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Distinguish cancer cells based on targeting turn-on fluorescence imaging by folate functionalized green emitting carbon dots

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ABSTRACT

Developing efficient methods for visual detection of cancer cells has the potential to contribute greatly to basic biological research and early diagnosis of cancer. Here, we report facile and one-step synthesis of green fluorescence carbon dots (CDs) with the help of a new passivating agent – poly(acrylate sodium) (PAAS). Based on the as-prepared CDs, a novel turn-on fluorescence probe was designed for targeting imaging of cancer cells via hydrogen-bond interaction between folic acid and CDs (FA-CDs). Intracellular experiments indicated that FA-CDs probe could accurately distinguish folate receptor (FR)-positive cancer cells in different cell mixtures with turn-on mode. In particular, combining the targeting of FA-CDs probe with the excellent photostability of CDs has inestimable meaning for fluorescence-assisted surgical resection and acquisition real-time information about tumor cells. Obviously, the as-prepared FA-CDs probe may have great potential as a high-performance platform for accurately recognizing special cancer cells, which may provide new tools for cancer prognosis and therapy.

1. Introduction

Realizing cancer cells detecting sensitively and specifically has significant meaning both for early prognosis and therapy, and researching metastasis of cancer (Chen et al., 2008; Lee et al., 2014; Yin et al., 2013). Traditional techniques including computed tomography, magnetic resonance imaging, and radionuclide imaging, require sophisticated instrument and are mainly fited for biological tissues (Feng et al., 2013). For cellular visual detection, fluorescence imaging has attracted considerable attention owing to its high sensitivity and spatiotemporal resolution (Chen et al., 2006; Zhang et al., 2012a). Recently, a few fluorescent probes for cancer cells detecting have been reported, for instance, by using fluorescent dyes or quantum dots (QDs) modified with targeting ligands to target specific cells (He et al., 2008; Qiao et al., 2013; Song et al., 2012; Wang et al., 2011). Nevertheless, most of these systems are always fluorescent, which not only needs many washing operations for accurate imaging of cells but also is not favorable to afford high signal/background ratio (Shi and Ma, 2012). Moreover, fluorescence always-on probe in the nontarget cells due to unspecific endocytosis can lead to a false positive signal (Zhang et al., 2012b). Comparable to always-on signals, an "on–off" or "off–on" switch mechanism should greatly help to address these issues and be more suitable for targeting cells detection because of enhancing optical spatial resolution (Bianying et al., 2013; Pan et al., 2013; Silvers et al., 2012; Wang et al., 2013).

Considerable efforts have been devoted to these fluorescence activated probes yet such probes are still rare (Mizusawa et al., 2012; Pan et al., 2013; Silvers et al., 2012; Wang et al., 2013). It is worth noting that these turn-on probes reported have two main problems: the preparation time- and labor-consuming and considerable toxicity from fluorescent reporters, which both limit the development of fluorescent activated probes for specific cells imaging and the advance of cancer diagnosis technology. Clearly, simplifying the work of fabricating the "turn-on" probes and selecting a fluorescent reporters with excellent biocompatibility and low toxicity are highly necessary.

In recent years, carbon dots (CDs) have attracted considerable attention because of their fascinating physical properties, low toxicity and outstanding biocompatibility (Dong et al., 2012; Lu et al., 2012; Shi et al., 2013; Zong et al., 2014). In view of these

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exciting features, CDs have been widely explored as fluorescent markers or reporters for intracellular imaging (Jaiswal et al., 2012; Sahu et al., 2012; Xu et al., 2013; Yang et al., 2012). However, the fluorescence of these CDs is almost blue under maximum excitation in which there exists two tough problems for bio-imaging. First, blue light from carbohydrate in cells would interfere with the fluorescent signals from CDs (Wang et al., 2014). Then, biological tissues display higher absorption in blue fluorescence region than in longer-wavelength regions (Anderson and Parrish, 1983). Though the emitting of some CDs may produce red-shift when the excitation is in longer-wavelength, the intensity of the emitting decreases significantly. Considerable efforts have been devoted to shift the maximum emission light of CDs to longer-wavelength regions (Fang et al., 2011; Wang et al., 2014), but these works exist some problems, such as low quantum yield (QY), complex syntheses procedure, and using toxic components. Moreover, almost all the probes based on CDs for cell imaging are always fluorescent (Song et al., 2012; Xu et al., 2013; Yang et al., 2012). Now, it is still a challenge to develop an turn-on fluorescence probe for distinguishing cancer cells based on longer-wavelength emitting CDs.

Herein, we report a new passivating agent – poly(acrylate sodium) (PAAS) for facile synthesis of CDs with strong green fluorescence. With the as-prepared CDs, the folic acid-CDs (FA-CDs) nanocomposite was designed as a turn-on fluorescence probe for targeting imaging of cancer cells overexpressed folate receptor (FR) with a negligible fluorescence background. The general design strategy is shown in Scheme 1. The way of fabricating probe is via a kind of noncovalent bond – hydrogen-bond, which can be applied widely and provide a new direction for preparing turn-on fluorescence probes. Moreover, not only the FA-CDs probe can target specific cancer cells for imaging but also the activated fluorescence of FA-CDs probe has the excellent photostability, which has significance for fluorescence-assisted surgical resection and acquisition real-time information about tumor cells. In view of these advantages, the as-prepared FA-CDs probe may have great potential as a high-performance platform for recognizing special cancer cells via fluorescence turn-on imaging, which may provide a new tool for cancer diagnosis and therapy.

2. Materials and methods

2.1. Materials

PAAS and Glucose (Glu) purchased from Sigma-Aldrich were used to prepare the CDs. Poly(methacrylate sodium) (PMAS), Polyacrylamide (PAM), 3-(4,5-dimethylthiahizol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), fluorescein isothiocyanate (FITC), FA, and FR were also obtained from Sigma-Aldrich. The HeLa human cancer of cervix cell line, HepG2 human hepatic carcinoma cell line, HEK-293 human embryonic kidney 293 cell line, Dulbecco's modified eagle media (DMEM), minimum essential medium (MEM), fetal bovine serum (FBS), and Penicillin–Streptomycin Solution (PSS) were obtained from Peking Union Medical College Hospital. All other chemicals were of analytical grade and used as received. Phosphate-buffered saline (PBS) solutions with required pH values were obtained with NaOH solution (1 mol L^{-1}) titrating H_3PO_4 solution (0.01 mol L^{-1}). All the experiments were carried out with ultrapure water.

2.2. Synthesis of CDs

CDs were synthesized by microwave assisted hydrothermal carbonization the mixture of Glu and PAAS. In a typical procedure, 2 g Glu and 1 g PAAS were dissolved with 10 mL water and then heated with microwave (300 W) for 4 min. The prepared CDs solution is brownish yellow and stored at 4 °C.

2.3. Preparation of the turn-on fluorescent probe

FA (1 mL, 2 mg mL^{-1}) was added into 1 mL of the CDs stock solution and then mixed fully. After being set for 10 min, the mixture was dialyzed against PBS (0.01 M, pH 7.4) for 2 h thrice to remove superfluous FA. The purified solution of FA-CDs was keeped in the dark place for further experiments.

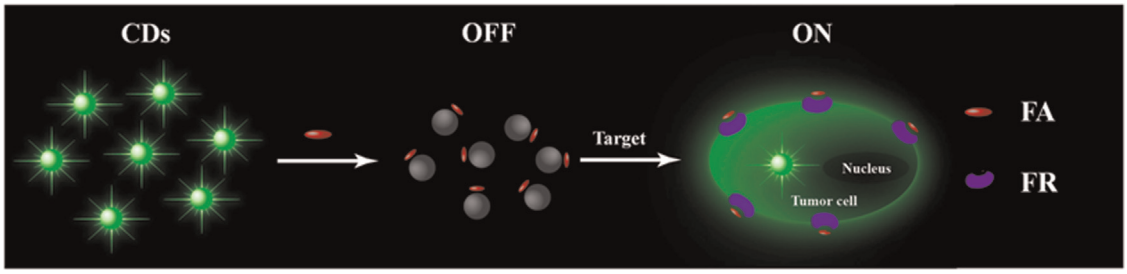
In the experiment of isothermal titration calorimetry (ITC), the reaction cell was totally filled with FA solution and the titration injector syringe was also full filled with the CDs. During the titration, the CDs was titrated to FA with 30 injections of 10 μL each spaced 2 min apart.

2.4. Cytotoxicity

MTT assay was carried out to evaluate cellular toxicity with HeLa cells. HeLa cells were seeded in a 96-well plate for 24 h to adhere. Then, serial dilutions of CDs with known concentrations were added into cells for incubation of another 24 h. According to the formula, the relative viabilities of cells were calculated with absorption values at 490 nm. Three independent experiments were performed under identical conditions. The cytotoxicity of PAAS was obtained following the same operations as described above.

2.5. Fluorescence imaging

Three cell lines, HeLa, HepG2, and HEK-293, were used for fluorescence imaging. The HeLa and HepG2 cells were cultured in a DMEM medium with 10% FBS and 0.01% PSS, and HEK-293 cells were cultured in an MEM medium with 10% FBS and 0.01% PSS at 37 °C in a 5% CO_2 incubator. For the in vitro imaging studies, cells were incubated in a confocal dish 24 h to adhere, and then the CDs were added to dish for incubating another 4 h under standard conditions. After being washed with PBS (0.01 M, pH 7.4) three times, the cells were imaged.



Scheme 1. Scheme illustration of the designed fluorescence turn-on probe for imaging cancer cells overexpressed FR.