Available online at www.sciencedirect.com

ScienceDirect

Procedia Engineering 168 (2016) 97 – 100

Procedia Engineering

www.elsevier.com/locate/procedia

30th Eurosensors Conference, EUROSENSORS 2016

Highly sensitive Electrochemical BioMEMS for TNF-α detection in humansaliva: Heart Failure

Alessia Longo[1,2], Abdoullatif Baraket[6,*], Monica Vatteroni[1,6], Nadia Zine[6], Joan Baussells[3], RogerFuoco[4], Fabio Di Francesco[4], Georgia S. Karanasiou[5], Dimitrios I. Fotiadis[5], Arianna Menciassi[2],Abdelhamid Errachid[6]

[1]*Biomedical Engineering University of Pisa, Italy.*
[2]*BioRobotics Institute Scuola Superiore Sant'Anna, Viale R. Piaggio 34, 56025 Pontedera (PI Italy).*
[3]*Instituto de Microelectronica de Barcelona, IMB-CNM (CSIC), Campus UAB, 08193, Bellaterra, Spain.*
[4]*Department of Chemistry and Indurstrial Chemistry, University of Pisa, Pisa, Italy.*
[5]*Department of Biomedical Research, Institute of Molecular Biology and Biotechnology, FORTH, Ioannina, Greece and Department of Materials Science, Unit of Medical Technology and Intelligent Information Systems, Ioannina, Greece.*
[6]*Université de Lyon, Institut de Sciences Analytiques (ISA) – UMR 5280, 5 rue de la Doua, 69100, France.**

Abstract

Prediction of disease progression using saliva as a diagnostic medium has roused the interest of scientific researchers in the 10 last past years. Potentially important biomarkers are increased in saliva during local and systemic inflammation. In the present study we have developed a highly sensitive biosensor for TNF-α detection in human saliva of patients suffering from heart failure. Therefore, a fully integrated electrochemical BioMEMS was developed in order to increase the sensitivity of detection, decrease the time of analysis, and to simultaneously detect varying cytokine biomarkers using eight gold working microelectrodes (WE). The monoclonal antibodies (mAb) anti-human Tumor Necrosis Factor alpha (anti-TNF-α) were immobilized onto gold microelectrodes through functionalization with carboxyl diazonium. Cyclic voltammetry (CV) was applied during the microelectrode functionalization process to characterize the gold microelectrode surface properties. Finally, electrochemical impedance spectroscopy (EIS) characterized the modified gold microelectrodes, and the detection range of TNF-α cytokines was from 1pg/mL to 15 pg/mL.

Peer-review under responsibility of the organizing committee of the 30th Eurosensors Conference

* Corresponding author. Tel.: +33617990332;
E-mail address: a.baraket@gmail.com

Peer-review under responsibility of the organizing committee of the 30th Eurosensors Conference
doi:10.1016/j.proeng.2016.11.156

*Keywords:*BioMEMS; heart failure; saliva; TNF-α.

1. Introduction

Heart failure (HF) is a condition where the heart fails in its duties of circulating blood through the lungs and back out to the tissues. Diagnosis of acute rejection is a complex and persistent problem in heart and ventricular assisted device (VAD) transplantation. To address this problem, measuring specific biomarkers can produce immediate information about the first signs of inflammation [1]. A wide spectrum of compounds (e.g. TNF-α cytokines) present in saliva may provide information for clinical diagnostic applications. Saliva is a good medium because its collection is not invasive and the donation process is relatively without stress. In recent years, different techniques have been used for the detection of protein biomarkers [2]. However, a majority of these techniques require adequate transducing elements, for instance fluorescent dyes or enzymes, to generate a signal which give rise of this interaction event. Increasing need for a fast, real-time and reliable medical diagnosis has led to growing interest in new point-of-care biological sensors capable for the sensitive and specific detection of biomolecules. For this interest we report in the present work on the fabrication of an electrochemical BioMEMS for multiple cytokine detection. This BioMEMS contains eight gold working microelectrodes (WEs) allowing a simultaneous detection (Fig. 1c). Cyclic voltammetry (CV) was applied during the microelectrode functionalization process to confirm the mAb immobilization and to characterize the gold microelectrode surface properties. Finally, EIS characterization was applied onto the gold WEs for TNF-α detection by the corresponding immobilized antibodies anti-TNF-α. The BioMEMS was highly sensitive to TNF-α within the range 1-15 pg/mL when compared to other interferences in artificial human saliva.

2. Experimental

2.1. Chemical and Reagent

Reagents used in this research are: 4-aminophenylacetic acid (4-carboxymethylaniline CMA), sodium nitrite ($NaNO_2$), hydrochloricacid (HCl), Ethanol, N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N-ethyl-carbodiimide hydrochloride (EDC), phosphatebuffered saline (PBS), phosphatebuffered saline Tween (PBSTween) and ($Fe^{2+}Fe^{3+}$) were purchased from Sigma Aldrich. Antibodies Tumor necrosis factor TNF-α, Recombinant Human TNF-α, Recombinant Human IL-10, Recombinant Human IL-8 and Recombinant Human IL-1, Antibodies TNF-α with fluorescence were purchased from R&D system France.Artificial saliva has been prepared by dissolving 0.6 g/L Na_2HPO_4, 0.6 g/L anhydrous $CaCl_2$,0.4 g/L KCl, 0.4 g/L NaCl, 4 g/L mucin and 4 g/L urea in deionized water, adjusted to pH 7.2, sterilized by autoclaving and stored in the refrigerator until use.

2.2. Antibodies and cytokine preparation

Antibodies and cytokines have been diluted in PBS buffer, aliquoted, and stored at −20°C following the protocol of the supplier. The cytokines TNF-α, IL-1 and IL-8 have been aliquoted before EIS measurements at different concentrations from 1, 5, 10 and 15 pg/mL respectively and stored at 4°C. For the detection in artificial saliva, the cytokine TNF-α has been diluted directly in artificial saliva.

2.3. Fluorescence microscopy

Fluorescence images were taken using a fluorescence micro-scope (Zeiss Axioplan 2 Imaging apparatus). Samples were observed by fluorescence light: Fluorescein, excitation was made with a 470 (±40) nm band-pass filter and fluorescence was observed with a 525 (±50) nm band-pass filter (Fig. 1a).

2.4. *Bio-Functionalization of Gold Surface*

Before functionalization, the surface of biosensor's WEs has been pre-cleaned by sonication for 10 min in acetone