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Theranostic liposomes of TPGS coating for targeted co-delivery of docetaxel and quantum dots

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ABSTRACT

The aim of this work was to develop a new type of D-alpha-tocopheryl polyethylene glycol 1000 succinate mono-ester (TPGS) coated multi-functional (theranostic) liposomes, which contain both docetaxel and quantum dots (QDs) for cancer imaging and therapy. Non-targeting and folate receptor targeting TPGS coated theranostic liposomes were prepared by the solvent injection method and characterized for their particle size, polydispersity, zeta potential, surface chemistry and drug encapsulation efficiency. MCF-7 breast cancer cells of folate receptor overexpression were employed as an *in vitro* model to assess cellular uptake and cytotoxicity of the drug and QDs loaded liposomes. The mean particle size of the non-targeting and the targeting liposomes was found to be 202 and 210 nm, respectively. High resolution field emission transmission electron microscopy (FETEM) confirmed the presence of quantum dots in the peripheral hydrophobic membranes of the liposomes. The qualitative internalization of multi-functional liposomes by MCF-7 cells was visualized by confocal laser scanning microscopy (CLSM). The IC50 value, which is the drug concentration needed to kill 50% cells in a designated time period, was found to be 9.54 ± 0.76, 1.56 ± 0.19 and 0.23 ± 0.05 μg/ml for the commercial Taxotere®, non-targeting and targeting liposomes, respectively after 24 h culture with MCF-7 cells. The targeting multi-functional liposomes showed greater efficacy than the non-targeting liposomes and thus great potential to improve the cancer imaging and therapy.

1. Introduction

Liposomes are lipid bilayer vesicles that were first prepared in the 1960s. Now, they are used for drug delivery to enhance solubility, permeability and stability of the drug and thus improve its pharmacokinetics and biodistribution [1–3]. Passive and active drug targeting can also be realized by liposomes with enhanced permeability and retention (EPR) and targeting ligand conjugation [3,4]. However, the main disadvantage of liposomes for drug delivery is their instability and short half-life in the blood circulation [5–8].

D-alpha-tocopheryl polyethylene glycol 1000 succinate mono-ester (TPGS) is a PEGylated vitamin E, which can greatly improve the pharmaceutical properties of vitamin E and thus has been widely applied in the food and drug industry. TPGS, prepared from the esterification of D-alpha-tocopheryl acid succinate and polyethylene glycol 1000 (PEG 1000), is an amphiphilic vitamin E, quite stable under normal conditions without hydrolysis. Owing to its hydrophilic-lipophilic balance (HLB) value being between 15 and 19, TPGS has excellent water solubility and it is suitable to serve as an effective surfactant, which can emulsify hydrophobic molecules [9,10]. The co-administration of TPGS has been shown to enhance the solubility, inhibit P-glycoprotein mediated multi-drug resistance, and increase the oral bioavailability of anti-cancer drugs [11–14]. Moreover, TPGS has been found to be an excellent emulsifier in the preparation of nanoparticles of biodegradable polymers such as poly (D, L, lactide-co-glycolide) (PLGA) [15]. TPGS can also be used as a component of new biodegradable copolymers of more desired HLB such as polylactide-TPGS (PLA-TPGS) for nanoparticle formulation of anti-cancer drugs [16]. As an effective emulsifier, TPGS can greatly enhance the performance of nanoparticles, resulting in much higher emulsification efficiency (67 times higher than polyvinyl alcohol) [17], drug encapsulation efficiency (up to

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100%) [17], cellular uptake, and *in vitro* cancer cell cytotoxicity, and more desirable *in vivo* pharmacokinetics (up to 360 h effective treatment for one shot *i.v.* administration) [18,19].

A landmark in liposome development was the invention of stealth liposomes, *i.e.* long circulating liposomes by PEGylation of the liposomes to increase the stability of the liposomes in the blood circulation. PEGylation was originally defined as conjugation of a bioactive molecule or polymer to polyethylene glycol (PEG) to enhance its solubility, permeability and stability. PEGylation can avoid quick recognition and elimination of liposomes by the immune system and thus prolong the circulation of liposomes in the body. An alternative or further development of PEGylation is conjugation of a bioactive molecule or polymer to TPGS. Recently, we have prepared conventional, PEG coated and TPGS coated liposomes. The cellular uptake and *in vitro* cytotoxicity of the liposomes were also assessed on brain cancer cells in comparison. Results of TPGS coated liposomes showed great advantages *in vitro* than PEG coated liposomes [20]. In one study, pharmacokinetic results of doxorubicin loaded TPGS coated liposomes in rats revealed that TPGS coated liposomes (based on the concentration of doxorubicin in plasma) have 24 h longer circulation time than PEG coated liposomes [21]. Further studies also showed the feasibility of forming TPGS containing liposomes, which showed improvement in the permeation of dextran through Caco-2 cells (Transwell® model) without any cytotoxicity effect [22,23]. Nevertheless, their investigation was focused only on oral drug delivery for better permeability and stability across the gastrointestinal (GI) tract [24].

Theranostic liposomes are generally designed to facilitate simultaneous imaging and therapy. The imaging agent such as quantum dots can be entrapped within the hydrophobic core or linked covalently to the surface of the liposomes and the therapeutic agent can be either encapsulated in the lipophilic core or embedded in the lipophilic bilayer shell. The liposomes can then be further conjugated with molecular probe for targeting. Such multi-functional liposomes may circulate for prolonged periods in the blood, evading host defenses, and gradually release drug by targeting and simultaneously facilitate *in vitro* or *in vivo* imaging [24,25]. Currently, quantum dots have become a popular imaging agent used in the multi-functional nanoparticles as they have significant advantages over organic dyes [26]. Furthermore, quantum dots emit highly intense signals, and they are photostable [27]. For example, Yang et al. have developed folate receptor targeted liposomes with quantum dots loaded, and their targeted cellular imaging properties are reported for the future cancer imaging applications [28]. Folate receptor targeting approach may also be suitable for a more specific liposomal drug delivery to cancer cells, which can prevent liposomes from being reached to normal cells. Cancer cells that vastly overexpress the folate receptor showed significant cellular uptake of folate targeted liposomes, while normal cells which do not express the folate receptor show less cellular uptake. Recently, differential expression of the folate receptor has been exploited to target liposomes to cancer cells [28,29]. It was reported that liposomes can be efficiently targeted to the folate receptor bearing tumor cells without any steric hinderence introduced by the PEG coating when conjugated to folate via a long PEG spacer, *e.g.,* 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-2000] (DSPE-mPEG-FA) [29].

We aim in this research to develop TPGS coated, folic acid-conjugated theranostic liposomes for targeted co-delivery of quantum dots and docetaxel to improve the cancer diagnosis and therapy. Docetaxel used as a model drug and quantum dots used as a model imaging agent are formulated in targeting (*i.e.* with folic acid conjugation) or non-targeting (*i.e.* with no folic acid conjugation) liposomes, which were investigated in close comparison. The multi-functional liposomes with or without targeting function were prepared by the solvent injection method, which were then characterized for their size and size distribution, surface morphology, surface charge, surface chemistry, drug encapsulation efficiency and drug release profile. Fluorescent quantum dots loaded multi-functional liposomes were studied for qualitative cellular uptake by folate receptor expressing MCF-7 cells. *In vitro* cytotoxicity of folate receptor expressing MCF-7 cells was assessed and the IC50 value, which is the drug concentration needed to kill 50% cancer cells in the designated period for example in 24 h, was assessed to evaluate the therapeutic effects of the multi-functional liposomes with or without targeting function.

2. Materials and methods

2.1. Materials

Docetaxel of purity 99.56% was purchased from Jinhe Bio-Technology Co. Ltd (Shanghai, China). Organic quantum dots (cadmium selenide quantum dots in the size range of 5–10 nm) dispersed in Decane (Qdot® 655 ITKTM, catalog number Q21721MP) were purchased from Invitrogen™ Corporation (Singapore). Before being used, quantum dots were treated according to the directions described by the manufacturer. 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) was acquired from Avanti Polar Lipids (Alabama, USA). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) was generous gift from Lipoid GmbH (Ludwigshafen, Germany). D-α-tocopheryl polyethylene glycol 1000 succinate mono-ester (TPGS) $C_{33}O_5H_{54}$ $(CH_2CH_2O)_{23}$ was from Eastman chemical company (Kingsport, TN, USA). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-2000] (DSPE-mPEG-FA) was synthesized by carbodiimide chemistry as previously reported [29]. Poly(ethylene glycol)-2000 bis-amine (PEG bis-amine), folic acid, cholesterol, acetone, methanol, ethanol, phosphate buffered saline (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), penicillin–streptomycin solution, trypsin-EDTA solution and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tween 80 was from ICN Biomedicals, Inc. (OH, USA). Fetal bovine serum (FBS) was purchased from Gibco Life Technologies (AG, Switzerland). Dulbecco's Modified Eagle's Medium (DMEM) was from Invitrogen Corporation. MCF-7 cells were provided by American Type Culture Collection. Clinical formulation Taxotere® was supplied by Aventis Pharmaceuticals, USA. All solvents such as acetonitrile, methanol and ethanol were of high performance liquid chromatography (HPLC) grade. All chemicals were used without further purification. Millipore water was prepared by a Milli-Q Plus System (Millipore Corporation, Branford, USA).

2.2. Preparation of TPGS coated multi-functional liposomes

TPGS coated multi-functional liposomes with or without folate targeting were prepared according to the solvent injection method [20,21]. In brief, docetaxel, DPPC, cholesterol, and TPGS (for non-targeting liposomes) plus DSPE-mPEG-FA (for targeting liposomes) were dissolved in 0.3 ml of ethanol at 60 °C, according to the formulae (Table 1). In addition, 0.2 μM of quantum dots (dispersed in THF as 1 μM/ml concentration) were also added in the ethanol system in both of the preparation. The mixture was then injected into 2.7 ml of 1 mM phosphate buffered saline (PBS), pH

Table 1
Formulas of liposomes.

Batches	Lipid compositions	Molar ratio	Weight ratio (mg)	Docetaxel (mg)	Quantum dots
DTX-QD	DPPC:Cholesterol:TPGS	8:7.7:1	86:43:22	1.0	0.2 μM
DTX-QDFA	DPPC:Cholesterol:TPGS: DSPE-mPEG2000-FA	8:7.7:0.9:0.1	86:43:19.8:4.7	1.0	0.2 μM

DTX: Docetaxel.
DTX-QD: Docetaxel loaded liposomes prepared with quantum dots.
DTX-QDFA: Docetaxel loaded liposomes prepared with quantum dots loading and folate targeting.