent apoptosis (Lee et al., 2011). We used the D-(KLAKLAK)$_2$ proapoptotic peptide as a model drug (Kim et al., 2011; Ko et al., 2009; Lee et al., 2011). Here, we hypothesized that AuNPs trapped in a lipid bilayer would generate sufficient heat energy to vaporize PFH in liposomes. As a result, the vaporization of PFH by light irradiation is expected to trigger D-(KLAKLAK)$_2$ proapoptotic peptide release (Fig. 1), allowing for the expansion of application of liposomes into various tumor sites in which a local laser probe can be positioned.

We preferentially investigated light-induced liposomal destabilization, the peptide release behaviors, the photothermal

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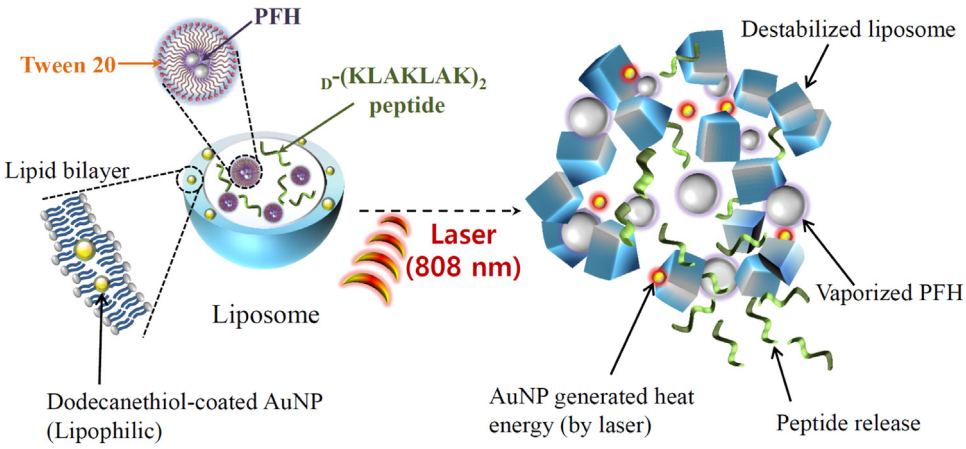

Fig. 1. Schematic concept of the light-driven gas-forming liposomes.

activity of AuNPs and the tumor cell cytotoxicity of D-(KLAKLAK)$_2$ proapoptotic peptide against CAOV-3 tumor cells.

2. Materials and methods

2.1. Materials

$HAuCl_4$, dodecanethiol, tetraoctylammonium bromide, toluene, sodium borohydride, sulfuric acid, chloroform, phosphatidylcholine (PC), 1H-1H-2H-perfluoro-1-hexene (PFH), acetone, dimethyl sulfoxide (DMSO), paraformaldehyde, and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) were obtained from Sigma-Aldrich (USA). Tween 20 was purchased from Daejung Inc. (Korea). ICP100-HCl was obtained from JKC (Korea). D-(KLAKLAK)$_2$ and D-(KLAKLAK)$_2$ tagged with tetramethylrhodamine (D-(KLAKLAK)$_2$-TAMRA, >95% purity) were purchased from Peptron, Inc. (Korea). A BCA protein assay kit was purchased from Thermo Scientific Inc. (USA). Minimum Essential Medium (MEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), penicillin, trypsin, ethylenediaminetetraacetic acid (EDTA), and streptomycin were purchased from Welgene Inc. (Korea). Mitotracker® was purchased from Invitrogen Inc. (USA). A Cell Counting Kit-8 (CCK-8) was obtained from Dojindo Molecular Technologies Inc. (Japan).

2.2. AuNP preparation

To prepare dodecanethiol-coated AuNPs, $HAuCl_4$ (300 mg) dissolved in deionized water (30 mL) was vigorously mixed with tetraoctylammonium bromide (2 g) dissolved in toluene (80 mL) for 2 h. After removing the water phase, 25 mL of aqueous sodium borohydride (0.4 M) was slowly added to the organic phase (toluene). After stirring the solution for 24 h, the organic phase was extracted and washed using first 0.1 M sulfuric acid and then deionized water. The solution was mixed with excess dodecanethiol (2400 μL) for 2 h. The toluene in the solution was evaporated, resulting in the production of a dried powder. The obtained powder was dispersed in chloroform and ultracentrifuged at 25,000 rpm for 600 s to separate the AuNPs from the unreacted chemicals. Next, the dried precipitates were again dissolved in chloroform (10 mg/mL), and then stored at 4 °C (Lee et al., 2014b).

2.3. Liposome characterization

PC (20 mg) and AuNPs [4 mg or 0 mg (as a control)] dissolved in chloroform (15 mL) were added to a round-bottom flask. The solvent in the round-bottom flask was removed using a rotary evaporator (EYELA, N-1000, Fisher Scientific Inc., USA) (Kwag et al., 2015). The resulting thin PC and AuNP film was rehydrated with 150 mM PBS (pH 7.4, 8 mL) containing PFH nanoemulsions [15 mg or 0 mg (as a control), which were emulsified in an 80/20 vol. (%) solution of PBS/tween 20 (150 mM, pH 7.4) after vigorously vortexing at 4000 rpm] and D-(KLAKLAK)$_2$ peptide [2 mg or 0 mg (as a control)] using a sonicator (60 Hz for 5 min) at 25 °C, finally yielding the liposomes [hereafter denoted as Lipo, Lipo-P (with PFH), Lipo-G (with AuNPs), and Lipo-GP (with both PFH and AuNPs), Table 1].

The loading efficiency of PFH in the liposomes was measured using a Lambda 1050 UV–vis spectrophotometer (Perkin Elmer, ISA) at 210 nm after extracting PFH in a supernatant solution from the liposomes centrifuged at 4000 rpm for 20 min (Chu et al., 2013; Kwag et al., 2015; Pornpattananangkul et al., 2010). The AuNPs trapped in the liposomes were measured using an ICP-MS (ICAP Q, Thermo Scientific Inc., USA) (Lee et al., 2014b; Rengan et al., 2015). Next, to evaluate the loading efficiency of the D-(KLAKLAK)$_2$ peptide, the liposomes were dissolved in a 99/1 (vol.%) solution of DMSO/PBS (150 mM, pH 7.4) to prepared the stock solution (Lee et al., 2011). The resulting solution was used for the BCA protein assay test in PBS solution. The loading efficiency (%) for the PFH, AuNPs, or D-(KLAKLAK)$_2$ peptide was defined as the weight percentage of PFH, AuNPs, or D-(KLAKLAK)$_2$ peptide trapped in the liposomes relative to the initial amount of D-(KLAKLAK)$_2$ peptide, PFH, or AuNPs. The loading content (%) of PFH, AuNPs, or D-(KLAKLAK)$_2$ peptide was calculated as the weight percentage of D-(KLAKLAK)$_2$ peptide, PFH, or AuNPs in the liposomes (Kwag et al., 2014; Lee et al., 2011, 2014b).

2.4. Characterization of liposomes

The particle size distribution of liposomes (0.25 mg/mL, PBS) irradiated with an 808 nm near-infrared (NIR) light was measured

Table 1
Characterization of liposomes.

Liposomes	Loading content (wt.%)			Loading efficiency (wt.%)		
	AuNP	PFH	Pepetide	AuNP	PFH	Pepetide
Lipo	–	–	–	–	–	–
Lipo-P	–	39.1 ± 0.2	7.7 ± 0.2	–	91.2 ± 0.6	85.4 ± 1.8
Lipo-G	9.6 ± 0.2	–	8.0 ± 0.2	47.7 ± 1.2	–	85.5 ± 2.3
Lipo-GP	9.0 ± 0.1	36.7 ± 2.0	7.8 ± 0.2	45.1 ± 0.1	85.7 ± 4.7	85.5 ± 2.6