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A fluorescence turn-on biosensor based on graphene quantum dots (GQDs) and molybdenum disulfide (MoS_2) nanosheets for epithelial cell adhesion molecule (EpCAM) detection

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ABSTRACT

This paper presents a "turn-on" fluorescence biosensor based on graphene quantum dots (GQDs) and molybdenum disulfide (MoS_2) nanosheets for rapid and sensitive detection of epithelial cell adhesion molecule (EpCAM). PEGylated GQDs were used as donor molecules, which could not only largely increase emission intensity but also prevent non-specific adsorption of PEGylated GQD on MoS_2 surface. The sensing platform was realized by adsorption of PEGylated GQD labelled EpCAM aptamer onto MoS_2 surface via van der Waals force. The fluorescence signal of GQD was then quenched by MoS_2 nanosheets via fluorescence resonance energy transfer (FRET) mechanism. In the presence of EpCAM protein, the stronger specific affinity interaction between aptamer and EpCAM protein could detach GQD labelled EpCAM aptamer from MoS_2 nanosheets, leading to the restoration of fluorescence intensity. By monitoring the change of fluorescence signal, the target EpCAM protein could be detected sensitively and selectively with a linear detection range from 3 nM to 54 nM and limit of detection (LOD) around 450 pM. In addition, this nanobiosensor has been successfully used for EpCAM-expressed breast cancer MCF-7 cell detection.

1. Introduction

Epithelial cell adhesion molecule (EpCAM) is a glycosylated membrane protein expressed on the surface of circulating tumor cells (CTCs) (Baeuerle and Gires, 2007). EpCAM protein is considered to be the most frequently and intensely studied tumor-associated antigens because of its overexpression in most cancer cells, including colorectal cancer (Dalerba et al., 2007), breast cancer (Gastl et al., 2000), gallbladder cancer (Varga et al., 2004), pancreatic cancer (Fong et al., 2008) and liver cancer (Yamashita et al., 2010). In a perspective view, EpCAM has been regarded as a prognostic tumor biomarker for cancer diagnosis, prognosis and therapy (Baeuerle and Gires, 2007). Previously, cytometry technique, polymerase chain reaction (PCR), or a combination or both approaches have been widely used for EpCAM based CTCs detection (Ntouroupi et al., 2008; Lambrechts et al., 1999). However, these methods suffer from the disadvantages of long analytical time, labor-intensive operation and expensive instruments, which hamper the application of point of care diagnosis (Ntouroupi et al., 2008; Lambrechts et al., 1999; Gubala et al., 2012). Besides, a majority of EpCAM-based diagnostic and therapeutic approaches are relied on anti-EpCAM antibody, which fails to provide objective clinical response because of the large size and instability in physiological environment (Schwartzberg, 2001; Armstrong and Eck, 2003). Therefore, small sized aptamer with the merits of ease of synthesis, good stability, fast tissue penetration and low toxicity has raised much attention as a perfect alternative of antibody (Tan et al., 2013). The specific affinity interaction between aptamer and different biomolecular targets also stimulates the extensively application of aptasensor (Song et al., 2013).

Fluorescence resonance energy transfer (FRET), relying on the energy transfer between donor and acceptor, is a powerful tool to monitor biomolecular interactions in nano-scale. The FRET based aptasensor is very promising due to its direct response and feasible quantification (Choi et al., 2006; Bagalkot et al., 2007; Shi et al., 2015a; Tsang et al., 2016). Traditional FRET pairs, such as fluorescent dyes and proteins, are mainly limited by poor photobleaching resistance and low chemical stability. Novel fluorescence nanoparticles, such as semiconductor quantum dots (QDs) (Zhang et al., 2005) and upconversion nanoparticles (UCNPs) (Ye et al., 2014), are photo-stable fluorescence probes but hampered by the high toxicity for biological applications. The emergence of graphene quantum dots (GQDs) as fluorescence probes perfectly solves these problems (Sun et al., 2013). GQDs are well-confined

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0D graphene fragments with high brightness, good photo-stability and excellent biocompatibility, which ensure their application as FRET donors in long-term bio-detection (Zhu et al., 2012; Shi et al., 2015b; Qian et al., 2015; Bhatnagar et al., 2016).

In addition, graphene oxide (GO), the water-soluble graphene derivative equipped with super quenching ability, has been used as efficient fluorescence acceptors in FRET assay (Shi et al., 2015c). Recently, much focus has been paid to other 2D materials such as transition metal dichalcogenides (TMD) due to their graphene-analogous structure (Xu et al., 2013; Yang et al., 2015). Among them, molybdenum disulfide (MoS_2) has attracted tremendous attention due to its easy exploitation, unique electronic and optical properties. Single-layered MoS_2 nanosheet is made up of a hexagonal layer of molybdenum atoms sandwiched between two layers of sulfur atoms (Ataca and Ciraci, 2011). Due to its high quenching capability and good biocompatibility, MoS_2 nanosheets started to be used as quenchers in FRET assays for biosensing applications. Zhu et al. (2013) firstly reported the high fluorescence quenching capability of MoS_2 nanosheet for fluorescent dye labelled ssDNA and demonstrated the different affinity to ssDNA versus dsDNA. After that, MoS_2 nanosheet based FRET platforms have been used for protein detection including DNA methyltransferase (MTase) activity (Deng et al., 2015), human a-thrombin (Ge et al., 2014) and prostate specific antigen (PSA) (Kong et al., 2015). However, the current MoS_2 FRET assays mainly used fluorescent dyes as donor molecules. The FRET assay with GQD as donor and MoS_2 as acceptor for protein detection has not been explored.

Herein, we reported a novel GQD-PEG-aptamer/MoS_2 based FRET assay for EpCAM protein detection with GQD as donor and MoS_2 nanosheet as quencher. The FRET assay was established by attachment of GQD labelled aptamer on MoS_2 nanosheets, triggering FRET phenomena due to close proximity between GQD and MoS_2 nanosheets. The fluorescence signal was maintained at "off" status due to the fluorescence quenching of GQD by MoS_2 nanosheets. In the presence of EpCAM protein, the specific affinity between aptamer and EpCAM protein would detach GQD labelled aptamer from MoS_2 surface, which turned "on" the fluorescence signal. By monitoring the change of fluorescence signal, EpCAM protein could be detected sensitively. The proposed FRET assays displayed a linear range from 3 nM to 54 nM with a limit of detection (LOD) around 450 pM.

2. Materials and methods

2.1. Materials

Carboxylated graphene quantum dot (GQD-COOH) solution was purchased from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, Jiangsu, China). Molybdenum disulfide (MoS_2) nanosheets were purchased from Nanjing Mknano Science and Technology Co., Ltd. (Nanjing, Jiangsu, China). Amine-PEG-Amine (MW = 2000) was purchased from Laysan Bio, Inc. (Arab, Al, USA). 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS) were purchased from Sigma Aldrich (St. Louis, Mo, USA). All of these chemicals were used as received without further purification. Aptamer was synthesized and purified by Integrated DNA Technologies (IDT) Inc. (Coralville, IA, US). A 48-base hairpin-structured aptamer modified with carboxyl group was used as the probe (5′-/5carboxy1/-CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG-3′) (Song et al., 2013). EpCAM recombinant human protein (hIgG1-Fc Ta) purchased from Sino biological Inc. (North Wales, PA, USA) was used as EpCAM-positive protein target. All the aptamer and protein were dissolved in ultrapure water to prepare stock solution.

2.2. Synthesis of PEGylated GQDs

Briefly, 2.4 mg of 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) and 3.6 mg of N-hydroxysulfosuccinimide (Sulfo-NHS) were added into 1 mL carboxylated graphene quantum dot (GQD-COOH) solution (1 mg/mL) and the mixture was stirred for 15 min. Then, 10% Amine-PEG-Amine diluted with PBS was added and incubated with activated GQD-COOH overnight at room temperature Finally, the PEGylated GQDs solution was purified and concentrated by ultrafiltration (Amicon Ultra-0.5, 3KD, Millipore). The possible large-sized aggregates could be filtered out by using microporous millipore membrane (0.22 μm) to get small GQD-PEG particles.

2.3. Synthesis of aptamer conjugated PEGylated GQDs

Carboxyl modified aptamer was then covalently conjugated onto the amine functionalized GQDs-PEG through EDC/NHS method. Initially, the carboxyl modified aptamer was pretreated with EDC/NHS for 15 min. After that, the activated aptamer with final concentration of 1.95 μM was added into amine functionalized GQDs-PEG solution. The mixture was then shaken overnight at room temperature. In order to remove excessive EDC, NHS and aptamer, the final product, GQD-PEG-aptamer, was purified and concentrated by ultrafiltration (Amicon Ultra-0.5, 30 KD, Millipore).

2.4. FRET assay establishment and EpCAM protein detection

Fluorescence spectra of FRET quenching efficiency and EpCAM protein detection were recorded by an Edinburgh FLSP920 spectrophotometer equipped with a 450 W steady-state xenon lamp at room temperature. In a typical experiment, the final product of GQD-PEG-aptamer (50 μL) was incubated with MoS_2 nanosheets (50 μL) against a series of final concentrations ranging from 10 μg/mL to 400 μg/mL. After 20 min incubation, the sample was measured with the excitation and emission wavelengths fixed at 360 nm and 466 nm, respectively. For EpCAM protein detection, GQD-PEG-aptamer/MoS_2 nanocomplex was incubated with an increasing concentration of target protein (3 nM, 9 nM, 18 nM, 36 nM, 54 nM). After 2 h incubation at 37 °C in the dark environment, the fluorescence signal was measured, respectively. The control experiments were conducted by measuring the fluorescence signal of sample after addition of the same volume of PBS. All the fluorescence spectra were recorded under the same condition.

2.5. Characterization

The morphology and size of GQDs, GQD-PEG and MoS_2 nanosheets were characterized using a JEOL-2100F transmission electron microscopy (TEM) equipped with an Oxford Instrument EDS system, operating at 200 kV. The absorption spectra of GQDs, GQD-PEG, MoS_2 nanosheets were characterized by a UV–vis spectrophotometer (Ultrospec 2100 pro). The Zeta potential and size distribution of MoS_2 nanosheets were determined at neutral pH environment with a Zetasizer Nano Z system from Malvern Instruments Ltd.

3. Results

3.1. Mechanism of GQD-PEG-aptamer/MoS_2 based FRET biosensor

The sensing mechanism of GQD-PEG-aptamer/MoS_2 based FRET biosensor for EpCAM protein detection is shown in Scheme 1