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Development of a molecularly imprinted polymer tailored on disposable screen-printed electrodes for dual detection of EGFR and VEGF using nano-liposomal amplification strategy

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ABSTRACT

This work demonstrates the development of a gold screen-printed electrode (Au-SPE)-based biosensor modified with a molecularly imprinted polymer and amplified using antibody-conjugated nano-liposomes. The developed biosensor was utilized for dual determination of epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) as cancer biomarkers. To prepare this biosensor, Au-SPE was modified with 3,3′-dithiodipropionic acid di(N-hydroxysuccinimide ester) via self-assembly method and then the target proteins (EGFR and VEGF) were covalently attached to the modified SPE. To synthesize the molecularly imprinted polymer, monomers of acrylamide and N,N′-methylenebis(acrylamide) were polymerized around the EGFR and VEGF templates, and to characterize the prepared biosensor, electrochemical impedance spectroscopy was used for analyses of surface changes in the engineered electrodes.

To produce reliable electrochemical signals, nano-liposomes which were loaded with Cd(II) and Cu(II) cations and decorated with antibodies specific for EGFR and VEGF were used as an efficient tool for detection of target biomarkers. In the analysis step, potentiometric striping analysis (PSA), as an electrochemical technique, was utilized for sensitive determination of these cations. The limits of detection (LODs) of EGFR and VEGF analyses were found to be 0.01 and 0.005 pg mL^{-1} with the linear dynamic ranges (LDRs) of 0.05–50000 and 0.01–7000 pg mL^{-1}, respectively. Moreover, the proposed biosensor was successfully used for sensitive, reproducible, and specific detection of EGFR and VEGF in real samples. Due to the SPE nature of the developed biosensor, we envision that this sensing tool has capability of being integrated with lab-on-a-chip (LOC), microfluidics, and micro total analysis systems.

1. Introduction

Miniaturized biosensors based on screen printed electrodes (SPE) for detection of multiple cancer biomarkers are an emerging tool for rapid, easy, and cost-effective detection of varieties of analytes (Arduini et al., 2016; Moreira et al., 2016; Patris et al., 2014). This type of sensors and biosensors often offer advantages over conventional methods. They allow researchers even non-specialist personnel to perform a large number of experiments with tiny volume of samples (Karami et al., 2017; Majidi et al., 2016). Since SPE can be successfully integrated with lab-on-a-chip (LOC), microfluidics, or micro total analysis systems, these are fascinating tools for analytical scientists (Punter-Villagrasa et al., 2014; Tüdős et al., 2001).

Cancer biomarkers are valuable in the early diagnosis, evaluation of chemotherapy, and demonstration of recurrence following chemotherapy (Afsharan et al., 2016; Johari-Ahar et al., 2015; Ludwig and Weinstein, 2005). For robust detection and evaluation process, physicians often request the screening of multiple markers to reach adequate sensitivity and selectivity of disease diagnosis. Technically, this multiple detection is a significant challenge for scientists in biosensor development; therefore, it is of great interest for varieties of analytical scientists.

Among cancer biomarkers, epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) have been recently proposed as valuable cancer biomarkers. Analysis of EGFR in the serum sample made possible early prediction of probability of response, progression-free survival (PFS), and overall survival (OS) in cancer patients (Gaafar et al., 2010; Hudelist et al., 2006), and VEGF is also a

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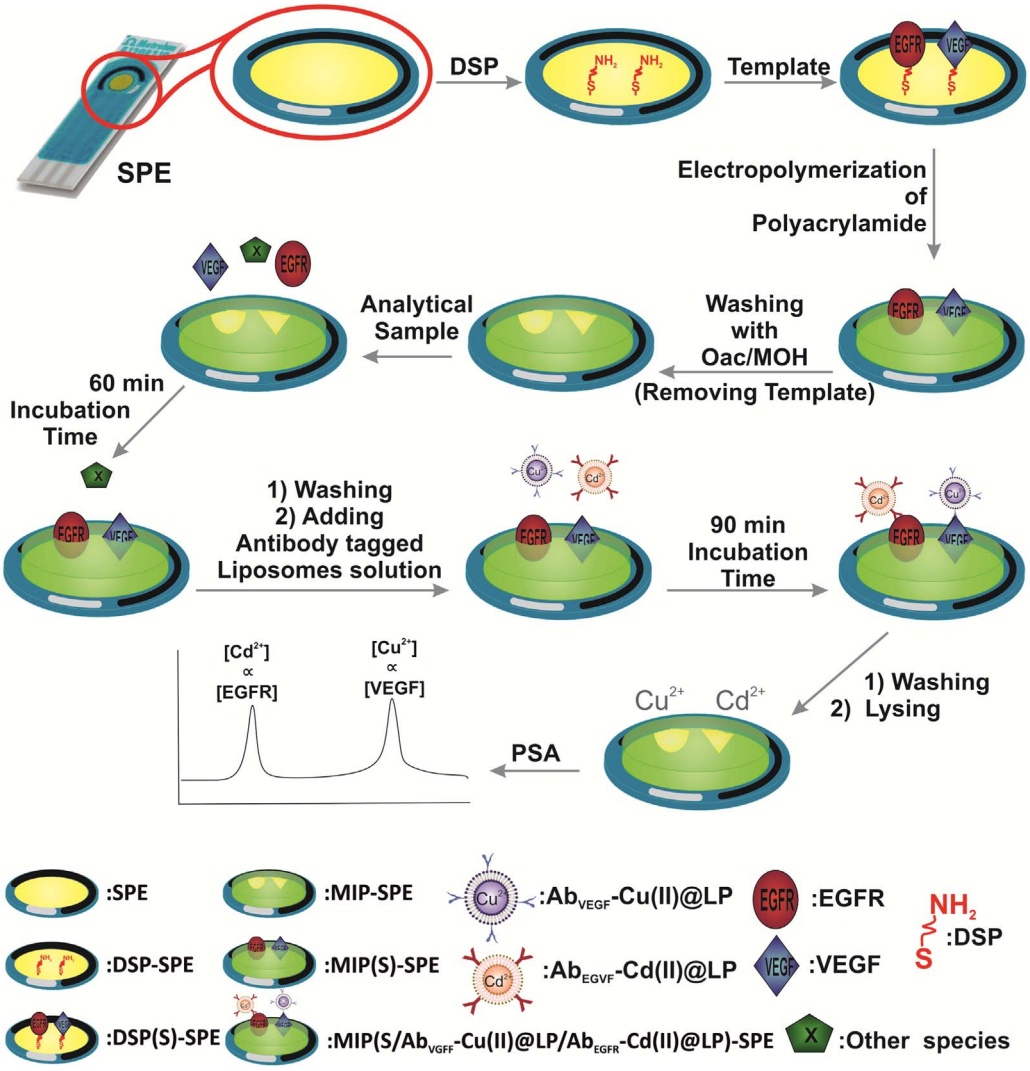

Scheme 1. Representation for the engineering processes of EGFR/VEGF biosensor.

signaling protein that is used in early diagnosis and metastasis detection of cancer (Herrlinger et al., 2004; Tamura et al., 2001; Zhou et al., 2009). However, concurrent detection of COX-2, EGFR, and VEGF-C have been proposed as an index for early diagnosis, recurrence prediction, and outcome evaluation for patients with endometrial carcinoma (Cai et al., 2017).

To date, various techniques have been reported for detection of EGFR and VEGF. For the detection of EGFR, quantum dot-based optical sensing (Ren et al., 2016; Wegner et al., 2014), surface plasmon resonance (Liu et al., 2011), scanning electrochemical microscopy (Takahashi et al., 2009), microfluidic biosensors (Regiart et al., 2017; Tian et al., 2017), impedimetric immunosensor (Elshafey et al., 2013b), nanoparticle-based detection (Mousavi et al., 2017; Vasudev et al., 2013), aptamer (Ilyas et al., 2012; Wan et al., 2012; Wang et al., 2012), peptide ligand (Li et al., 2013b), and aptamer-antibody based sandwich immunosensing (Ilkhani et al., 2015) have been proposed. For VEGF detection, electrochemical (Fu et al., 2016b), field effect transistor (Lee et al., 2009), optical (Freeman et al., 2012), capacitive (Qureshi et al., 2015) aptamer-based biosensors and molecular fluorescence endoscopy (Tjalma et al., 2016) have been proposed. However, there are few works reported for simultaneous detection of EGFR and VEGF. Until now, Kim et al. utilized fluorescence-Raman endoscopy in colorectal cancer imaging (Kim et al., 2017). In this study, we investigate the application of molecularly imprinted polymers (MIP) as the capture element and antibody-conjugated liposomes as a detection element for concurrent detection of VEGF and EGFR in serum samples. This work represents a novel strategy for simultaneous detection of biomarkers via efficient liposomal (LP) amplification strategy.

Molecularly imprinted polymers (MIPs) are emerging recognition elements that are selective to the analyte template, which can be considered as plastic antibody. In contrast to antibodies, MIPs have higher chemical and thermal stability, while being cost-effective and user-friendly (Canfarotta et al., 2016).

Recently, MIP-based electrochemical sensors/biosensor(s), which utilize MIP as recognition element, have been extensively used (Chen et al., 2011; Kryscio and Peppas, 2012; Song et al., 2014). However, there is no report on the development of electrochemical biosensors based on both MIP and antibodies for sandwich assay in dual detection of EGFR and VEGF.

2. Materials and methods

2.1. Chemicals

$K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$, K_2HPO_4 and KH_2PO_4 were purchased from Merck Co., (Darmstadt, Germany). Acrylamide (AAM) and N, N′-methylenebisacrylamide (NNMBA), ammonium persulphate (APS), oxalic acid (OXA), and sodium chloride were from Fluka. Potassium chloride, sodium chloride, cadmium (II) nitrate and copper (II) sulfate pentahydrate were purchased from Sigma-Aldrich Chemical Co. (St.