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Short communication

Label-free detection of HIV-2 antibodies in serum with an ultra-high frequency acoustic wave sensor

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ABSTRACT

Herein is described a label-free immunosensor dedicated to the detection of HIV-2. The biosensor platform is constructed as a mixed self-assembled monolayer-coated quartz wafer onto which HIV-2 immunodominant epitopes are immobilized. The biosensing properties, in terms of specific *vs.* non-specific antigen–antibody interactions, are evaluated with the electromagnetic piezoelectric acoustic sensor (EMPAS) using equimolar serum solutions of HIV-2 or HIV-1 monoclonal antibodies, respectively. This immunosensor constitutes the first real-world application of the EMPAS technology in the bioanalytical field.

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1. Introduction

Over a number of years, biosensor technology has received considerable attention in connection with potential applications in environmental and food analysis, drug detection and clinical diagnostics [1]. In essence, biosensors are analytical devices that are composed of proteins, nucleic acids or cells imposed on a transducer surface in order to allow for the detection of biomarkers in a real-time, non-destructive and label-free manner [1,2]. Self-assembling monolayer (SAM) chemistry is regularly regarded as a method of choice for the fabrication of biosensors. In fact, such chemistry offers one of the highest quality routes for the preparation of chemically stable and structurally well-defined functionalizable organic surfaces, onto which biomolecules such as proteins, antibodies or oligonucleotides can attach in a subsequent immobilization step [1]. The detection of biomolecular interactions and their conversion into a measurable signal have resulted in the development of transducing technologies, such as the well-established surface plasmon resonance technique, and devices based on acoustic physics such as the thickness-shear mode sensor, that are capable of being interfaced within the biosensor in an intimate overall structure [3]. Recently, we have introduced the electromagnetic piezoelectric acoustic sensor (EMPAS), which is capable of performing real-time and highly sensitive measurements at tunable, ultra-high frequencies (over 1 GHz) in an electrode-free environment as demonstrated by our latest work that aimed at developing and testing the concept of SAM-based biosensing platforms for the EMPAS using buffered target-analyte solutions [1]. It is now our goal to further exploit this technology using biological fluids in real-world scenarios for the clinical diagnostic field.

Biosensor devices have not found a prominent place in this sector despite considerable promise in terms of throughput and possible multiplexing of analytical application. Among a number of reasons for this, the interfering role played by the cellular and protein-based components of blood and urine is a crucial factor. We recently addressed this issue of non-specific adsorption (NSA) for the EMPAS through new antibiofouling surface chemistry [1]. The particular focus of the present work is HIV detection, for which the well-established enzyme-linked immunosorbent assay (ELISA) has been the laboratory standard screening technique since 1985 [4]. Until recently, few truly innovative improvements/alternatives have indeed been proposed [5–8]. Herein, we introduce an EMPAS-based immunosensor for the serological detection of HIV-2, which constitutes the first real-world extension of our technology in the bioanalytical field. The biosensing platform is constructed as a mixed SAM-coated quartz wafer onto which HIV-2 immunodominant epitope probes are immobilized. Specific *vs.* non-specific antigen–antibody interactions are evaluated with the EMPAS system incorporated into a flow-injection configuration. Responses are measured by flowing over our biosensing surfaces HIV-2 (specific) or HIV-1 (non-specific)

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monoclonal antibodies deliberately introduced into serum solutions.

2. Experimental

2.1. Quartz wafer cleaning

The quartz wafers (Laptech Precision Inc. (Bowmanville, ON, Canada), AT-cut, 13.5 mm in diameter, 20.0 MHz fundamental frequency) were first sonicated in 20 mL of concentrated dishwashing soap for 30 min. The wafers were then thoroughly rinsed with hot water followed by distilled water then gently dried with forced air. Subsequently, the wafers were individually soaked in 6 mL of Piranha solution (3/1 (v/v) mixture of concentrated H_2SO_4 and 30% H_2O_2) pre-heated to 90 °C using a water bath (CAUTION: Piranha solutions are corrosive. Handle with care!). After 30 min, the wafers were rinsed with distilled water (3X) followed by spectrograde methanol (3X). Next, the wafers were sonicated in spectrograde methanol for 2 min then individually transferred into vials, which were subsequently placed in an oven maintained at 150 °C for drying. After 2 h, the vials were rapidly transferred into a humidity chamber, maintained at 60% relative humidity using a saturated solution of $MgNO_3{\cdot}6H_2O$, and set aside overnight.

2.2. CATD/HTS mixed SAM formation

Neat CATD [9] (10 μL) and neat HTS (10 μL) were separately diluted with anhydrous toluene (10 mL) in a glovebox maintained under inert (N_2) and anhydrous (P_2O_5) atmosphere. 500 μL of each solution were mixed in individual test tubes into which cleaned quartz wafers were then soaked (NOTE: to prevent any undesired reaction of CATD and HTS with the walls of our test tubes during silanization, glassware was pre-treated overnight with a 1/20 (v/v) solution of octadecyltrichlorosilane in anhydrous toluene). The test tubes were sealed with rubber stoppers, removed from the glovebox and placed on a spinning plate for 2 h. The wafers were then rinsed twice with dry toluene and finally sonicated in toluene for 2 min. After a final rinse with one portion of toluene, the previous procedure was repeated with dry chloroform. Finally, the wafers were rinsed twice with dry chloroform, gently dried under forced air then immediately transferred back to the glovebox for the subsequent chlorine/iodine exchange reaction.

XPS analysis (Supplementary data: Fig. 1) revealed that this silanization process is initially very fast as shown by the sharp increase of the XPS signal for chlorine and carbon, which are the two elements characteristic of CATD and HTS. Conversely, the XPS signals for oxygen and silicon (which are elements mainly present in the underlying quartz wafers) simultaneously decreased. The silanization process was completed after 2 h when all percentages reached a steady value.

2.3. Chlorine/iodine exchange

In a dry 100 mL beaker placed in the glovebox, anhydrous NaI (EMD Biosciences Inc.®, 3.0 g, 20 mmol) was dissolved in freshly distilled anhydrous and deaerated acetone (30 mL). The resulting solution was portioned (2 mL) in dry test tubes into which freshly prepared CATD/HTS mixed SAM-coated quartz wafers were individually soaked. The test tubes were then sealed with rubber stoppers, removed from the glovebox and placed on a spinning plate for 1.5 h, sheltered from light. The wafers were then rinsed with anhydrous acetone (5X), gently dried under forced air then transferred back to the glovebox for immediate HIV-2 probe immobilization.

XPS analysis (Supplementary data: Fig. 2) showed that the chlorine/iodine exchange was successful as proven by the disappearance of the chlorine signal at 200 eV and the appearance of a signal at 621 eV corresponding to covalently-bound iodine.

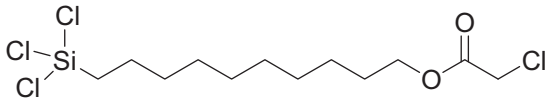

Fig. 1. Structure of CATD linker.

2.4. HIV-2 probe immobilization

The desired 0.1 mg/mL HIV-2 probe solution was prepared in the glovebox by dissolving 1.0 mg of undecapeptide NSWGCAFRQVC (MedMira Inc., Halifax, NS, Canada) into freshly distilled anhydrous DMF (10 mL). This solution was portioned (800 μL) in dry test tubes into which freshly prepared iodo-modified CATD/HTS mixed SAM-coated quartz wafers were individually soaked. The test tubes were then sealed with rubber stoppers, removed from the glovebox and set aside overnight, sheltered from light. The wafers were then rinsed with anhydrous DMF (3X) followed by spectrograde methanol (3X). They were finally dried under a gentle N_2 stream then stored in screw-cap vials under atmospheric conditions for EMPAS analysis.

2.5. EMPAS measurements

Anti-gp36 and anti-gp41 monoclonal antibody solutions (0.1 mg/mL) were prepared by diluting 4 μL of the appropriate 2 mg/mL antibody stock solution with 76 μL of fetal bovine serum (FBS, Sigma–Aldrich®). After the standard set-up of EMPAS [10], an HIV-2 probe-functionalized mixed SAM-coated quartz wafer was inserted into the flow-through cell and Dulbecco's phosphate buffered saline (PBS, Sigma–Aldrich®) was flown at a rate of 50 μL/min. Once the frequency signal stabilized at the desired ultra-high resonating frequency of 1.06 GHz (53rd harmonic), 50 μL of freshly prepared anti-gp36 or anti-gp41 antibody FBS solution were injected into the flow-through system using a low-pressure chromatography valve. Once the antibody serum solution completely passed over the biosensing platform (causing the resonating frequency to shift), the uninterrupted PBS buffer flow rinsed the surface off of any loosely bound material. The frequency signal stabilized again, the experiment was stopped and the frequency shift was calculated. The experiment was then repeated with another HIV-2 probe-functionalized mixed SAM-coated quartz wafer.

3. Results and discussion

Our strategy first involved the preparation of robust and highly reactive mixed SAMs onto our EMPAS quartz wafers that can be readily functionalized with HIV-2 epitope probes in a subsequent single and pre-activation free step. For that purpose, we selected CATD (Fig. 1), a linking molecule (or linker) we had recently used with success for the fabrication of SAM-based microarrayed platforms capable of detecting target proteins with high selectivity using Kelvin physics [9]. CATD possesses a highly reactive trichlorosilyl tail function, for strong siloxane anchorage onto the quartz wafers, as well as a reactive α-chloro-acetyl head function that can be directly used for additional surface attachments. Shorter hexyltrichlorosilane (HTS) was chosen as the diluent molecule to space out CATD linker molecules within the SAM. The purpose was to decrease the detrimental steric hindrance around the head function of neighbouring CATD molecules thereby enhancing SAM reactivity and binding affinity [1].

Mixed SAMs were prepared upon treatment of cleaned quartz wafers with 1/1/2000 (v/v/v) CATD/HTS solutions in anhydrous

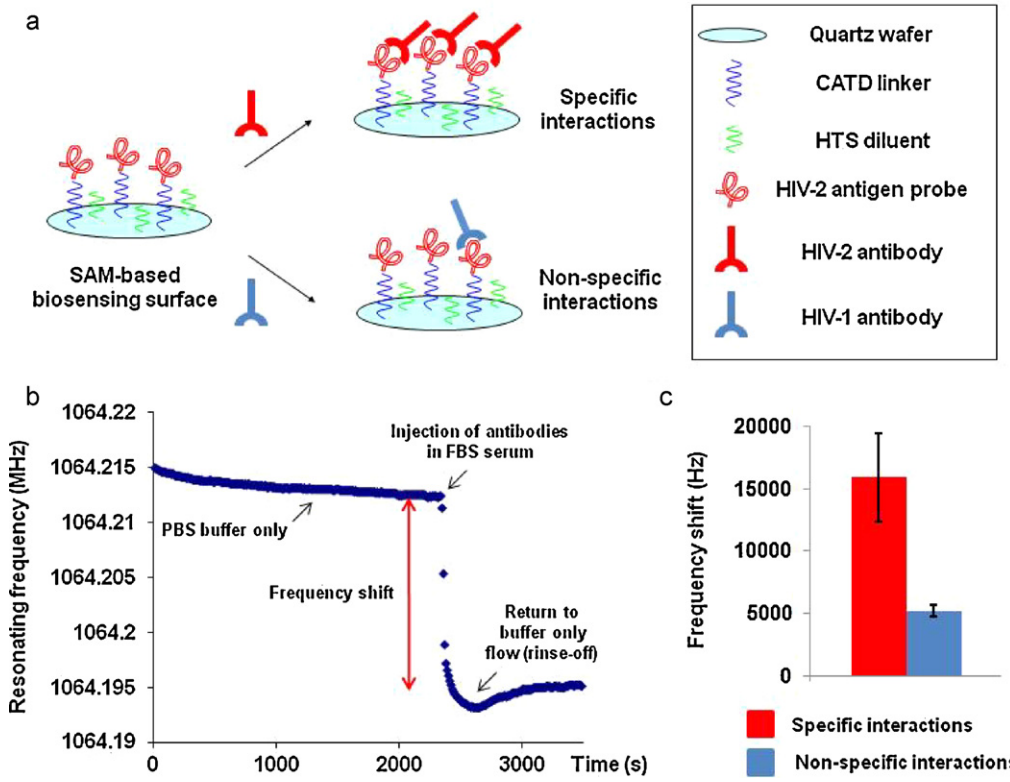

Fig. 2. (a) Schematic representation of the HIV-2 epitope-functionalized mixed SAM-based biosensing platform and of the general EMPAS experiment depicting the two sets of independent measurements. (b) Representative EMPAS graph showing the initial resonating frequency stabilization (with PBS buffer flow only), the injection of the antibody serum solution, the frequency shift and the final rinse-off with the uninterrupted buffer flow. EMPAS measurements were recorded at the ultra-high resonating frequency of 1.06 GHz. (c) EMPAS frequency shifts for the specific (red) and non-specific (blue) antigen–antibody interactions respectively measured upon flowing HIV-2 gp36 or HIV-1 gp41 monoclonal antibody solutions over the biosensing surfaces (5 replicates per set). Both anti-gp36 and antigp41 antibody solutions were prepared as 0.1 mg/mL solutions in fetal bovine serum (FBS). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

toluene at room temperature. X-ray photoelectron spectroscopy (XPS) analysis showed that this silanization process is initially very fast but only completed after 2 h. The mixed SAM α-chloro-acetyl head functions were then modified into their more reactive α-iodo-acetyl analogs in order to further increase the binding affinity of our SAMs for the subsequent HIV-2 probe immobilization [11]. As shown by XPS analysis (see Supplementary data), this was readily achieved by immersing CATD/HTS mixed SAM-coated quartz wafers into NaI solutions in acetone for 1.5 h. The biosensing surfaces (Fig. 2a) were finally completed upon overnight immersion of freshly prepared iodo-modified CATD/HTS mixed SAM-coated quartz wafers into 0.1 mg/mL DMF solutions of HIV-2 antigenic probe [12]. The latter is undecapeptide NSWGCAFRQVC, that has been shown to be the immunodominant epitope of HIV-2 transmembrane glycoprotein gp36 [13].

The biosensing surfaces were then analyzed with the EMPAS system at an ultra-high resonating frequency of 1.06 GHz. EMPAS experiments involved two sets of independent frequency shift measurements (5 replicates per set), where monoclonal antibodies against our HIV-2 probe (anti-gp36) were used to record specific antigen–antibody interactions whereas solutions of monoclonal antibodies against the corresponding immunogenic region of HIV-1 gp41 transmembrane glycoprotein (anti-gp41) were employed to simultaneously assess non-specific adsorption (NSA) and cross-reactivity (Fig. 2a). Both anti-gp36 and anti-gp41 antibody solutions were prepared as 0.1 mg/mL solutions in fetal bovine serum (FBS). By conducting our experiments with antibody-spiked serum solutions, we created for the EMPAS a real-world detection scenario, where the analytes of interest (antibodies) are present in low concentration and must be distinguished from an excess of interfering species (serum matrix). The frequency shifts (Fig. 2b) for the specific antigen–antibody interactions (16 kHz) were substantially greater than those recorded for the non-specific interactions (5 kHz) (Fig. 2c). However, the measurements for the specific interaction experiments only displayed modest reproducibility with a relative standard deviation (RSD) of 22%. This result is likely connected to the issue of control over the probe spatial complementarity, which is required for molecular recognition by its antibody [1]. With respect to the non-specific interaction experiments, anti-gp41 antibody serum solutions interacted quite reproducibly (9% RSD) with the device surface in a manner sufficient to resist a final PBS buffer rinse-off. We believe that cross-reactivity between the HIV-2 antigenic probe and HIV-1 antibodies, along with NSA of interfering species present in the serum matrix, is also responsible for such an observation [13–15]. Regardless, this work clearly shows that this SAM surface chemistry-EMPAS combination is effective in detecting and distinguishing HIV-2 anti-gp36 from HIV-1 anti-gp41 antibodies with good selectivity in a real-world (serum) detection scenario.

4. Conclusion

Herein, we have described our promising preliminary results for a label-free immunosensor dedicated to the detection of HIV-2. The biosensor platform was constructed as a highly reactive functionalizable mixed SAM-coated quartz wafer onto which HIV-2 immunodominant epitopes were subsequently immobilized in a single, pre-activation free step. The biosensing properties of this assembly, in terms of HIV-2 specific *vs.* HIV-1 non-specific antibody-antigen interactions, were evaluated in serum using the sensitive EMPAS system. Results demonstrated that the presented

immunosensor is effective in detecting and distinguishing HIV-2 from HIV-1 monoclonal antibodies with good selectivity in a real-world detection (serum) scenario. Current efforts are devoted to lower our present detection level (100 μg/mL) to the medically relevant level (pg/mL) displayed by modern state-of-the-art fourth generation antigen/antibody-combined HIV laboratory tests [16].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.04.008.

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