New Tool for Screening of Whole Blood for Early Detection of Breast Cancer Antigen (CA153)

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Abstract: Nanostructured materials play an important role in the design of new tools for medical use. Therefore materials such as maltodextrins were excellent candidates for the design of stochastic microsensors used in the screening of whole blood for breast cancer biomarker. The results obtained for the assay of breast cancer antigen in whole blood using this microsensor (by applying a potential of 125mV vs Ag/AgCl and performing measurements specific to stochastic sensing described in detail in the article) were in agreement with those obtained using the standard method, ELISA (Enzyme-Linked Immunosorbent Assay utilized a monoclonal anti-CA 15-3 antibody directed against intact breast cancer antigen, CA 153 for solid phase immobilization - on the microtiter wells. The concentration of CA 153 was directly proportional to the color intensity of the test sample.) for serum samples. The limit of determination obtained for breast cancer antigen (0.5mU/mL) when assayed in whole blood, proved that the microsensor is a good tool (in terms of sensitivity, selectivity, and fast response) for early screening of whole blood for breast cancer.

Keywords: Breast cancer antigen, screening, stochastic microsensor.

INTRODUCTION

Accordingly with the WHO, early stage detection of cancer is the only way of surviving for almost all types of cancer. Although, breast cancer was known for its important survival, due to the advances in therapies and medication, the rate of morbidity and mortality is still increasing due to late detection. Therefore, a fast, high accurate, and low cost screening test is a real need for early detection of breast cancer antigen (CA 153) – the biomarker with the highest incidents in breast cancer [1, 2] - in whole blood.

For the quantification of polymorphic epithelial mucin (PEM) (breast cancer biomarker, CA 153) expressed at the surfaces of the human mammary tumoral cells, as well as of the tumoral cells were proposed to date impedimetric methods [3], biosensors based on gold nanoparticles [4], Raman spectroscopy [5], fluorescence [6], calorimetry [7], suspended microchannel resonators [8] and opto-fluidic ring resonator biosensors [9].

To make the screening test affordable, there is a need to use a low cost method which at the same time will be able to determine accurately the breast cancer antigen (CA153). Electrochemically microsensors are well known for their high sensitivity and accuracy as well as for the low cost of measurements. To be able to accurately perform a qualitative analysis, the sensors of choice should be the stochastic microsensors, because this type of sensors can gave the "signature" of the breast cancer antigen (CA 153) as t_{off} in the diagram recorded, as well as the amount of the antigen in the sample – measured using t_{on} [10-13].

Therefore, in the present article we propose a stochastic microsensor based on maltodextrin with dextrose equivalent between 4 and 7. This maltodextrin has a compact helix structure. To make sure that one does not destroy the naturally occurred pores, the maltrodextrin was physically immobilized on a natural diamond paste matrix (monocrystallin diamond particle size of 1μm), because in previous experiments it gave the best sensitivity, selectivity and S/N ratio for amperometric measurements [14].

EXPERIMENTAL

Materials

Natural monocrystallin diamond powder having a particle size of 1μm (99.9%), maltodextrin (dextrose equivalent 4-7) and breast tumor antigen (CA 153) (0.1mol/L solution in phosphate buffer, pH=7.4 containing 0.1% NaN_3) were purchased from Aldrich (Milwaukee, USA); paraffin oil was purchased from Fluka (Buchs, Switzerland). 0.1mol/L Phosphate buffer solution (pH=7.4), and NaN_3 were purchased from Merck. Deionised water obtained from a Millipore Direct-Q 3 System (Molsheim, France) having a conductivity less than 0.05μS/cm, was used for the preparation of all solutions. The solutions of biomarkers were all made in 0.1 mol/L phosphate buffer (pH=7.4) containing 0.1% NaN_3 in order to have the medium

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from the vials on which the antigen was purchased. The range of concentrations for sensors' evaluation was obtained using serial dilution technique, from 0.00005 to 5000U/mL.

Stochastic Microsensor Design

200 mg of natural monocrystalline diamond powder was mixed with 20 µL paraffin oil to form a diamond paste. 100µL from the solution of maltodextrin with dextrose equivalent 4-7 (10^{-3}mol/L) was added to the diamond paste to give the modified diamond paste. The modified paste was placed into a plastic tube (Figure **1**). The diameter of the microsensor was about 100µm. Electric contact was obtained by inserting an Ag/AgCl wire into the modified diamond paste. The surface of the microsensor was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion) before using. When not in use, the microsensor was stored in a dry state at room temperature.

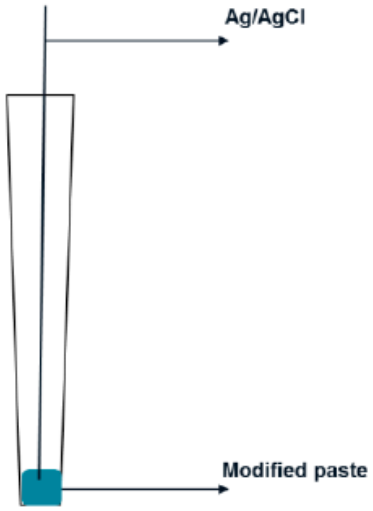


Figure 1: Stochastic microsensor based on maltodextrin.

Apparatus

A PGSTAT 12 (a very sensitive electrochemical instrument capable of measuring very low currents – up to fA) and software Ecochemie (version 4.9) were used for all electrochemical measurements. A Pt electrode and an Ag/AgCl electrode served as the counter and reference electrodes in the cell. A Cyberscan PCD 6500 pH/mV-meter from Eutech Instruments was employed for all pH measurements. Agilent 5500 SPM system, described by PicoSPM controlled by a MAC Mode module and interfaced with a PicoScan controller from Agilent Technologies, Tempe, AZ, USA (formally Molecular Imaging) was used for active surface analysis of stochastic microsensor. A multipurpose large scanner and Point Probe Plus Silicon SPM Sensor cantilevers (PPP-FM cantilevers), n+- silicon material with no coating, of about 227 µm length, 1.8 N m-1 spring constants, with the tips oscillated near their resonant frequencies in air, of about 64 kHz were used for all measurements. Scanning of the surface was done at a rate of 0.8-1.2 lines per second, at room temperature in the tapping mode.

Stochastic Method of Analysis

A chronoamperometric technique was used for the measurements of t_{on} and t_{off} at 125mV. At this value of potential the microsensor had a stochastic behavior. The electrodes were dipped into a cell containing solutions of biomarker (buffered with a solution containing 0.1 mol/L phosphate buffer (pH=7.4) and 0.1% NaN_3) of different concentrations. The unknown concentrations of biomarker in blood samples were determined from the calibration equations 1/ton = f(Conc.).

RESULTS AND DISCUSSIONS

High-resolution imaging (topography of the surface obtained in tapping mode in the range of scan lengths from 50µm to 1µm) using atomic force microscopy (AFM) has been applied to observe the presence of pores in the diamond paste based maltodextrin (Figure **2**). The presence of the round pores in the modified paste justified the stochastic behavior of the microsensor when a constant value of potential (125mV vs Ag/AgCl) was applied and the current value vs. time was recorded.

Typical diagrams obtained for a microsensor based on plane and modified diamond paste are shown in Figures **3** and **4**. The signature of breast tumor antigen is given by the value of t_{off} which is 3.3s when both standard biomarker solutions (Figure **4**) and whole blood samples were measured. This value can be used for the qualitative analysis of CA 153 in whole blood, because it did not change when the biomarker was assayed from whole blood samples.

The equation of calibration for the stochastic microsensor was determined using the values of t_{on} – (Figure **4**) defined as the binding time [10] (binding constant (determined from the experimental data) between maltodextrin and breast tumor antigen on the channel being $0.011U^{-1}/mL^{-1}$) obtained for all concentration in the linear concentration range,

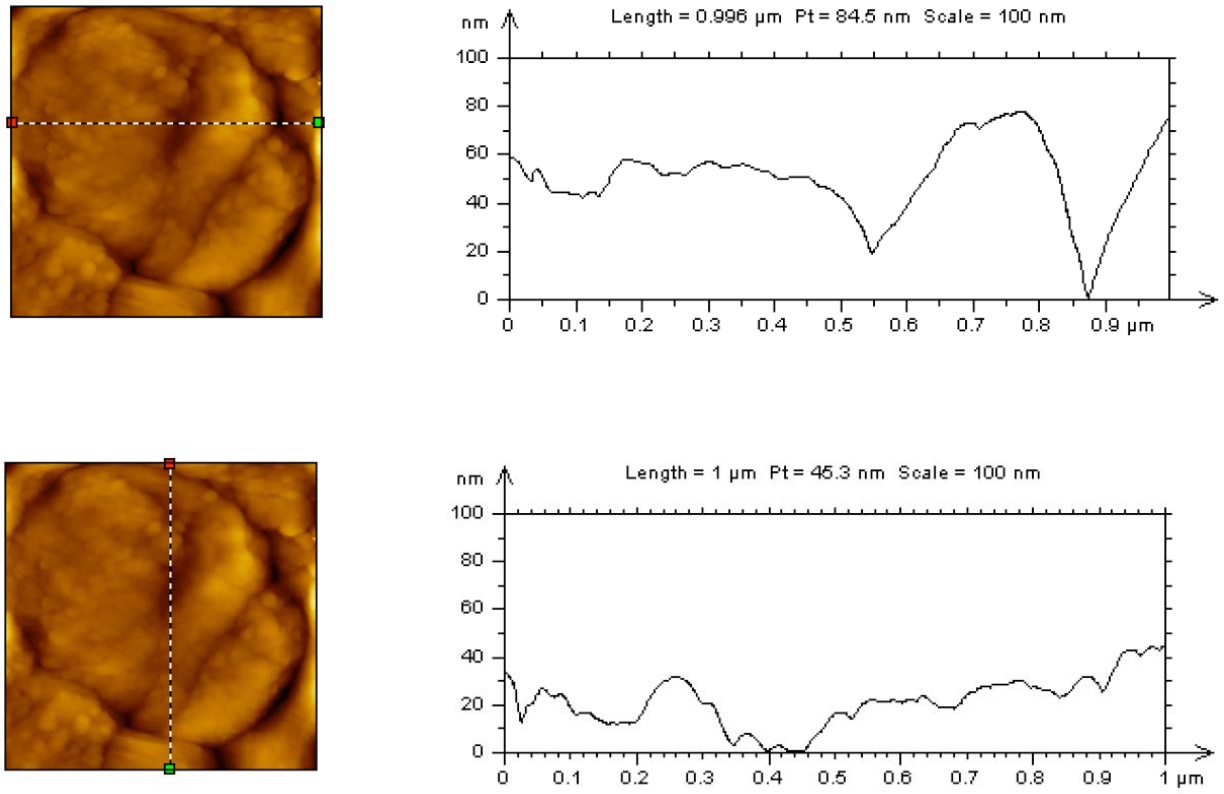


Figure 2: AFM topographical images of maltodextrin based diamond paste microsensor (Extracted profiles (1x1µm)).

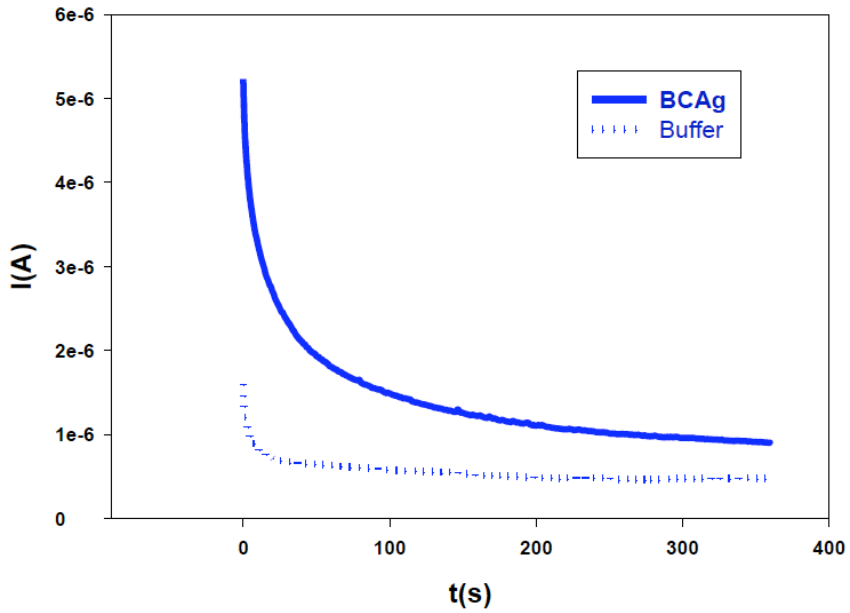
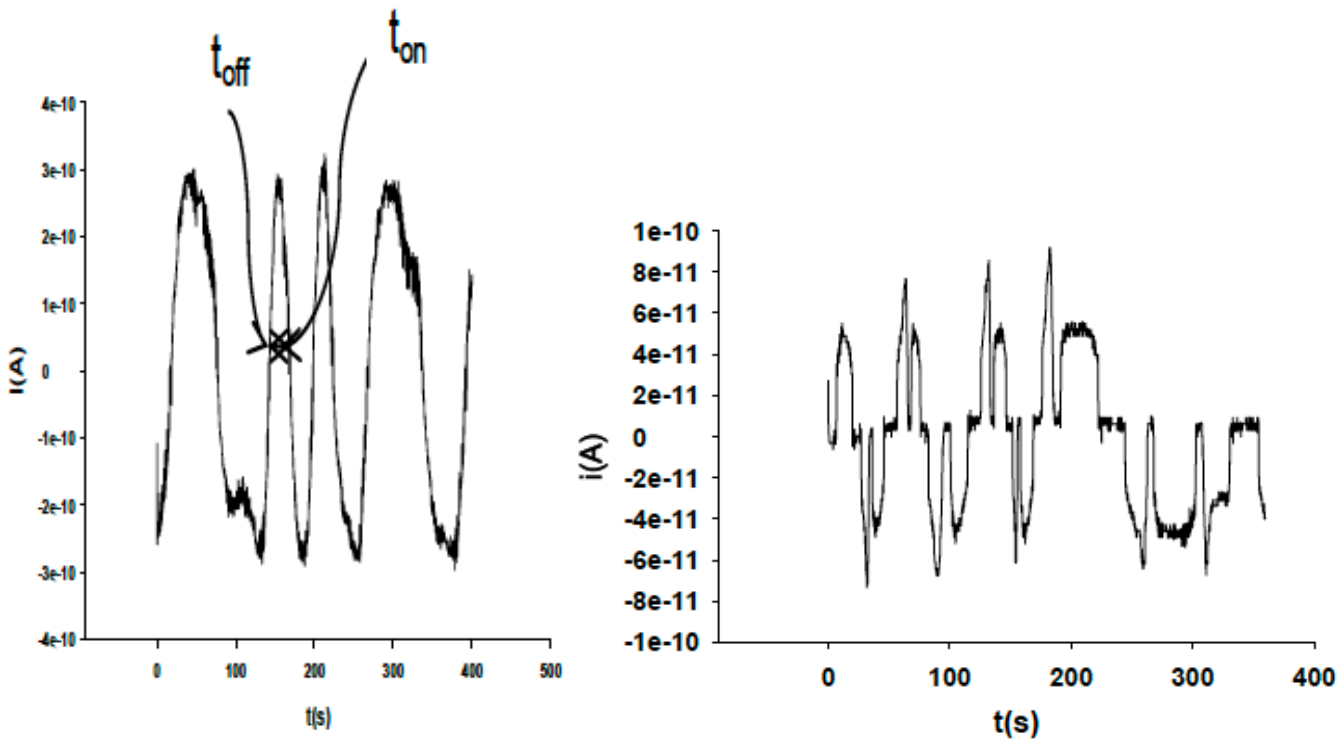


Figure 3: Diagrams obtained using plain diamond paste based microsensor for buffer (PBS, pH=7.4 containing 0.1% NaN_3) solution and for breast cancer antigen solution (C = 50U mL^{-1}).

measured in seconds obtained for different concentrations of CA 153, measured in U/Ml (r=0.9878):

$$1/t_{on} = 20.11 + 0.044 \times C$$

The linear concentration range was between 0.5mU/mL and 500U/mL, covering patients who are on a very early stage as well as patients on late stages of breast cancer. The sensitivity was 0.044U s/mL, with a limit of detection of 0.1mU/mL. The relative standard



(**a**) (**b**)

Figure 4: Diagrams obtained using modified diamond paste with maltodextrin based stochastic microsensor for (**a**) breast cancer antigen solution (conc. = 50U mL^{-1}) and (**b**) for buffer (PBS, pH=7.4 containing 0.1% NaN_3) solution.

deviation (RSD%) per sensor was less than 0.1% for the response/sensitivity of the sensor, values being recorded for the 6 months. 20 Sensors were designed and tested during this period; while the results obtained in terms of sensitivity for 6 months had a RSD value less than 0.1%, proving the reliability of the design proposed.

For ovarian cancer antigen, gastrointestinal cancer antigen, and hepatitis B virus antigen, HER1, HER2 the diagrams showed different signatures (t_{off}), than the one recorded for CA 153. Accordingly, one can distinguish between the biomarkers specific to different cancers and hepatitis B. Furthermore, the biggest advantage of stochastic sensors is that the signatures of the biomarkers (t_{off} values) are independent on the matrix from where they are determined.

The stochastic microsensor was used for qualitative and quantitative analysis of the breast tumor antigen from 12 whole blood samples obtained from patients (The study was approved by the institutional review committee - Ethics Committee approval nr. 11/2013. Informed consent was obtained from the patients.). The results obtained were compared with those provided by the clinical laboratories that used for the analysis of CA 153 in serum samples ELISA technique (used in clinical laboratory as standard method for the assay of CA153).

Qualitative assay of the breast tumor antigen was done in whole blood samples using the value of t_{off}. Accordingly, t_{off} values specific for the breast cancer antigen were found in ten out of 12 diagrams obtained for real whole blood samples. As a result, there were 10 positive results and 2 negative result for the screening test performed with the proposed stochastic sensor. These results correlated good with the results obtained using the standard method (ELISA) used in accredited clinical laboratories.

The results obtained for the quantitative assay of breast tumor antigen performed for the 10 positive samples are shown in Table **1**. ELISA method is always providing lower values for CA 153, if compared with the method based on utilization of stochastic microsensor. This is because ELISA is using serum samples – separated from whole blood samples, and a part of biomarker is lost during processing of samples. The correlation between the quantitative values is good, proving that the stochastic microsensor can be used for the quantification of CA153 in whole blood – being able to provide to the medical doctor valuable information about the stage of illness.

Table 1: Quantitative Analysis of CA 153

Sample Nr.	Stochastic sensor based on maltodextrin (U/mL)		ELISA (standard method, serum samples) (U/mL)
	Whole blood samples	Serum samples	
1	97.0	95.8	96.0
2	70.0	68.1	68.0
3	35.2	33.7	33.0
4	76.2	75.9	76.0
5	25.8	24.6	24.0
6	45.8	43.5	43.0
7	67.5	65.0	66.0
8	135.9	133.4	133.0
9	259.7	258.6	258.0
10	21.8	19.9	20.0

CONCLUSIONS

This article opens a new direction on the screening tools technologies used for early detection of lesion. The possibility of determining the quality as well as the quantity of the analyte in one run made the method comparable with the chromatographic methods, but at higher sensitivities, reliability, in a faster time, and at a low cost. Compared with ELISA, it is using the sample as taken from patients, it is faster, the same microsensor can be used for more than 100 whole blood samples per day, and it is cheaper.

The results obtained proved that the microsensor is a good screening tool for early detection of breast cancer antigen in whole blood. The features are its utilization as a screening tool for different biomarkers, making possible early detection, in a fast time, and at a price affordable by any insurance company.

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REFERENCES

[1] Price MR, Tendler SJB. Polymorphic epithelial mucins (PEM): molecular characteristics and association with breast cancer. The Breast 1993; 2: 3-7. http://dx.doi.org/10.1016/0960-9776(93)90028-E

[2] Hanisch FG, Muller S. MUC1: The polymorphic appearence of a human mucin. Glycobiology 2000; 10: 439-49. http://dx.doi.org/10.1093/glycob/10.5.439

[3] de la Rica R, Thompson S, Baldi A, Fernandez-Sanchez C, Drain CM, Matsui H. Label-free cancer cell detection with impedimetric transducers. Anal Chem 2009; 81: 10167-71. http://dx.doi.org/10.1021/ac9021049

[4] de la Escosura-Muniz A, Sanchez-Espinel C, Diaz-Freitas B, Gonzalez-Fernandez A, Maltez-da Costa M, Merkoci A. Rapid identification and quantification of tumor cells using an electrocatalytic method based on gold nanoparticles. Anal Chem 2009; 81: 10268-74. http://dx.doi.org/10.1021/ac902087k

[5] Hdegaard M, Krafft C, Ditze HJ, Johansen LE, Hassing S, Popp J. Discriminating isogenic cancer cells and identifying altered unsaturated fatty acid content as associated with metastasis status, using K-means clustering and partial least squares-discriminant analysis of Raman maps. Anal Chem 2010; 82: 2797-802. http://dx.doi.org/10.1021/ac902717d

[6] Ding C, Li X, Ge Y, Zhang S. fluorescence detection of telomerase activity in cancer cells based on isothermal circular strand-displacement polymerization reaction. Anal Chem 2010; 82: 2850-5. http://dx.doi.org/10.1021/ac902818w

[7] Wang C, Ma L, Chen LM, Chai KX, Su M. scanning calorimetric detections of multiple DNA biomarkers contained in complex fluids. Anal Chem 2010; 82: 1838-43. http://dx.doi.org/10.1021/ac902503j

[8] von Muhlen MG, Brault ND, Knudsen SM, Jiang S, Manalis SR. label-free biomarker sensing in undiluted serum with suspended microchannel resonators. Anal Chem 2010; 82: 1905-10. http://dx.doi.org/10.1021/ac9027356

[9] Gohring JT, Dale PS, Fan X. Detection of HER2 breast cancer biomarker using the opto-fluidic ring resonator biosensor. Sens Actuators B 2010; 146: 226-30. http://dx.doi.org/10.1016/j.snb.2010.01.067

[10] Bayley H, Cremer PS. Stochastic sensors inspired by biology. Nature 2001; 413: 226-30. http://dx.doi.org/10.1038/35093038

[11] Hladky SB, Haydon DA. Discreteness of conductance change in bimolecular lipid membranes in the presence of certain antibiotics. Nature 1970; 225: 451-3. http://dx.doi.org/10.1038/225451a0

[12] Stefan-van Staden RI, Moldoveanu I, Sava DF, Kapnissi-Christodoulou C, van Staden JF. Enantioanalysis of pipecolic acid with stochastic and potentiometric microsensors. Chirality 2013; 25: 114-8. http://dx.doi.org/10.1002/chir.22119

[13] Batchelor-McAuley C, Dickinson EJF, Rees NV, Toghill KE, Compton RG. New electrochemical methods. Anal Chem 2012; 84: 669-84. http://dx.doi.org/10.1021/ac2026767

[14] Stefan RI, Bairu SG. Monocrystalline diamond paste based electrodes and their applications for the determination of Fe(II) in vitamins. Anal Chem 2003; 75: 5394-8. http://dx.doi.org/10.1021/ac026300b

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