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Synthesis and characterization of a bimodal nanoparticle based on the host-guest self-assembly for targeted cellular imaging

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ABSTRACT

Multimodal imaging provides distinct advantages over traditional single modal imaging. The combined modalities of magnetic resonance imaging (MRI) and near-infrared imaging (NIR), in particular, provide a powerful tool for tumor diagnosis. In this study, a bimodal MRI and NIR self-assembled supramolecular nanoparticle was developed via the self-assembly of host-guest interactions between hyaluronic acid–β-cyclodextrin (HA–CD) and amantadine (Ad)-modified imaging agents (Gd–DOTA and NIR cyanine dye Cy7). The supramolecular HA–CD–GC nanoparticles (NPs) were characterized by transmission electron microscopy (TEM), Zeta potential, and dynamic light-scattering (DLS) experiments. The relaxivity and fluorescent properties of the NPs were also determined. HA–CD–GC NPs exhibited an enhanced relaxivity of 11.4 $mM^{-1}S^{-1}$, which was three-fold higher than that of clinical Gd^{3+}-chelated complex, for MRI imaging. Moreover, HA–CD–GC NPs displayed excellent fluorescence. In addition, HA–CD–GC NPs were internalized into tumor cells via HA-receptor CD44-mediated endocytosis. Therefore, the self-assembled HA–CD–GC NPs are effective targeted tumor cell imaging systems and have potential applications in cancer diagnosis and treatment.

1. Introduction

Molecular imaging is a powerful diagnostic approach for detecting diseased organs or tissues and studying treatment effects [1]. In the past few decades, positron emission tomography [2], computed tomography [3], magnetic resonance imaging (MRI) [4], ultrasound [5], and optical imaging [6] have emerged as crucial imaging modalities in the medical field. Although these current modalities have many advantages, several limitations restrict their widespread clinical use. The combination of the complementary advantages of these modalities to overcome imaging limitations has drawn the interest of many investigators [7,8]. Among all imaging modalities, bimodal MRI and near-infrared (NIR) nanoprobes stand out because of the high spatial resolution of MRI and the high sensitivity of NIR. Melancon et al. synthesized a dual magneto-optical imaging probe (PG–Gd–NIR813) to non-invasively assess tumor-associated macrophages [9]. Yeh et al. conjugated a lipophilic heptamethine cyanine MHI-148 on porous Gd silicate@$mSiO_2$ to obtain bimodal NPs for tumor-targeted imaging [10]. Enhanced MRI signals or NIR fluorescence intensities are correlated with several parameters, particularly the loading capacity of imaging molecules in NPs and the interaction among the functional molecules. When MRI and NIR imaging agents are covalently grafted on the macromolecular skeleton, balancing the effect of the two imaging modalities becomes more challenging because of space-steric effect and reaction efficiency. Thus, there is a need to establish a simple and convenient approach to optimize the synergy of MRI enhancement and NIR performance in bimodal nanoprobes, as well as the effective enhancement of MRI relaxivity and fluorescent performance.

Hyaluronic acid (HA), a (β–1,4) -linked D-glucuronic acid and (β–1,3) *N*-acetyl- D-glucosamine polysaccharide, is ubiquitous in the extracellular matrix and connecting joints. Given its biocompatibility, biodegradability, and non-immunogenicity, HA is a useful building block for drug delivery and bioimaging. Moreover, HA is a ligand for cluster determinant 44 (CD44) hyaluronan receptors, which are over-expressed in some tumor diseases [11,12]. Thus, HA has considerable potential as a tumor-targeting delivery system. β-Cyclodextrin (CD) is a

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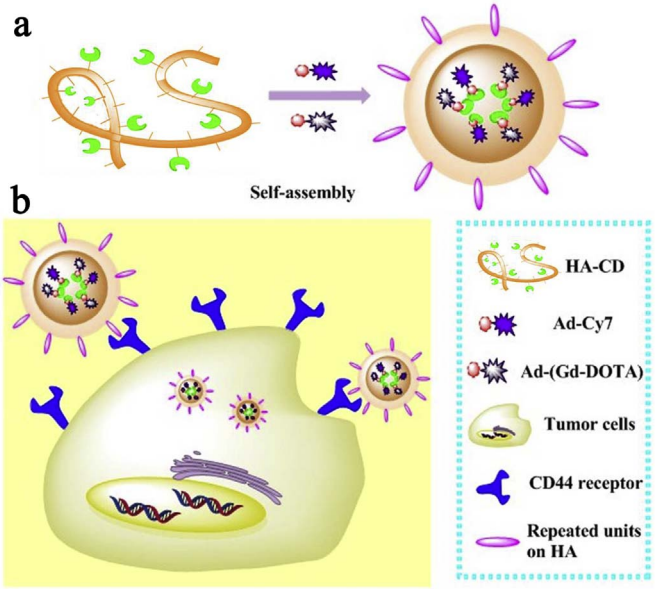
cyclic oligosaccharide with a hydrophobic interior that encapsulates small hydrophobic molecules and forms clathrates through host-guest interactions. Numerous CD derivatives have been used as drug delivery systems because of their excellent biocompatibility and loading efficiency. Chen et al. reported that encapsulating Gd (III) in CD-mediated nanoparticles produced a MRI contrast agent with improved relaxivity and sensitivity [13]. Dong et al. reported that CD-encapsulated Ad-modified calcein fluorescent dyes formed supramolecular fluorescent nanoparticles with excellent fluorescent performance and cancer-specific properties [14]. Li et al. [15] and Yang et al. [16] reported on the cancer cell-selective delivery of anticancer drugs encapsulated by HA–CD nanoparticles. Based on these corresponding investigations, we hypothesized that the HA–CD could be self-assembled with Ad-modified MRI and NIR molecules via host-guest interaction to produce bimodal MRI and NIR supramolecular nanoprobes with enhanced relaxivity and fluorescence performance.

In this study, we developed a novel bimodal MRI and NIR self-assembled supramolecular nanoparticle (HA–CD–GC NPs) via host-guest interactions between HA–CD (as host section) and Ad-modified imaging agents (Gd–DOTA and near-infrared cyanine dye Cy7 as guest sections) (Scheme 1). The HA–CD–GC NPs were characterized by transmission electron microscope (TEM), Zeta potential, and dynamic light-scattering (DLS). The relaxivity and fluorescent properties of HA–CD–GC NPs were also evaluated. Moreover, a cellular uptake study was performed to evaluate the specific identification of HA–CD–GC NPs on CD44 expressed on the surface of tumor cells.

2. Materials and methods

2.1. Materials and reagents

Hyaluronic acid sodium salt (HA , 10 KD) was purchased from the Bott Import and Export Trade Co., Ltd (Shenzhen, China). Amantadine (Ad), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl), Dicyclohexylcarbodiimide (DCC), P-toluene sulfonyl chloride and N-hydroxysuccinimide (NHS) were purchased from Aladdin (Shanghai, China) and Sigma-Aldrich (Shanghai, China). Ethylenediamine was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Tert-butyloxycarbonyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was purchased from Synpartner PharmTech Co., Ltd (Zhejiang, China). β-Cyclodextrin (β-CD) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The dialysis process was performed in a dialysis bag (500 Da) purchased from Source Biological Technology Co., Ltd (Shanghai, China). 200–400 mesh silica gel was purchased from Qingdao Ocean Chemical Inc. (Qingdao, China). Cell lysis solution was purchased from Thermo scientific (Massachusetts, USA). BCA protein quantitation assay was purchased from KeyGEN BioTECH (Nanjing, China). CD44 monoclonal antibody, β-actin antibody and Horseradish Peroxidase-conjugated AffiniPure goat anti-mouse IgG (H+L) were purchased from Proteintech China Branch (Wuhan, China). Horseradish Peroxidase-conjugated AffiniPure Goat Anti-rabbit IgG (H+L) and allergic ECL chemiluminescence reagent kit were purchased from Beyotime Biotechnology (Shanghai, China). Cell Counting Kit-8 was purchased from Biotool Biological Technology Co., Ltd (Shanghai, China). All the organic chemicals were of analytical grade or higher.



Scheme 1. (a) Design of bimodal MRI and NIR HA–CD–GC NPs self-assembled via host-guest interactions of β-cyclodextrin with amantadine modified Gd–DOTA and Cy7. (b) Schematic diagram of HA–CD–GC NPs as tumor targeted imaging probes, entering cytoplasm by CD44-mediated endocytosis.

2.2. Methods and instruments

The characterization of synthesized compounds was conducted on a matrix-assisted laser desorption ionization time-of-light (MALDI-TOF) mass spectroscopy (5800, AB SCIEX, Gottingen, Germany) with a-cyano-4-hydroxycinnamic acid as a matrix or on a fourier-transform mass spectrometry (FT-MS, SolariX XR-15T, Bruker, Germany) with 2,5-dihydroxybenzoic acid as a matrix. NMR spectra were detected on a Bruker AV400 Instrument (AVANCE III, Bruker Biospin, Swiss). The NIR fluorescence emission spectra were measured on a cary eclipse fluorescence spectrophotometer system (Agilent Technologies, Palo Alto, USA) and the UV–vis absorption spectra were obtained on a UH5300 spectrophotometer (Hitachi, Tokyo, Japan). The distribution of particle size and zeta potential were recorded by the laser particle size and zeta potential analyzer (Zetasizer Nano, Malvern, England). TEM samples were obtained by dropping the water dispersion onto a Cu grid, which was coated with a lacey carbon film and dried under surrounding environment. TEM observations were performed using a Tecnai Spirit transmission electron microscope operating at 120 kV (FEI, Hillsboro, USA). Inductively coupled plasma-optical emission spectroscopy (ICP-OES 7300DV, PerkinElmer, Fremont, USA) was used for measuring the contents of Gd element in the solution. MRI experiments were performed on a 0.5 T magnet scanner (MiniMR-Rat, Shanghai Niumag Co., Ltd, Shanghai, China). The cells images were observed on a confocal microscope system (FV1000MPE, Olympus, Tokyo, Japan).

2.3. Synthesis and characterization of compounds

2.3.1. Synthesis and characterization of mono- (6-ethanediamine-6-deoxy) –β-CD

Compound mono-(6-ethanediamine-6-deoxy)–β-CD was synthesized by two sections. First, mono-6-(*p*-toluenesulfonyl)-6-deoxy–β-CD (6-OTs–β-CD) was obtained according to the literature [17]. Second, 6-OTs–β-CD (3 g, 2.33 mmol) was added into ethylenediamine (10 ml) under N_2. Then the mixture was kept stirring at 60 °C for 12 h. After that, the solution was cooled to room temperature and poured into a large amount of ethanol to give the white product. The resultant product was obtained in 75% yield by filtered, washed with ethanol and dried under vacuum. MALDI-TOF mass spectroscopy [(*m/z*, $[M+H]^+$): 1177.43 (calculated), 1177.6953 (observed); (*m/z*, $[M + Na]^+$): 1199.43 (calculated), 1199.6816 (observed); (*m/z*, $[M + K]^+$): 1215.43 (calculated), 1215.6567 (observed)].

2.3.2. Synthesis of β-CD-modified HA (HA–CD)

The COOHs of HA were activated through EDC and NHS systems. EDC (9.5–38.9 mg, 0.0496–0.203 mmol) and NHS (5.9–23.1 mg, 0.0513–0.201 mmol) were added into a solution of sodium hyalur-