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Glycosylated liposomes loading carbon dots for targeted recognition to HepG2 cells

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ABSTRACT

It is important to obtain the targeted nanocarriers during cancer treatment. Herein, mannosylated liposomes encapsulating carbon dots (CDs) are provided for targeted recognition of liver cancer HepG2 cells, which is based on the specific interaction between D-mannose and glycoprotein on the surface of HepG2 cells. CDs were prepared by hydrothermal method. Then, they were encapsulated into liposomes by hydrophobic force. The encapsulation of CDs into liposomes increases their stability and fluorescence intensity. Furthermore, D-mannose can also be inserted into liposomes by the aldehyde amine reaction between aldehyde groups of mannose molecules and amino groups of liposomes. The obtained D-mannose-CDs-liposomes (Man-CDs-liposomes) exhibit selectively tracking and efficiently labelling for cancer cells. The work highlights the potential application of CDs for bioimaging and diagnostic.

1. Introduction

A main challenge for cancer research is how to recognize the cancer cell by targeted molecules on nanocarriers and achieve sensitive release at target sites [1–4]. The recognition of tumor cells at the molecular level is particularly important for cancer treatment. Different targeted recognition approaches have been developed by the interaction between biomarker molecules and cell surface receptor proteins including protein-protein interaction and protein-peptides interaction [5–8]. During the target recognition process, it is important to obtain the specific molecules for recognize cancer cell. Although the recognition of protein is very strong, it is difficult to obtain specific protein on cancer cell surface. As known, the carbohydrates have important biological functions such as specific molecular recognition by the interaction between carbohydrate and glycoprotein [9–12]. The cancer cells are covered with many glycans [13–16]. The glycans can express the corresponding carbohydrate-binding receptor glycoprotein. Different glycoproteins on the cancer cell surface can recognize specific saccharides molecules, which may forms the "fingerprint" of the cancer cell. So, glycosylated nanocarriers are thought to have great potential as cancer biomarkers [17–19]. Thus, it is feasible to achieve a targeted recognition by modifying the appropriate carbohydrate on the surface of drug carrier.

Liposomes have been widely used as carrier for delivering drugs in both vitro and vivo, which exhibits good biocompatibility and biodegradability. Liposomes are composed of phospholipids, cholesterols molecules to form the bilayer membrane structure including hydrophilic and hydrophobic regions [20–22]. Different drug and biomarker molecules can be loaded into the bilayer membrane of liposomes by hydrophilic-hydrophilic and hydrophobic-hydrophobic interaction [23–25]. Glycosylated liposomes may be formed by incorporating carbohydrate molecules into liposomes. After bioactive materials are incorporated into liposomes, they can be carried to the specific cancer cells by the interaction of targeted recognition [26–28]. To trace the target recognition process, the fluorescence label for glycosylated liposomes may provide a chance to observe the interaction between carbohydrates and glycoprotein.

Carbon dots (CDs) are small carbon nanoparticles with different surface passivation including organic or polymeric molecules, which displays bright fluorescence emission in visible regions [29–31]. CDs have attracted more focus due to their excellent luminescent properties including bioimaging, drug delivery and biosensor. Compared to conventional semiconductor quantum dots, CDs show good biocompatibility, low toxicity, excellent photostability and versatile surface modification [32–34]. Krysmann et al. reported that the fluorescence of CDs originates from both the carbogenic core and surface states [35]. So, the fluorescent characteristics of CDs can be adjusted by changing their surface functional groups [36–38]. Based on the hydrophilic

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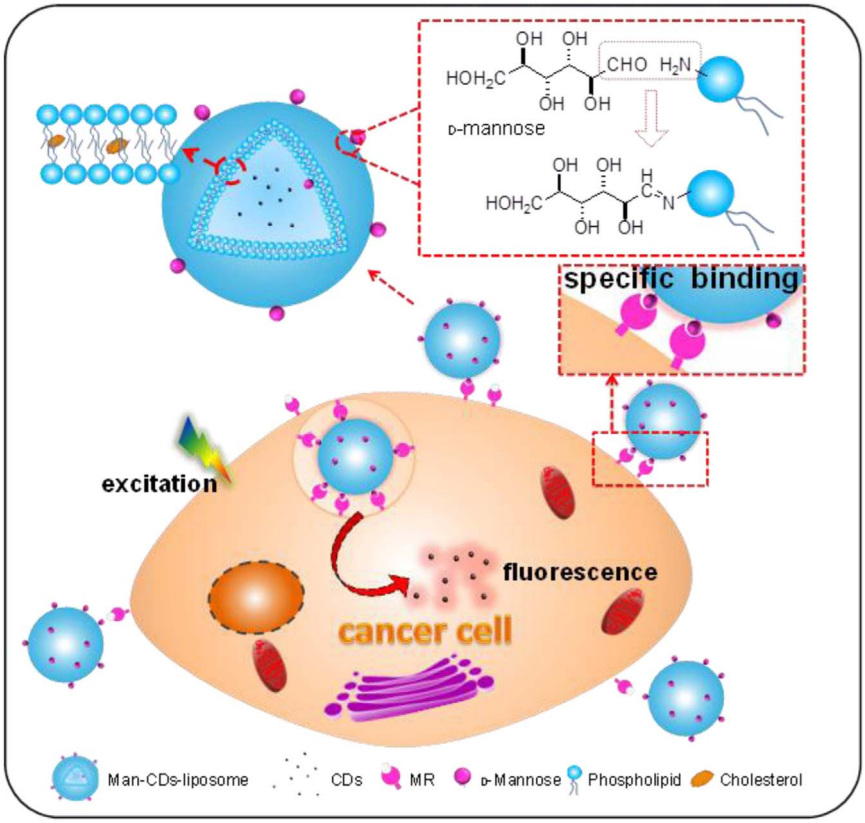

Scheme 1. Schematic illustration depicts the structure of Man-CDs-liposome. The process that Man-liposomes loading is displayed. The aldehyde groups of D-mannose can react with the amino on the hydrophilic head of liposomes to form imine by aldehyde-amide condensation reaction. Man-CDs-liposome can targeted recognition of HepG2 cells.

surface functional groups, CDs can be encapsulated into liposomes. The groups of liposomes can passivate the surface defect state of CDs, which increase their efficiency of fluorescence.

Herein, glycosylated liposomes encapsulating CDs are prepared for targeted recognition of cancer cells as shown in Scheme 1. The aldehyde groups of D-mannose can easily react with the amino groups of liposomes to form imine. It is well known that the expression protein of mannose receptor (MR) existed on the cell surface of HepG2 [16,39,40]. The mannose moiety on liposomes can recognize the HepG2 cell. Therefore, the modification of D-mannose on liposome surface can achieve the purpose of targeted recognition. Schematic illustration depicts the structure of Man-CDs-liposomes. The aldehyde groups of D-mannose can react with the amino on the hydrophilic head of liposomes in a weak alkaline environment to form imine by aldehyde-amide condensation reaction. CDs as fluorescence probe may track and image the targeted-recognition process. The CDs in liposomes have higher fluorescence quantum yields and stability than pure CDs because of their surface passivation by organic groups of liposomes. This provides a platform to targeted recognize cancer cell by the interaction between carbohydrate and glycoprotein.

2. Experimental section

2.1. Chemicals and reagents

Cholesterol was purchased from Aladdin Reagents Co. Ltd. (Shanghai, China). Soya lecithin and cephalin was obtained from Shanghai Tywei Co. Ltd. (Shanghai, China). Chloroform ($CHCl_3$), p-phenylenediamine ($C_6H_8N_2$), urea (CH_4N_2O), D-mannose, phosphotungstic acid (PTA) and hydrogen peroxide (H_2O_2, 30%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). 3-(4, 5-dimethy1-2-thiazoyl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma-Aldrich. All chemicals and reagents were used without further purification. Milli-Q water was used throughout the experiment.

2.2. Instrumentations

The morphology of sample was characterized with a transmission electron microscopy (TEM, Philips CM200 LaB6 microscope) operating at 200 kV and an atomic force microscope (AFM, Bruker Dimension Icon microscope, Scanasyst mode). And the lattice spacing in high-resolution TEM image was analyzed using Digital Micrograph 3.5. UV–vis absorption spectra were recorded with a Hitachi UH 4150 UV–visible-NIR spectrophotometer at room temperature. Fourier transform infrared (FT-IR) spectra were collected on a Nicolet iS10 FT-IR spectrometer. Fluorescence spectra and fluorescence lifetimes were obtained on a fluorescence spectrophotometer (PTI-QuantaMaster 400), assembling with a Xenon lamp used for excitation. The zeta potential and dynamic light scattering (DLS) were analyzed using a zeta potential analyzer (Zetasizer Nano ZS90) at room temperature. X-ray photoelectron spectroscopy (XPS) measurements were carried out on an ESCALAB250 X-ray photoelectron spectrometer (Thermo Fisher) by Al (kalpha) X-rays as radiation source (150 W and 15 kV). X-ray diffraction (XRD) patterns were collected on a Rigaku D/max-2500 ×-ray diffractometer, using Cu Kα radiation (λ = 1.5406 Å). The cell imaging was collected by a Nikon Inverted Microscope Eclipse Ti-U Main Body (Nikon, Tokyo, Japan) equipped with Nikon DS-U3 CCD, assembling with 375 and 532 nm laser devices used for excitation.

2.3. Synthesis of carbon dots

CDs were synthesized through hydrothermal conditions [37]. First, p-phenylenediamine (0.12 g), urea (0.12 g) and 30 mL of H_2O were added into a reaction kettle (50 mL). Then the mixed solution was