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Carbon dot/TAT peptide co-conjugated bubble nanoliposome for multicolor cell imaging, nuclear-targeted delivery, and chemo/photothermal synergistic therapy

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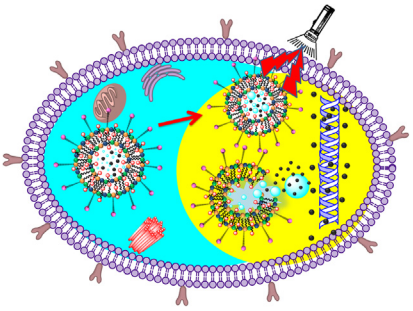
HIGHLIGHTS

- Designing of CDs & TAT conjugated multifunctional bubble liposome has been reported.
- CDs have been incorporated for optical monitoring as well as NIR absorbing agent.
- CDs/TAT@NBLs enabled the optical monitoring of the cells multicolor fluorescence.
- Biocompatible, economic & stable liposome shows improved therapeutic efficacy.
- It is a multifunctional platform with low cytotoxicity and efficient cancer cell-killing.

GRAPHICAL ABSTRACT



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ABSTRACT

A pioneering nucleus-targeting dual-functional thermosensitive bubble-generating liposome as drug carrier has been developed with conjugated cell penetrating peptide and photothermal agent (CDs) for multi-color cell imaging and combination (chemo- and photothermal) therapy of cancer, using doxorubicin as drug molecule. The liposome was prepared by 'in-house' synthesized precursors molecules, to reduce the cost of production in comparison to the earlier reported liposomes. This drug carrier was proposed to target nucleus of the cancer cells, owing to specific and selective cell-penetrating property of TAT, followed by burst but stable drug release due to decomposition of bubble forming agent present inside the liposome core, by being subjected to near-infrared (NIR) irradiation (i.e. photothermal conversion of radiation to heat). The in-vitro temperature and/or NIR-triggered release study indicated that liposome was sensitive towards heat and able to generate the sufficient temperature of 68 °C in the presence of NIR-laser source. The in vivo experiment was also performed to explore the NIR-responsive hyperthermia in the mice body. The as prepared bubble containing liposome-based drug delivery system exhibits superior stability, no drug leakage and enhanced in vitro and in vivo drug delivery with efficient cancer cell killing via combination therapy. The multicolor fluorescence obtained via CDs improves the accuracy of the cell imaging study as well as works as a key component for photothermal treatment of cancer. The results obtained in the study demonstrated that the designed smart drug carrier have great potential in the field of cancer theragnosis i.e. imaging, targeted drug delivery, and treatment.

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1. Introduction

The first and foremost requirement to treat incurable diseases is targeted delivery of appropriate drugs using an efficient drug carrier. Various nanoforms have already been used as drug delivery system, starting from albumin, gelatin, metals, oxides, semiconductors, polymers, liposomes to nanomagnets [1–3]. However, most of them start their journey from blood vessels and end them in the close vicinity of a tumor cell or at the cell membrane, extreme to the cytoplasm and very rarely reached to the nucleus. However, the main target of these carriers was tumor cell nucleus, which should be destroyed to actually kill the disease. For this, the anti-cancer drugs are required to enter the cell nucleus, but in a real situation, it is very difficult for drugs to remain in their active form after arriving at the nucleus [4,5]. Therefore, we required a nucleus targeted drug delivery system (NTDDs) to enhance the efficacy of currently available drugs.

Temperature-sensitive liposomes (TSLs) and/or polymer-modified thermosensitive liposomes (PTSLs) are the most commonly employed nanosystem for drug delivery owing to their excellent pharmacokinetic profiles, low toxicity, membrane compatibility and hyperthermia-triggered site-specific drug release mechanism [6]. Some of them have already entered into the market, for example, Doxil®, Daunoxome® for doxorubicin delivery. Drug release from TSLs/PTSLs can be induced by a very mild increase in the temperature of the specific site i.e. 39–40 °C (few degrees above physiological temperature) [7,8]. The anciently used sources of hyperthermia to trigger drug release from TSLs are microwave, IR laser, or radiofrequency heating, which require the specific handling and painful for the patients too [9–11]. In addition, the TSLs are also subject to some limitations including fast burst release of drugs, low encapsulation efficiency, poor storage stability and lack of tunable triggers for drug release [12]. But the main disadvantage lies with the liposomes is their low permeation through tumor cells resulting in drug release in the extracellular fluid. To make the liposomes specifically targeted to the nucleus, they must be functionalized with the proper targeting ligand like nuclear localization signaling (NLS) peptides. In spite of this, the liposome carriers are unable to a show the fluorescence property; therefore, they should also be attached with some fluorescent dye for tracking up their drug delivery path and process. In the recent years, liposomes were well modified with various targeting as well as fluorescence probes, to make them multifunctional [13]. For example, recently, Yu et al. have designed a tumor targeting and locally triggered-release nanocarrier system based on gold nanorods (GNRs) and thermosensitive bubble liposomes (TSBLs) [14]. Herein, GNRs were used to absorb near infrared (NIR) radiation and polyethylene glycol (PEG) for targeting and better stability in the blood [14]. The GNRs can able to absorb NIR light and convert it into heat, resulting in an elevated temperature of nanocarrier and drug release. The elevated temperature and drug release synergistically kill the tumor cells and lower down the anti-cancer drug dose, which is well known for their side effects. Similarly, Qin et al. have also reported an NIR-triggered drug delivery system based on the amphiphilic chitosan derivative-coated single-wall carbon nanotubes (CNT) encapsulated in the poly(N-isopropyl acrylamide). After that PEG diacrylate was applied in the present work to tune the nanoparticles with the phase transition temperature [15]. Another work is reported which is based on the dipalmitoylphosphatidylcholine (DPPC) liposome, with gold nanoparticle and poly(N-isopropyl acrylamide-co-butyl methacrylate). This modified liposome was used to study the release profile of calcein [16].

To increase the target specificity and improved cell permeation, some of the researchers have also employed the cell-penetrating peptides (CPPs) modified liposomes. The CPPs (example R9, TAT etc.) are derived from proteins and able to cross biological membranes very efficiently. Among the various types of CPPs, the TAT peptides are one of the most promising and most studied CPPs. According to Pan et al. TAT peptide has been shown to be an efficient molecule for translocating nanoparticles into cell nuclei via the binding import receptors importin α and β (karyopherin) and subsequently targeting the nuclear pore complexes of cancer cells and entering their nuclei [17]. Recently, Yang et al. have reported NIR-responsive liposome which is modified with asparagine-glycine-arginine peptide for targeted drug delivery [9]. Xie et al. have reported the liposomes bearing a photolabile-caged peptide for targeted delivery into cancer cells [10]. In our previous work, we have also tried to use R9 and carbon dots modified liposome for transdermal delivery of curcumin. The result shows that the R9 modified nanosized liposome has greater stability, long circulation time with higher cytocompatibility than conventional liposomes.

Herein, for the first time, we have developed a carbon dot (CDs) and TAT-conjugated bubble liposome (CDs/TAT@NBLs) for multiple applications i.e. multi-color cell imaging, nucleus-targeting, NIR-triggered drug delivery of doxorubicin (DOX) and NIR photothermal therapy, simultaneously. For nucleus targeting, we have used the most popular cell-penetrating peptides i.e. TAT and CDs were used as NIR absorbing nanomaterial to trigger the drug release and also involve in the photothermal killing of tumor cells. In addition to these, to reduce the overall cost of liposome synthesis, we have used some laboratory prepared precursors, derived from very cost-effective chemicals/reagents, namely, (1) (1,3-distearamidopropan-2-yl) ethyl 2-((2- aminoethyl) dimethylammonio) phosphate (DSAEP), (2) (1,3-dipalmitamidopropan-2-yl) ethyl 2-((4-aminophenyl)dimethyl ammonio) phosphate (DPAEP), and (3) 2,2-dimethyl-3-tetradecanamidopropyl (2-((tris(2-hydroxypropyl) ammonio) oxy) ethyl) phosphate (EAHEP). The synthesis process of all the precursors was shown in various Schemes (Schemes 1 and 2). The CDs/TAT@NBLs DDS constructed by the non-covalent grafting of anticancer drug (DOX), not only efficiently accelerated nuclear and tumor accumulation of DOX, but also markedly enhanced the cytotoxicity for cancer cells in both conditions i.e. in vitro and in vivo, which is found superior to many other nanoparticle/liposome-based DOX delivery systems. The proposed liposome shows several advantages over the commonly reported drug carriers like: (1) The CDs/TAT@NBLs was stable enough for circulation in the blood without any leakage and targeted to reach the cell nuclei by the help of TAT peptide; (2) NIR-mediated heat stimulus generated via CDs shows dual performance i.e. increases the tumor cell temperature and triggered the drug release via bursting of bubble present inside the liposomes; (3) CDs modification enables the liposomes for proper and multi-colored cell imaging.

2. Experimental

2.1. Materials and instrumentation

2-Chloro-1,3,2-dioxaphospholane-2-oxide (COP), tri-isopropanol amine, N,N'-dimethyl paraphenylenediamine, TAT, N,N′-Dicyclo hexylcarbodiimide (DCC) and DOX were purchased from Sigma-Aldrich (India) and TCI chemicals (India). Carboxymethylcellulose, myristic acid, thionyl chloride, stearic acid, cholesterol, palmitic acid, triethylamine (TEA), glacial acetic acid and the solvents like methanol, ethanol, tetrahydrofuran (THF), dimethyl sulphoxide (DMSO) and chloroform were purchased from Spectrochem Pvt. Ltd. (India) and Loba Chemie Pvt. Ltd. (India).