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Combined photodynamic and antibiotic therapy for skin disorder via lipase-sensitive liposomes with enhanced antimicrobial performance

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ABSTRACT

A lipase-sensitive singlet oxygen-producible and erythromycin-loaded liposome (LSSPL) was developed for combination antibacterial therapy for skin disorder. The LSSPL was synthesized by coating pullulan-pheophorbide a (PU-Pheo A) conjugates onto erythromycin-loaded liposomes composed of 1,2-dipalmitoyl-sn-phosphatidylcholine (DPPC) and cholesterol. Lipase activity was chosen as the environmental-stimulus for the controlled release of erythromycin and Pheo A from LSSPL because skin inflammation-inducing *Propionibacterium acnes* (*P. acnes*) secrete extracellular lipases. The presence of *P. acnes* lipases disrupted LSSPLs by selective cleavage of their ester linkages, liberating erythromycin and Pheo A. Along with the antibacterial effect of erythromycin, additional laser irradiation onto Pheo A further achieved the inhibition of *P. acnes* growth and treatment of *P. acnes*-infected inflammation in nude mice back skin. Therefore, antimicrobial therapy, using a stimulus-responsiveness moiety, presents a feasible way to treat bacteria-induced skin disorders.

1. Introduction

Acne is a common chronic skin disorder that affects adults and adolescents. It can significantly lead to psychological abnormalities and social problems [1–4]. Various factors have been identified as causes of acne, including excess sebum secretion, disturbed keratinization within the follicle, and duct colonization with *Propionibacterium acnes* (*P. acnes*) [5]. *P. acnes* is the predominant inhabitant of follicles of sebum-rich regions in human skin. The lipase secreted by *P. acnes* is associated with the pathogenesis of acne by hydrolyzing sebaceous triglycerides into free fatty acids. The released free fatty acids lead to skin inflammation by stimulating the follicular epithelium and causing follicular rupture [6,7]. Various antibiotics and therapeutic agents, such as tetracycline, erythromycin, benzoyl peroxide, clindamycin, and tretinoin, have been used to treat *P. acnes* [8–12]. However, antibiotic resistance of bacteria and side effects have become significant problems [13–15].

Recently, antimicrobial photodynamic therapy (aPDT) has attracted attention as a non-invasive therapeutic modality for treating bacterial infections. aPDT employs a light source, oxygen, and photosensitizer (PS). Light irradiation onto the PS generates reactive oxygen species (ROS), such as free radical and singlet oxygen, which are toxic to living organisms. ROS interact with cellular components and cause critical damage to DNA and membranes, resulting in bacterial cell death [16–19]. Most of the developed PSs have been found to be non-invasive therapeutic agents. However, for clinical use, their hydrophobic nature, low solubility, and lack of target specificity should be improved [20–22]. To overcome these drawbacks, PSs were delivered to their targets using polymer conjugates, lipid-based nanoparticles, polymeric nanoparticles, and polymeric micelles [23–27].

We developed a lipase-sensitive singlet oxygen-producible and erythromycin-loaded liposome (LSSPL) to treat *P. acnes*-infected inflammatory skin dermis (Fig. 1). First, erythromycin-loaded liposomes were synthesized by the thin film hydration method using 1,2-dipalmitoyl-sn-phosphatidylcholine (DPPC), cholesterol, and erythromycin. Second, introduction of a singlet oxygen production functionality was achieved by surface coating with pullulan-pheophorbide a (PU-Pheo A) conjugates. Pullulan was chosen owing to its biocompatible and biodegradable properties, high solubility, and the ease of chemical substitution. Pheo A was chosen as a PS because of its high stability and wide applicability in various

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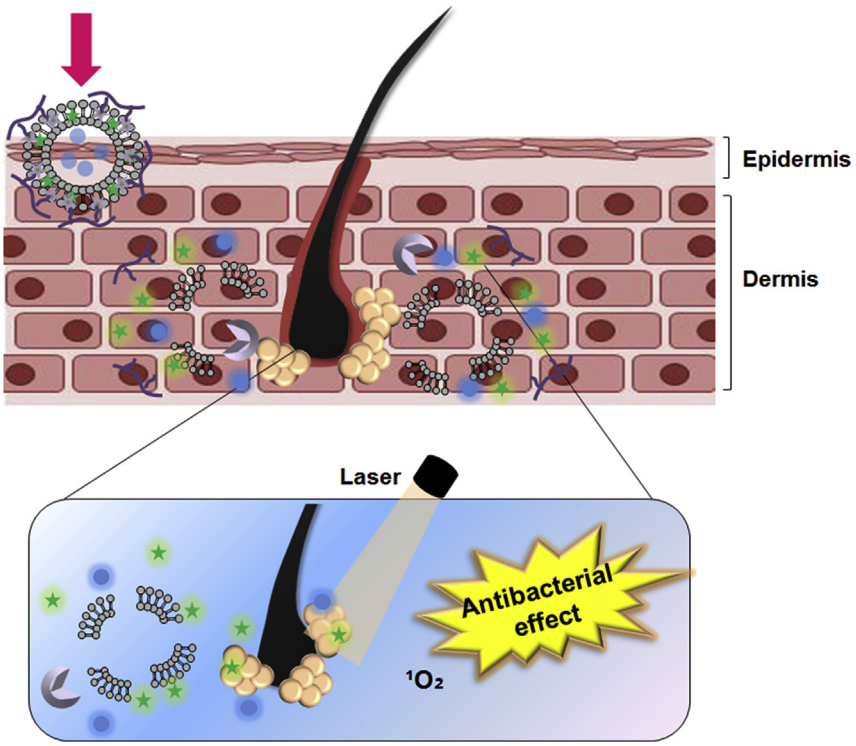

Fig. 1. Schematic representation of the lipase-sensitive singlet oxygen producible liposome (LSSPL). *P. acnes* lipases in the inflammatory skin dermis induce disruption of the LSSPLs, resulting in the escape of trapped antibiotics and surface-coated photosensitizers. Laser irradiated photosensitizers produce singlet oxygen. Combined antibacterial effects can be achieved by singlet oxygen and antibiotics.

diseases. The structure of LSSPL was designed to be disrupted by *P. acnes* lipases. Lipases specifically break the ester bonds in DPPC as well as between PU and Pheo A. Disruption of LSSPL leads to a distribution of the free forms of erythromycin and Pheo A in the surrounding environment. In addition to the erythromycin-mediated antimicrobial effects, laser irradiation onto Pheo A further enhances the antimicrobial effect via singlet oxygen production. Consequently, LSSPL can be efficiently used for combination therapy for *P. acnes* and *P. acnes*-infected inflammatory skin dermis.

2. Materials and methods

2.1. Materials

PU (number-average molecular weight of 100,000) was purchased from the Hayashibara Company (Okayama, Japan). Erythromycin, *Candida rugosa* lipase (700 units/mg), Pheo A, 1,3-dicyclohexyl carbodiimide (DCC), 4-dimethylaminopyridine (DMAP), DPPC, cholesterol, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Chloroform and dimethylsulfoxide (DMSO) was purchased from Junsei Chemical (Tokyo, Japan). Dialysis membranes (molecular weight cut off (MWCO): 1,000, 3500 and 12,000–14,000) were obtained from Spectrum Laboratories, Inc. (Rancho Dominguez, CA, USA). A singlet oxygen sensor green (SOSG; S-36002) was purchased from Molecular Probes, Inc. (Eugene, OR, USA). *P. acnes* (ATCC®6919™) and WI-38 human fibroblast (ATCC® CCL-75™) were purchased from ATCC. Dulbecco's modified Eagle's medium (DMEM) was purchased from Thermo Scientific Hyclone (Utah, USA). Fetal bovine serum (FBS) and antibiotic solution (penicillin/streptomycin) were purchased from Gibco (Grand Island, NY, USA).

2.2. Synthesis of PU-Pheo A conjugate

To prepare PU-Pheo A conjugates, PU (50 mg) and Pheo A (10 mg) were dissolved in DMSO (10 ml) separately. Then, DMAP and DCC were added to the Pheo A solution as a catalyst and coupling reagent, respectively, at a molar ratio of 1:1:1.5. After 4 h, the Pheo A solution was dropped into the PU solution and stirred for 2 days. Unreacted materials were removed by dialysis using a membrane with 12,000–14,000 MWCO for 3 days. Purified PU-Pheo A conjugates were lyophilized, and 500 MHz ^{1}H NMR spectroscopy (Bruker, Germany) was used to evaluate the conjugation between PU and Pheo A.

2.3. Preparation and characterization of the liposomes

Liposomes were prepared by the thin film hydration method. First, only erythromycin-loaded liposomes (OELLs) were prepared. DPPC (10 mg), cholesterol (1.5 mg), and erythromycin (1 mg) were dissolved in chloroform (5 ml). Then, the solution was transferred to a round-bottom flask and evaporated at 40 °C to produce thin films. The thin films were then hydrated using 1 mM ammonium sulfate (5 ml) and sonicated for 3 min to obtain OELLs. Second, to prepare LSSPLs, the PU-Pheo A conjugates (1 mg/ml of Pheo A) were added dropwise to OELLs and stirred overnight. The prepared LSSPL dispersion was lyophilized with sucrose. Sucrose was used as a lyoprotectant to increase the stability and integrity of LSSPLs during the lyophilization process [28]. A UV/Vis spectrophotometer (UV-2450, Shimadzu, Japan) was used to quantify the concentrations of erythromycin and Pheo A in 1 mg of LSSPLs. Erythromycin and Pheo A were detected at 215 and 671 nm, respectively. In addition, as a control group, only PU-Pheo A coated liposomes (OPCLs) were prepared by dropping PU-Pheo A conjugates onto DPPC-cholesterol liposomes.