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Development of a bifunctional nanobiosensor for screening and detection of chemokine ligand in colorectal cancer cell line

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ABSTRACT

Highly sensitive detection of chemokines in various biological matrices and its interaction with a natural receptor molecule has tremendous importance in cell signaling, medical diagnostics, and therapeutics. In this direction, we have designed the first bifunctional nanobiosensor for chemokine screening and detection in a single experimental setting. The sensor probe was fabricated by immobilizing CXCR2 on the gold nanoparticles (AuNPs) deposited 2,2′:5′,2"-terthiophene-3′ (p-benzoic acid) (TBA) nanocomposite film. The interaction between CXCR2 and chemokines was studied using electrochemical impedance spectroscopy (EIS) and voltammetry. CXCL5 among three ligands showed the strongest affinity to CXCR2, which was further utilized to develop an amperometric CXCL5 biosensor. Analytical parameters, such as CXCR2 receptor concentration, temperature, pH, and incubation time were optimized to obtain the high sensitivity. A dynamic range for CXCL5 detection was obtained between 0.1 and 10 ng/mL with the detection limit of 0.078 ± 0.004 ng/mL (RSD < 4.7%). The proposed biosensor was successfully applied to detect CXCL5 in clinically relevant concentrations in human serum and colorectal cancer cells samples with high sensitivity and selectivity. Interference effect and the stability of the developed biosensor were also evaluated. Method verification was performed by comparing the results using commercially available ELISA kit for CXCL5 detection.

1. Introduction

Chemokines are a family of small cytokines or signaling proteins secreted by cells (Le et al., 2004). They have tremendous importance in cell signaling, diagnostics, and therapeutics (Cyster, 1999). It has been reported that chemokine ligands (CXCLs) levels are elevated in various tumors isolated from cancer patient's tissue samples (Bersini et al., 2014). The role of CXCL expression is also directly correlated with tumor growth, tumor derived angiogenesis, and metastasis (Balkwill, 2004; Bersini et al., 2014). The interaction of various CXCLs (eg: CXCL5, CXCL8, CXCL13) with the natural receptor molecule is very important, and possess various biological functions (Sun et al., 2008), even though no comparative binding study between ligands and receptor has been done till date. Among all the CXCLs, CXCL5 has been extensively studied over the years and it has been identified as a novel prognostic factor in various cancers (Ben-Baruch, 2012; Sun et al., 2008; Kowalczuk et al., 2014). Therefore, CXCL5 is a valuable biomarker to study the prognosis of various cancer which may provide valuable information's to clinicians, eventually helpful in cancer therapy. In view of such an important clinical biomarker, CXCL5 or its expression levels has been analyzed using enzyme-linked immunosorbent assay (Agarwal et al., 2013), immunohistochemistry (Kawamura et al., 2012), and quantitative real-time PCR (Li et al., 2011) in various biological samples. These methods are important; however, they have limited point-of-care clinical applications because they are lengthy, require highly trained professionals, and in ability of point-of-care analysis. Thus, the development of a sensitive, robust, and alternate method for CXCL5 detection in the clinically relevant range in body fluids as well as cancer cell model is extremely important and clinically significant, which may provide valuable information's to clinicians to understand cancer progression, eventually assisting in cancer therapy. To do this, an electrochemical biosensor can be preferred over other detection methods due to its robustness, user friendly mode of operation, and ability of miniaturization for on-site medical diagnosis (Rahman et al., 2008; Hussain et al., 2017). It is worthwhile to indicate here that in this work we aim to design a bifunctional electrochemical biosensor which can perform dual task in a single experimental design. First, finding the best ligand (CXCL5) for its interaction with the natural receptor and second quantitative detection of CXCL5 in human serum and cancer cell model to show its clinical

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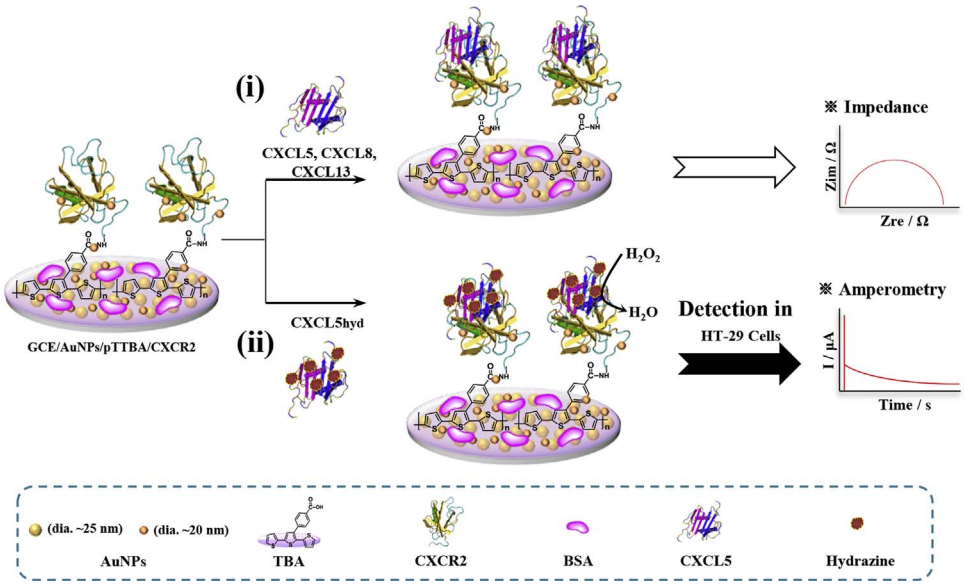

Scheme 1. Schematic diagram of the bifunctional CXCR2 sensor probe for (i) chemokine selection and (ii) CXCL5 detection in human serum and colorectal cancer cells.

importance.

Among various bioreceptors for CXCL5 detection, a chemokine receptor molecule "CXCR2", having natural affinity (Thomson and Lotze, 2003) would be interesting to attempt, which may offer high binding affinity, eventually assisting in highly sensitive CXCL5 biosensor development. We have experimentally observed that CXCL5 has the highest affinity to CXCR2 sensor probe than CXCL8 and CXCL13. To achieve a highly sensitive detection of CXCL5, chronoamperometry based nanobiosensor is worthy to attempt due to its high selectivity, stability, and sensitivity (Bardea et al., 1999; Chandra, 2016; Zhu et al., 2012). To do this, the stable immobilization of bioreceptors on the sensor probe surface is very important and critical in biosensor fabrication (Chandra et al., 2011; Kwon et al., 2006; Lee and Shim, 2001). Electrochemical biosensors fabricated using conducting polymers-AuNPs composite (Koh et al., 2011; Kim and Shim, 2013; Chandra et al., 2015) are known to be highly stable and ultrasensitive because the receptor biomolecules can be covalently immobilized on the polymer backbone comprising -COOH or -NH_2 groups (Lee and Shim, 2001; Rahman et al., 2004; Kim et al., 2009).

In the present study, we have systematically studied the interaction between a chemokine receptor and chemokines using a bifunctional nanobiosensor. The fabricated biosensor was characterized by various methods. The experimental parameters were optimized and the detection limit of a target chemokine ligand was determined. The developed sensor was operated in amperometric format to detect CXCL5 directly in human serum and colorectal cancer cells for the first time to show its real clinical value. The selectivity of the biosensor was also examined toward various non-target proteins and biochemicals present in the real sample matrix. The sensing results were also validated by comparing them with commercial ELISA.

2. Experimental

2.1. Materials

2,2′:5′,2"-terthiophene-3′ (p-benzoic acid) (TBA) was synthesized using the Paal-Knorr pyrrole condensation reaction (Kim et al., 2009). Tetrabutylammonium perchlorate (TBAP, electrochemical grade) was purchased from Fluka (USA) and purified according to a general laboratory method followed by drying under vacuum at 1.33×10^{-3} Pa. CXCR2 receptor, CXCL5, CXCL8, CXCL13 ligands were purchased from Abcam (USA). 1-Ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), dichloromethane (99.8%, anhydrous, and sealed under N_2 gas), sodium citrate ($C_6H_5Na_3O_7$), sodium borohydrate ($NaBH_4$), and hydrogen peroxide (H_2O_2), bovine serum albumin (BSA), IgG, myoglobin, haptoglobin, citrulline, fibrinogens were purchased from Sigma-Aldrich (USA). The CXCL5 ELISA kit was purchased from R & D Systems (USA) and was used as per the manufacturer instructions. Phosphate buffer (desired pH) was prepared using monobasic and dibasic sodium phosphates following general laboratory procedure. All other chemicals were of extra pure analytical grade and were used without further purification. Human breast adenocarcinoma (SK-BR3), cervical cancer (HeLa), human colorectal cancer (HT-29) cell lines were obtained from American Type Culture Collection. Dulbecco's Modified Eagle's Medium (DMEM) was purchased from BioWhittaker®, whereas RPMI-1640 medium, fetal bovine serum (FBS) and other cell culture materials including Dulbecco's phosphate buffered saline (PBS) were purchased from Sigma® (USA)

2.2. Apparatus

All electrochemical experiments were done in a conventional three electrode cell system using a potentiostat/galvanostat, Kosentech, model KST P-2 (South Korea). The modified glassy carbon electrode (GCE) (dia. 3.0 mm), Ag/AgCl (in saturated KCl), and a platinum (Pt) wire were used as working, reference, and counter electrodes, respectively. The QCM experiment was performed using a SEIKO EG & G model QCA 917 and a PAR model 263 A potentiostat/galvanostat (USA). An Au-coated working electrode (area: 0.196 cm^2; 9 MHz; AT-cut quartz crystal) was used to perform the quartz crystal microbalance (QCM) experiment in a noise free cabinet. The impedance spectra were obtained at an open circuit voltage between 100 Hz and 1 MHz and a sampling rate of five points per decade using an EG & G Princeton Applied Research PARSTAT2263. X-ray photoelectron spectroscopy (XPS) was performed using a VG Scientific XPSLAB 250 XPS spectrometer and a monochromated Al Kα source with charge compensation at KBSI (Busan). The scanning electron microscopy (SEM) images were obtained using a Cambridge Stereoscan 240 at KBSI (Busan).

2.3. Biosensor fabrication

The diagram of bifunctional nanobiosensor for (i) chemokine ligand selection and (ii) CXCL5 detection has been shown in Scheme 1. In the first step, AuNPs were electrodeposited onto the GCE before the polymerization of TBA in a 0.5 M H_2SO_4 solution containing 0.001%