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Robust fluorescence sensing platform for detection of CD44 cells based on graphene oxide/gold nanoparticles

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ABSTRACT

Gold-coated graphene oxide hybrid material (GO/AuNPs) has exceptional physical and chemical properties like π–π stacking interaction and plays a role in quencher of fluorescence dye. Therefore, GO/AuNPs could enhance the signal-to-background ratio with fluorescence dye that was the point in this fluorescent biosensor. In this study, tetramethyl-6-carboxy-rhodamine (TAMRA)-labeled aptamers that specifically interact with the hyaluronic acid binding domain of CD44 were used as targets to investigate the applicability of the method. GO/AuNPs–TAMRA-aptamer complexes could detect CD44 target cancer cells within a concentration range of 1×10^1 to 1×10^7 CFU/mL. A linear relationship was observed between target cell concentration and relative fluorescence intensity. The more mounted up CD44 target cell concentrations, relative fluorescence intensity of GO/AuNPs–TAMRA-aptamer complexes was increased even more, which was superior to that of GO alone. Sensitivity of the detection system displayed a low detection limit of 1×10^1 CFU/mL. Additionally, this method is specific in that fluorescence is not much enhanced in CD44 negative cancer cell line. Thus, the fluorescence sensing based on GO/AuNPs could be developed to receptive and robust detection tool for various target molecules.

1. Introduction

In recent decades, research and development of DNA biosensors has attracted considerable attention in the fields of clinical diagnosis, screening for food poisoning, and environmental monitoring [1–3]. As a result, numerous types and techniques of DNA biosensors based on fluorescence, colorimetry, electrochemistry, and surface plasmon resonance spectroscopy have been investigated and developed for DNA detection [4–9]. For example, fluorescence-based DNA biosensors exhibit relative ease of operation, high specificity and sensitivity, as well as high-throughput analysis [10–13]. Despite of these advantages, a low signal-to-background ratio may occur due to the intrinsic signal of fluorescence dyes in the absence of targets [14]. Therefore, there is a need for a fluorescence-based DNA biosensor that eliminates the high background while maintaining target specificity.

Recently, nanomaterials have been used in the field of biosensor systems. Especially, graphene oxide (GO) has various interesting features like as large surface area, volume ratio, and quenching effect by surface energy transfer with fluorescence dyes [15–18]. Moreover, GO offers abundant carboxyl, hydroxyl, and epoxy groups on the surface of a two-dimensional layered carbon sheet from a graphene precursor [19]. It can provide unique characteristics such as via π–π stacking and weak electrostatic attractions between single-stranded DNA (ssDNA) and the GO surface [20,21]. This is a key factor of enhancing the sensitivity of fluorescence-based DNA detection system. In spite of their merits and capabilities of GOs, however, serious level of agglomeration may reduce their surface area and stability. Consequently, these phenomena limit the interaction between GO and ssDNA [22,23].

To overcome such problems, hybrid nanomaterial has been provided an alternative choice to meet the requirements. Hybrid materials with nanoparticles such as silica, silver, and platinum as coating materials have been used on the surface of GO [24–26]. However, these nanoparticles are inappropriate for DNA-based biosensor because of repulsive force or high reactivity with other reagents [27–29]. Conversely, AuNPs have many outstanding properties including biocompatibility, chemical inertness, ability to immobilize the ssDNA and enhancement of solubility [30,31]. Therefore, combining of the AuNPs onto the surface of GO (GO/AuNPs) can improve the stability in solution and maintain a certain distance from GO. Moreover, GO/AuNPs allows higher quenching efficiency than GO or AuNPs alone that can provide a good signal-to-background ratio for improving the performance of fluorescent methods [32–35].

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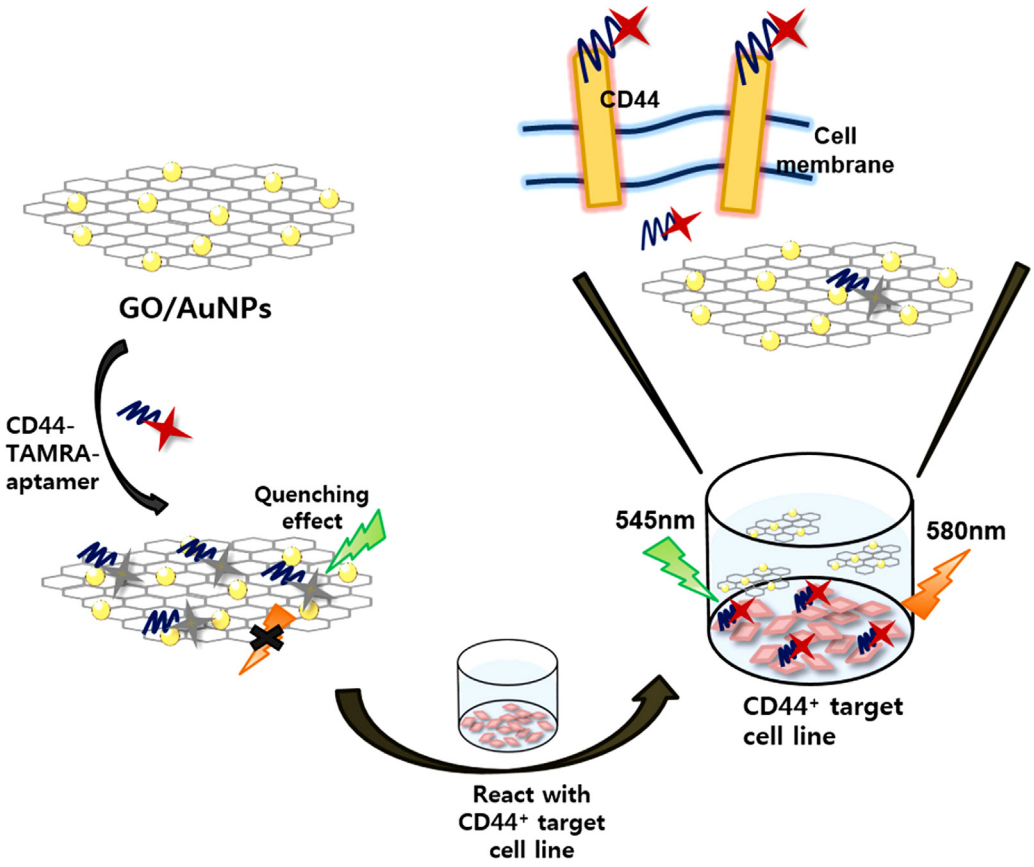

Fig. 1. Schematic illustration of GO/AuNPs sensing-based fluorescent detection.

The integral transmembrane protein CD44 is the primary receptor for hyaluronic acid (HA). It promotes proliferation, migration, invasion, and tumor-associated angiogenesis, which result in cancer development [36]. CD44 can therefore be used as a biomarker for cancer diagnosis or prevention. Conjugation of chemotherapeutic drugs or other anticancer agents to HA or anti-CD44 antibodies has demonstrated for successful delivery to the tumor site as well as increased efficacy in animal tumor models. This suggests that inhibition of tumor growth and metastasis occurred by CD44–HA interactions, thus indicating that these interactions may provide a new approach to the targeted diagnosis and treatment of specific cancers [36–38]. However, this hypothesis is difficult to evaluate because the interaction between HA and CD44 is very weak. Aptamer has become increasingly important diagnostic and therapeutic molecular tools [39] and an aptamer that specifically binds to the HA-binding domain of CD44 with high affinity was reported [37,40].

Here, we describe a simple and sensitive fluorescence-sensing platform based on GO/AuNPs, which constructs for fluorescence detection of CD44 with CD44-specific aptamer (Fig. 1). Initially, tetramethyl-6-carboxyrhodamine (TAMRA) dye-labeled CD44 aptamer strongly binds to the surface of GO/AuNPs to form a self-assembled structure via π–π stacking and electrostatic attractions. Accordingly, the fluorescence of TAMRA dye has almost perfectly quenched. When GO/AuNPs–aptamer complexes add to target cell lines, hybridization occurs between the CD44 aptamer and HA-binding domain of $CD44^+$ target cells. Finally, the fluorescence intensity of TAMRA markedly increases by its release from the GO/AuNPs surface compared with GO alone. This fluorescence-sensing platform based on GO/AuNPs can be adapted to provide accurate, sensitive, and specific detection of diverse targets, and serve as a signal-to-background ratio enhancer with improved efficacy compared to GO alone.

2. Materials and methods

2.1. Reagents and apparatus

Graphite powder was obtained from Kanto Chemical (Kanto, Japan). Potassium permanganate ($KMnO_4$), phosphorus pentoxide (P_2O_5), concentrated sulfuric acid (H_2SO_4), potassium persulfate ($K_2S_2O_8$), gold(III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), trisodium citrate ($Na_3C_6H_5O_7$), and phosphate-buffered saline (pH 7.4) were purchased from Sigma–Aldrich (St. Louis, MO). Hydrazine solution (NH_2NH_2), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) were obtained from Samchun (Seoul, Korea). Trypsin-EDTA solution was purchased from Welgene (Gyeongsan, Korea). Filter papers were purchased from Whatman (Dassel, Germany). The TAMRA-labeled aptamer (5′-TAMRA-GAG ATT CAT CAC GCG CAT AGT CCC AAG GCC TGC AAG GGA ACC AAG GAC ACA GCG ACT ATG CGA TGA TGT CTT C-3′) was synthesized by MACROGEN (Seoul, Korea). Five types of human cancer cell lines (SKOV3, MDA-MB-231, A549, MCF-7, and T98G) were obtained from Korean Cell Line Bank (Seoul, Korea). All other reagents were analytical reagent grade and were used as received without further purification or treatment. Ultrapure water obtained from a Millipore water purification system (Milli-Q, Billerica, MA) was used in all assays. UV/vis spectrophotometer analysis was conducted with OPTIZEN POP spectroscopy (Mecasys, Daejeon, Korea). Fluorescence spectroscopic analysis was performed using the OLYMPUS DP73 microscope (Tokyo, Japan) and transmission electron microscope (TEM) analysis was conducted with a Tecnai G2 F30 S-Twin microscope (FEI, Hillsboro, OR). Atomic force microscope (AFM,