

Capacitance Polypyrrole-based Impedimetric Immunosensor for Interleukin-10 Cytokine Detection

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Abstract: In the present study, we developed a novel label-free capacitance impedimetric immunosensor based on the immobilization of the human monoclonal antibody anti-interleukin-10 (anti-IL-10 mAb) onto polypyrrole (PPy)-modified silicon nitride (Si_3N_4) substrates. The immunosensor was used for the detection of the recombinant interleukin-10 antigen (rh IL-10) that may be secreted in patients at the early stage of inflammation. The immunosensor was created by chemical deposition of PPy conducting layer on pyrrole-silane (SPy)-treated $\text{Si}/\text{SiO}_2/\text{Si}_3\text{N}_4$ substrates ($\text{Si}/\text{SiO}_2/\text{Si}_3\text{N}_4\text{-SPy}$), followed by anti-IL-10 mAb immobilization through carboxyl-functionalized diazonium (CMA) protocol and carbodiimide chemistry. The surface characterization and the biofunc-

tionalization steps were characterized by SEM, FTIR and cyclic voltammetry (CV) while the detection process was carried out by using electrochemical impedance spectroscopy (EIS) analyses. The created immunosensor showed two linear fittings ($R^2=0.999$) for the detection of rh IL-10 within the concentration range from 1–50 pg/mL. It exhibited high sensitivity ($0.1128 (\text{pg/mL})^{-1}$) with a very low limit of detection (LOD) = 0.347 pg/mL, more particularly, at the low concentration range (1–10 pg/mL). Thus, this developed polypyrrole-based immunosensor represents a promising strategy for creation of miniaturized label-free, fast and highly sensitive biosensors for diagnosis of inflammation biomarkers at very low concentrations with reduced cost.

Keywords: Polypyrrole • Capacitance immunosensor • Interleukin-10 (IL-10) cytokine • Detection • Electrochemical impedance spectroscopy

1 Introduction

Interleukin-10 (IL-10) is the prototype of the anti-inflammatory cytokines, which are secreted during the pro-inflammatory process and during the acute stage of inflammation from T-cells, macrophages, B cells and keratinocytes [1]. IL-10 is a potent regulator of immunosuppression and plays an important role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis [2,3]. Its inhibitory action is exerted primarily towards the most typical cytokines markers of inflammation, such as IL-1, IL-6, IL-8, and anti-human tumor necrosis factor- α (TNF- α) cytokine that causes cell apoptosis. Thus, IL-10 is considered a potential therapeutic and detection tool for various diseases especially for autoimmune diseases associated with inflammatory components [4–6]. Recently, some studies showed that increasing the level of IL-10 in blood plasma is an effective biomarker for the risk prediction of future cardiovascular events in patients with acute coronary syndrome which may lead to heart failure (HF) and sometimes death [7]. Thus, the early diagnosis and treatment of inflammatory-related diseases are urgent via fast detection and quantification of these biomarkers at the early stages of inflammation. In this regard, the recombinant human antigen (rh IL-10) has been tested in healthy volunteers, and patients with

Crohn's disease, rheumatoid arthritis, psoriasis, hepatitis C, and HIV using various immunodiagnostic techniques such as enzyme-linked immunosorbent assay (ELISA)

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