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Phospholipid-chitosan hybrid nanoliposomes promoting cell entry for drug delivery against cervical cancer

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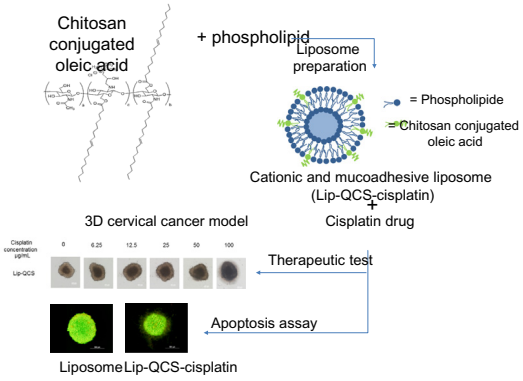
GRAPHICAL ABSTRACT



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ABSTRACT

This study emphasizes the development of a novel surface modified liposome as an anticancer drug nanocarrier. Quaternized N,O-oleoyl chitosan (QCS) was synthesized and incorporated into liposome vesicles, generating QCS-liposomes (Lip-QCS). The Lip-QCS liposomes were spherical in shape (average size diameter 171.5 ± 0.8 nm), with a narrow size distribution (PDI 0.1 ± 0.0) and zeta potential of 11.7 ± 0.7 mV. *In vitro* mucoadhesive tests indicated that Lip-QCS possesses a mucoadhesive property. Moreover, the presence of QCS was able to induce the cationic charge on the surface of liposome. Cellular internalization of Lip-QCS was monitored over time, with the results revealing that the cell entry level of Lip-QCS was elevated at 24 h. Following this, Lip-QCS were then employed to load cisplatin, a common platinum-containing anti-cancer drug, with a loading efficiency of 27.45 ± 0.78% being obtained. The therapeutic potency of the loaded Lip-QCS was investigated using a 3D spheroid cervical cancer model (SiHa) which highlighted their cytotoxicity and apoptosis effect, and suitability as a controllable system for sustained drug release. This approach has the potential to assist in development of an effective drug delivery system against cervical cancer.

1. Introduction

Development of effective nanocarriers for drug delivery has become an important factor dictating advances in the field. Several

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types of nanocarriers having novel features aiding drug delivery to specific target sites, and to overcome biological barriers, have been designed [1,2]. The advanced nanocarriers devised especially for tumor treatment include those that are stimuli-responsive, are active targeting, and have site-specific triggers for localized drug delivery [3,4]. Many lipid and polymer-based nanoparticles have been studied as the basis for nanocarriers, and liposomes, neosomes, chitosan, poly (lactic-*co*-glycolic acid), poly-L-lysine and hybrid classes of these have been investigated to this end. Among lipid-based nanocarriers, liposomes are considered as the most conventional for use as drug carriers due to their practicality and biocompatibility, and the ability to regulate drug release. Additionally, both hydrophilic and hydrophobic drug cargo systems can be encapsulated within liposomes. However, liposomes have some limitations being that they are rapidly cleared and suffer from non-specific targeting, therefore, surface modification of these remains a key strategy for tailoring these for therapeutic use. In the polymeric nanocarrier class, chitosan is widely used in drug delivery as it is naturally occurring and biocompatible, allowing non-parenteral routes of delivery (nasal, ophthalmic, buccal, vaginal, transdermal and pulmonary) [5,6]. The outstanding advantage of chitosan over others is its excellent mucoadhesive properties, which occur via the opening of epithelial tight junctions resulting in transient permeability of mucosal barriers [7,8]. Therefore, chitosan is considered as a mucosal penetration enhancer for drug delivery applications [9,10].

Mucus membranes lining cavities and canals in the respiratory, digestive and urogenital tracts act as barriers to noninvasive drug administration routes. Drugs need to pass though mucosal surfaces to avoid hepatic first pass metabolism before reaching their target sites. Therefore, fabricating novel drug delivery systems to overcome these issues is a major theme of research in medical science. Mucus membranes have been recently highlighted as a focal point for targeted delivery in mucus-associated diseases [11,12]. A mucoadhesive nanocarrier designed for tumor therapy showed potential, with the drug-carrier system being able to pass through mucosal membranes such as pollen-mimetic porous microspheres [13] and PVA-coated PLGA [14]. The advantages of this type of cancer treatment are most obvious in cervical cancer, as the endocervix and exocervix have extensive amounts of mucosal lining [15]. Most invasive cervical cancers are induced by Human Papilloma Virus (HPV) type 16 or 18, which are the high-risk types [16]. HPV infection in keratinocytes at the basal layer of the mucosal cervix epithelium causes abnormal cells to cover the opening of the cervix muscular canal where the mucous membranes of the vagina and cervix meet. Therefore, mucous membranes are a potential target site for drug delivery against cervical cancer since the location of the cervix allows ease of access and the vagina is a favorable site for both local and systemic delivery [17,18]. Recently, Yang et al. (2014) demonstrated that incorporating PVA into polystyrene particle resulted in enhanced mucoadhesion [14]. This experiment demonstrated the use of a surfactant to formulate the drug carrier, and investigated the transport of nanoparticles in human cervicovaginal mucus. Berginc et al. (2014) developed mucoadhesive liposomes for vaginal delivery of curcumin. An *in vitro* model of vaginal mucus was developed allowing monitoring of curcumin permeability under conditions mimicking the vaginal environment [19]. This study showed that coating the liposomes with bioadhesive polymers chitosan and carbopol significantly increased the level of curcumin permeability, resulting in a superior drug delivery formulation over conventional vaginal delivery systems.

Cisplatin is a platinum-based anti-cancer drug which induces cytotoxicity by interfering with transcription and/or DNA replication. The interaction of cisplatin and DNA forms platinum-DNA adducts, resulting in the activation of several signal transduction pathways involved in apoptosis [20]. Although cisplatin has been selected as a primary drug for treating cervical cancer, it has some significant drawbacks. Firstly, cisplatin has been proved to be extremely toxic [21] such that treatment often induces severe side effects including nephrotoxicity, neurotoxicity, ototoxicity, nausea and vomiting [22]. Additionally, as cisplatin is a low molecular-weight drug the retention time in tumor tissue is very short and it can be easily circulated causing damage to normal cells [23]. Resistance to cisplatin can also develop [20,24]. Several approaches have been developed to reduce the side effects of cisplatin such as using it in mixed drug formulations, and chemical modification of the cisplatin structure to produce less toxic analogs [25,26]. Efficient targeting of cisplatin to the required tissue would greatly enhance treatment effectiveness; directly targeting the tumor tissue using specific nano-drug delivery systems would prevent some of the aforementioned problems.

This research aimed to prepare and investigate the therapeutic effects of mucoadhesive liposomes containing cisplatin for cervical cancer treatment. The modification of phospholipids through chemical conjugation with chitosan generates quaternized N,O-oleoyl chitosan (QCS) for incorporation in liposome. The QCS-incorporated liposome (Lip-QCS) is a hybrid of polymer and lipid-based drug delivery system. The core of Lip-QCS is considered as a lipid-based delivery system which has fast and high endocytosis capacity. Therefore, our system is designed to be an active and biocompatible nanocarrier which possesses a mucoadhesive property as well. Encapsulation of cisplatin within Lip-QCS results in high therapeutic efficiencies. This approach is applicable for the development of an effective drug delivery system for local or topical administration against cervical cancer which is a mucus-associated disease. The purposed system is able to be further incorporated into cervix and vaginal delivery formulations such as gels [27], fibers [12], tablets [28] or creams [29] to increase the drug retention of cisplatin and promote active drug internalization into the target tissue.

2. Materials and methods

2.1. Materials

Phosphatidylcholine of soybean origin (Epikuron 200) was purchased from Cargill (Hamburg, Germany) which contains phosphatidylcholine about 92%. Chitosan (Mw of 600 kDa) was purchased from Seafresh Chitosan Lab, Thailand. Triton X-100 was supplied by Merck (Darmstadt, Germany). Phosphate buffer saline pH 7.4 (PBS) used in this study contained the following concentrations: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4; 2 mM KH_2PO_4. Minimum Essential Medium-Alpha (MEM-α) was supplied by GIBCO Invitrogen (NY, USA). *cis*-Diamineplatinum(II) dichloride (cisplatin) was purchased from Sigma-Aldrich (MO, USA). Fetal bovine serum (FBS) was obtained from Biochrom AG (Berlin, Germany). Trypsin–EDTA, L-glutamine, penicillin G sodium, streptomycin sulfate, and amphotericin B were obtained from Invitrogen Corp. (NY, USA). Mucin type III extracted from porcine stomach was obtained from Sigma-Aldrich (MO, USA). CellTiter-Glo Luminescent Cell Viability kits were purchased from Promega (WI, USA). SiHa (HPV type 16 positive cells) were purchased from American Type Culture Collection (ATCC number HTB-35). ApoLive-Glo™ Multiplex kits were obtained from Promega (WI, USA).

2.2. Synthesis of quaternized N,O-oleoyl chitosan (QCS)

To synthesize QCS, two chemical modification steps were performed. N,O-oleoyl chitosan was first synthesized, and then