Biosensors and Bioelectronics 26 (2011) 2595–2600

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Direct immobilisation of antibodies on a bioinspired architecture as a sensing platform

Yi Wan[a,b], Dun Zhang[a,*], Yi Wang[a], Peng Qi[a,b], Baorong Hou[a]

[a] Key Lab of Corrosion Science, Shandong Province, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China
[b] Graduate School of the Chinese Academy of Sciences, 19 (Jia) Yuquan Road, Beijing 100039, China

ARTICLE INFO

Article history:
Received 14 September 2010
Received in revised form 28 October 2010
Accepted 9 November 2010
Available online 26 November 2010

Keywords:
Dopamine
Impedimetric immunosensor
Sensing platform
Bioinspired architecture
Bacteria detection

ABSTRACT

A sensitive and selective immunosensor for the nonlabeled detection of sulfate-reducing bacteria (SRB) is constructed using a self-polymerised polydopamine film as the immobilisation platform. Self-polymerisation of dopamine is used as a powerful approach for applying multifunctional coatings onto the surface of a gold electrode. The polydopamine film is used not only as the immobilisation platform, but also as a cross-linker reagent for the immobilisation of the anti-SRB antibody. The polydopamine film is loaded with a high density of anti-SRB antibodies linked to the substrate to obtain high response signals. The formation and fabrication of the biosensor and the quantification of antibody anchoring are monitored, and SRB detection is performed by either quartz crystal microbalance (QCM) or electrochemical impedance spectroscopy (EIS). After modeling the impedance Nyquist plots of the SRB/anti-SRB/polydopamine/gold electrode for increasing concentrations of SRB, the electron transfer resistance (R_{ct}) is used as a measure of immunocomplex binding. The R_{ct} is correlated with the concentration of bacterial cells in the range of 1.8×10^2 to 1.8×10^6 CFU mL^{-1}; the detection limit is 50 CFU mL^{-1}. This work demonstrates a new immobilisation platform for the development of a sensitive and label-less impedimetric and piezoelectric immunosensor. This immunosensor may be broadly applied in clinical diagnoses and the monitoring of water environmental pollution. The method proposed is distinct in its ease of application, use of a simple protocol, and mild reaction conditions. These allow it to be applied to a wide variety of materials.

1. Introduction

The determination, identification, and quantification of pathogenic bacteria for clinical diagnosis, water environmental analysis, and food safety are crucial for public health protection. Sulfate-reducing bacteria (SRB) are anaerobic microorganisms that use sulfate as a terminal electron acceptor, resulting in the production of sulfide. Sulfide is highly corrosive and toxic; thus, it can be a serious problem for industries, economies, and ecological systems, such as the offshore oil industry. Therefore, a rapid and easy method for the detection of pathogen is essential for the control of public health and environmental contamination.

Conventional methods for monitoring microbial populations, such as the most probable number (MPN) method (Abd-El-Malek and Rizk, 1958), involve a pre-enrichment step or a selective enrichment step followed by a biochemical test, and the sophisticated series of assays required can take up 15 days to complete. A variety of protocols have been presented for monitoring microbial populations, including plating and culturing (Swaminathan and Feng, 1994), biochemical tests (Louis et al., 2005), enzyme-linked immunosorbent assays (Gaylarde and Cook, 1990), and pertinent molecular techniques, such as polymerase chain reaction (Cook et al., 2008) or fluorescence in situ hybridisation (Lücker et al., 2007). Although promising, these techniques are still under development, and several problems, such as the consumption of long periods of time, high costs, and necessity of sophisticated procedures, occur when they are used in situ and in real time.

Over the past decade, many attempts have been made to use efficient and cost-effective biosensor technology as a rapid and reliable detection approach. A variety of alternative methods have been reported for the detection of pathogens, including differential pulse voltammetry (He et al., 2009), electrical conductivity (Lu et al., 2008), field effect transistors (Villamizar et al., 2008, 2009), mass spectrometry (Russell et al., 2007), surface enhanced Raman scattering (Jarvis et al., 2008), infrared spectroscopy (Ravindranath et al., 2009), multiplexed chemiluminescent immunoassay (Karsunke et al., 2009), fluorescence spectroscopy (Klodzinska and Buszewski, 2009), and capillary electrophoresis (Lantz et al., 2007). The feature characteristics of these biosensors result from the simplification of the sample preparation

* Corresponding author. Tel.: +86 532 82898960; fax: +86 532 82898960.
E-mail address: zhangdun@ms.qdio.ac.cn (D. Zhang).

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doi:10.1016/j.bios.2010.11.013

steps, or from their high specificity and sensitivity, which allow the detection of specific pathogens in complex matrices. However, whilst these detection methods are relevant for laboratory use, they cannot adequately serve the needs of health practitioners or enable large-scale monitoring of environmental pollution.

Given the abovementioned drawbacks, several efforts to develop a facile and simple method for the nonlabeled determination of pathogens with increased sensitivity and selectivity that is both labour- and time-saving have been proposed. Some sensitive, reliable, and more rapid biosensor techniques have been recently reported for the nonlabeled detection of microorganisms, including surface plasma resonance (SPR) (Balasubramanian et al., 2007), quartz crystal microbalance (QCM) (Shen et al., 2007), and electrochemical impedance spectroscopy (EIS) (Pournaras et al., 2008; Varshney and Li, 2009; Wan et al., 2009, 2010; Yang et al., 2009). Among them, QCM and EIS have been paid increasing attention. The approach often shows unique and considerably advantages compared to other biosensor analytical methods. For example, SPR-, QCM- and EIS-based feature simplicity, low costs, and ease of operation. Moreover, QCM-based and EIS-based approaches are nondestructive methods for the characterisation of biological elements, making them extremely expedient for the nonlabeled detection of pathogens.Dopamine is a catecholamine neurotransmitter that occurs in both vertebrates and invertebrates (Benes, 2001). It contains catechol and amine functional groups that are also found in high concentrations in mussel adhesive proteins (Waite, 2000). At pH = 8.5, chemical changes occur in the dopamine molecule, resulting in polymerisation of the molecules to form polydopamine, which coats the solid substrates (Lee et al., 2007). The polymerised film is fairly similar to that found the mussel adhesive protein. The polydopamine coating, in turn, provides an extremely chemically reactive surface onto which other chemical/biological molecules can be deposited. Because the surface is so reactive in many different ways, a wide variety of second coatings can be applied to it. An important feature of polydopamine and its analog, dopamine, is their ortho-dihydroxyphenyl functional group, which forms strong bonds with various inorganic/organic surfaces that have been shown to be stronger than biotin–streptavidin interactions (Lee et al., 2006). Therefore, self-polymerisation of dopamine can be used as a powerful approach to applying multifunctional coatings onto many surfaces, including noble metals, metal oxides, ceramics, and polymers (Lee et al., 2007). Polydopamine can be used not only used as an immobilisation platform, but also as a cross-linker reagent for the immobilisation of biomolecules. The immobilisation platform is essential for the operation of the biosensor, and plays a key role in its selectivity, sensitivity, and stability. Various platforms, such as self-assembled monolayers (Wan et al., 2009), organic polymer materials (Sai et al., 2006), and nanomaterial-doped polymer films (Gniadek et al., 2009) for immobilising antibodies or other protein molecules have been reported in the literature. These materials have been investigated extensively due to their high stability, benign biocompatibility, and oriented localisation, all of which may be exploited to provide model surfaces for investigating cell behaviours in bioanalysis and pathogenic detection. As well, the immobilisation of antibodies or other biomolecules onto surfaces is important in many analytical biochemical procedures, such as pathogen detection for human health and biosecurity and environmental monitoring for the inadvertent contamination of food and water. Approaches for the immobilisation of antibody or other biomolecules onto surfaces involve either noncovalent or covalent cross-linkered reactions. Noncovalent reactions, such as physical adsorption and protein–ligand binding though ionic interactions, hydrogen bonds, van der Waals interactions, and hydrophobic interactions, allow the direct immobilisation of antibodies or other biomolecules on substrate surfaces for long periods of time (>12 h) (Balasubramanian et al., 2007). In contrast, covalent immobilisation of antibodies or other biomolecules on substrates may be undertaken via chemical groups activated using 1-ethyl-3[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC)/*N*-hydroxisuccinimide (NHS), glutaraldehyde, or dithiobis(sulfosuccinimidylpropionate) to yield stable, reproducible, and reliable data within a short period of time (ca. 2 h) (Hao et al., 2009; Mantzila et al., 2008; Poitras and Tufenkji, 2009). However, Lee et al. reported the facile two-step covalent conjugation of trypsin onto a variety of substrates, such as cellulose, titanium oxide, copper, and polycarbonate, via mussel adhesive protein-inspired coatings without other cross-linker reagents (Yuhan et al., 2008).

In this work, a thin adherent polydopamine film is synthesised via self-polymerisation at alkaline pH and used as an immobilisation platform. Anti-SRB antibody conjugation on a polydopamine-modified gold electrode is easily achieved. We investigate a bioinspired architecture relevant to biosensor research to anchor and detect SRB. Strong quinine–amine group interactions between polydopamine and anti-SRB antibody make this immobilisation strategy much more efficient than chemistry-based methods. The formation and fabrication of the biosensor and the quantification of antibody anchoring are monitored by the QCM technique, and SRB detection is performed by EIS analysis. We also investigate the immobilisation chemistry and characterisation of surface topography via FT-IR spectroscopy, water contact angle measurements, and scanning electron microscopy. Finally, the proposed immunosensors are successfully used for the detection of SRB in experimentally inoculated bacteria samples.

2. Materials and methods

2.1. Chemicals and solutions

Dopamine hydrochloride, albumin bovine V (BSA), and fluorescein isothiocyanate (FITC) were purchased from Sigma–Aldrich Corp. (MA, USA). Rabbit antibody against SRB was provided from by Abmart Inc. (Shanghai, China). Other chemicals used in this paper were purchased from Sinopharm Chemical Reagent Co., Ltd, China. $MgSO_4$, NH_4Cl, Na_2SO_4, $CaCl_2$, Na_2HPO_4, NaOH, sodium lactate, and yeast extract were used to prepare the modified Postgate's medium. The Postgate's medium was filtered with a 0.2 μm pore size filter. All chemicals were of the highest purity available and were used as received without further purification. All solutions were prepared using Milli-Q water obtained from a Milli-Q filtration system.

2.2. Instrumentation

QCM measurements were performed recorded in a custom-built cell setup equipped with a Ti-gold plated AT-cut quartz crystal (CH Instruments, Inc.) 5 mm in diameter with a resolution frequency of 7.995 MHz. The frequency output from the oscillator was measured with using a CHI 430C equipment (CH Instruments, Inc.). Mass changes due to the binding reactions has been calculated by using the Sauerbrey equation (Sauerbrey, 1959):

$$\Delta m = -\frac{A\sqrt{\rho_q \mu_q}}{2f_o^2}\Delta f \quad (1)$$

where a mass change (Δm) in the crystal results in a subsequent change (Δf) in its resonance frequency (f_o); A, the piezoelectrically active crystal area, is 0.196 cm^2; ρ_q, the density of quartz, is 2.648 g/cm^3, and μ_q, the shear modulus of quartz for AT-cut crystals, is 2.947×10^{11} $g/(cm\,s^2)$. The temperature of the QCM cell was thermostated at 25 °C.

Electrochemical measurements were made using a CHI760C (CH Instruments, Inc.) conventional three electrode system, which

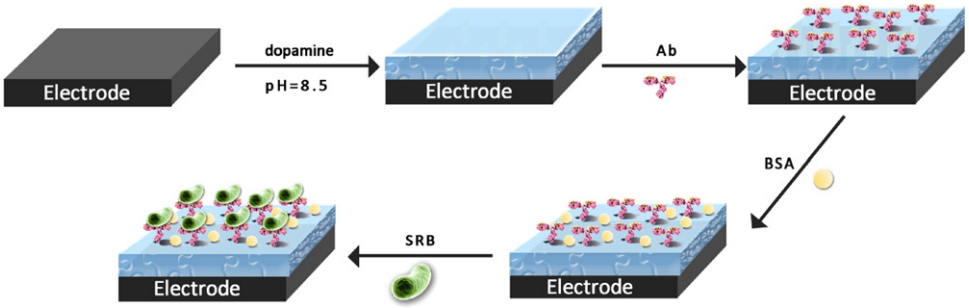

Fig. 1. Schematic description of polydopamine coating, biomolecule immobilisation through reaction between amines and polydopamine-coated substrates, and subsequent bacteria detection.

included a platinum wire and an Ag/AgCl, 3 M KCl wire as the counter and reference electrodes, respectively. A functionalised Au electrode was used as the working electrode. EIS was used to characterise the stepwise modification process of the functionalised electrode. After preparing the multilayer biosensor surface, SRB detection was performed by EIS. The functionalised electrode was immersed in a culture containing SRB in a series of concentrations ranging from 1.8 to 1.8×10^7 CFU mL^{-1}; the incubation time was 1 h. The functionalised working electrode was cleaned by PBS, and Faradic impedance spectra were recorded at the open circuit potential. EIS was conducted in 10 mL PBS in the presence of the redox probe with 5 mM $K_3Fe(CN)_6$ and 5 mM $K_4Fe(CN)_6$ (1:1) at the open circuit potential vs. Ag/AgCl, 3 M KCl and an alternating potential with amplitude of 10 mV at the frequency range from 0.1 Hz to 100 kHz. The experiments were repeated three times. The reproducibility of the impedance responses is represented by the error bar, which was obtained from the standard deviation for the three replicates.

2.3. Bacterial cultivation

A pure SRB culture was grown in modified Postgate's medium containing 2 g magnesium sulfate, 1 g ammonium chloride, 0.5 g sodium sulfate, 0.1 g calcium chloride, 0.5 g dipotassium hydrogen phosphate, 2 mL sodium lactate, and 7.5 g yeast extract/L at 30 °C for 4 d. The bacterial cells were then isolated by centrifugation (6000 rpm, 20 min) and rinsed three times with PBS. Prior to measurements, SRB samples were stored in the refrigerator at 4 °C to decrease the growth rate. The culture was serially diluted with physiological saline solution, and the viable cell number was determined by the most probable number (MPN) method, according to the American Society of Testing Materials Standard D4412-84. The bacterium, *E. coli*, was provided by Dr. Li Su and used as a control organism.

2.4. Detection of bacteria

The fabrication of the sensor and detection of bacteria are shown in Fig. 1. Before EIS measurements, an Au electrode (CH Instruments, Inc.) was polished with silicon carbide paper and alumina powder, cleaned ultrasonically in absolute ethanol, rinsed with Milli-Q water, and then dried in an air flow. The electrode was subsequently electrochemically pretreated by cycling the potential range from −0.2 to 1.6 V in a 0.05 M H_2SO_4 solution at a scan rate of 10 V s^{-1} until a steady cyclic voltammogram was obtained. Dopamine (3.33 mg mL^{-1}) was dissolved in 10 mM Tris–HCl (pH 9.0), and substrates were dipped into the solution for overnight (10 h). pH-induced oxidation changed the solution colour to dark brown. Vertical sample orientation was necessary to prevent nonspecific microparticle deposition on surfaces. The coated surfaces were rinsed with ultrapure water and dried by nitrogen gas before storage or treated as described below for the direct immobilisation of antibodies. The polydopamine-coated substrates were transferred into an antibody solution (1 mg mL^{-1}, pH 7.4) for 1 h. The electrode was then rinsed with water to remove the physically absorbed antibodies prior to use in the following procedure. As shown in Fig. 1, 1% BSA was used to block the nonspecific sites on the surface of the functionalised electrode. Finally, a series of SRB solutions (50 μL) were added and incubated for 1 h. After each step, the electrode was rinsed thoroughly with Milli-Q water to avoid the physical adsorption of SRB and then dried with a stream of nitrogen. In addition, fluorescence microscopy analysis was used to characterise the number of SRBs on the surface of the bioinspired film (refer to Fig. 2S in the supplementary material).

3. Results and discussion

3.1. Fabrication and characterisation of bioinspired architecture

Immobilisation of biomolecules, such as protein or DNA, onto surfaces is important in many biochemical areas, including analytical chemistry, medical diagnostics, and gene analysis. In our work, chemical changes in the dopamine molecule resulted in the polymerisation of the molecules to form polydopamine, which coats the solid substrates at pH = 8.5. The colour of the dopamine solution changed from colourless to charming pink within several minutes, and then to black an hour later. The growth of bioinspired polydopamine was monitored via QCM methods to track the variation of the mussel-inspired film. The results indicate that the growth conditions of film reach a stationary phase after immersion for 6 h. We selected pH = 8.5 to produce mild conditions, which are advantageous for the fabrication of biosensors and immobilisation of other biomolecules. Polydopamine is used not only as the immobilisation platform, but also as a cross-linker reagent for the immobilisation of biomolecules. This method avoids the need to develop complex self-assembled monolayers, thus allowing straightforward and complete surface functionalisation for the direct immobilisation of recognition elements within a relatively short period of time.

The biocompatibility of a sensing platform for chemical/biological molecule loads and bioactivity is nearly related to its hydrophilicity. Contact angle measurements of the sensing platform were carried out on bare gold and polydopamine-coated substrates to study their hydrophilicity. The water contact angle of the polydopamine film significantly decreased to $45.34 \pm 2.19°$ from $68.67 \pm 2.33°$ without self-polymerisation of dopamine. This indicates that the substrate was relatively hydrophilic and thus suitable for antibody immobilisation, providing a favorable microenvironment for living bacteria. Such an environment prolonged the life of adhered bacteria for the next experiment.The FT-IR spectrum of the antibody-functionalised polydopamine substrate was observed to be distinctly different from that of a

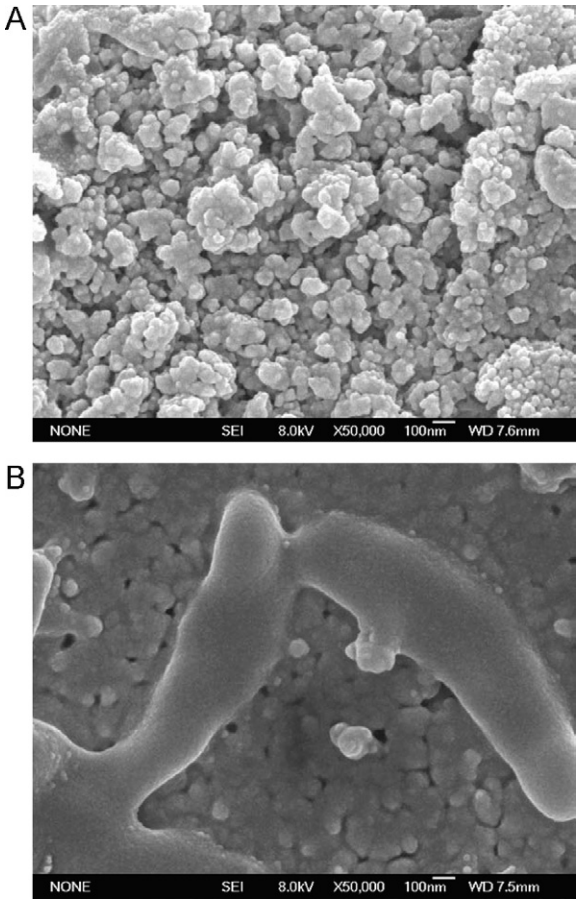

Fig. 2. SEM images of (A) polydopamine film and (B) SRB-Ab-polydopamine film.

pure polydopamine surface (Fig. S1). For polydopamine film, the peak at 1608 cm^{-1} represents the strong and overlaid absorption band of the stretching vibrations of C=C aromatic rings and N–H bending, the peaks at 3349, 3312, and 2922 cm^{-1} represent the stretching vibrations of O–H, N–H, and C–H of dopamine, respectively, and the peak at 1181 cm^{-1} represents the stretching vibrations of C–O. These polydopamine bands were observed at 1608, 1496, and 1325 cm^{-1}. A series of bands in the fingerprint area, which are attributed to the spectra of adsorbed aromatics, was also observed. Compared to the FT-IR spectra of polydopamine, a distinct difference for the antibody functionalised polydopamine substrate is found; a large number of peaks disappeared due to surface modifications. The functional layer of the anti-SRB antibody was spontaneously formed through the simple immersion of polydopamine-coated substrates. Functional layer formation on the polydopamine layer is believed to involve reactions between terminal catechols that react with amines via Schiff base reactions (Lee et al., 2007).

Meanwhile, SEM was used to affirm the binding of bacteria cells to antibodies immobilised on the surface of the bioinspired surface, as shown in Fig. 2. SEM images were taken after the self-polymerisation of dopamine immobilisation (Fig. 2A) and following the binding of bacteria (Fig. 2B). The SEM of the bioinspired film prepared from the treatment of dopamine at pH = 8.5 displayed a laced, three-dimensional, noncompact structure. Such a surface was favorable for the loading and adhesion of bacterial cells. After antibody immobilisation, 0.1 mL of bacteria (10^8 CFU mL^{-1}) was incubated on the electrode surface for 60 min, followed by rinsing with buffer and acquisition of SEM images. Fig. 3B is a higher magnification image showing two single bacteria captured by immobilised antibodies on the surface of the mussel-inspired

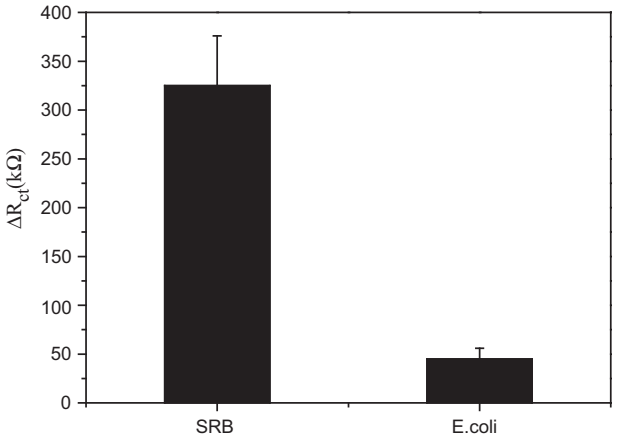

Fig. 3. Comparison of responses of impedimetric immunosensor to (a) 1.8×10^8 CFU mL^{-1} sulfate reducing bacteria (SRB) and (b) 2.1×10^8 CFU mL^{-1} *E. coli*.

coating. Few bacteria were observed to bind to the immobilised on the surface of the mussel-inspired coating when *E. coli* was used in a control experiment.

3.2. Sensor fabrication

The QCM technique, a nonlabeled biosensor, offers promising advantages over conventional detection techniques, from the viewpoints of system miniaturisation, cost effectiveness, and simplicity of operation. Following the theory of Sauerbrey, QCM is a very sensitive tool for detecting changes in weight and is thus a helpful method for sensing binding events between an analyte and a sensing layer. Polydopamine was self-polymerised onto the gold electrode of quartz crystals as described earlier. Anti-SRB antibodies were directly immobilised following the optimised protocol. Table 1 shows the quantified parameters of the fabrication of the biosensor. Experimental conditions were first studied so that we could demonstrate that the anti-SRB antibody on the surface of the bioinspired coating facilitates the binding of SRB to the antibody. As shown in Table 1, the addition of 50 μL anti-SRB antibody to the polydopamine-QCM after 1 h generated 79 Hz frequency change at equilibrium. The immobilisation of the antibody on the polydopamine matrix resulted in the binding of 3.53×10^{-6} nmol mm^{-2} anti-SRB antibody to the substrate. The binding ratio of antibody to immobilised bioinspired film was 6.78×10^{-3}. The nonspecific sites were then blocked using BSA, resulting in a response signal shift of 119 Hz. The subsequent addition of SRB provided a large binding signal (128 Hz). The relationship between the QCM sensor response and bacteria concentration was qualitative rather than quantitative. This is due to the combination of both effects. QCM measurement determines only those materials that are bound to the sensor surface and requires that the surface layer be rigid. However, the potential of piezoelectric mass sensing devices for the detection of bacteria or other biological materials remains undefined due to the nonrigid structure of the bacterial cells. Bacterial cell or biological material binding often involves energy dissipation, which causes damping of the oscillation of the crystals due to internal friction or trapping of water by the cells or other biological materials. The immunoreaction between the antibody and target bacterial cells also showed different stoichiometric ratios at different bacterial cell concentrations.

3.3. Selectivity of the developed immunosensor

Specificity is usually an important criterion for immunosensors. The selectivity of the immunosensor against other bacteria (*E. coli*)

Table 1
Quantification of immobilisation and sensing parameters.

Modification procedures	ΔF (Hz)	Δm (ng)	ng mm^{-2}	nmol mm^{-2}	Binding ratio
PDOPA	12.32	16.08	0.08	5.20×10^{-4}	
Ab/PDOPA	79.10	105.9	0.53	3.53×10^{-6}	Ab/PDOPA 6.78×10^{-3}
BSA/Ab/PDOPA	111.9	149.9	0.75	1.13×10^{-5}	BSA/PDOPA 2.17×10^{-2}
SRB/BSA/Ab/PDOPA[a]	128.4	172.2	–	–	–

[a] Bacterial binding often involves energy dissipation due to internal friction or trapping of water by the cells, which causes damping of the oscillation of the crystals because of the nonrigid nature of bacterial cells.

was evaluated by impedimetric signal shifts via EIS detection at the same concentration (2.0×10^8 CFU mL^{-1}) under the experimental conditions specified above. Fig. 3 shows the resistance changes at fixed concentrations of the target bacteria and contrast analyte. In this sensor, the ΔR_{ct} values increased less than 45 ± 11 kΩ when *E. coli* were analysed, whereas a distinct increase (330 ± 54 kΩ) was obtained after incubating with SRB, indicating that the observed changes in electron transfer resistance with SRB were due to specific antibody–antigen recognition. This makes the impedimetric immunosensor feasible for the detection of SRB.

3.4. EIS measurement

As a sensitive and reliable tool, EIS has been applied to a wide range of areas, such as biosensors, to measure changes at electrode interfaces for the recognition of biochemical molecules. EIS detection can be used in the absence or presence of a redox couple, such as $Fe(CN)_6^{3-/4-}$, in non-faradic and faradic impedance measurements, respectively. The impedance response measured without the redox couple is ascribed to the detection of substances attached to the electrode surface that provide insulation. Compared to non-faradic impedance, the faradic method in the presence of a redox couple identifies recognition events that occur on the functionalised electrode surface as changes in faradic impedance spectra. The latter approach is regarded as effective and efficient for characterising changes in the interfacial electrical properties of a modified electrode. Therefore, faradic impedance measurement was chosen for the determination of SRB in the present study.

Faradic impedance data are often simulated using the modified Randles circuit and a model that combines electron transfer kinetics and diffusion control. The ohmic resistance of the electrolyte solution between the modified working electrode and the Pt wire counter electrode is represented by a resistance (R_s), C_{dl} is the double layer capacitance, and R_{ct} is the charge transfer resistance of the redox couple. The Warburg impedance (Z_w) (semi-infinite diffusion mechanism) in the measurement system arises from the diffusion resistance of the $Fe(CN)_6^{3-/4-}$ redox probe between the bulk solution and the interface of the modified working electrode.

For faradic impedance measurements, R_{ct} is the most sensitive and straightforward parameter used to characterise recognition events occurring on the surface of the working electrode. The diameter of the semicircle in the Nyquist plots is measured, since the parameters R_s and Z_w are hardly influenced by chemical transformations on the electrode surface. Moreover, variations in C_{dl}, as opposed to changes in R_{ct}, are barely detectable. In addition, R_{ct} can be directly extracted from Nyquist plots based on the intercept of the semicircle with the real axis.

EIS has been used to monitor the adhesion of bacterial cells on modified electrodes. When bacterial cells were adhered onto the mussel-inspired film, the modified electrode showed increased charge-transfer impedance (R_{ct}). Fig. 4A shows the Faradic impedance spectra obtained on the SRB/Ab/polydopamine/Au electrodes prepared with different SRB concentrations. The diameter of the Nyquist plots increased regularly with increasing SRB concentrations, indicating that SRB was absorbed onto the surface of the Ab/polydopamine/Au electrode to produce a packed film that showed electron transfer for the redox probe. A linear relationship between the electron-transfer resistance and logarithmic value of SRB concentrations was found for bacterial concentrations ranging from 1.8×10^2 to 1.8×10^6 CFU mL^{-1}, with a slope of 54.6 and a correlation coefficient of 0.984 (Fig. 4B). As shown in Fig. 4B, when the R_{ct} value of the antibody-immobilised bioinspired electrode was taken as the threshold of the signal, the detection limit of this impedimetric immunosensor was 50 CFU mL^{-1}, which is comparable with other label-free immunosensors for the detection of pathogenic bacteria using different techniques. When the antibody physically absorbed onto the surface of gold electrode is used as a control experiment, the signal change shows very low variation compared to data obtained from the electrode coated

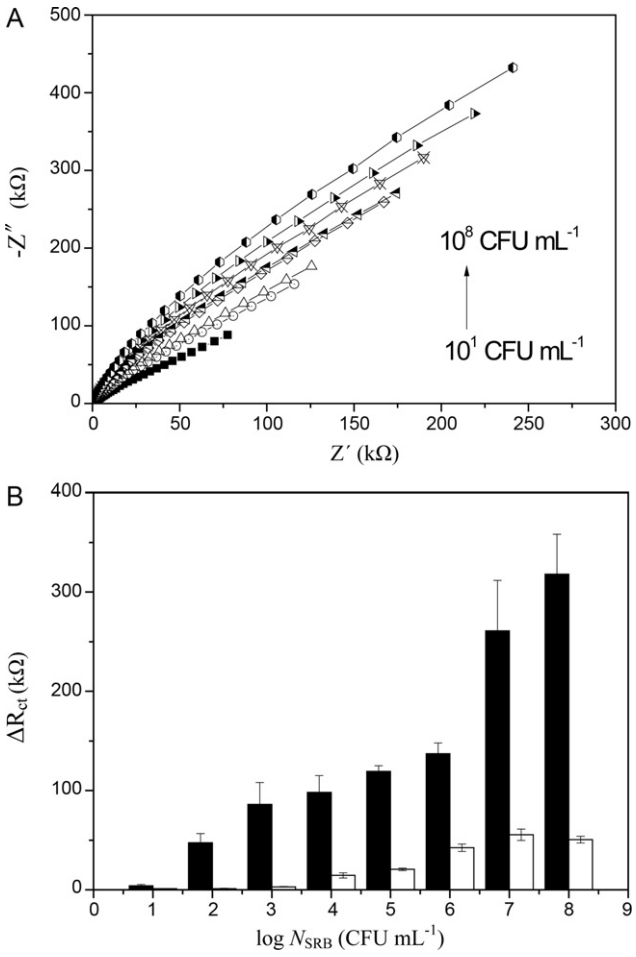

Fig. 4. (A) Nyquist plots and (B) calibration curve with antibody-immobilised gold electrode (■) with and (□) without bioinspired coating were incubated in the presence of SRB from 1.8×10 to 1.8×10^8 CFU mL^{-1}. The R_{ct} of the BSA/Ab/polydopamine/gold electrode was considered as the background. The impedance spectra were recorded within the range of 100 kHz to 100 mHz at the formal potential of $[Fe(CN)_6]^{3-/4-}$. The amplitude of the alternate voltage was 5 mV.

with the bioinspired architecture. The results further indicate that the bioinspired architecture is capable of directly immobilising the antibody for the specific recognition of targeted bacteria without a cross-linker reagent. When 1.8×10^8 CFU mL^{-1} SRB cells were bound onto the bioinspired architecture surface, the increase in R_{ct} value was 1068%, and the detection limit of EIS measurement was 50 CFU mL^{-1}. The range of linearity covering five orders of magnitude is comparable with other sensors based on various sensing platforms. In conclusion, the impedimetric immunosensor reported based on the mussel-inspired film in this work could have potential applications in diagnoses and environmental monitoring.

The fluorescence observations tend to confirm the quantification of bacteria bound onto the surface of bioinspired coating, as monitored by EIS measurements. The direct detection of microorganisms and their traces using fluorescence microscopes as a control experiment is one of the most promising techniques to obtain decisive evidence of bacteria cells. The most significant points of this technique are high sensitivity and spatial information with high resolution. Information on local environments and microscopic ecology can also be obtained. The images presented in Fig. 2S show a progressive increase in fluorescence intensity due to intact bacteria cells with increasing SRB concentrations after 60 min incubation. Serially diluted solutions of SRB were incubated with the antibody-functionalised bioinspired film platform, washed to remove unbound SRB, stained with FITC dyes, and imaged using fluorescence microscopy to estimate the number of bacteria. Results show that fluorescence-stained clusters of bacterial cells can be observed from as little as 10^2 CFU mL^{-1}. This is similar to the detection limit that was observed using the impedance method.

4. Conclusion

This work employs a self-polymerised polydopamine film as an immobilisation platform for the preparation of a sensitive immunosensor based on EIS in a marine environment. FT-IR, water contact measurement, and SEM images indicate that the interactions between the ortho-dihydroxy-phenyl functional group of the polydopaime substrate and the reactive amino groups of the anti-SRB antibody result in the formation of the functionalised immunosensor. The proposed sensor for SRB detection shows good fabrication reproducibility and detection precision. Applicability of the proposed method in environmental pollution and food sample safety analyses is under investigation. Using antibodies selective to other pathogenic bacteria, the immunosensor based on the self-polymerised polydopamine sensing platform holds great potential for the rapid detection of other pathogens. However, the specificity of the biosensor system requires further improvement.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 41076047), Chinese Academy of Sciences (Grant KZCX2-YW-205-03) and Shandong Provincial Natural Science Foundation, China (Grant No. ZR2010DM004).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.11.013.

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