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Electrochemical immunosensor for tumor necrosis factor-alpha detection in undiluted serum

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ABSTRACT

An immunosensor for the sensitive detection and estimation of tumor necrosis factor-alpha (TNF-α) in undiluted serum has been developed via an electrochemical enzyme-linked immunosorbent assay (ELISA) process. Electrochemical sensing was performed using a TNF-α specific monoclonal antibody modified self-assembled monolayer of dithiobis(succinimidyl propionate) on a comb-shaped gold electrode microarray. After anti-TNF-α antibody binding, unreacted active groups of DTSP were blocked using ethanol amine (EA) and nonspecific binding was prevented using phosphate buffer based starting block T20 (SB). Sensitive and disposable SB–EA–anti-TNF-α/DTSP/Au electrodes were exposed to solutions with different TNF-α concentrations for 20 min in undiluted serum. Conversion of 4-aminophenyl phosphate to 4-aminophenol and its electrochemical oxidation was utilized for indirect estimation of TNF-α. Results for SB–anti-TNF-α/DTSP/Au electrodes indicate that the sensors can be used for the sensitive estimation of TNF-α in undiluted serum in the range 500 pg/ml to 100 ng/ml with a detection limit of 60 pg/ml and sensitivity of 0.46 $(ng/ml)^{-1}$. Negligible interference from serum and other biomarker proteins was observed. The described electrochemical ELISA is much faster than conventional ELISA and can be applied for sensing of a range of analytes in real patient samples.

1. Introduction

With an ever increasing demand for improved healthcare, informative biomolecules known as biomarkers are gaining much attention for accurate and faster diagnosis and prognosis of a specific disease [1,2]. These can be found at the early stage of diseases and quantification of these biomarkers from blood, urine or other tissues can provide vital information regarding patient health [3]. Thus, detection and estimation of their level accurately and sensitively is critical for early stage identification and proper recovery. Several approaches and protocols for their detection, such as optical (fluorescence, chemiluminescence, UV–visible), biophotonic, electrochemical sensors, electro-chemiluminescence, etc. have been reported in literature [3–5]. Among these, optical methods such as ELISA, fluorescence and bio-photonic have provided high quality data and are most often been employed for detection. However, such optical methods require complex equipment and trained personnel for operation; thus the development of alternative methods that give high quality data with simple procedures is desired [4,6]. Owing to their accuracy, higher sensitivity, portability, low cost and simplicity, electrochemical immunosensors have recently gained significant attention [1,3,4,7,8]. Among electrochemical immunosensors, electrochemical ELISA, which uses the selectivity of an antigen–antibody interaction on an electrochemical platform, is seen as one of the most promising methods. Electrochemical ELISA has the added advantage of not being affected by particles, chromophores, and fluorophores that might be present in a sample and which might cause interference with optical detection [9–11]. However, a simpler method for developing a convenient, stable and sensitive electrochemical ELISA still requires significant research.

In the development of an immunosensor, the immobilization of a capturing molecule, such as an antibody, onto a surface is crucial and can determine the stability of the sensor [10–14]. In attempts to immobilize and stabilize capturing biomolecules, a number of approaches such as the use of direct physical adsorption, self-assembled monolayers (SAM), polymer membranes, etc. have been investigated [1,10,11,15]. Among the covalent immobilization methods, use of thiol SAMs has shown a number of advantages such as an easy and efficient immobilization with uniform surface coverage. However, most of the SAMs require either activation or further modification before immobilization of the bioreceptor,

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which might disturb the integrity of the biomolecule [16]. Recently, SAMs based on dithiobissuccinimidyl propionate (DTSP) have shown that they can be used for facile covalent binding of biomolecules under very mild conditions, thus preserving their activity [17–19]. For successful development, a desirable biosensor should have capability to identify the desired biomarker in an unbiased manner in complex samples (e.g. serum) with various potentially interfering materials. Eliminating serum background is critical for biosensor development as serum contains high levels of cells, proteins, electrolytes, lipids, etc. which may interfere with the detection signal and may give rise to false positive signals in real samples [4]. For point-of-care (POC) applications, where both sample collection and the test are done on site, the use of blood or serum as a sample is unavoidable. Researchers have described various methods to reduce the serum background interference [4,20,21]. Among these, the use of serum dilution before testing has proven to be quite an effective method; however it also causes dilution of the target protein, thus lowering its accurate detection.

In the present work, an electrochemical ELISA based system has been developed by solving various issues involved in their development. DTSP has been used to immobilize antibody on comb-shaped gold micro array electrodes. A phosphate buffer saline/tween20 based starting blocker (SB) has been utilized for preventing false signals from detection of target tumor necrosis factor-alpha (TNF-α) protein in undiluted serum. SB contains a proprietary protein that provides exceptional performance and broad compatibility with ELISA assays. Furthermore, alkaline phosphatase that can biocatalyze 4-aminophenyl phosphate (4-APP), resulting in enhanced redox signal by enriching the catalysed product on the working electrode during sandwich assay, has been employed to get higher response. During the immunoassay, increasing TNF-α concentration causes increased antigen binding with immobilized antibodies on the sensor surface, which upon interaction with a second antibody and ALP-streptavidin results in higher conversion of 4-APP to 4-amino phenol. On electrochemical oxidation, this releases two electrons to produce quinonimide and is therefore detected on the surface. Fig. 1b shows the schematic steps of the immunoassay and detection process. The chosen TNF-α protein is a cytokine serving as potent protein biomarker for various physiological and pathological processes such as apoptotic cell death, fever and cachexia. It is also known to be involved in immune system regulation and in systemic inflammation [3]. In healthy individuals, cytokine levels, like TNF-α, usually exist from pg/ml to ng/ml levels, however in pathological conditions such as rheumatoid arthritis (RA), its level rises by ten to hundred times causing local cell apoptosis, thus necessitating rapid detection method for early disease diagnosis and prognosis. Its elevated levels to ng/ml in serum has also shown to be associated with a wide range of diseases, such as systemic erythema nodosum leprosum, Crohn's disease, endotoxic shock, HIV infection, stroke, neonatal listeriosis, severe meningococcemia and osteosarcoma [1,3,7,15,18,22,23], thus making it an important biomarker necessitating the development of immunosensors for its sensitive and selective detection. Current methods of detecting TNF-α include optical enzyme-linked immunosorbent assay (ELISA) along with chemiluminescence, radio-immunoassays, mass spectrometry/laser desorption, flow cytometry, enzyme-linked immunosorbent spot assay, and immuno-PCR [3–5,15,24]. Among these commercially available products, ELISA is quite sensitive and mainly employed; however it suffers from relatively high background signal and long procedure, bulky instrumentation and requirement of trained personnel. Other methods are also expensive, laborious and suffer from similar problems. In the present work attempt, a self-assembled monolayer based electrochemical ELISA for protein biomarker detection in undiluted serum has been developed.

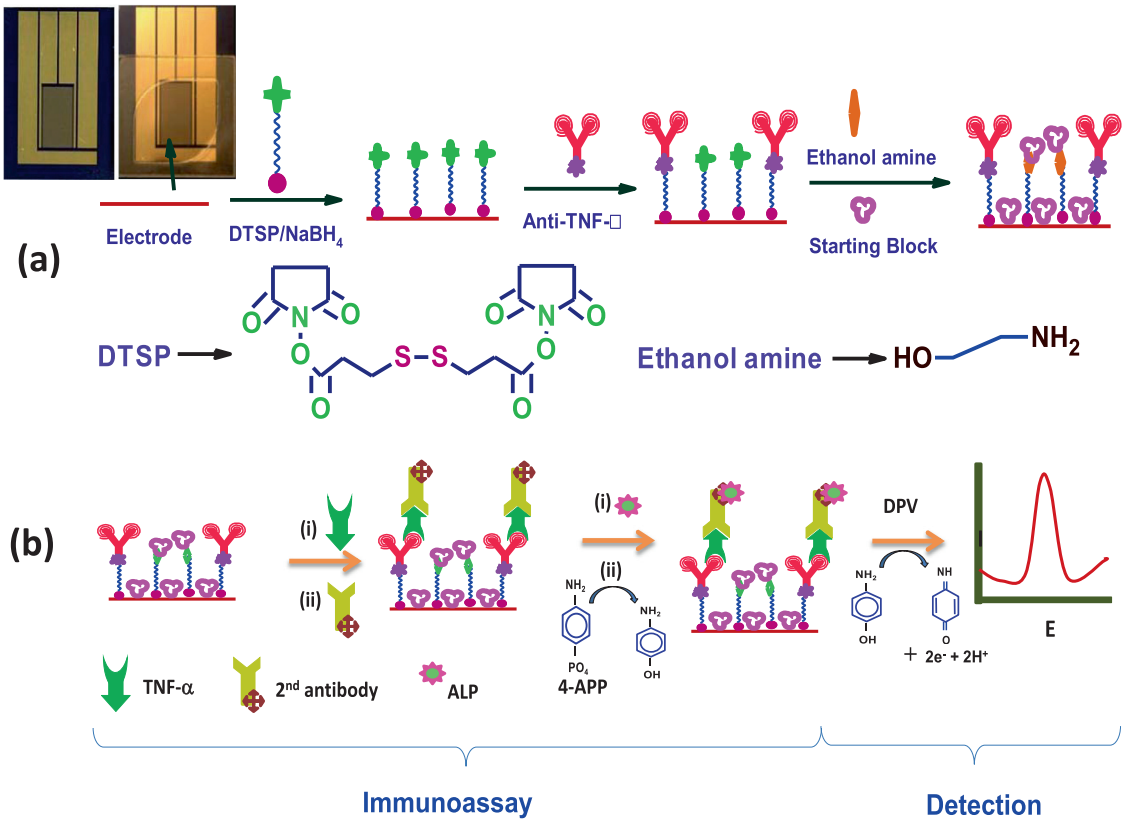

Fig. 1. (a) Schematic of the SB–EA–anti-TNF-α/DTSP/Au fabrication. (b) Schematic for immunoassay and testing.