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Label-free Impedimetric Immunosensor for the Detection of Fibulin 2 as a Novel Biomarker for the Diagnosis of Hypertrophic Cardiomyopathy in Human Saliva

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Summary:

a novel label-free impedimetric immunosensor was developed for the detection of a recently recognized as a new promising cardiac biomarker, fibulin 2 (FBLN2), for the diagnosis of hypertrophic cardiomyopathy. The approach is based on immobilization of the corresponding antibody, anti-Fibulin 2 (anti-FBLN2), onto array of gold microelectrodes functionalized with 16-mercaptohexadecanoic acid (MHDA). The assembly of 16-MHDA onto the gold working microelectrodes was confirmed by cyclic voltammetry (CV), while the interaction between FBLN2 and anti-FBLN2 was monitored using electrochemical impedance spectroscopy (EIS). Additionally, the different stages of surface modification were characterized using contact angle measurement (CAM), FTIR and Atomic Force Microscopy (AFM) in order to confirm the success of immobilization process. The analytical performance of the proposed immunosensor was evaluated and the immunosensor exhibited a high sensitivity with a good linearity in the range from 5 to 25 pg/mL with R^2 of 0.976. The developed immunosensor will be used for the detection of FBLN2 in human saliva using standard addition method.

Keywords: Electrochemical impedance spectroscopy, Impedimetric immunosensor, Fibulin 2, Hypertrophic Cardiomyopathy, Human saliva.

Introduction

A distinctive hallmark of Hypertrophic Cardiomyopathy (HCM) is the thickening of the left ventricle accompanied by Extracellular Matrix (ECM) remodeling, typically characterized by increased interstitial fibrosis [1]. The disease exhibits heterogeneity in clinical and pathological presentations, as well as in genetic backgrounds [2].

The ECM constitutes an intricate network of proteins essential for preserving the structural integrity and functional homeostasis of cardiac tissue. Maintaining a balance in collagen synthesis and degradation, protease activity, and the presence of fibulins, cytokines, and chemokines is critical for preserving cardiac function [3]. These myocardial changes in ECM composition can partially be monitored in the circulation due to the shedding and solubility of a group of relevant ECM proteins. These shed proteins can then be utilized as biomarkers for HCM. To date, limited numbers of gold standard biomarkers have been assigned for cardiovascular conditions prognosis and diagnosis, such as B-type natriuretic peptide (BNP), Cardiac troponin (cTNT) and N-terminal pro-BNP (NT-pro-BNP).

Fibulin-2 (FBLN2), an ECM glycoprotein, has recently reported to be upregulated in the myocardium of HCM patients and further been suggested as a potential biomarker for HCM patients [4]. FBLN2 is present in the ECM and is involved in elastic fiber assembly and stabilization [5]. Previous reports also indicated that FBLN2 plays an essential role in Ang II-induced TGF-β signaling and subsequent myocardial fibrosis. Therefore, accurate measurement of FBLN2 in the circulation of HCM patients will support these findings and further help introduce a non-invasive and effective platform for patients follow up.

An fast and sensitive detection of FBLN2 is needed to replace the ELISA which is a lab-based technique and requires a qualified personnel. In this study, we developed a novel label-free impedimetric immunosensor for the detection of a recently recognized cardiac biomarker, FBLN2, in saliva. To the best of our knowledge, the obtained sensor is the first to electrochemically measure the newly announced HCM biomarker FBLN2.

Materials

Anti-Fibulin 2 antibody was purchased from abcam. Recombinant human fibulin 2 protein was purchased from R&D SYSTEMS. 16-Mercaptohexadecanoic acid, ethanolamine, N-hydroxysuccinimide (NHS), and PBS tablets were purchased from Sigma-Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from CARL ROTH.

Apparatus

Electrochemical measurements were carried out using an electrochemical workstation, Bio-Logic SP-200 potentiostat. The developed chip produced by silicon technology integrtes four gold microelectrodes with four gold working electrodes, two Ag/AgCl reference μelectrodes and one Pt counter μelectrode.

Method

The gold microelectrodes were firsly cleaned using KOH and H_2O_2, followed by washing with acetone and dried under nitrogen. They were then incubated in MHDA. The carboxylic groups were activated using EDC/NHS then allowed to react with the amino groups of the anti-FBLN2 for 1 hr. The anti-FBLN2-modified electrodes were then immersed in different concentrations of FBLN2 and the EIS was recorded for each.

Results

The performed characterization proved the success of the immobilization process as the peaks of redox couple disappeared after assembly of MHDA onto the surface due to the blocking of the charge transfer (Fig.1).

The analytical performance of the proposed sensor was evaluated by EIS and the sensor exhibited a high sensitivity with a good linearity from 5 to 25 pg/mL with R^2 of 0.976 (Fig. 2).

Ongoing work

We are working on the interference study and the detection of FBLN2 in human saliva.

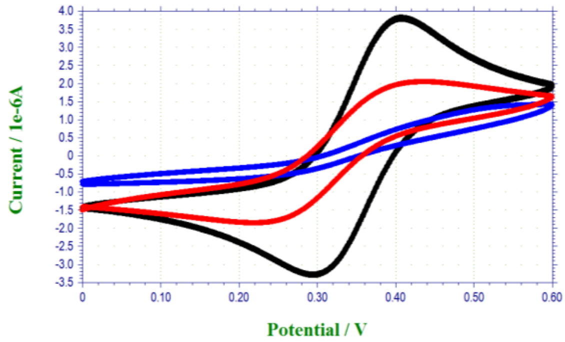

Fig. 1. Electrochemical characterization by CV recorded at each stage of electrode modification in 5 mM of K3[Fe(CN)6]/K4[Fe(CN)6] in PBS pH 7.4.

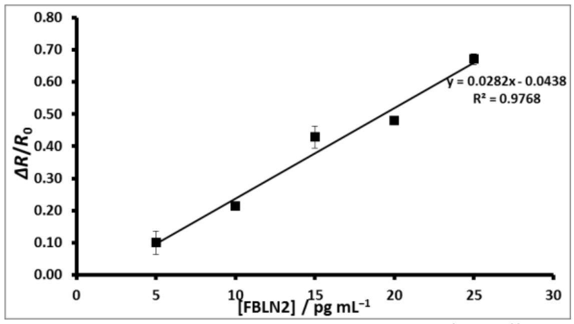

Fig. 2. The calibration curve obtained for different concentrations of FBLN2 using EIS .

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