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Exploring the optimal ratio of D-glucose/L-aspartic acid for targeting carbon dots toward brain tumor cells

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ABSTRACT

Targeting imaging to the desired site of action can increase the accuracy and effectiveness of diagnostic and treatment. In this work, a series of fluorescent carbon dots (CDs) were prepared by varying molar ratios of D-glucose (Glu) to L-aspartic acid (Asp). Their photophysical properties, morphologies and structures were investigated in detail. More important, the targeting ability was screened by confocal laser scanning microscopy and flow cytometry. The results indicate that CDs prepared from the optimal molar ratio of Glu/Asp (7:3) exhibit the highest targeting ability on C6 glioma cells. This work highlights the interplay of molecular design and corresponding functions, and open new possibility of developing state-of-art nanoparticles for biomedical applications.

1. Introduction

Central nervous system (CNS) disorders could give rise to much significant hazardous for human health, for example, brain tumors, Alzheimer's disease and so on. Among them, glioma has been treated as one of the most deadly killers which is malignant primary brain tumors, because it is not only very difficult to find but also untoward to completely eradicate by surgery [1–5]. The main barriers involved the blood-brain barrier (BBB), which can prevent most substances from the blood into the brain tissue [6]. Thus, early diagnosis becomes the urgent demand [7]. Recently, fluorescence imaging technology has attracted increasing interest due to unique advantages in terms of good contrast, high sensitivity and easy operability [8–27].

As a new type of fluoresce imaging agents, carbon dots (CDs) open up a new avenue for fluorescence imaging because of the significant physical and chemical properties, such as tunable color, high stability, water solubility, pronounced biocompatibility as well as low toxicity [28–45]. Especially, some CDs were reported to have self-targeting abilities toward cancer cells, which is a great advantage over other fluorescent agents. The fluorescent materials usually need extra conjugation with targeting molecules for improving the selectivity and imaging contrast in the tumor site [46–52].

In the present work, a series of CDs (named CD_{19}, CD_{28}, CD_{37}, CD_{46}, CD_{55}, CD_{64}, CD_{73}, CD_{82} and CD_{91}) were obtained by varying the molar ratios of D-glucose (Glu)/L-aspartic acid (Asp) (1,9, 2,8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1). Laser confocal scanning microscopy (LCSM) imaging and flow cytometry analysis were conducted to compare the selectivity of the CDs on C6 glioma cells. The results indicate that CD_{73} has the highest targeting ability on C6 cells and can act as a perfect imaging and targeting agent for noninvasive glioma diagnosis (Scheme 1).

2. Materials and methods

2.1. Materials

D-Glucose and L-aspartic acid (Asp) were purchased from Aladdin Reagent Co. Ltd. (Shanghai), other reagents were purchased from Beijing Chemical Reagent Co. Ltd. (Beijing). All the reagents were used without further treatment.

2.2. Characterizations

High-resolution TEM (HR-TEM) images were recorded with a FEI-TECNAI G2 transmission electron microscope operating at 200 kV. X-ray photoelectron spectra (XPS) were obtained on a Thermo Scientific

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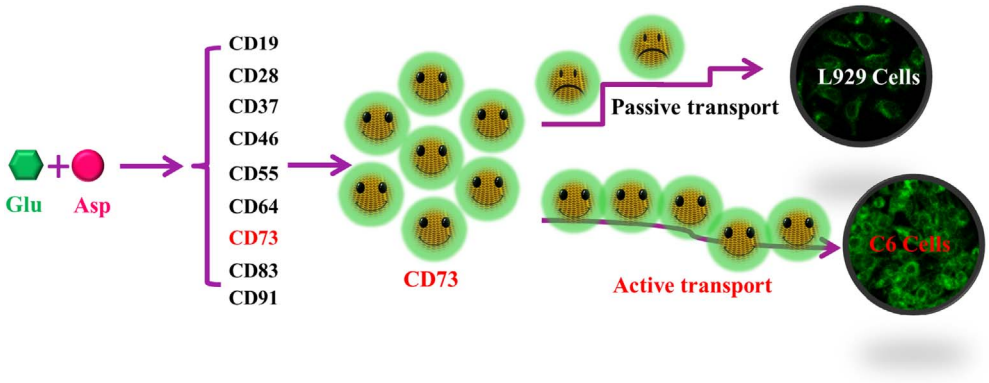

Scheme 1. Schematic illustration of exploring the optimal ratio of Glu/Asp to prepare CDs for targeted brain tumor cells imaging.

ESCALAB 250 Multitechnique Surface Analysis. Fourier Transform Infrared (FT-IR) spectra were recorded on a Bruker Vertex 70 spectrometer. The crystalline structure was recorded by using an X-ray diffractometer (XRD) (Bruker AXS D8 Focus), using Cu Ka radiation (λ = 1.54056 Å). Raman spectra were conducted on Horiba JY LabRAM HR Evolution Raman spectrometer. Fluorescence emission spectra were recorded on a PerkinElmer LS-55 phosphorescence spectrophotometer. UV–Vis absorption spectra were conducted on Shimadzu UV-2450 spectrophotometer. Zeta potential of the nanoparticles was analyzed using a Zetasizer Nano-ZS (Malvern Instruments Ltd.). Atomic force microscope (AFM) images were captured on the Multimode 8 (Bruker Co.) in tapping mode. CLSM images were conducted on Zeiss LSM 780 imaging system. Flow cytometry assay was carried out on Becton Dickinson FACSAria sorting flow cytometer (Becton-Dickinson, Mountain View, US).

2.3. Synthesis of CDs

The CDs were prepared through a thermal pyrolysis method with Glu and Asp as raw materials at different molar ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1). The mixtures of Glu and Asp were loaded into nine breakers respectively and 1 M NaOH were added. The reaction mixtures were placed inside the oven and heated to 125 °C and kept for 30 min. Then heated to 200 °C and maintained for 20 min. After that, the crude products were cooled to room temperature naturally. The products were dissolved into 20 mL of water and subjected to dialysis. Solid CDs were obtained via freeze-drying method.

2.4. Cell culture and (3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay

L929 mouse fibroblast cells (L929 cell) and C6 Brain Glioma cells (C6 cell) were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. The cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% heat inactivated fetal bovine serum (FBS, GIBCO), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma), and the culture medium was replaced once every day.

2.5. Biocompatibility test

L929 and C6 cell lines harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^5 cells per well and incubated in DMEM for 24 h, respectively. The medium was then replaced by CDs at a concentration from 10 to 750 μg/mL. The incubation was continued for 48 h. Then, 20 μL of MTT solution in PBS with the concentration of 5 mg mL^{-1} was added and the plates were incubated for another 4 h at 37 °C. Followed by removal of the culture medium containing MTT and addition of 150 μL of DMSO to each well to dissolve the formazan crystals formed. Finally, the plates were shaken for 10 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader.

2.6. Confocal laser scanning microscope (CLSM) studies

Cellular uptakes by L929 and C6 cells were examined using a CLSM. L929 cells and C6 cells were seeded in 6-well culture plates (a sterile cover slip was put in each well) at a density of 5×10^4 cells per well and allowed to adhere for 24 h. After that, the cells were treated with CDs (2 mg mL^{-1}) for 1 h at 37 °C. And then the supernatant was carefully removed and the cells were washed three times with PBS. Subsequently, the cells were fixed with 800 μL of 4% formaldehyde in each well for 20 min at room temperature and washed twice with PBS again. The slides were mounted and observed with a CLSM imaging system.

2.7. Statistical analysis

The data were expressed as mean ± standard deviation (SD). Student's *t*-test was used to determine the statistical difference between various experimental and control groups. Differences were considered statistically significant at a level of $p < 0.05$.

2.8. Flow cytometry

Flow Cytometry Scanning (FCS) Cellular uptake by C6 and L929 cells was examined with FCS. Each cell line was seeded in six-well culture plates at a density of 5×10^4 cells per well and allowed to adhere for 24 h. Hereafter the cells were treated with CD-Asp for 1 h at 37 °C. Soon afterwards, the culture medium was removed, and the cells were washed with PBS three times and treated with trypsin. Then, 1.0 mL of PBS was added to each culture well, and the solutions were centrifuged for 5 min at 5000 rpm. After the removal of the supernatants, the cells were suspended in 0.5 mL of PBS. Data for the 10,000 gated events were collected, and analyses were performed by flow cytometry (Beckman, California).

3. Results and discussion

3.1. Characterizations of CDs

CD_{19}, CD_{28}, CD_{37}, CD_{46}, CD_{55}, CD_{64}, CD_{73}, CD_{82} and CD_{91} were synthesized through a thermal pyrolysis method with Glu and Asp as raw materials at different molar ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1, respectively. The reactions parameters and yields of products were listed in Table S1. Taking CD_{73} as an example, 1 M of NaOH solution were added to the aqueous of Glu (1.26 g, 7 mmol) and Asp (0.339 g, 3 mmol) to adjust pH to 8.0. The reaction mixture was heated to 125 °C and kept for 30 min, then continue to be heated up to 200 °C and maintain at 200 °C for 20 min. The crude product was cooled to