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Multi-functionalized hyaluronic acid nanogels crosslinked with carbon dots as dual receptor-mediated targeting tumor theranostics

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ABSTRACT

Hyaluronic acid (HA)-based theranostic nanogels were designed for the tumor diagnosis and chemotherapy, by crosslinking the folate-terminated poly(ethylene glycol) modified hyaluronic acid (FA-PEG-HA) with carbon dots (CDs) for the first time. Due to the extraordinary fluorescence property of the integrated CDs, the theranostic nanogels could be used for the real-time and noninvasive location tracking to cancer cells. HA could load Doxorubicin (DOX) via electrostatic interaction with a drug-loading capacity (DLC) of 32.5%. The nanogels possessed an ideal release of DOX in the weak acid environment, while it was restrained in the neutral media, demonstrating the pH-responsive controlled release behavior. The cytotoxicity and cellular uptake results clearly illustrated that most DOX was released and accumulated in the cell nuclei and killed the cancer cells efficaciously, due to their dual receptor-mediated targeting characteristics.

1. Introduction

Chemotherapy remains one of the cornerstones in cancer treatment. However, the cytotoxic drugs cause severe side effects in the therapeutic process, due to their non-specific delivery. To reduce the side effects and improve the antitumor activity of such drugs, the nanoscaled smart drug delivery systems (DDS) with desired stimuli-responsive controlled release performance have been intensively studied in the recent years (Chacko, Ventura, Zhuang, & Thayumanavan, 2012). They could accumulate in the tumor sites through the passive targeting function via enhanced permeability and retention (EPR) effect due to their small size (Maeda, Nakamura, & Fang, 2013), as well as the active targeting effect by modification with targeting ligands, such as folate, which could recognize the folate receptor, a glycosylphosphatidylinositol-linked membrane glycoprotein, overexpressed in tumor tissues (Low, Henne, & Doorneweerd, 2008).

Most recently, integrating the diagnosis and chemotherapy functions into a theranostic platform makes it easily to real-time and noninvasively monitor the therapeutic effects during the chemotherapy treatment course (Pei et al., 2015), by introducing the fluorescent organic molecules (Jia et al., 2016) or inorganic nanomaterials (Chen et al., 2010), luminescent semiconductor nanocrystals (Ho & Leong, 2010), MRI contrast agents (Guo et al., 2015), and so on.

The excellent biodegradability and biocompatibility of the polysaccharides, as well as plentiful chemical functionalities, make them most promising carriers for DDS (Goodarzi, Varshochian, Kamalini, Atyabi, & Dinarvand, 2013). Especially for hyaluronic acid (HA), it could target the tumor tissues as an ideal biologically active substance for drug delivery, owing to the specific recognization by the glycoprotein CD44 overexpressed in tumor tissues (Tripodo et al., 2015).

Carbon dots (CDs), a kind of biologically compatible carbon nanomaterial, has been recognized as an emerging class of fluorescent materials and provide a broad application potential in biomedicine (Hola et al., 2014), due to its downconversion photoluminescent (PL) properties, inherently low cytotoxicity, and amphiphilic characteristics with abundant functional groups such as amino, hydroxyl and carboxyl. They have been recently used as a bioimaging substance in tumor theranostics. For example, Zheng et al. designed a theranostic nanomedicine by means of the condensation reaction between the amino groups on the surface of fluorescent CDs and the carboxyl group of the oxaliplatin derivative Oxa(IV)-COOH, integrating the optical properties of CDs and the anticancer function of oxaliplatin for the simultaneous drug delivery and fluorescent tracking (Zheng et al., 2014).

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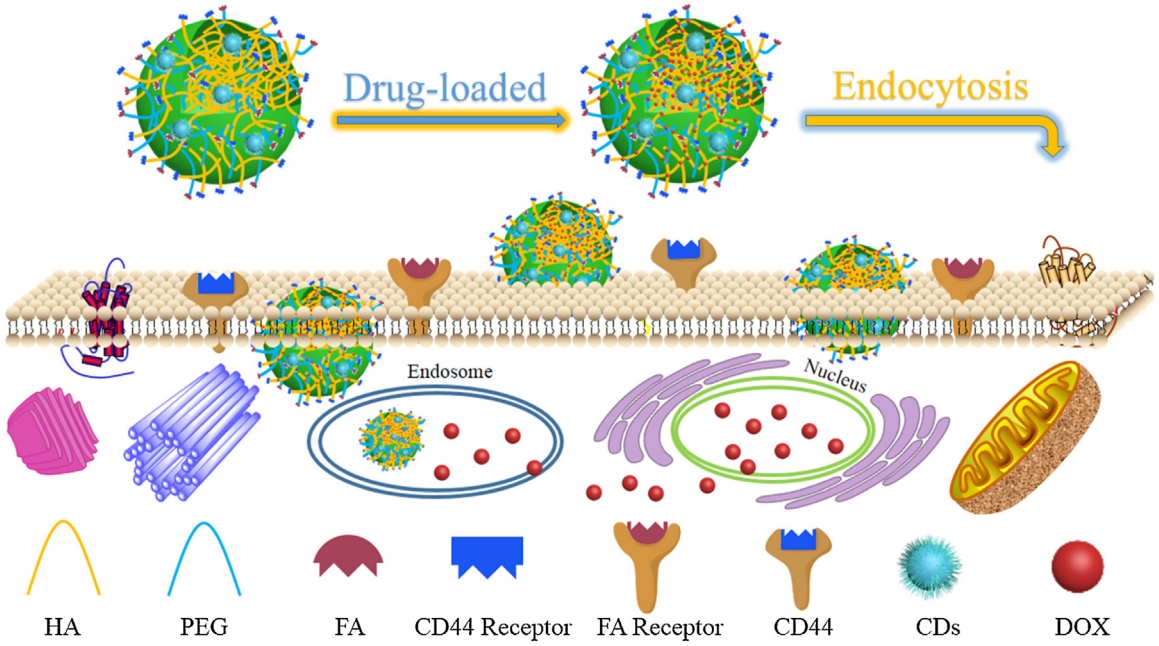

Scheme 1. Illustration of the proposed DOX-loaded FA-PEG-HA-CDs theranostic nanogels for the intracellular release.

In the present work, extraordinary hyaluronic acid (HA)-based theranostic nanogels (FA-PEG-HA-CDs) were prepared by crosslinking the folate-terminated poly(ethylene glycol) modified HA (FA-PEG-HA) with CDs. This system was designed for the PL imaging and dual receptor-mediated targeting controlled delivering DOX (Scheme 1).

2. Materials and methods

2.1. Materials

Citric acid anhydrous (99.5%) was bought from Sinopharm Chemical Reagent Co., Ltd. Low molecular weight sodium hyaluronate (M_W = 400,000, cosmetic grade) was provided by Shandong Freda Biopharm. Co., Ltd. Ethylenediamine (99%) was purchased from Rionlon Bohua (Tianjin) Pharmaceutical Chemical Co. Ltd. Poly(ethylene glycol) diamine (H_2N-PEG-NH_2, Mn = 4000, 99%) was got from Beijing Kaizheng Biotech Development Co. Ltd. Folic acid (FA, 97%) was got from Tianjin Guangfu Technology and Development Co., Ltd. Dicyclohexyl carbodiimide (DCC, 95%) was bought from Shanghai KEFENG Chemical Reagent Co., Inc. *N*-Hydroxysuccinimide (NHS, 98%) was purchased from Aladdin Chemistry Co. Ltd. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 99%) was provided by was provided by J&K Chemical co., LtD. Doxorubicin hydrochloride (DOX·HCl, 99.4%) was provided from Beijing Huafeng United Technology Co. Ltd. Double-deionized water was used throughout.

2.2. Synthesis procedure

2.2.1. Carbon dots (CDs)

Carbon dots (CDs) were synthesized via a one-step microwave-assisted pyrolysis as reported previously (Cheng et al., 2014). Citric acid anhydrous (2.0 g, 10.41 mmol) was dissolved in 20 mL water in a 100 mL beaker. Then, ethylenediamine (0.936 g, 15.57 mmol) was added dropwise into the abovementioned solution with vigorous stirring, obtaining a clear, transparent solution. The beaker was placed in a domestic microwave oven (700 W) and sustained for 4 min. After cooling down to room temperature, the product was dissolved in water, and dialyzed (MWCO of 1000) for 3 days to remove the impurity. Finally, the CDs were collected by lyophilization.

2.2.2. Folate-terminated poly(ethylene glycol) (FA-PEG-NH_2)

Folic acid (0.2207 g, 0.5 mmol) was dissolved in 10 mL of anhydrous DMSO, and then triethylamine (0.11 mL, 0.79 mmol) was added with stirring. Furthermore, DCC (0.1032 g, 0.5 mmol) and NHS (0.0575 g, 0.5 mmol) were added into the abovementioned solution. The reaction mixture was stirred at ambient temperature in the dark for 8 h and filtered off the generated precipitate (Dicyclohexylurea, DCU). 10 mL DMSO solution of H_2N-PEG-NH_2 (2 g, 0.5 mmol) was added into the filtrate. The reaction was conducted for 48 h at room temperature. Then, the mixture was disposed by dialysis (MWCO of 1000) with water for 3 days to remove DMSO. Finally, the product of FA-PEG-NH_2 was collected by lyophilization (Singh, Gupta, Asthana, & Jain, 2008). The substituting degree of product was calculated as 85.08 mol% by measuring the absorption with a UV–vis spectrophotometer at 360 nm.

2.2.3. Folate-terminated poly(ethylene glycol) modified hyaluronic acid (FA-PEG-HA)

1 g of sodium hyaluronate was dissolved in 100 mL of water. Then, EDC·HCl (0.4753 g, 2.48 mmol) and NHS (0.2854 g, 2.48 mmol) were added into the solution and the pH value of the solution was adjusted to 4.5 with HCl aqueous solution. The activation process was lasted for 4 h at room temperature. And then, FA-PEG-NH_2 (1.1011 g, 0.25 mmol) was added into the mixture solution with vigorous stirring, and the mixture was stirred for 48 h at room temperature. Finally, it was dialyzed (MWCO of 14,000) with water for 3 days to remove the impurities and the resultant product (FA-PEG-HA) was collected by lyophilization (Zhao et al., 2014). It was found that average 5.1 FA-PEG molecules were grafted onto per 100 repeating units of HA, by measuring the absorption with a UV–vis spectrophotometer at 360 nm.