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Carbon nanosphere-based fluorescence aptasensor for targeted detection of breast cancer cell MCF-7

Dandan Yang[a], Mei Liu[a], Jing Xu[a], Chao Yang[a], Xiaoxiao Wang[a], Yongbing Lou[a,*], Nongyue He[b,*], Zhifei Wang[a,*]

[a] School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China
[b] School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, China

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ABSTRACT

In this work, carbon nanosphere (CNS)-based fluorescence "turn off/on" aptasensor was developed for targeted detection of breast cancer cell MCF-7 by conjugation with FAM (a dye)-labeled mucin1 (MUC1) aptamer P0 (P0-FAM), which can recognize MUC1 protein overexpressed on the surface of MCF-7. Different from other carbon based fluorescence quenching materials, CNSs prepared by the carbonization of glucose not only have the high fluorescence quenching efficiency (98.8%), but also possess negligible cytotoxicity (in the concentration range of 0–1 mg/mL, which is 10 times higher than that of traditional carbon nanotubes or graphene oxide (0–100 μg/mL)). As for the detection of the mimic of the tumor antigen MUC1, the resulting fluorescence intensity increases nearly linearly in the range of 0–6 μM with the limit of detection (LOD) of 25 nM.

1. Introduction

The fluorescence cell imaging detection [1–3] is very prevalent for in vitro biological studies due to its minimal perturbation to living systems and the advantage of the convenience in operation. To improve sensitivity and specificity in the cell imaging, it is necessary to maximize fluorescence signal from the target cell and minimize the fluorescence from the background at the same time. With the discovery of the quenching effect brought by nanomaterials, many nanomaterials (such as carbon nanotubes [4], graphene oxide (GO) [5,6], gold nanoparticles [7]) have been widely exploited in the fabrication of ideal fluorescence "turn-off/on" probes. Unfortunately, due to the cytotoxicity, most of existing nanomaterials are still far away from the requirement for constructing ideal probe. As we know, after in situ fluorescence imaging, the living cells sometimes will be sorted for further biological studies. So it will be better that the nanomaterials involved in cell imaging should have less effect on the growth behavior of the studied cells. For instance, as a recent attempt in overcoming the limitation of traditional carbon nanotubes and GO, Huang et al. [8] replaced them with carbon nanospheres, which were synthesized by the reaction of phenol and formalin aqueous solution, to construct the fluorescent sensor. However, due to the possible toxicity brought by formalin in their works, continuing efforts in pursuit of non-cytotoxic carbon-based nanomaterials will be encouraged. Meanwhile, nucleic acid aptamer created from a large random sequence pool can specifically bind to various molecular targets and has been widely employed as an alternative to antibody to construct sensors in bioanalysis [9–12]. So the design of aptamer based fluorescent probes (also called fluorescent aptasensor) which can only be turned on when binding a target cell is highly desired in the fluorescence cell imaging.

Considering the above background, in this contribution, we studied the feasibility of CNSs obtained by the carbonization of glucose [13,14] as a novel sensing platform for the construction of sensitive "turn off/on" fluorescent nanoprobe. It has been reported that such amorphous CNSs might even present in carbohydrate containing foods such as bread, corn flakes [15]. Breast cancer is becoming the main cause of cancer deaths for women [16]. MUC1, a large molecular weight transmembrane glycoprotein aberrantly overexpressed in diseased tissues compared with normal tissues, is closely associated with many types of cancers and can be considered as tumor biomarker in the diagnosis of breast cancer [17]. As a proof of concept, the detection of MCF-7 breast cancer cell and tumor antigen MUC1 is selectively studied. As illustrated in Fig. 1A, the aptasensor is fabricated by the adsorption of FAM (a dye)-labeled MUC1 aptamer P0 (P0-FAM), which can recognize the MUC1 on the surface of MCF-7, onto the surface of CNSs via the electrostatic force. To enhance the adsorption of MUC1 aptamer on CNSs, their surfaces are modified with branched polyethylenimine (PEI) in advance, which can endow CNSs' surfaces with the positive charge. Under this condition, the aptasensor is "turn off" due to the quenching effect induced by graphitization domains in CNSs

* Corresponding authors.
E-mail addresses: lou@seu.edu.cn (Y. Lou), nyhe@seu.edu.cn (N. He), zfwang@seu.edu.cn (Z. Wang).

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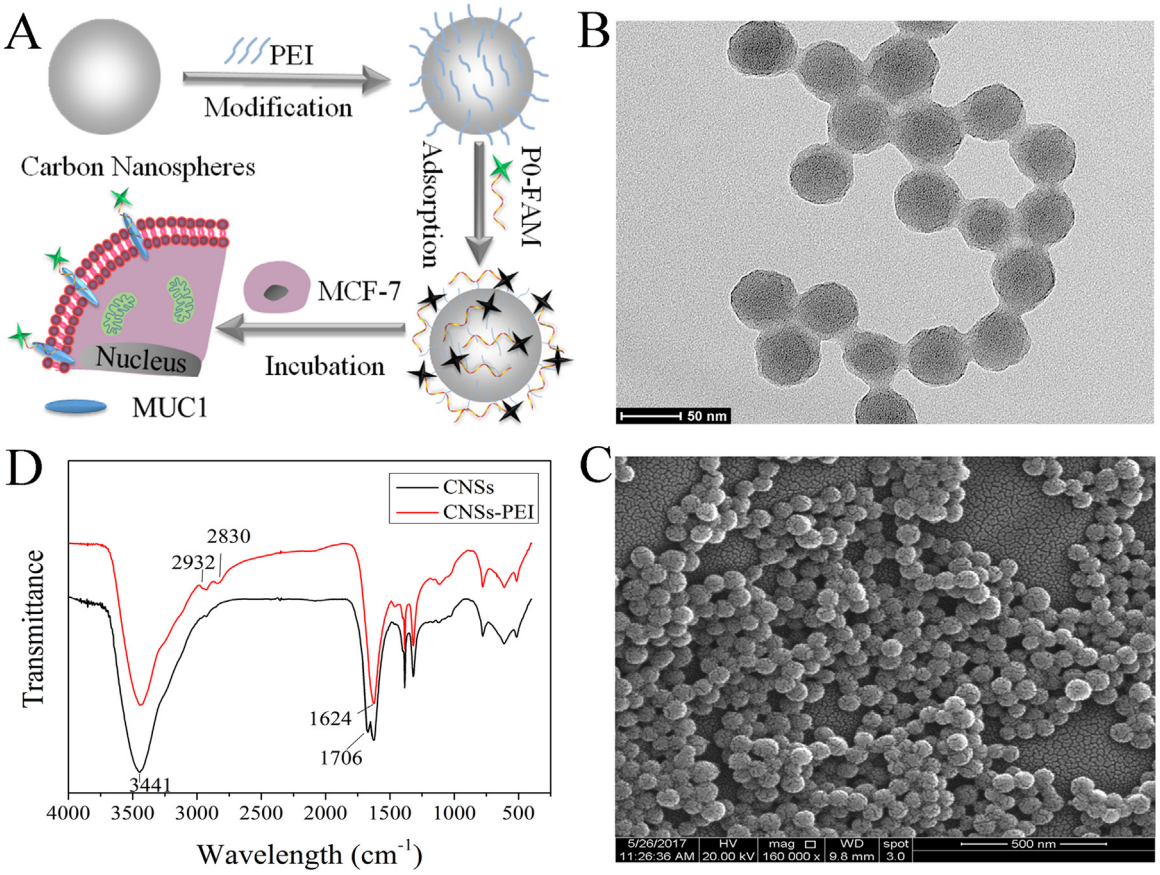

Fig. 1. Schematic illustration of the sensing principle based on the CNSs/P0-FAM aptasensor (A); TEM (B) and SEM (C) images of CNSs; FT-IR spectra (D) of CNSs and CNSs-PEI.

and no fluorescence signal is observed. However, in the presence of MCF-7 cells, the fluorescence of P0-FAM will be "turn on" due to the detachment of P0-FAM from CNSs by means of the stronger interaction between P0-FAM and MUC1 than that between P0-FAM and CNSs.

2. Material and methods

2.1. Materials and reagents

Mammary Epithelial Cell Growth Medium (MEGM) was obtained from Beijing Bitab Biotech Co., Ltd. Roswell Park Memorial Institute-1640 (RPMI-1640) culture medium, fetal bovine serum (FBS), lysozyme and Cytochrome C were obtained from Sangon Biotech Co., Ltd. Trypsin was obtained from Beyotime Biotech Co., Ltd. FAM-labeled MUC1 aptamer P0 (5'-FAM-GCAGTTGATCCTTTGGATACCCTGG-3') was synthesized by Sangon Biotech. The MUC1 peptide (N terminus → C terminus: PDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSA) was synthesized and purchased from the DGpeptides Co., Ltd. (Hangzhou, China). MCF-7 cells and MCF-10A cells were obtained from cell bank in Shanghai Institute for Biological Science. Branched polyethylenimine (MW = 600 Da) was purchased from Aladdin.

2.2. Apparatus

TEM measurements were conducted on an Ultra 55 electron microscope (Germany). SEM images were taken using an FEI Inspect F50 scanning electron microscope (FEI, U.S.A). FT-IR spectrum was measured using Nicolet 5700 spectrometer (U.S.A). Zeta potential was measured by a Zeta Potential Analyzer (Brookhaven Instruments, U.S.A). XPS spectrum was recorded on a Thermo ESCALAB 250XI. Fluoromax-4 (Horiba, Japan) was used to measure and record the fluorescence emission spectra. The cell images were obtained by a Leica confocal scanning laser microscope (CSLM) (TCS SP8, Germany) with the laser excitation at 488 nm.

2.3. Synthesis of CNSs and PEI functionalized CNSs (CNSs-PEI)

CNSs were first synthesized by directly heating 10% aqueous glucose solutions at 170 °C according to previous reports [13,14]. Next, a sufficient amount of PEI was added into the aqueous solution of CNSs (1 mg/mL), and the resulting mixture was kept at room temperature for 36 h. After the reaction, the excess PEI was removed by the centrifugation and the resulting CNSs-PEI were finally dispersed in water (1 mg/mL).

2.4. Preparation of CNSs/P0-FAM nanocomplex

CNSs/P0-FAM nanocomplex was prepared by adding CNSs-PEI (30 μg/mL) into PBS containing 70 nM P0-FAM. And then the resulting solution was incubated at room temperature for 0.5 h. Finally, CNSs/P0-FAM nanocomplex was washed and stored at 4 °C. To test the fluorescence quenching ability of CNSs, other amounts of CNSs-PEI (0–35 μg/mL) were also used. The corresponding fluorescence intensity of solution was detected with Fluoromax-4 (Ex. 488 nm, Em. 515 nm).

2.5. Extracellular detection of MUC1 peptide with CNSs/P0-FAM nanocomplex

MUC1 peptide was used as the mimic of the tumor antigen MUC1 to test the recognition ability of CNSs/P0-FAM nanocomplex. To 300 μL of PBS containing CNSs/P0-FAM nanocomplex (30 μg/mL), different amounts of MUC1 peptide was added. After incubation for 0.5 h, the