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Novel textile material based disposable sensors for biomedical analysis

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A novel textile material was used for the design of new disposable stochastic sensors for biomedical analysis. The textile material used had a veil texture, and was plasma coated with a layer of silver; the sensing part was covered using a plasma technique with a second layer of carbon like diamond. The silver side was used as an electrical contact. The diamond side was modified with three types of maltodextrines having different dextrose equivalence (DE) (MD-1 (DE 4.0–7.0), MD-2 (DE 13.0–17.0), MD-3 (DE 16.5–19.5)). Interleukin-6 (IL-6), a pro-inflammatory cytokine that plays a key role in the pathophysiology of cardiovascular diseases, was used as a model analyte. These stochastic microsensors can be used reliably for both qualitative and quantitative analysis for the assay of IL-6 in whole blood samples with limits of determination as low as 1 fg mL^{-1}.

Introduction

In biomedical fields, the detection of biomarkers is the most researched aspect, in the last few years. Biomarkers are used as indicators of biological and pathological processes in the body and their concentration levels may provide information about a specific disease. It is essential to have a high sensitive method of analysis in order to diagnose diseases at a very early stage (asymptomatic patients). This kind of method requires simple and small devices for sensitive and reliable measurement of biomarkers, to reduce costs and to facilitate diagnosis by eliminating the need for trained personnel to perform the analysis.[1]

Interleukin-6 (IL-6) is a polypeptide chain of 185 rests of amino acids, which form a bunch of 4 α-helixes,[2] a major inflammatory mediator that presents both pro- and anti-inflammatory effects and it is a regulator of immune responses.[3] IL-6 it is also a cytokine type of biomarker for cardiovascular diseases,[4] and a potential indicator of leukemia[5] and of visceral and abdominal obesity.[6] It is known that in circulating blood, 15–35% of total IL-6 concentration originates from adipose tissue,[7] which is why adiposity contributes to the pro-inflammatory state.[8] Serum IL-6 levels are high in humans with excess adiposity.[9] Moreover, IL-6 was fully connected by Park and his coworkers, with visceral adiposity.[6] Besides overall obesity, the deposition of visceral adipose tissue is a major factor responsible for the control of chronic inflammation state in obese patients. Therefore, a decrease in visceral adiposity may prevent patients from cardiovascular diseases.[6]

To date, for the expression of IL-6 in confirmed patients there were proposed methods such as: electrochemical immunoassay,[10] enzyme-linked immunosorbent assay (ELISA),[11,12] fluorescent microarray,[13] conductometric immunosensor,[14] chemiluminescence immunoassay,[15] and fluorescence-based fiber-optic biosensors.[16] All these methods need complicated instrumentation, expensive reagents and extra-qualified personnel. Their limits of detection are sometime too high for the normal levels in fluids such saliva or cerebrospinal liquid, especially if these samples are belonging to children. Therefore, it is a real need to develop new methods of analysis of such IL-6 in biological fluids.

In the past years, electrochemical methods have attracted the interest of the researchers, as a response to these concerns. The advantages of electrochemical methods are: high sensitivity, good selectivity, very low limits of detection, simple use and cheaper reagents. Some electrochemical methods and their low limit of detection are presented in Table 1. Furthermore, developments of a new class of sensors – stochastic sensors allowed reliable identification of the analytes in the biological fluid based on their signature, followed by their quantification.[17]

In this paper we proposed three stochastic sensors based on a novel fabric material which can detect with high sensitivity IL-6 in whole blood samples, based on its signature.

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Table 1 Electrochemical methods used for IL-6 detection

Sensor	Method	Limit of detection	Sample	Reference
SWCNTs/AbIL-6/HRP	Electrochemical sandwich immunoassay	0.5 pg mL^{-1}	Calf serum	Malhotra *et al.*, 2010
AuNPs	Electrochemical immunosensor	2.0 pg mL^{-1}	Human serum	Deng *et al.*, 2011
AbIL-6/SWCNTFs	ECL immunosensor	0.25 pg mL^{-1}		Sardesai *et al.*, 2011
AuNPs/HCPE	Electrochemical immunosensor	0.033 pg mL^{-1}		Zhang *et al.*, 2011
ERGO-AuPdNPs/AgNPs/HCPE	Electrochemical immunosensor	0.059 pg mL^{-1}		Lou *et al.*, 2014
PS@PDAmetalNCs-GNR/HSPCE	Electrochemical multi-analyte immunoassay	0.1 pg mL^{-1}		Shi *et al.*, 2014

Experimental

Materials and reagents

All chemicals were of analytical grade. Interleukin-6 (IL-6) and maltodextrins (MD-1, MD-2, MD-3) were obtained from Sigma Aldrich. IL-6 was reconstituted in 0.1 mL of deionized water. IL-6 solutions of different concentrations (10^{-6} to 10^{-15} g mL^{-1}) were prepared in buffer solution (PBS, pH = 7.4) using serial dilution method. The IL-6 solutions were used for 3 months, when stored in the fridge (2–8 °C) when not used for measurements.

Apparatus and methods

All measurements were performed with an AUTOLAB/PGSTAT 302N (Utrecht, The Netherlands) connected to a personal computer with a GPES software, used to record the measurements. A three electrode system electrochemical cell was employed, formed from the working electrode, Ag/AgCl (0.1 mol L^{-1} KCl) as the reference electrode in the cell and a platinum wire as the counter electrode in the cell, respectively.

Design of stochastic microsensors based on veil material

The design of the sensor based on veil textile was made to be suitable also for applications like *in vivo* analysis and also in baby's pampers for fast screening of urine for IL-6. Therefore, we used a silver layer to serve as electric contact, and carbon like diamond (one of the most biocompatible materials) to serve as matrix for the sensor. Maltodextrin (having a structure varying from a closed helix to a coil) are also biocompatible, and non-toxic substances, which can assure good channels for applications in stochastic sensing.

The veil textile was covered first with a layer of silver, and the active side was covered with a layer of carbon like diamond (Fig. 1). Layer-by-layer deposition was used for the active side of the sensor. Polyester textile was coated with a layer of diamond-like carbon on top of a conductive silver layer using

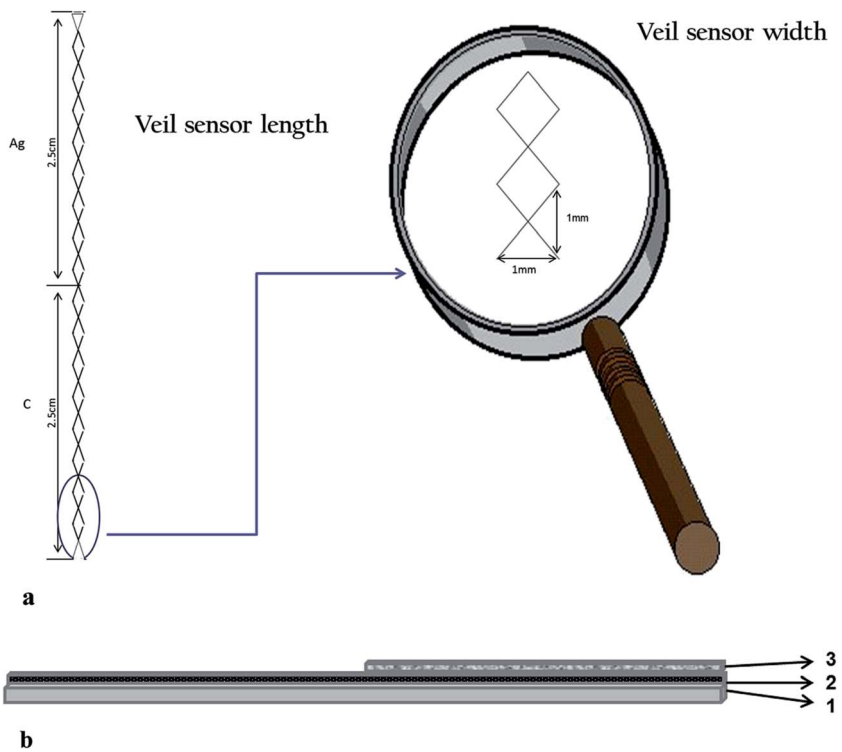

Fig. 1 Design of the veil based stochastic sensor. (a) General shape; (b) layer-by-layer deposition: 1 – textile fiber; 2 – silver layer; 3 – carbon like diamond layer.

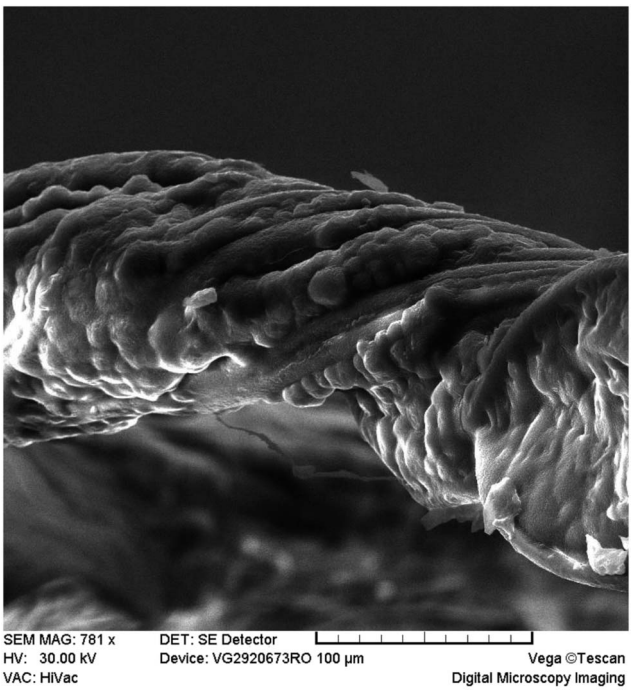

Fig. 2 SEM image of the textile fiber.

anodic arc plasma, in vacuum. The main asset of this type of plasma is the fact that the plasma plume does not fill the vacuum chamber volume (it is localized). Temperature sensitive substrates such as polyester are not immersed into the plasma and can therefore be coated. The operation principle of this plasma source consists on evaporation of the material to be deposited by electron-bombardment and ignition of a plasma in the vapors thus created, using high voltage.[18,19]

A SEM image of coated polyester textile is presented in Fig. 2. As can be observed, the coating material penetrates through the fine fibers composing a textile thread and bonds them such that a continuous conductive fiber is obtained.

The silver side was used as electrical contact, while the carbon side was used as the sensing part. The carbon side was modified with three types of maltodextrines having different dextrose equivalences, MD-1, MD-2, and MD-3 by immersion into 10^{-3} mol L^{-1} maltodextrines solutions (MD-1, MD-2, or MD-3) for 24 hours. After the immersion, the fabric pieces were left to dry, at room temperature, for another 12 hours. A C-veil piece of fabric, modified with maltodextrine can be used for one day with high reliability (RSD (%) for sensitivity is lower than 0.1%). After a day, the sensitivity drops, RSD (%) values recorded for the sensitivity for between day's measurements being higher than 47.0%. Before each measurement, the fabric-based sensors were cleaned with deionized water. When not in use, they were kept at room temperature, in a dry place.

Stochastic mode

All measurements were carried out at 25 °C. The applied potential was 125 mV *vs.* Ag/AgCl. The signatures of IL-6 (t_{off} value) in Table 2 were used for its identification in the diagrams obtained from whole blood samples. Calibration equations: $1/t_{on} = a + b \times Conc_{IL\text{-}6}$ were recorded and used for the assay of the unknown concentration of IL-6 in whole blood samples.

Samples

Whole blood samples were obtained from obese patients, collected at the University Hospital in Bucharest. All experiments for method validation involving real whole blood samples were performed in compliance with the relevant laws and institutional guidelines, with the University of Medicine and Pharmacy "Carol Davila" Bucharest ethics committee approval no. 11/2013. Informed consent was obtained for any experimentation with human subjects.

The whole blood samples (1–2 mL) were analyzed as collected from patients, without any pretreatment.

Results and discussions

Response characteristics of stochastic sensors

Three modified C-veil based sensors were tested for the assay of IL-6 using stochastic mode. A potential of 125 mV *vs.* Ag/AgCl and the response was recorded. The response of the sensors is based on current conductivity: on the first stage, IL-6 is blocking the channel and the current is dropping to zero until all the molecule is entering in the channel – the time spend on the first stage is the signature of IL-6 (t_{off} value, Fig. 3 and 4). The second stage is related to quantitative measurement of the concentration of IL-6 – binding process with the wall of the channel is taking place:

Table 2 Response characteristics of stochastic sensors based on C-veil and different types of maltodextrines, for the assay of IL-6[a]

Sensor based on C-veil and	Calibration equation and correlation coefficient (r)*	Linear concentration range (g mL^{-1})	Limit of determination (g mL^{-1})	Sensitivity (s^{-1} g^{-1} mL^{-1})
MD-1	$1/t_{on} = 0.038 + 3.31 \times 10^4 \times C$, $r = 0.9996$	10^{-8} to 10^{-6}	1×10^{-8}	$3.31(\pm 0.01) \times 10^4$
MD-2	$1/t_{on} = 0.050 + 6.20 \times 10^{11} \times C$, $r = 0.9997$	10^{-15} to 10^{-13}	1×10^{-15}	$6.20(\pm 0.03) \times 10^{11}$
MD-3	$1/t_{on} = 0.133 + 6.62 \times 10^4 \times C$, $r = 0.9993$	10^{-8} to 10^{-6}	1×10^{-8}	$6.62(\pm 0.03) \times 10^4$

[a] All measurements are the average of ten determinations. * $\langle 1/t_{on} \rangle = s^{-1}$; $\langle C \rangle = g\ mL^{-1}$.

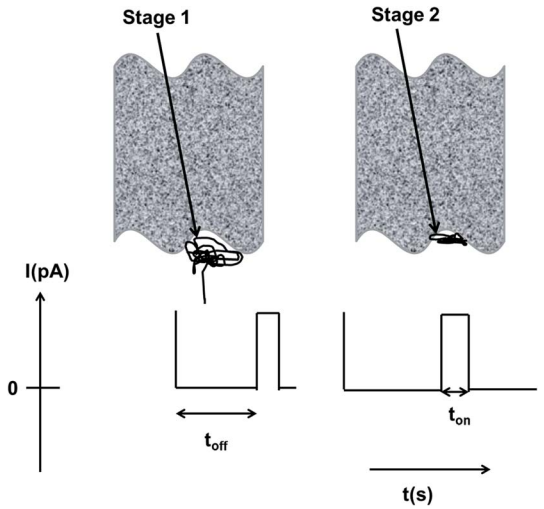

Fig. 3 Current development for stochastic sensors.

$$Ch_{(i)} + IL\text{-}6_{(i)} \Leftrightarrow Ch \cdot IL\text{-}6_{(i)}$$

where Ch is the channel, and i is the interface, followed by redox processes; the time spend in the channel by IL-6 is the t_{on} value (Fig. 3 and 4). This value is used accordingly with the stochastic mode described above to evaluate the performances of the stochastic sensors such as: linear concentration range, limit of determination, sensitivity.

The response characteristics of the proposed stochastic sensors were shown in Table 2. The signatures of IL-6 determined using different stochastic sensing assays are given by the values of t_{off} (Table 2) which are different for each sensor, and depend on the length, size, geometry of the molecule, as well as its velocity to go inside the channel.

As shown in Table 2 the lowest limit of determination (1 fg mL^{-1}) and the highest sensibility (6.20×10^{11} s^{-1} g^{-1} mL^{-1}) were obtained when the MD-2 modified C-veil based sensor was used. Low values for the RSD (%) values of sensitivity were recorded, these proving the stability and accuracy of the proposed sensors. This sensor showed a better stability and a better signal than the other two sensors, in experimental measurements. This response of the sensor based on MD-2 can be correlated with the structure of MD-2 which is between tide helix (MD-1) and coil (MD-3), and favorized a better chemistry inside its channel.

Comparing the results presented in Tables 1 and 2, the sensor based on MD-2 present a lower limit of determination than those shown by the methods in Table 1.

Each sensor can only be used for one day, when more than 50 measurements were performed; for these measurements the

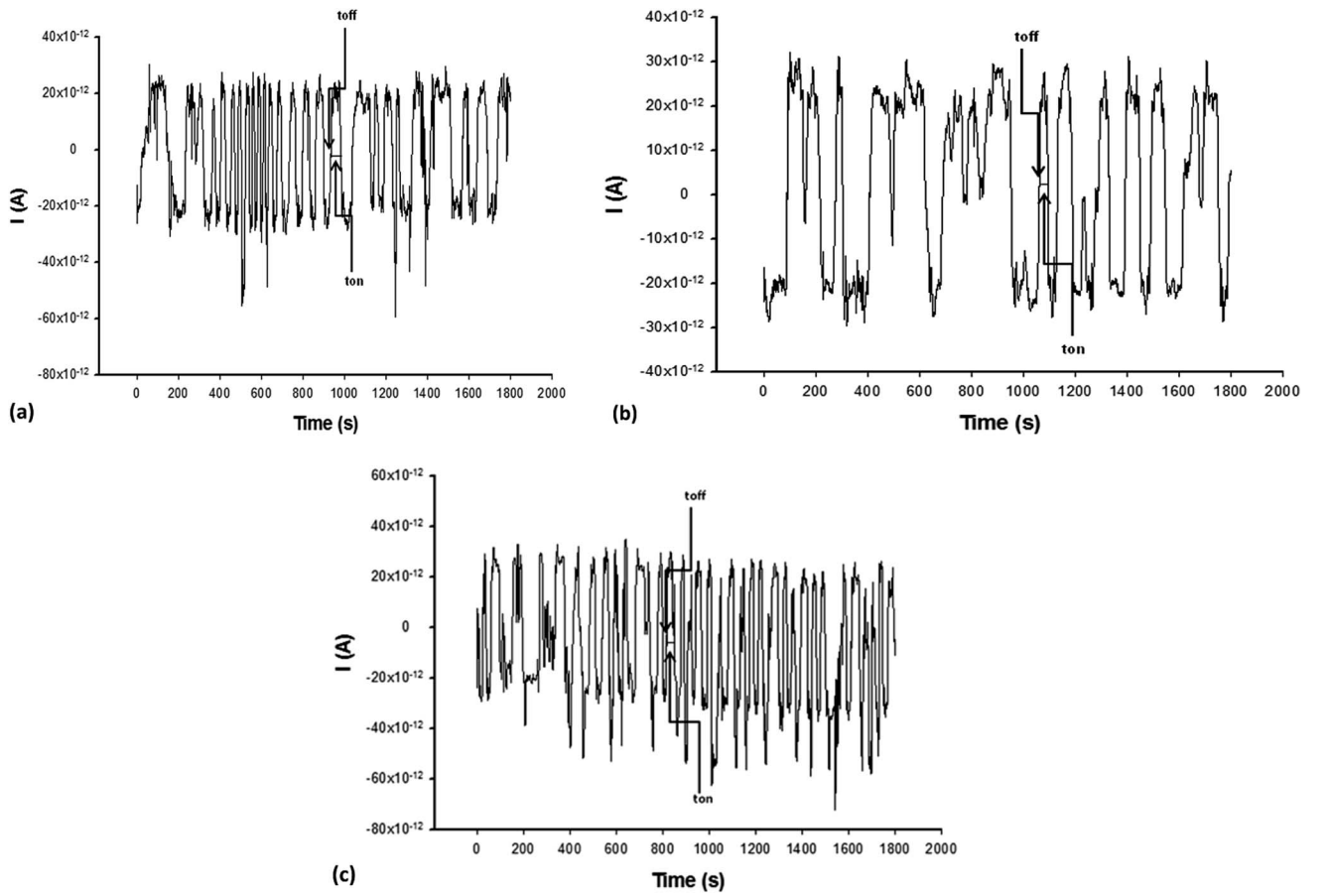

Fig. 4 Diagrams obtained for the assay of interleukin-6 in whole blood samples using the sensors based on diamond-like carbon based veil impregnated with: (a) MD-1, (b) MD-2, and (c) MD-3.

Table 3 Determination of IL-6 in whole blood samples[a]

	Modifier			
Sample no.	MD-1	MD-2	MD-3	
IL-6, ng mL^{-1}				Paired *t*-test
1	4.0 ± 0.1	3.4 ± 0.2	4.0 ± 0.2	2.04
2	3.0 ± 0.2	2.2 ± 0.2	3.1 ± 0.1	2.56
3	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.34
4	3.0 ± 0.1	3.2 ± 0.1	3.5 ± 0.2	1.25
5	6.0 ± 0.1	6.3 ± 0.1	5.2 ± 0.2	3.02
6	6.2 ± 0.2	6.8 ± 0.2	6.0 ± 0.2	0.96
7	18.0 ± 0.3	20.0 ± 0.3	17.1 ± 0.1	3.07
8	1.7 ± 0.1	1.7 ± 0.1	1.1 ± 0.1	1.01
9	11.0 ± 0.3	10.9 ± 0.2	10.7 ± 0.2	2.23
10	14.0 ± 0.2	13.8 ± 0.2	13.0 ± 0.2	2.20
Recovery%	99.98 ± 0.11	98.65 ± 0.10	97.62 ± 0.13	—

[a] All results are the average of four measurements.

RSD (%) recorded for sensitivity were less than 0.1%. In between days, the RSD (%) recorded for the sensitivity was higher than 47%, showing that the sensors based on veil cannot be used. Accordingly, the lifetime of the sensors designed is 1 day.

Selectivity was checked *versus* two other cytokines: MCP-1 and TNF-α, as well as *versus* some adipokines like leptin and PAI-1. For these substances were obtained different t_{off} values, proving that the proposed sensors are selective.

Analytical applications

The proposed sensors were used for the assay of IL-6 in whole blood samples. Diagrams were recorded and values of t_{off} and t_{on} were measured (Fig. 4). First of all was identified in the diagram the signal given by IL-6 based on its signature (t_{off} value), followed by the assay of t_{on} value used accordingly with stochastic mode shown above, to determine its concentration.

Recovery tests were performed for the assay of IL-6 in whole blood samples. Different amounts of IL-6 were added to the whole blood samples to give certain concentrations. The results shown in Table 3 proved that IL-6 an be reliable assaied in whole blood samples: the accuracy of measurements were demonstrated by recoveries of IL-6 which were higher than 97.00%, while the precision was given by the RSD (%) values for recovery tests and for the assay of IL-6 in whole blood samples – RSD (%) values being lower than 0.40%.

Paired *t*-test at 99.00% confidence level was performed for the results obtained for the assay of IL-6 in ten whole blood samples (Table 3). All values calculated for pair-*t* test at the 99.00% confidence level are less than the tabulated theoretical value: 4.032. Accordingly, there is no statistically significant difference between the results obtained using the proposed sensors at 99.00% confidence level, for the assay of IL-6 in whole blood samples.

Conclusions

This paper proposed three sensors based on a novel fabric material used for stochastic sensing of interleukin-6 in whole blood samples. Three maltodextrines (MD-1, MD-2 and MD-3) with different dextrose equivalence were used to modify the diamond-like carbon based veil sensor. The best performances were recorded for the sensors obtained using MD-2: a limit of determination of 1 fg mL^{-1} and a high sensitivity. Even though these proposed novel stochastic sensors are disposable sensors (they can be used one day), they showed very good response characteristics, making them reliable for the assay of IL-6 in whole blood samples. The attractive performance of these novel stochastic sensors suggested potential application towards the early evaluation of cancer, inflammation disorders and obesity.

Abbreviations

SWCNTs	Single walled carbon nanotubes
SWCNTFs	Single walled carbon nanotubes forests
ECL	Electrochemiluminescence
Ab	Anti-body
HRP	Horseradish peroxidase
HCPE	Heated carbon paste electrode
NPs	Nanoparticles
ERGO	Electrochemically reduced graphene oxide
GNR	Graphene nanoribbon
PS	Polymer
NCs	Nanocomposites
HSPCE	Heated screen-printed carbon electrode

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