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Fast screening of biological fluids for cytokines and adipokines using stochastic sensing

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ABSTRACT

Two stochastic sensors based on metal grapheme nanocomposites paste modified with protoporphyrin-IX were tested for the assay of adipokines (leptin, PAI-1) and cytokines (IL-6, MCP-1). Both stochastic sensors showed reliable response characteristics and very low determination limits: 10 pg L^{-1} for IL-6, 1.25 pg L^{-1} for leptin, 1 fg L^{-1} for PAI-1 and 1 pg L^{-1} for MCP-1. Screening tests were performed for saliva, serum, and cerebrospinal fluids with high reliabilities, in a very short time. The panel of adipokines and cytokines assayed from biological fluids may serve for fast detection of diseases related to obesity.

1. Introduction

The past years have recorded a continuous evolution of stochastic sensors [1,2]. These sensors, due to their ability to detect a single molecule at a time, can be used reliably for multianalyte determinations in biomedical analysis [3,4]. The main advantages of using these sensors are: their response does not depend on the composition of matrix, reliable qualitative and quantitative analyses can be performed, the time of analysis is short, and the cost is low.

Several biological trails have been involved in the development of cardiovascular diseases (CVDs), including obesity and inflammation [5]. Biomarkers responsible for obesity are also involved in inflammation [interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1)], coagulation and fibrinolysis [plasminogen activator inhibitor-1 (PAI-1)], appetite (leptin) insulin resistance and diabetes, atherosclerosis and certain forms of cancer [6]. In humans, plasma levels of PAI-1 correlate with fatal atherosclerotic events, and some studies have implied that PAI-1 is a self-regulating risk factor for coronary artery disease [7].

IL-6 levels correlate with human obesity, insulin resistance [8], and are known to predict death caused by CVDs [9]. IL-6 and MCP-1 are the major cytokines involved clinically in acute coronary syndromes as biomarkers [10].

We have tested two stochastic sensors used for the screening of biological fluids for cytokines and adipokines. Two cytokines (IL-6 and MCP-1) and two adipokines (leptin and PAI-1) have simultaneously been assayed from different biological fluids: serum, cerebrospinal fluid, and saliva.

2. Experimental

2.1. Materials and reagents

Interleukin-6 (IL-6), leptin, plasminogen activator inhibitor-1 (PAI-1), monocyte chemotactic protein-1 (MCP-1), protoporphyrin IX (PIX), hydrochloric acid, sodium chloride, and sodium hydroxide were purchased from Sigma Aldrich (Milwaukee, USA) and paraffin oil (d_4^{20}, 0,86 gxcm^{-1}) from Fluka (Buchs, Switerland). Graphene powders (Graphene-Pt-3, and Graphene-Au-3) were synthesized by a group from the National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania,

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Table 1
Response characteristics of stochastic microsensors for the assay of IL-6, leptin, and PAI-1.

Microsensors based on	Calibration equation and correlation coefficient (r)	Linear concentration range (g/mL)	t_{off}	Sensitivity (s^{-1}/g mL^{-1})	Limit of determination (g/mL)
IL-6					
PIX/Graphene-Pt-3	$1/t_{on} = 0.048 + 1.65 \times 10^{7} \times C$ r = 0.9999	10^{-11}–10^{-9}	2.5	1.6×10^{7}	1×10^{-11}
PIX/Graphene-Au-3	$1/t_{on} = 0.030 + 1.51 \times 10^{8} \times C$ r = 0.9999	10^{-11}–10^{-9}	4.5	1.5×10^{8}	1×10^{-11}
Leptin					
PIX/Graphene-Pt-3	$1/t_{on} = 0.033 + 4.25 \times 10^{7} \times C$ r = 0.9999	1.25×10^{-11}–1.25×10^{-9}	2.0	4.3×10^{7}	1.25×10^{-11}
PIX/Graphene-Au-3	$1/t_{on} = 0.032 + 4.59 \times 10^{7} \times C$ r = 0.9999	1.25×10^{-12}–1.25×10^{-10}	3.0	4.6×10^{7}	1.25×10^{-12}
PAI-1					
PIX/Graphene-Pt-3	$1/t_{on} = 0.024 + 2.67 \times 10^{8} \times C$ r = 0.9998	10^{-15}–10^{-13}	4.5	2.7×10^{8}	1×10^{-15}
PIX/Graphene-Au-3	$1/t_{on} = 0.038 + 4.47 \times 10^{5} \times C$ r = 0.9999	10^{-12}–10^{-10}	2.0	4.5×10^{8}	1×10^{-12}

All measurements are the average of ten determinations. $<1/t_{on}> = s^{-1}$; $<C>$ = g/mL.

according to the procedures proposed earlier [11,12]. All chemicals were of analytical grade.

The leptin solution (1.25 mg/mL) was prepared using a solution containing 0.5 mL 15 mmol/L HCl, 0.3 mL 7.5 mmol/L NaOH and phosphate buffer solution (PBS) pH = 5.2. Interleukin-6 (IL-6) was prepared with 0.1 mL of deionized water; 0.05 mL of it was added to 4.95 mL of PBS pH = 7.4, to obtain a concentration of 1 μg/mL. The plasminogen activator inhibitor type 1 (PAI-1) solution was prepared in a solution containing 0.5 mol/L NaCl and 20 mmol/L CH_3COONa, to a concentration of 10^{-3} mg/mL. For the preparation of leptin solutions of different concentrations (1.25×10^{-1} mg/mL–1.25×10^{-10} mg/mL), we used the serial dillution method, in PBS and deionized water. For the preparation of IL-6 solutions of different concentrations (10^{-6} g/mL–10^{-15} g/mL), we used serial dillution method in PBS pH = 7.4. For the preparation of PAI-1 solutions of different concentrations (10^{-4} mg/mL–10^{-13} mg/mL), we used serial dillution method in a mixture of NaCl 0.5 mol/L and CH_3COONa 20 mmol/L. Monocyte chemotactic protein-1 (MCP-1) solution was prepared in buffer solution (PBS pH = 7.4). For the preparation of MCP-1 solutions of different concentrations (10^{-6} g/mL–10^{-18} g/mL), we used serial dillution method in the same buffering conditions. When not in use, PAI-1 solutions were stored in the freezer at −20 °C, while MCP-1, leptin and IL-6 solutions were stored in the fridge at 2–8 °C.

2.2. Apparatus and methods

All measurements were performed with AUTOLAB/PGSTAT 302N (Methrom) connected to a computer with a GPES software, used to record the measurements. A three electrode system electrochemical cell was employed. Ag/AgCl (0.1 mol L^{-1} KCl) electrode was used as a reference electrode in the cell and a platinum wire as a counterelectrode in the cell, respectively. A stochastic method was used for the measurements of t_{off} and t_{on}, at a constant potential (125 mV vs. Ag/AgCl).

Table 2
Signatures of IL-6, leptin, PAI-1 and MCP-1.

Nanostructured material	Matrix	t_{off} (s)			
		IL-6	Leptin	PAI-1	MCP-1
PIX	Graphene-Pt-3	2.5	2.0	4.5	1.4
	Graphene-Au-3	4.5	3.0	2.0	2.4

2.3. Design of stochastic microsensors based on graphene–metal nanoparticle composites

The graphene powder was mixed with paraffin oil until a homogenous paste was obtained. A solution of PIX 10^{-3} mol L^{-1} (prepared in THF) was added to each paste in a ratio of 1:1 (μL:mg). Two plastic tips (diameter of the active surface, 300 μm) were separately filled with the graphene modified pastes and the electric contact was acquired by inserting a silver wire into the tip. Before each measurement the surface of the microsensor was cleaned with deionized water and it could be renewed with polishing alumina paper. When not it used, the stochastic microsensors were kept in a dry and dark place, at room temperature.

2.4. Stochastic mode

All measurements were carried out at 25 °C. The chronoamperometric technique was used for the measurements of t_{on}, which represents the quantitative parameter, and t_{off}, which is called the signature of the analyte, and represents the qualitative parameter. Stochastic mode was used for both qualitative and quantitative assays of the IL-6, leptin, PAI-1 and MCP-1. The measurements were carried out at a constant potential of 125 mV, in 360 s for calibration measurements of each analyte and 1800 s for sample measurements of all four analytes. Measuring the t_{off} values for each analyte it was possible to identify them in the diagram recorded for the stochastic sensors, and further to be able to detect the quantity of each biomarker. The unknown concentrations of the analytes were determined from the calibration graphs $1/t_{on} = f(conc.)$ recorded with every sensor for each analyte.

2.5. Samples

16 saliva samples (6 control samples and 10 samples collected from patients confirmed with lupus) with their correlated serum samples were obtained from the Clinical Hospital Colentina, Bucharest, Romania. Saliva collection was done in the morning around 8 am before eating or drinking for all subjects included in the study. Saliva sampling was performed following a mouth rinse with 5 ml of water to wash out any debris or exfoliated cells. From each subject around 1 ml of unstimulated whole saliva was collected. The saliva samples were divided in two: one part was used for the assay of IL-6, leptin, PAI-1 and MCP-1 using stochastic sensing, and the other one was centrifuged at 2000 rpm for 10 min, and IL-6 from saliva was analyzed using ELISA standard method.