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Rapid detection of *Bacillus anthracis* using monoclonal antibody functionalized QCM sensor

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ABSTRACT

Since the anthrax spore bioterrorism attacks in America in 2001, the early detection of *Bacillus anthracis* spores and vegetative cells has gained significant interest. At present, many polyclonal antibody-based quartz crystal microbalance (QCM) sensors have been developed to detect *B. anthracis* simulates. To achieve a simultaneous rapid detection of *B. anthracis* spores and vegetative cells, this paper presents a biosensor that utilizes an anti-*B. anthracis* monoclonal antibody designated to 8G3 (mAb 8G3, IgG) functionalized QCM sensor. Having compared four kinds of antibody immobilizations on Au surface, an optimized mAb 8G3 was immobilized onto the Au electrode with protein A on a mixed self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid (11-MUA) and 6-mercaptohexan-1-ol (6-MHO) as adhesive layer. The detection of *B. anthracis* was investigated under three conditions: dip-and-dry, static addition and flow through procedure. The results indicated that the sensor yielded a distinct response to *B. anthracis* spores or vegetative cells but had no significant response to *Bacillus thuringiensis* species. The functionalized sensor recognized *B. anthracis* spores and vegetative cells specifically from its homophylic ones, and the limit of detection (LOD) reached 10^3 CFU or spores/ml of *B. anthracis* in less than 30 min. Cyclic voltammogram (CV) and scanning electronic microscopy (SEM) were performed to characterize the surface of the sensor in variable steps during the modification and after the detection. The mAb functionalized QCM biosensor will be helpful in the fabrication of a similar biosensor that may be available in anti-bioterrorism in the future.

1. Introduction

Bacillus anthracis vegetative cells and their spores have gained great concern since "the anthrax powder incident" in America in the fall of 2001 (Atlas, 2002; Robinson-Dunn, 2002; Schmid and Kaufmann, 2002). Due to its potential as a bioterrorism weapon, it is necessary to come up with a useful detection test. However, it needs many rigid requirements, such as specificity, sensitivity, speediness, stability, and simplification, among others (Campbell and Mutharasan, 2006a,b; Yemini et al., 2007). The traditional methods for detecting *B. anthracis* vegetative cells and their spores are mainly based on bacteriological, serology–immunological, and genetic methods (Pfisterer, 1991; Robinson-Dunn, 2002; Titball et al., 1991). These methods, which were frustrating and time-consuming, were widely used until the appearance of the biosensor in the middle of 20th century (Cooper and Hall, 1988; Dager et al., 1995; Tsoka et al., 1998). In a biosensor, the bioactive material, which can specifically interact with the analyte, is effectively immobilized on the surface of the transducer. The character of the biosensor is mainly determined by two factors: the sensitivity of its transducer, and the immobilized effect of the bioactive material (D'Souza, 2001; Starodub and Starodub, 2003). At present, the biosensor transducer has many types, such as evanescent wave based on optics, nano-wires based on electric–chemical (Arduini et al., 2007), thermal biosensors (Bossi et al., 2000; Chaniotakis, 2004), and piezoelectric biosensors (Arce et al., 2007), among others.

Since Sauerbray obtained the relationship between the change of resonance frequency of quartz and its mass increase due to adsorption (Sauerbray, 1959), quartz crystal microbalance (QCM) has been widely used as a sensor transducer. It is possible that a

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great amount of energy would be lost in liquid media, hence the detection using QCM should be only carried out in the gas phase until Shons first smeared antigen on quartz crystal to detect the antibody in solution. This was commonly considered as the first application of QCM as biosensor (Shons et al., 1972). However, QCM was widely applied as a sensor in the liquid phase until the 1980s due to its capacity to reach steady resonance in liquid along with the enhancement in the circuit designation and detection setup technique. Currently, more attention has been paid to QCM as a sensor due to its rapid analysis speed, satisfactory sensitivity, simple instrumentation, and low cost. QCM has been developed as automatic, real time and in situ detection. In addition, many commercial products based on QCM have been exploited and used in agriculture and other industries (Grill et al., 2005; Marx, 2003; Seibert et al., 1996; Wegener et al., 2001). In the biosensor field, however, QCM is still mainly being studied in the laboratory. Nonetheless, once it has been sufficiently studied, it will have many useful applications on the basis of a mature technique of commercial QCM (Kurosawa et al., 2006). In addition, the performance of QCM is usually influenced by its state of use, such as the procedure of sample detection, which deserves to be studied systematically.

The antibody plays a very important role in the fabrication of immunosensor, and the immobilization of the antibody on transducer surface has also become another focus of study in the biosensor field. In view of the fact that Au is usually used as a surface for QCM, many studies have been reported on antibody immobilization on the Au surface. These studies include the following: direct physical adsorption of the antibody (Liu et al., 2003; Zhang et al., 2002), cross-linking with glutaraldehyde (Babacan et al., 2000), self-assembling monolayer (SAM) (Su and Li, 2004), oriented immobilization of the antibody on protein A in assistance with mixed SAM (Wang et al., 2004), and the engineered antibody (Kim et al., 2007), among others. According to the strength of the interaction of the antibody with transducer, immobilizing methods could be classified into covalent and noncovalent combinations. For covalent combinations, sulf–Au bond is mainly formed between the mercapto-group and the Au surface, and the mercapto-group may come from a native thiolate group of the antibodies or from the materials conjugated to the antibodies, such as 11-mercaptoundecanoic acid (11-MUA) (Brogan et al., 2003). Regardless what kind of immobilizing method is chosen, it is necessary that more antibodies should be immobilized on the transducer surface. Furthermore, significant activities of antibodies should be preserved and that more active sites should be exposed outwards. However, it is not possible to simultaneously satisfy the abovementioned requirements. Thus, we should choose the proper immobilizing method that can satisfy them. At present, many polyclonal antibody-based QCM sensors have been developed to detect *B. anthracis* spores, vegetative cells, or its simulates, but they could not simultaneously detect *B. anthracis* spores and vegetative cells with one antibody functionalized sensor (Ayela et al., 2007; Halamek et al., 2002; O'Daly et al., 6 1992).

In this study, an mAb 8G3 functionalized QCM biosensor was fabricated to simultaneously detect *B. anthracis* spores and vegetative cells. Four different methods of antibody immobilization on Au surface were studied to investigate their response to the same target and to optimize an efficient immobilization of antibody on QCM surface. Three detection procedures were carried out to study the influence of detection method on the response of the QCM biosensor, namely, dip-and-dry, static addition, and flow through. Cyclic voltammogram (CV) and scanning electronic microscopy (SEM) were performed to characterize the surface of the sensors in variable steps during the modification and after the detection.

2. Materials and methods

2.1. Apparatus and materials

2.1.1. Apparatus

6 MHz AT-cut quartz crystals with Au and chromium covered electrode surface on both sides were purchased from Factory 707 in Beijing. The thickness of the quartz, chromium, and Au was 0.3 mm, 20 nm, and 50 nm, while their diameter was 12.5, 6, and 6 mm, respectively. Likewise, the crystal was fastened with two O-rings to ensure that only one side of the crystal would touch the solution. The CHI127 detection cell composed of Teflon with a volume of about 270 μl was obtained from the CHI Company (USA). It was modified to adapt the flow through procedure. Hence, a sealed bottom with a shorter inlet and a longer outlet was attached, and the QCM wafer was used as a transducer on the top to avoid the influence of the analytes' weights. A peristaltic pump with pipes connected to sample tube was used to cyclically transport the analytes. The resonant frequency was monitored with an Agilent 53131A frequency counter connected to a computer. Fig. 1 shows the schematics of the whole QCM detection system.

2.1.2. Preparation of B. anthracis spores and mAb

B. anthracis spores were prepared by growing the bacteria at 37 °C on modified Difco sporulation medium (DSM) (Sonenshein et al., 1974), containing 6 g tryptone, 3 g yeast extract, 10 g NaCl, 1 g KCl, 0.25 g $Mg_2SO_4 \cdot 7H_2O$, 0.23 g $Ca(NO_3)_2$, 0.197 g $MnCl_2 \cdot 4H_2O$, 0.0002 g $FeSO_4$, and 15 g agar/l.

Preparations containing 10^6 spores of *B. anthracis* strain A16 and inactivated by 1.5% formaldehyde were injected subcutaneously into 6-week-old BALB/c mice. The immunization was repeated 3× at 2-week intervals before boosting by intraperitoneal injection. The spleen cells were removed 3 d later and fused with myeloma cells, according to the procedures of Kohler and Milstein (Kohler and Milstein, 1975). The hybridomas were cloned by limit dilution, screened using the enzyme linked immunosorbent assay (ELISA). The mAbs were purified by caprylic acid-ammonium sulfate pre-

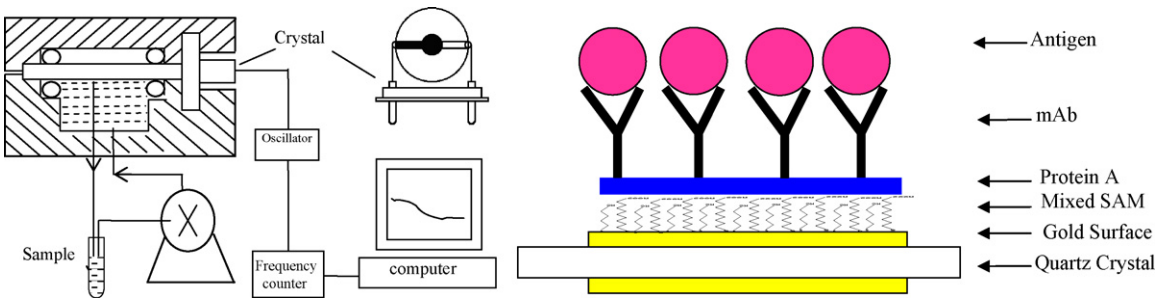

Fig. 1. Schematic illustration of QCM detection system (left) and mAb functionalized QCM sensor (right) (not drawn to scale).

cipitation of ascites (Perosa et al., 1990), and analyzed by SDS–PAGE and ELISA to determine the purity, activity and species-specificity.

2.1.3. Other materials

B. thuringiensis spores and vegetative cells, and *E. coli* were stored in our laboratory. The mouse IgG (Boster, China) was used as a negative antibody in our study. Glutaraldehyde, 11-mercaptoundecanoic acid (11-MUA), 6-mercaptohexan-1-ol (6-MHO), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), sulfur *N*-hydroxysuccinimide (S-NHS), protein A from *Staphylococcus aureus*, bovine serum albumin (BSA), pepsin, and chitosan, were purchased from Sigma–Aldrich. Phosphate buffered saline (PBS): 137 mmol/l NaCl, 2.7 mmol/l KCl, 4.3 mmol/l Na_2HPO_4, 1.4 mmol/l KH_2PO_4, pH 7.4; acetic acid, KCl, $K_4Fe(CN)_6$, $K_3Fe(CN)_6$ were obtained from the Country Medicine Group (China). The ultrapure water (18.2 MΩ cm) was produced by Millipore-Q system. All the other agents used in this work were analytical grade.

2.2. Immobilization of mAb on QCM surface

The QCM crystal was first cleaned with piranha solution (H_2SO_4: 30% H_2O_2 (v/v) was 7:3) for 2 min. Care was taken to keep the electrode welding points out of the solution. (Note: piranha solution has strong corrosive properties; operation with it should be carried out carefully.) Thereafter, the crystal was rinsed with ultrapure water and ethanol, followed by drying with N_2. In turn, the recycled crystal should be dealt with 1 M NaOH and 1 M HCl, and then disposed according to the abovementioned procedure.

After cleaning up, the QCM wafer was fixed into the detection cell with two O-rings on both sides. Then, 100 μl mixed solution of 2.5 mM 11-MUA and 7.5 mM 6-MHO in ethanol was injected into the detection cell and incubated in room temperature for 24 h to form a mixed self-assembled monolayer on the Au surface. Subsequently, the mixed SAM was activated with 100 mM EDC and 50 mM S-NHS in mixed aqueous solution for 2 h after washing the crystal with ethanol and ultrapure water. The activated mixed SAM surface was then incubated in 10 mg/ml protein A for 5 h in room temperature. The crystal was then incubated with 0.23 mg/ml mAb 8G3 against *B. anthracis* spore for 10 h at 4 °C. After coating with 1% BSA to passivate unspecific sites for 1.5 h, an mAb functionalized QCM sensor was prepared, as schematically shown in Fig. 1. Between each operation and after the passivation with BSA, the QCM wafer was washed twice with ultrapure water, and the changes in frequencies of QCM during each procedure were recorded with the 53131A frequency counter.

Other than the orientated immobilization of mAb via protein A on mixed SAM, three other antibody immobilization methods on Au surface based on the available literature were also studied in this work to compare their response effect on analytes. These methods were the following: mAb on SAM of 11-MUA (Su and Li, 2004), cross-linking of mAb with glutaraldehyde (Babacan et al., 2000), and site-directed immobilization with Fab′ fragment of mAb (Brogan et al., 2003).

2.3. Detection of B. anthracis vegetative cells and spores

The detection of *B. anthracis* vegetative cells and spores was carried out with QCM sensor in the following three types of procedure (Su and Li, 2004):

Dip-and-dry procedure: 10 μl of *B. anthracis* spore or vegetative cells suspension in different concentration were allowed to come in contact with the Au area for 1 h, then rinsed with PBS and ultrapure water, after which it was dried in N_2 flow. The resonant frequencies of the same sensor were measured in gas phase before and after immersion, and the frequency shift was recorded as the signal response to the analyte.

Static addition procedure: The sensor was mounted onto the Teflon static detection cell and equilibrated with 99 μl of PBS buffer with one side of the crystal exposed to the solution, then 1 μl of *B. anthracis* spore or vegetative cell suspension in different concentration was gently injected into the PBS buffer. Finally, the frequency shift of the sensor was recorded as a function of time until it reached a plateau.

Flow through procedure: A flow cell was modified from CHI127 electrolyte cell, as shown in Fig. 1. First, the PBS buffer was inputted through the chamber by the peristaltic pump at a flow rate of 300 μl/min, and the frequency shift was recorded as a function of time. After the baseline of PBS buffer reached stable, the liquid flow was switched to sample solution in a tube, and the solution from outlet was also guided to this tube to form a cycle. After running the sample solution for a given time, the liquid flow was switched back to the PBS buffer. The sensor was then rinsed in situ with PBS buffer until another stable baseline was obtained, and the frequency shift between two plateaus was correlated with the concentration of sample solution.

2.4. Characterization of QCM surface

After conducting the detection experiment of analytes, the QCM wafer was observed using scanning electronic microscopy (SEM) to investigate the combination between the immuno-QCM biosensor and the antigen. During the fabrication procedure of the immuno-QCM biosensor, the Au surface was also characterized in an electrochemical method, that is, cyclic voltammogram (CV), after each decorated step.

3. Results and discussion

3.1. Optimization for immobilization of antibody on QCM surface

Fig. 2 shows the comparison of response of mAb 8G3 functionalized QCM biosensor to 7×10^8 CFU/ml of *B. anthracis* vegetative cells using four different methods of antibody immobilization on QCM surface. Among the four immobilization methods, orientated immobilization of mAb via protein A on mixed SAM yielded the maximum frequency shift of -150 ± 6 Hz. This is shown as Fig. 2(c). In contrast, site-directed immobilization with Fab′ fragment of mAb, presented the minimum frequency shift of -9 ± 0.5 Hz. This is presented as Fig. 2(d). For (c), as protein A could combine with F_c fragment of antibody, a greater amount of antibody was immobilized onto the QCM surface and most active sites were also directionally exposed outwards. Consequently, this combined with more targets, and more frequency shifts were produced. On the other hand, for (d), although Fab′ of antibody could be directionally immobilized on Au surface, its capability to combine with antigen was reduced in contrast to the whole IgG. Furthermore, due to the removal of the F_c fragment, it was also easy to bring space hindrance to interact with analytes. For (a) and (b), the direction of antibody (intact IgG) contacting with the QCM surface was random. As such their frequency shift presented between (c) and (d), while the effect of (a) was better than (b), that is, the immobilization of mAb on SAM had greater efficiency than that utilizing cross-linking of mAb with glutaraldehyde. To summarize, method (c) had a short response time and yielded the maximum response signal to the same analyte. Therefore, it was chosen to fabricate the mAb functionalized QCM biosensor. Moreover, the immobilization in the succeeding section in this paper was presented according to (c), except when especially pointed out. It was also indicated

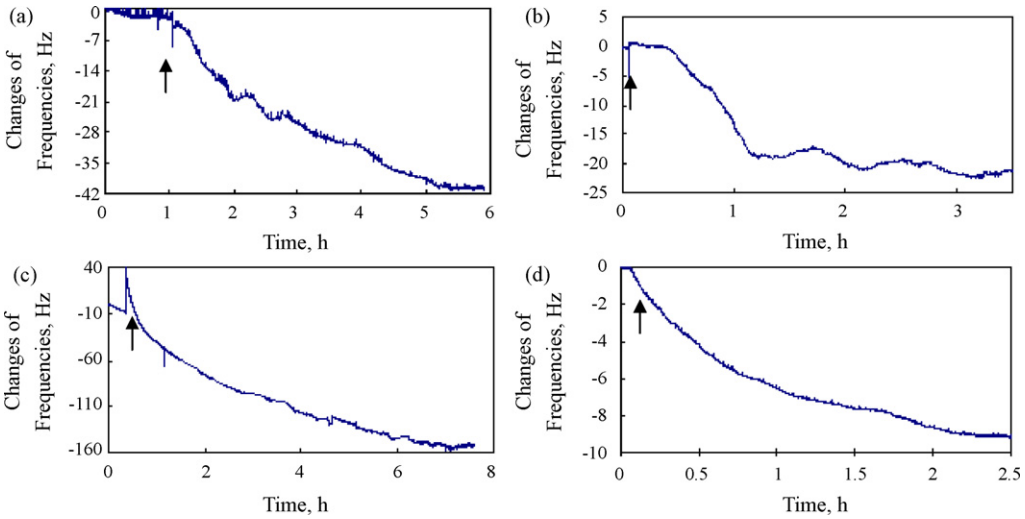

Fig. 2. Comparison of response of antibody functionalized QCM biosensor to the same antigen (7×10^8 CFU/ml of *B. anthracis* vegetative cells) by different methods of immobilization of antibody on QCM surface; ↑represents the addition of antigen. Conditions: 7×10^8 CFU/ml of *B. anthracis* vegetative cells, static injection procedure. Immobilization method: (a) mAb on self-assembling monolayer (SAM), (b) cross-linking of mAb with glutaraldehyde, (c) orientated immobilization of mAb via protein A on mixed SAM, and (d) site-directed immobilization with Fab′ fragment of mAb.

that the character of immuno piezoelectric biosensor was mainly determined by the immobilization method of the antibody.

3.2. Specificity of the QCM biosensor to B. anthracis

In order to study the specificity of this biosensor, we investigated the response of the mAb functionalized QCM biosensor to *B. anthracis* and *B. thuringiensis* spores using the dip-and-dry procedure. The QCM biosensor had a distinctive response to *B. anthracis* spores, -227 ± 13 Hz ($n = 3$, ±S.D.) to 2×10^5 spores/ml, but had little but contrasting response to *B. thuringiensis* spores, only 9 ± 3 Hz ($n = 3$, ±S.D.) to 2×10^5 spores/ml. The positive shift of frequency of *B. thuringiensis* spores was probably due to the loss of a part of the antibody or BSA on the Au surface during the washing procedure. This was a further indication that *B. thuringiensis* spores could not specifically combine with the QCM biosensor. The fluctuation of the QCM biosensor in atmosphere was less than 0.5 Hz within about 30 min. If the signal-to-noise ratio was set to 3, that is, 1.5 Hz frequency shift could be considered as the signal, then the limit of detection of this QCM biosensor to *B. anthracis* spores could theoretically reach 10^3 spores/ml.

Fig. 3 presents a comparison of the response of mAb functionalized QCM biosensor to 7×10^4 CFU/ml *B. anthracis* and *B. thuringiensis* vegetative cells in the flow through procedure. The results show that the QCM biosensor had a fast and distinct response to *B. anthracis* vegetative cells. However, it had no obvious response to *B. thuringiensis* vegetative cells. The frequency shift corresponding to *B. anthracis* vegetative cells reached the maximum, −70 Hz, in less than 10 min, after which a fluctuation of about 15 Hz appeared probably due to the unspecific interaction between the analyte and the biosensor surface. We also studied the response of this QCM sensor to 1×10^5 CFU/ml of *E. coli* bacteria. Though not shown in this figure, the frequency fluctuation of about 1.3 Hz, less than 1.5 Hz, during the cycle of sample solution in 1 h was only presented when the frequencies became steady. In addition, the results of the recognition of *B. anthracis* specific mAb to its antigen in ELISA also proved that the mAb used in this QCM sensor could distinguish the *B. anthracis* spores or vegetative cells from its homophylic ones or other non-related bacteria, such as *E. coli*. Moreover, we also tested the specificity of the antibody–*B. anthracis* interaction with a non-related antibody (mouse IgG) functionalized QCM sensor, and no response signal was found in this sensor to 1×10^7 spores/ml of *B. anthracis* except for a fluctuation of ±10 Hz when the sample was injected, as shown in supplementary information S2. Therefore, it could be concluded that the mAb functionalized QCM biosensor could specifically distinguish the *B. anthracis* spores or vegetative cells from its homophylic ones.

3.3. Response of the QCM biosensor to B. anthracis in a range of concentrations

Fig. 4 presents the response of the QCM biosensor to a range of concentrations of *B. anthracis* vegetative cells, from 7×10^2 to 7×10^8 CFU/ml, and their calibration curve in log–log plot. It was shown that the frequency shift of the QCM biosensor increased, along with the concentration of *B. anthracis* vegetative cells. The biosensor had a response to low concentration of *B. anthracis* vegetative cells, -5 ± 0.7 Hz to 7×10^2 CFU/ml. The standard deviation of frequency shift was 0.53 Hz in PBS ("zero" cell concentration). This

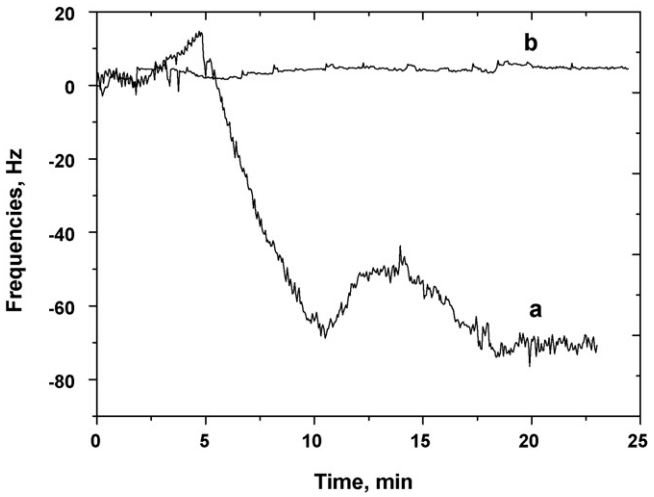

Fig. 3. A comparison of response of mAb functionalized QCM biosensor to 7×10^4 CFU/ml *B. anthracis* and *B. thuringiensis* vegetative cells in flow through procedure with a flow rate of 300 μl/min.

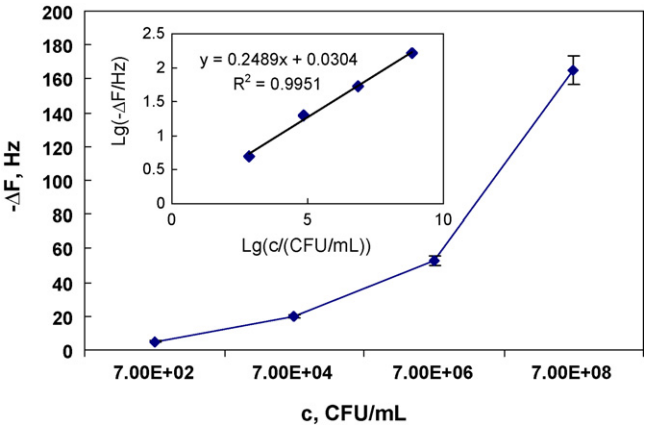

Fig. 4. Changes of frequencies of mAb functionalized QCM biosensor response to *B. anthracis* vegetative cells in range of concentrations. (inset: calibration curve for frequency shift along with *B. anthracis* concentration in log–log plot). Error bars represent one standard deviation of three parallel data.

is shown in supplementary information S1. Moreover, a standard deviation of 3 is usually adopted as the limit of detection (LOD), namely 1.6 Hz, less than 5 Hz. Hence, the LOD of this QCM sensor should be below 7×10^2 CFU/ml of *B. anthracis* vegetative cells. The sensitive response might lie in the detection pattern of static addition procedure, which could preserve some weak interaction between the analyte and this biosensor. This can also enhance the response signal, though it might bring some unspecific sorption onto the transducer surface and produce false positive signal.

3.4. Characterization of the mAb functionalized QCM surface

After the detection of mAb functionalized QCM sensor for *B. anthracis*, we observed the QCM surface using scanning electronic microscopy (SEM). Thereafter, we found the morphologies of *B. anthracis* spores and vegetative cells captured by QCM sensor surface, as presented in Fig. 5. This verified the combination between the QCM sensor and its analytes. In addition, it should be pointed in particular, that nearly no analyte for control species, such as *B. thuringiensis* and *E. coli*, detected in QCM sensor functionalized by mAb against *B. anthracis*, was found in the same method, and neither for the detection for *B. anthracis* in QCM sensor functionalized by non-related antibody (mouse IgG). It was indicated that the QCM sensor functionalized by mAb against *B. anthracis* could distinguish *B. anthracis* species specifically.

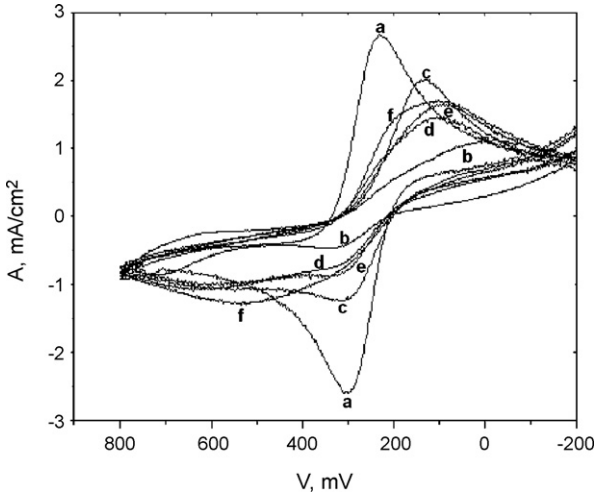

Fig. 6. Characterization of QCM surface during its decoration procedure in cyclic voltammograms: (a) bare Au surface, (b) mixed SAM, (c) activation with EDC and S-NHS, (d) coating with protein A, (e) coating with mAb, and (f) combination with *B. anthracis* spores. Working solution PBS in the presence of 10 mM $K_3Fe(CN)_6$ and 10 mM $K_4Fe(CN)_6$, scan rate 50 mV/s.

To investigate the surface of QCM during the decoration of Au surface, cyclic voltammogram (CV) was performed on a same Au surface after each decorating procedure. This is presented in Fig. 6. It was shown that the current of redox on bare Au surface was the maximum, and presented symmetrical CV (shown in Fig. 6(a)). This indicates that the redox reaction of $Fe(CN)_6^{3-/4-}$ occurred on a very clean surface. Fig. 6(b) presents the CV after the formation of mixed SAM on Au surface, with a sharp decrease of current. This implied the formation of an insulating layer on this surface, and was in accordance with the fact that the long alkyl chain of mixed SAM could reduce the penetration of a redox pair near the surface. The current of the redox kept an uptrend (shown in Fig. 6(c)), which was due to the decrease of cross-linking degree of carboxylic group of 11-MUA after the activation with EDC and S-NHS, and convenient for the redox pair to travel on the surface. After the coating of protein A, the current of the redox kept a downtrend (shown in Fig. 6(d)) indicating more insulation on the surface because of the coating of protein. An examination of the previous literature shows that the coating of mAb was expected to decrease the current of the redox (Su et al., 2008; Zeravik et al., 2007); in contrast, our work showed that it increased the current (shown in Fig. 6(e)). This was probably due to the fact that some protein A or SAM

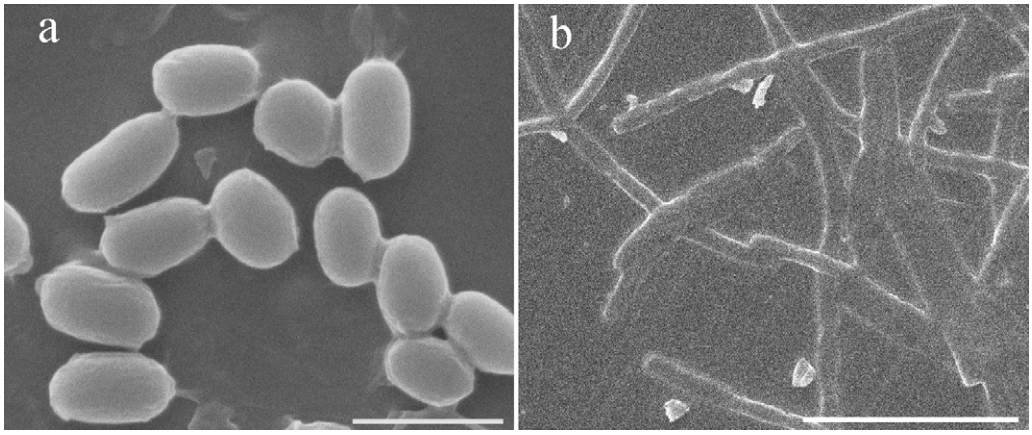

Fig. 5. Characterization of morphologies of *B. anthracis* spores (a, scale bar 2 μm) and vegetative cells (b, scale bar 10 μm) captured by QCM sensor surface in scanning electronic microscopy (SEM).

was washed and lost by the PBS buffer, and it led to the abnormal increase of the current because of the penetration of more redox pairs through the protein gap. Likewise, Fig. 6(f) shows that the redox current increased abnormally. Except for the surface of bare Au, other cyclic voltammograms were not symmetrical. This indicated various changes in the Au surface during its modification.

All the results in this work were obtained on a QCM sensor surface with freshly immobilized mAb. Limited experiments have been carried out to determine the repeat application of the sensor. We tested the stability for storage of our QCM sensor; it could preserve its capacity to specifically distinguish its antigen after storage at 4 °C for 1 month. However, its response signal could only reach about 70% (to 7×10^6 CFU/ml of *B. anrhracis*) of the new QCM sensor that was fabricated on the same condition. For regeneration, we could release the antigen from the sensor that was used by decreasing pH (commonly to pH 1) of the buffer over the surface of the QCM sensor whereas minor antibody immobilized would also be released from the sensor. Inevitably, this would reduce its capability to detect the analyte. Currently, an investigation is being conducted on a systematic study of loss of activity, as well as methods to improve the regeneration of sensors.

4. Conclusion

An mAb 8G3 functionalized QCM biosensor was fabricated to simultaneously detect *B. anthracis* spores and vegetative cells. The results indicate that the sensor yielded a distinct response to *B. anthracis* spores and vegetative cells but had no obvious response to *B. thuringiensis*. The QCM sensor's limit of detection (LOD) could reach 10^3 CFU or spores/ml of *B. anthracis* in less than 30 min. It was advantageous or comparable to the currently available immunosensors reported for *B. anthracis* detection (Lee et al., 2005; Campbell and Mutharasan, 2006a,b). Furthermore, QCM could simultaneously recognize *B. anthracis* spores and vegetative cells from its homophylic ones. Four immobilizing methods of antibodies on Au surface were studied to investigate their response on the same target. It was shown that orientated immobilization of mAb via protein A on mixed SAM had a short response time, and yielded the maximum response signal to the same analyte. Three detection procedures, namely dip-and-dry, static addition, and flow through, were carried out to study the influence of detection method on the response of the QCM biosensor. It was revealed that the dip-and-dry method had a higher sensitivity than the flow through and static addition method, while the latter were more rapid and convenient for real time detection. On the other hand, the flow through procedure could avoid the influence of the analyte weight.

Acknowledgements

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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.bios.2008.07.071.

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