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Photochemical tissue penetration via photosensitizer for effective drug penetration in a non-vascular tumor

Daehong Min [a, 1], Dooyong Jeong [a, 1], Myung Gyu Choi [b], Kun Na [a, *]

[a] Center for Photomedicine, Department of Biotechnology, The Catholic University of Korea, 43 Jibong-ro, Wonmi-gu, Bucheon-si, Gyeonggi-do 420-743, Republic of Korea
[b] Division of Gastroenterology, Department of Internal Medicine, College of Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, 222, Banpo-daero, Seoucho-gu, Seoul 137-701, Republic of Korea

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ABSTRACT

To improve the tissue penetration efficiency ($PE_{\%}$) of hydrophilic-drugs in non-vascular drug eluting stents (DES), we designed photochemical tissue penetration (PTP) invested DES (PTP-DES). The PTP technology was applied to the stent as a covering membrane to generate singlet oxygen. Singlet oxygen damages the epithelial layer, so the $PE_{\%}$ of released drugs could be improved. To prepare the PTP-DES membrane, chlorin e6 (Ce6, photosensitizer) was incorporated in a gemcitabine (GEM) eluting polyurethane (PU) membrane (Ce6–GEM–PU). Ce6–GEM–PU has smooth surface that is ~40 μm thick. The photoactivity of Ce6 was maintained for 2 weeks (*in vitro* GEM releasing period). In a separate cell culture system, both 1.5 folds higher $PE_{\%}$ and an improved tumor cell growth inhibition effect were shown after light exposure. Additionally, in tissue penetration experimental system, 2 folds increased in the $PE_{\%}$ of GEM was induced by laser exposure at 80 J/cm^2. Additionally, improved $PE_{\%}$ of hydrophilic molecules (Fluorescein and GEM) was confirmed in colon tumor bearing mice. Consequentially, tumor growth, when implanted with Ce6–GEM–PU, was effectively inhibited without significant side effects. Based on these results, we believe that the PTP-DES system has great potential for improving the therapeutic effect of conventional DES.

1. Introduction

A non-vascular drug eluting stent (DES) is a biomedical device that is implanted in the gastrointestinal (GI) tract for stabilizing the flow of body fluids when obstructive symptoms are shown due to tumor growth [1–3]. Many of the early reports revealed that non-vascular DES contributes to decrease in malignant obstructive cells and a reduction in tumor size [4,5]. In an unresectable GI tract obstruction, implantation of non-vascular DES is the treatment of choice because other therapeutic methods such as intravenous chemotherapy or radiotherapy have been restricted due to very poor response [6,7]. For these reasons, the implantation of non-vascular DES has been known to be the most efficient local treatment method as it has the advantages of granting high local concentrations of the anticancer drug at the implantation site with lower systemic toxicity [8].

Recently, gemcitabine (GEM) eluting membrane covered stents have been developed and highlighted because of the strong therapeutic effect of GEM in GI tumor therapy [9,10]. However, GEM has a low tissue penetration efficiency ($PE_{\%}$) in the GI tract because of its hydrophilicity and the epithelial barrier function [11,12]. Actually, the structure of the epithelia protects the tissues from the external environment and acts as a selective barrier to inhibit the diffusion of drugs [13]. Barrier function of the epithelial layer against chemotherapy is an important factor for the limited tissue penetration of GEM against a GI tumor [14,15]. For this reason, some cells of a GEM treated tumor can continue to proliferate and allow tumor growth [16,17].

Indeed, the barrier alteration has been known as a common method to improve drug penetration in the pharmaceutical field [18]. The barrier alteration leads to decreased density of the epithelial layer and improved drug penetration [19]. From these previous researches, we believe that an alteration of the epithelial layer is required for improving drug penetration.

* Corresponding author. Tel.: +82 2 2164 4832; fax: +82 2 2164 4865.
E-mail address: kna6997@catholic.ac.kr (K. Na).
[1] These authors contributed equally to this work.

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With this, we assumed that both increased $PE_{\%}$ of the eluted GEM and improved therapeutic effect of the DES could be achieved by photochemical tissue penetration (PTP) technology. The PTP technology is based on the light activation of photosensitizer. The activated photosensitizer generates singlet oxygen ($^1O_2$), and then reduces the epithelial density [20–22]. Besides, this light triggered local treatment has been shown to be an effective and safe strategy for reducing tissue density [23–25]. Inspired by these observations, we designed a PTP technology invested non-vascular DES (PTP-DES) for improving the $PE_{\%}$ of GEM into the GI tumor (Scheme 1).

To prepare the PTP-DES system, chlorin e6 (Ce6, photosensitizer) was incorporated in the gemcitabine (GEM) eluting polyurethane (PU) membrane (Ce6–GEM–PU). Ce6 can be incorporated in implantable biomaterials because it absorbs near infrared (NIR) light without significant toxicity [26,27]. Through this method, we expected that this PTP-DES system could provide an opportunity for improving drug penetration and the therapeutic effect.

In this study, the characteristics of the Ce6–GEM–PU membrane such as surface morphology, drug contents, drug releasing behavior and photoactivity were confirmed. Additionally, the improvement of the $PE_{\%}$ of eluted GEM was confirmed via *in vitro* and *in vivo* experiments. Finally, the therapeutic potency of the membrane was demonstrated using colon cancer bearing mice.

2. Materials and methods

2.1. Materials

Gemcitabine (GEM) hydrochloride was purchased from TCI Chemical (Tokyo Chemical Industry, Tokyo, Japan). Chlorin e6 (Ce6) was purchased from Frontier Scientific, Inc. (Salt Lake City, UT, USA). Polyurethane (PU, Pellethane 2363 80A, Lubrizol) and Metallic stents were supplied by Taewoong Medical Co. (Goyang-si, Korea). Tetrahydrofuran (THF) was purchased from Junsei Co. (Tokyo, Japan). DMEM medium, RPMI 1640 medium, fetal bovine serum (FBS), antibiotics (penicillin and streptomycin), trypsin and Dulbecco's phosphate buffer saline (DPBS) were purchased from Gibco RBL (Invitrogen Corp., Carlsbad, CA, USA). 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazoliumbromide (MTT) and 9,10-dimethylanthracene (DMA) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Preparation of Ce6–GEM–PU

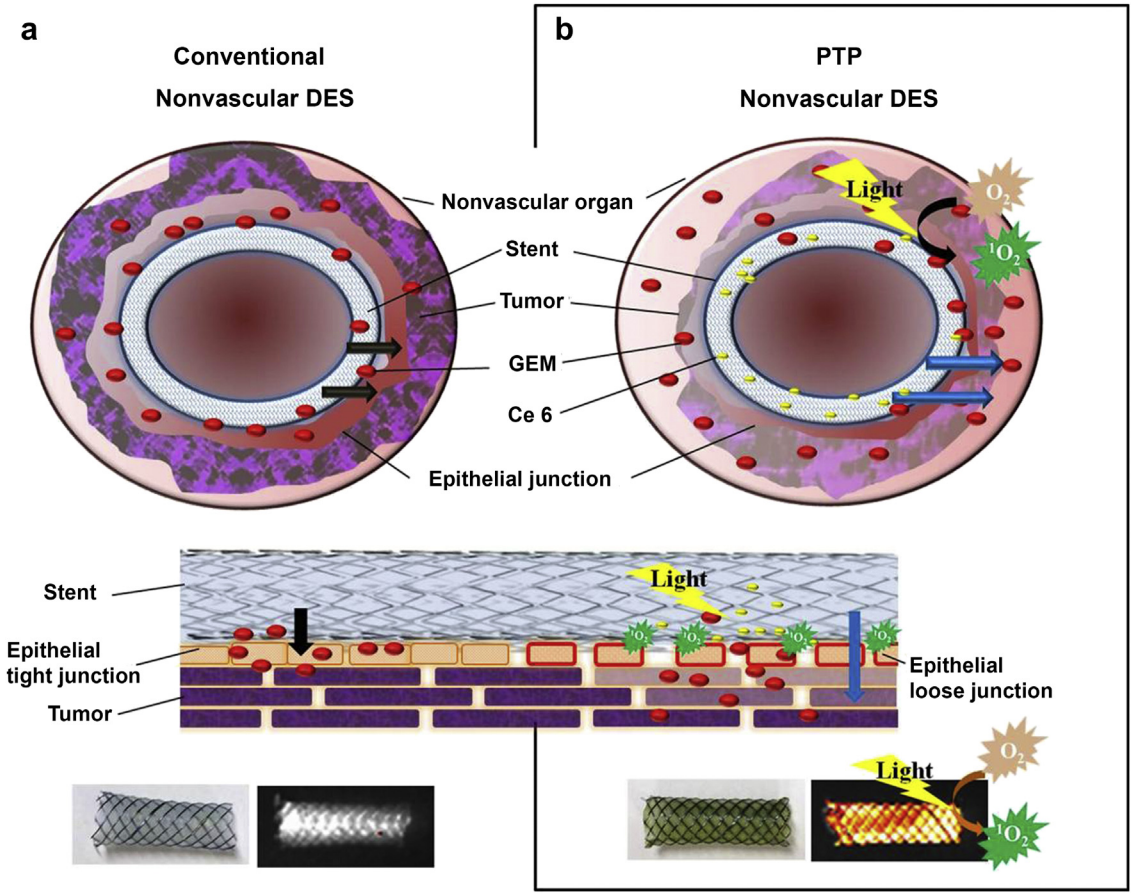
27 mg of GEM and 5 mg of Ce6 were dissolved in 1 mL of 50% ethanol and then added to 1 mL of THF. The GEM·Ce6 containing solution was sonicated (Ultra-turrax T-25®; IKA, Germany) for 2 min to make a homogenous solution. This solution was gently added to 8 mL of PU solution (500 mg of PU in 8 mL of THF) with magnetic stirring. To prepare the membranes, 200 μL of the above mixed solution was poured into the 1.76 cm^3 sized (d: 1.5 cm, h: 0.01 cm) polytetrafluorethylene (Teflon) mold and dried for 12 h at room temperature.

2.3. Characterization of membranes

The thickness of each membrane was determined by a micro-meter (Mitutoyo, Kawasaki, Japan). The drug content was analyzed after the GEM extraction process. The drugs in each membrane were quantified by UV-visible spectrophotometer (UV-2450; Shimadzu, Kyoto, Japan) at 268 nm.

2.4. Observation of the surface morphology

The surface morphologies of each membrane were observed with Field emission-scanning electron microscopy (FE-SEM, Hitachi S-4800; Tokyo, Japan). Each membrane was sliced into small pieces, mounted, sputter coated with platinum using an ion coater and then observed at an accelerating voltage of 10 kV. Additionally, each membrane was sectioned into slices that were 5 μm thick using a freezing microtome (cryotome FSE; Thermo scientific, USA). The sectioned



Scheme 1. Schematic illustration of the difference between Conventional DES and PTP-DES in non-vascular organs, (a) Conventional DES, the released GEM of DES cannot penetrate deep into the tumor tissue because of the epithelial tight junctions, (b) PTP-DES, the released GEM of light responsive-DES can penetrate deep into the tumor tissue by inducing damage to the epithelial junction.