Fuel cell sensor and biosensor based on Prussian blue nanotube membrane

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2016

Wei, Y. (2016). Fuel cell sensor and biosensor based on Prussian blue nanotube membrane.
Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/66035>

<https://doi.org/10.32657/10356/66035>

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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A thesis submitted to the Nanyang Technological University in fulfillment of
the requirement for the degree of Doctor of Philosophy

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Acknowledgements

I would like to express my most sincere gratitude to my supervisor associate professor Li Tianhu and my former supervisor Dr. Toh Chee Seng as I would not have chance to prepare this thesis if there were not them. Firstly, I offer my sincere thanks to Prof. Li for his generous help and selfless support to me for both my research work and daily life in the last two years. In addition, I also would like to offer my sincere appreciation to Dr. Toh Chee Seng for his wonderful teaching, patient guidance and continuous encouragement to me over the first two years of my Ph.D study. Their rigorous attitudes towards research, profound knowledge and great patience have brought positive effects both for my research work and daily life. Thank you, Prof. Li and Dr. Toh. Your kindness would be remembered forever.

I would like to express my sincere thanks to all members in Prof. Li group. I would like to thank Lei Qiong for her patient listening and giving advice to me in life. I would to thank Jasmine Lee, Ba Sai and Zhang Hao for their generous help to me when I join in this group. I would also like to thank lab-mates in Dr. Toh's group Chen Ming Soon, Ho Jia Shin and Varun Rai for their generous help. Especially, I would like to show my sincere thanks to Wong Lai Peng for her selfless help and patient guidance for my research work. I would like to thank my dear friend Deng Jiajia as she always supports me, believe me and help me both in my life and research work.

I also want to take this opportunity to thank Assoc. Prof. Zhao Yanli and Assoc. Prof. Chiba as they encouraged me to apply for Ph.D and gave me lots of support when I applied for my Ph.D. I would also like to express my thanks to Ms. Chen Yuchen for her generous and selfless help in preparation of this thesis.

I would like to thank my father, when I was pregnant he always did some delicious food for me. I would like to show my sincere appreciation to my mother, as she always took care of me and my daughter without any complaining. I would like to thank my parents in law for their selfless help me to take care of my daughter, then I can pay attention to my research work.

I would like to express my utmost gratitude to my husband. Just his selfless love, continuous encouragement and support are drawn me unceasing power and energy. I would like to thank my lovely girl, her smile and coquetry to me is my forever motive.

I want to thank NTU for giving me such a chance and scholarship to pursue my Ph.D. I want to thank CBC staffs, especially Lynette, Yean Chin and the technique staffs, for their support and help throughout my Ph.D study. I would thank all of you guys who ever helped me.

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Abstract

Due to the chemical stability and high electrocatalytical activity, Prussian blue (PB) has been explored in numerous applications including electronic devices, batteries and electrochemical sensors and biosensors. In the past two decades, applications of biofuel cells in electrochemical sensors and biosensors have aroused great interest among researchers. However, owing to high cost with employing precious metal catalysts and complicated cell designs, little attention has been paid to the fuel cell-based electrochemical sensors. In this thesis, we would present a system of fuel cell sensor and biosensor with simple two-compartment design based on a Prussian blue nanotubes membrane cathodic electrode.

In Chapter 1, we proposed a unique self-powered amperometric sensor based on PB nanotubes membrane, in which the current signal has been derived by the chemical energy of the hydrogen peroxide (H_2O_2) analyte. In the sensing process, PB was reduced to Everitt's salt (ES) by the electron flow from the reaction of water oxidation at counter electrode and the ES was itself oxidized by reduction of H_2O_2 in the sensing cell. Therefore, a steady-state of PB and ES in concentration could be maintained by the reaction of hydrogen peroxide reduction and the galvanic cell reaction at auxiliary electrode. This strategy could be further demonstrated in a model glucose biosensor with coupling glucose oxidase. The simple design initiates the studies on development of self-powered amperometric sensors by selecting anodic and cathodic reactions with suitable thermodynamic potentials.

In the following chapter, we described a H_2O_2 powered virus sensor for direct detection of unlabelled virus particles. The sensor has been fabricated based on the dual-compartment fuel cell and the formation of antibody-virus complexes within the sensor's membrane nanochannels. Employing a PB nanotubes membrane cathode and a platinum

mesh anode, this fuel cell virus sensor exploited the change in the membrane resistance of the powered system and offered an impressive short response time of ~5 min toward the specific virus target, at low concentration values of 3 to 45 pfu mL⁻¹. As low as 0.04 pfu mL⁻¹ of detection limit was achieved, which was comparable to state-of-the-art PCR based methods. The sensor incorporated specific virus antibody afforded a high selectivity for serotypes of virus. An integrated PB-*nt* membrane filled with Nafion[®] perfluorinated resin demonstrated powerful utilization as a standalone fuel cell based virus sensor, which offered promising potential to develop a sustainable, low cost and rapid low power virus detection tool.

In Chapter 4, we demonstrated a hand-held H₂O₂ fuel cell sensor based on Prussian blue nanotubes membrane. This H₂O₂ fuel cell sensor was constructed using four standalone nafion-filled PB-*nt* membranes connected in parallel, which employed the PB-*nt* membrane as both electrodes and fuel reservoir. The PB-*nt* membrane side was applied as cathode for reduction of H₂O₂ and reservoir cell, and the opposite of the PB- *nt* membrane coated by platinum was served as anode for oxidation of water to oxygen. The two-electrode system was adopted for the performance tests. An open-circuit potential (OCP) of 0.54 V with a maximum power density of 0.7 mW cm⁻² has been achieved at a low concentration of 10 mM H₂O₂. The analytical performance of the fuel cell sensor was monitored using a micro-current meter to record the responding signal towards varying H₂O₂ concentrations. An excellent linear relationship has been established between the responding current signal and low concentrations of H₂O₂.

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Chapter 1

General Introduction

Chapter 1 General Introduction

1.1 Prussian blue

1.1.1 Introduction of Prussian blue

Prussian blue (PB), or ferric hexacyanoferrate ($\text{Fe}_4(\text{Fe}(\text{CN})_6)_3$), has been considered as the oldest coordination compound with lots of interesting chemical and physical properties which have been actively investigated and applied into a number of functional devices. PB was accidentally discovered by a Berlin artist named Diesbach three centuries ago, which opens the remarkable transition metal cyanide chemistry.

Before identifying a satisfactory explanation of PB upon its chemical properties, it has been always employed as an important pigment for centuries. The structure of PB was firstly reported by Keggin and Miles in 1936, which involved the basis of powder diffraction patterns [1]. Half a century later, a more precise structure was further elucidated by Ludi and co-workers from the single crystal of PB by the electron and neutron diffraction measurements [2]. The structure of PB has been presented as a basic cubic structure with dimensions of 10.2 Å, in which the alternating ferric and ferrous ions are located on the face centred cubic lattice sites and are bridged by the cyano ligands as shown in figure 1.1 [3]. In this way, the ferric ions coordinate with the nitrogen atoms of the cyanide ions while the ferrous ions are surrounded by the carbon terminals of cyanides ions, which results in a low-spin and high-spin electron configuration, respectively. Interestingly, the basic cubic unit cell of PB has some defects, interstitial sites and vacancies which might introduce countercations or some other molecular species to intercalate [3, 4]. As a result, PB is considered to be a mixed-valence coordination compound with an open, zeolitic structure.

Figure 1.1 Diagrammatic drawing of the cubic unit cell of PB. Reproduced with the permission from American Chemical Society [3], copyright 1986 American Chemical Society, with modification.

PB itself is a definitely insoluble inorganic polycrystal. The term of PB with intercalation of K^+ was known as a “soluble” PB invented by dye makers and presented as $\text{KFeFe}(\text{CN})_6$ [3]. In fact, the so-called “soluble” PB is highly insoluble, and the term of “soluble” just refers to the ease with which the potassium can be peptized [5]. For the “soluble” PB, the cubic structure was presumed with the potassium ions occupying the octahedral interstitial lattice sites within PB [3].

1.1.2 Electrochemical properties of PB

PB in terms of both solubility and insolubility is chemically stable, but exhibits an active property in electrochemistry. In 1978, Neff firstly reported the preparation of thin film of PB onto a Platinum foil through the method of electrochemical deposition and presented its’ electrochemical behaviour by the cyclic voltammetry [6]. The cyclic

voltammogram of the PB film electrode has distinctly demonstrated the reversible redox reactions between PB and its redox forms [6]. Since then, a new field in the fundamental investigation of its electrochemical properties has been expanded [7–9], and numerous applications of this unique inorganic polycrystal have been developed. [10–12].

The electrochemical properties of PB are attributed to the redox active iron centres. It was found that two kinds of electron transfer channels are available in the Prussian blue due to redox reactions of the high spin $\text{Fe}^{2+/3+}$ ions and low spin $\text{Fe}(\text{CN})_6^{3-/4-}$ ions [8, 13]. Therefore, two sets of redox peaks are observed in the typical cyclic voltammogram of a PB film as shown in Figure 1.2. The redox peak around +0.2 V (vs Ag/AgCl) is demonstrated in terms of reduction of PB to Prussian white (PW) or Everistt's salt (ES), which refers to the reduction/oxidation of high spin $\text{Fe}^{2+/3+}$ ions. The set of redox peaks at more positive potentials (around +0.9 V vs Ag/AgCl) is explained in term of oxidation of PB to Berlin green (BG), which correspond to the low spin $\text{Fe}(\text{CN})_6^{3-/4-}$ ions. The reduction and oxidation for soluble form of PB are formulated as follows:

The redox reactions for insoluble form of PB are similar to ones for the soluble form of PB and have been proposed:

Figure 1.2 Voltammogram of PB film on a gold wire electrode in 1N K₂SO₄ solution, sweep rate $\nu = 50\text{mV} / \text{S}$. Reproduced with the permission from American Chemical Society [5], copyright 1986 American Chemical Society, with modification.

For the convenience of subsequent discussion, the term of PB would refer to soluble PB, and the redox reaction of PB system would point to the ES-PB redox system.

1.1.3 Preparation of PB electrode

Owing to its stable chemical properties, easy preparation, electrocatalytic activity and low cost, PB electrodes have drawn much attention. In general, the PB electrode is prepared through electrodeposition of PB on a conductive substrate. The deposition is usually performed in aqueous solution containing a mixture of ferric and ferricyanide ions, with either applying an electrical potential as the reductive driving force or spontaneously

in an open-circuit regime [12]. The amount of PB loading on the substrates can be controlled by changing the electrode potential, current density and time of the electrolysis.

1.1.4 Electrocatalytic activity for reduction and oxidation of hydrogen peroxide

PB and its redox forms have been found to exhibit an excellent electrocatalytic activity towards reduction and oxidation of hydrogen peroxide due to its zeolitic nature and the channel diameters of about 0.32 μm which support the small molecules of H_2O_2 enough space for movement and diffusion through the crystal lattice of PB [2, 8, 10].

1.1.5 Electrochemical applications of Prussian blue

Owing to its chemical stability, easy preparation and low cost, PB has been successfully applied into varieties of electrochemical devices, electrocatalysis, batteries, ion exchange, analytical sensors and biosensors. Especially, the application of PB-based electrochemical sensors and biosensors has achieved a significant development in the last decades.

1.1.5.1 PB-based H_2O_2 sensors

Since Kingo Itaya's seminal work about the electrocatalytic reduction and oxidation of H_2O_2 by the redox forms of PB [10], various PB-based electrochemical sensors have been developed for detection of H_2O_2 [14–16].

The electrochemical sensor is an important research field of analytical chemistry, which has been designed to transform electrochemical information into a detectable signal by oxidizing or reducing the target at the working electrode. Electrochemical sensor usually consists of a chemical recognition system and physiochemical transducer [17]. The Chemical recognition system has always been employed to produce chemical

response which would be converted into an electrical signal through the physiochemical transducer. The converted electric signal was finally detected by modern electrochemical instrument. The target detection could be achieved from the detected electric signals which contain the chemical information of the analyte.

As PB modified electrode can be used to detect H_2O_2 by both electrocatalytic reduction and oxidation, it can work in both cathodic and anodic manner as follows:

In the case of electrooxidation of H_2O_2 , a high potential of at least 0.6 V must be applied to initiate the oxidation. However, at this potential, a variety of biological compounds existing in the real biological sample, such as ascorbate, urate, bilirubin, acetaminophen, catecholoamines, and some thio-compounds can also be oxidized, which would affect the sensors' selectivity and sensitivity. Besides the interfering species, the large produced parasitic anodic current makes quantitative determination of H_2O_2 impossible. In contrast, the electrocatalytic cathodic activity of PB electrode exhibits a similar activity with noble metal electrode (exp. Pt electrode) toward reduction of H_2O_2 [18], whereas it shows insensitive toward molecular oxygen [19]. Therefore, considering the practical perspectives and applications, the determinations based on electroreduction of H_2O_2 in cathodic manner are much more useful. In this dissertation, we would talk about some work on the electroreduction of H_2O_2 at the PB modified membrane electrode.

1.1.5.2 PB-based biosensors

PB is also widely employed in electrochemical biosensors [20–23]. Electrochemical biosensor is actually a sub-set of electrochemical sensor which employs a biochemical element with transducers in recognition system to convert the biological information into an effective electrical signal for analysis [17].

The PB-based biosensors can be considered as the sub-sensors of the PB-based H_2O_2 sensors. Most enzymatic biosensors, which are based on the detection of oxidases, could change to detect the enzymatically generated H_2O_2 . Owing to the interference of some reducible species in real samples in its anodic measurements, PB-based biosensors with cathodic measurements have a big potential and possibility.

According to the reported PB-based enzymatic biosensors, the mechanism of oxidase enzyme modified PB electrode is shown in the following scheme (Scheme 1.1).

Scheme 1.1 Scheme of biosensor based on oxidase modified PB electrode.

1.1.5.3 PB-based fuel cells

In recent years, PB has been explored the application in the fuel cell systems due to its efficient catalytic activity for hydrogen peroxide. Therefore, as a catalyst and cathode

material for reduction of H_2O_2 , PB has presented a promising potential in H_2O_2 fuel cells. This part will be expounded in the following part.

1.1.5.4 Other applications based on PB modified electrode

Besides the H_2O_2 sensors, biosensors and fuel cells, PB has been successfully deployed in numerous applications as summarized in Table 1.1.

Table 1.1 Applications of PB modified electrode

Applications of PB
Ion exchange
Ion-selective sensors
Batteries
Electrochromic devices
Molecular-based magnets

1.2 Fuel cell and biofuel cell

Driven by the exhaustion of fossil fuels and the increasing energy demands, fuel/biofuel cells have been invented to utilize renewable fuel sources to produce electricity and displace the depleting fossil fuels.

1.2.1 Introduction of fuel cell

Fuel cell is an electrochemical device which can continuously convert chemical energy to electrical energy through chemical reactions in the cells [24]. The concept of fuel cell has been proposed over 200 years ago, and the first successful example of conversion of chemical energy to electricity in fuel cell was described 170 years ago. Despite this long interest, it was not until 1950s that the rapid development of fuel cell technology was made.

Typically, the fuel cell is constructed by three basic components: ion conducting electrolyte, an anode and a cathode [24–26]. The electrolyte provides a physical barrier to separate the fuels and the oxidants, but permits the ionic charges to transform between the anode and cathode. The electrodes of anode and cathode support place for chemical reactions to convert the chemical energy to the electrical energy. In addition, an electro-catalyst, which is used to speed up the chemical reaction at the electrode, is an essential part in the fuel cells. Generally, the active electro-catalysts are directly merged into the electrodes and therefore are not specified as an independent component. The schematic representation of fuel cell is shown in scheme 1.2.

Scheme 1.2 Schematic diagram of fuel cell

Comparing with conventional energy conversion devices, such as heat engines and batteries, fuel cells exhibit various advantages [27–31]. Firstly, fuel cells show higher efficiency in energy conversion and lower oxidation temperature compared to the thermal engines, thus provide zero or near zero carbon dioxide and nitrogen dioxide emissions. Compared to batteries, fuel cells have an unparalleled advantage that fuel cells can continuously provide electricity because the fuel cell reactions do not degrade over time. However, batteries require a continuous source of fuels to sustain the chemical reactions, otherwise they must be disposed once the chemical fuels are used up, which give rise to the problem of cost and pollution. In addition, fuel cells are not restrained by the size and can achieve high efficiency at any scale, therefore they can be located at almost any place, including factories, office towers and residences where air quality and transmission congestion may be of concern.

Due to these environmental benefits of high efficiencies, low emissions and constant power production, the fuel cell technology will be inevitable as a vital energy production technology in the future. What's more, from the aspect of economic, the technology of fuel cell represents a frugal approach to provide electricity because of its flexible fuels and quick installation.

1.2.2 Applications of fuel cells

With the fossil energy depleting, the technologies of fuel cells provide a promising alternative to the conventional technology of energy production. Due to the various advantages of clean, efficient and reliable energy generation, fuel cell technologies are now used in three broad areas including power for transportable devices, stationary power generation and portable power generation [26, 28–31].

1.2.2.1 Power for transportation

Due to the high efficiency of energy conversion and low emission, many fuel cell researchers in industry are inspired to develop the fuel cell-powered vehicles in replacement of traditional transports. The first fuel cell vehicle of a modified farm tractor fitted with a 15 Kilowatt fuel cell was presented around 1959. With the rapid and full development of the fuel cell technologies, currently, the fuel cell vehicles including automobiles, buses, motorcycles and bicycles have been already operated in service all around the world. Moreover, the fuel cell auxiliary power units have been ready sold as an optional accessory to power electronics in “caravans”, or campers, while parked in remote locations or campsites.

1.2.2.2 Stationary power generation

On account of quiet energy generation and low emissions, the stationary applications of fuel cell technologies, involving generation of both electrical power and heat can range from residential buildings, commercial sectors to industrial facilities. These applications include emergency back-up power supply, remote-area power supply, and distributed power or combined heat and power generation.

1.2.2.3 Portable power generation

Besides the large-scale power generation, the fuel cells with small size have been developed to replace batteries which aim to power the minimal electronic products, such as mobile phone, laptops, video cameras and portable computers. The minute fuel cells can produce a viable alternative power for biological applications as well. Besides application for hearing aids power supply, the low-powered fuel cell can be applied for long term pacemakers. It is noteworthy that several low-powered fuel cells are currently being manufactured for these applications. The most successful example of the application of the portable fuel cell is the Breath Alcohol Measurement.

1.2.3 Biofuel cell and applications

Biofuel cells, which are the abbreviation of biological fuel cells, are a device similar with the conventional fuel cells, except the biological catalysts instead of precious metal catalysts in the cells [34]. The research of biofuel cells is driven by the aspiration for fabrication of minute fuel cell devices which can be implanted within the body. According to the biocatalysts, biofuel cells can be classified in two main categories: microbial biofuel cells and enzymatic biofuel cells. The microbial biofuel cell is defined as a bio-electrochemical device that uses bacteria to convert the organic substrate into

electrical energy [33]. An enzymatic biofuel cell is a device that employs enzyme as catalyst for oxidation and reduction of specific fuels and oxidizer substrates at anodes and cathodes to generate electricity [34].

Along with the recent development of biofuel cells, there are several potential applications receiving more attention to be developed.

1.2.3.1 Implantable power sources

Enzymatic biofuel cells have been developed rapidly over the past few decades, especially in respect to the implanted medical devices. Previously, the implanted medical devices are commonly powered by the conventional battery systems which have an inherent drawback of short working life time. In contrast, the enzymatic biofuel cells employ physiological species as catalyst, and can directly acquire fuels and oxygen from their working environments, thus can be potentially operate in the living systems independently over prolonged periods of time without the need for external recharging or refueling [32, 34, 35].

1.2.3.2 Wastewater treatment

There is an urgent need for the sustainable treatment and utilization of wastewater due to the limitation of freshwater resources and environmental pollution. The biofuel cell technology, which has been shown to have the ability of recovery of energy from the wastewater, can provide the solution for the problems caused by most traditional wastewater treatment processes, such as energy consumption as well as the environmental pollution, to reduce the energy input and the unnecessary sludge production by removal of the organic compounds from the waste stream and generation of electrical power from wastewater [36, 37].

1.2.3.3 Self-powered sensors and biosensors based on biofuel cells

The concept of self-powered sensors was firstly introduced for the nuclear sensors. With the development of interdiscipline, the term of self-powered sensors has been expanded to biosensing system based on the electrochemical energy conversion. The first use of biological fuel cell for self-powered sensing was reported in 2001 by Katz et al. [38]. They detailed two different self-powered biosensors (as shown in figure 1.3) based on the enzymatic biofuel cells in their first seminal paper. One of the self-powered biosensors was a glucose biosensor employing glucose oxidase at the bioanode of biofuel cell and cytochrome c oxidase at the biocathode of biofuel cell, as shown in figure 1.3. This sensing system employs the analyte of glucose as fuel and there is no voltage output in the absence of glucose, but a voltage signal can be observed in the presence of glucose. The second self-powered biosensor developed by Katz and Willner was designed based on an enzymatic biofuel cell with lactate dehydrogenase at the bioanode and cytochrome c oxidase at the biocathode, as shown in figure 1.3. This system behaves similarly to the self-powered glucose biosensor, but employs a different substrate. Although these two biofuel cell sensors produce relatively low open circuit voltages (below 200 mV) and power densities (below 0.025 mW/cm^2), this research opens up the study of self-powered biosensors based on the biofuel cells design. Additionally, the self-powered biosensors do not require an external applied potential to drive the electrochemical signals, which will be significant for simplification of the sensor devices.

Figure 1.3 Configuration of the first self-powered biosensor based on biofuel cell. (A) PQQ-FAD/GOx-functionalized anode utilizing analyte of glucose as a fuel or (B) PQQ-NAD⁺/LDH-functionalized anode utilizing analyte of lactate as a fuel, and (C) Cyt *c*/COX-functionalized cathode utilizing O₂ as an oxidizer in combination with both anode A or B. Reproduced with the permission of the American Chemical Society.

Due to the simple design and reducing energy usage, fabrication of self-powered sensors and biosensors using the alternative chemical energy in biofuel cells has attracted great interest of researchers. In 2010, Dong's group introduced a new strategy of enzymatic inhibition effect in the self-powered sensors based on biofuel cells. In their report, they described a glucose/oxygen biofuel cell sensor employing a glucose dehydrogenase bioanode and a laccase biocathode for detection of cyanide through the cyanide inhibitive effect on the laccase immobilized biocathode, as shown in figure 1.4

[39]. A sample solution containing a constant glucose fuel and oxygen was injected into the biofuel cell sensor and this can produce an open circuit potential as well as current and power in the absence of cyanide. However, the current density and power density decrease in the presence of increasing concentration of cyanide. Based on this interesting study, in the following years, a series of works in similar direction have been reported including analytes such as Hg^{2+} [40] and acetaldehyde [41]. Different from Dong's self-powered sensor with the inhibition effect, another strategy of enzymatic reactivation effect in the self-powered sensors based on biofuel cells was introduced by Minter's group [42]. These new and interesting developments of the self-powered sensors exceed the limitation of sensing analytes which are only reduced or oxidized by the biological catalysts and expand the scope of detection analytes.

Figure 1.4 The mechanism of the enzymatic biofuel cell sensor for cyanide detection. The enzymatic biofuel cell sensor employs a bioanode for oxidation of glucose and a biocathode for reduction of oxygen. The cathodic enzyme laccase is inhibited and no longer catalyzes reduction of oxygen to water in the presence of cyanide. (A) Polarization power curves of the uninhibited control and regeneration with 30 min fresh PBS washing of the glucose/ O_2 biofuel cell. (B) Polarization power curve of the glucose/ O_2 biofuel cell sensor in the presence of 1 mM cyanide. The electrolytes solution contains 10 mM NADH, 40 mM glucose, and 0.2 M PBS (pH 6.5). Reproduced with the permission of the American Chemical Society.

1.3 PB-based Fuel cells

Albeit the high efficiency and low emission of the common fuel cells, these advantages have been partially offset by the high cost of catalysts and high operation temperature. Therefore, various types of nonprecious catalysts have been investigated to replace the noble metals to reduce the costs. Especially in recent years, the iron complex

as the catalyst and cathode materials in the fuel cell design has attracted researcher's attention. PB, as the classic iron cyanide polymer, has demonstrated great potential in the H₂O₂ fuel cells due to its' well-known catalytic activity for reduction of H₂O₂.

1.3.1 Hydrogen peroxide fuel cell

H₂O₂, as an environmental-friendly energy carrier, has a high energy density. It can act as the dual roles of fuel and oxidant in the fuel cell and is fairly nontoxic because it is either reduced to water or oxidized to water and oxygen in the fuel cells [43, 44]. Besides the handling in aqueous phase, H₂O₂ has a benefit for easy transport. Additionally, H₂O₂ has a high energy density which offers the potential for various applications. H₂O₂ fuel cells thus have received intensive attention.

Traditionally, H₂O₂ fuel cells, in which H₂O₂ represents as fuel and electron acceptor, need to be operated under the acidic and basic conditions, respectively [45, 46]. Therefore, two compartments are strictly required to provide an acidic medium for the anodic reaction and an alkali medium for the cathodic reactions. The anodic and cathodic reactions are given by the following equations:

A high theoretical driving force of 1.09 V can be achieved in such fuel cells. However, the demanding and complicated requirements for the two-compartment fuel cell design cause a major shortcoming of expensive cost in the construction of fuel cell design.

To keep with the requirement of equipment towards miniaturization and simplification, fabrication of single-compartment fuel cell has become a popular topic. Due to lack of specific electrocatalysts, the research about one-compartment fuel cell has been restricted. Additionally, oxygen, as the most commonly used electron acceptor in the fuel cells, brings some difficulties of dissolution and diffusion in one compartment, which restrained the studies on the one-compartment fuel cells. With the inspiration from one-compartment biofuel cell design, Yamazaki and co-authors firstly fabricated a one-compartment fuel cell in 2008, in which they made use of H_2O_2 as both a fuel and an electron acceptor. This one-compartment H_2O_2 fuel cell directly operated in alkaline solution and obtained a maximum OCP of 0.13 V using silver wire as cathode and Pt, Pd, Ni and Au wires as anode [44]. In contrast, a few years later, Yamada *et al* [47] reported a single-compartment H_2O_2 fuel cell running in acidic solution. An OCP of 0.5 V with a maximum power density of $10 \mu\text{W cm}^{-2}$ at 0.3 M H_2O_2 has been obtained when using $\text{Fe}^{\text{III}}(\text{Pc})\text{Cl}$ as cathode and nickel mesh as anode. Subsequently, an acidic H_2O_2 fuel cell working in one compartment and using PB as cathode was reported by Seyed [48], which gave an OCP of 0.6 V and a maximum power density of 1.55 Mw cm^{-2} at 0.5 M H_2O_2 .

1.3.2 PB as cathode materials in fuel cells

PB has exhibited various electrochemical applications in ion selective electrodes, charge storage devices and electrocatalysis. Recently, PB has been explored to be applied in fuel cell systems.

The most commonly used cathodic catalyst for reduction of oxygen material in a fuel cell was Pt electrode, which has been shown to be active for reduction of H_2O_2 . However, besides the problem of its high cost, Pt catalysts would facilitate the undesirable H_2O_2

chemical decomposition. Therefore, it is expected to find an efficient catalyst to decrease the excessive cost and simultaneously minimise the problem of H_2O_2 decomposition.

It is precisely due to the high electrocatalytic activity, easy preparation and low cost, the PB electrodes have shown great potential as cathodic materials in the peroxide fuel cells. In addition, comparing with the Pt electrodes, PB electrodes have a faster kinetics and lower overpotential for the H_2O_2 reduction reaction. The first demonstration on utilization of PB modified electrode as cathode catalyst in a direct borohydride-hydrogen peroxide fuel cell (DBPFC) has been reported in 2008 by Selvarani et al. [49]. Later, Santos has reported to employ a PB-modified Pt electrode as cathode catalyst for reduction of H_2O_2 in a DBP fuel cell in 2012 [50]. Although the above reports have shown a good indication featuring PB as cathode catalyst for reduction of H_2O_2 in fuel cell systems, it had not attracted people's interests and attention. To date, there are only a few reports employing PB as the cathode catalyst in fuel cell systems [48–51].

Therefore, the high cell performance with using PB as cathode makes the H_2O_2 fuel cell with PB employing as cathode appealing for further development.

1.4 Motivations and objectives

The lack of research on fuel cell sensing systems employing PB as cathode has prompted us to proceed with the projects. The main objectives of this thesis are to develop the novel fuel cell sensors and biosensors based on PB nanotubes membrane electrode.

Inspired from development of self-powered sensor device based on using the chemical energy in biofuel cells, we described a self-powered amperometric sensor for detection of H_2O_2 in the first project. The main focus was to introduce the self-powered

amperometric sensor without application of external electrical potential. The sensor was fabricated based on a dual-compartment galvanic cell which would derive the current signal from the chemical energy of the H_2O_2 analyte. In the sensing process, Prussian blue was reduced to Everitt's salt by the electron flow from the counter reaction, and the Everitt's salt was itself oxidized by reduction of H_2O_2 . Therefore, a steady-state of Prussian blue and Everitt's salt forms of the Prussian blue in concentration could be maintained by hydrogen peroxide reduction at the Prussian blue nanotubes membrane electrode and the galvanic cell reaction at auxiliary electrode.

In the second project, we have reported a H_2O_2 powered virus sensor for direct detection of unlabelled virus particles. The sensor was fabricated based on the dual-compartment fuel cell and the formation of antibody-virus complexes within the sensor's membrane nanochannels. With employing PB nanotubes membrane as cathode and a platinum mesh as anode, the virus sensor exploited the change in the membrane resistance of the powered system. A integrate PB-*nt* membrane filled with Nafion[®] perfluorinated resin demonstrated powerful utilization as a standalone fuel cell based virus sensor, which offered promising potential to develop a sustainable, low cost and rapid low-power tool for detection of virus.

In the final project, we demonstrated a hand-held H_2O_2 fuel cell based on Prussian blue nanotubes membrane (PB-*nt* membrane) as power source for a miniaturized amperometric sensor. This H_2O_2 fuel cell sensor was constructed using four standalone nafion-filled PB-*nt* membranes connected in parallel, which employed the PB-*nt* membrane as both electrodes and fuel reservoir. A micro-current meter was applied to record the response signals of the fuel cell towards varying concentrations of H_2O_2 . This

low power fuel cell sensing design offers a real sense for realization of the low cost and portable sensor devices.

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Chapter 2

Self-powered Amperometric Sensor and Biosensor

Based on Prussian Blue Nanotubes Membrane

Chapter 2 Self-Powered Amperometric Sensor and Biosensor Based on Prussian Blue Nanotubes Membrane

2.1 Introduction

The traditional electrochemical amperometric sensors and biosensors, which aimed to detect analytes by monitoring the flow of electrons through the sensors and biosensors, are used to require an electrical potential. In this way, an equivalent power magnitude of $I \cdot V$ was provided by the applied potential to drive the redox reactions between analytes and electrodes, mediators or electrocatalytic species including enzymes. Following Katz and Willner's first report on a self-powered biosensor based on a biofuel cell design [1], plenty of examples about utilization of this alternative chemical energy to replace the external applied electrical energy in the electrochemical sensors and biosensors have been developed, employing analytes such as glucose [2], cyanide [3], Hg^+ [4] and EDTA [5]. In this context, all of these reported self-powered sensors and biosensors are used to detect the analytes by measuring the open-circuit voltage (OCV) in response to the analytes concentration. Conversely, the reports about self-powered amperometric methods are very rare, especially for the methanol detection in fuel cells [6, 7].

In this chapter, we would describe electrical-potential-free methodology, employing a unique two-compartment cell, which derived the current signals from the chemical energy of analytes. Upon application of a porous alumina membrane, the sensing solution compartment has been separated from reference solution compartment as shown in Scheme 2.1. In this case, the alumina membrane coated with a ~50 nm platinum layer has been utilized as working electrode, which contacted directly with the sensing solution, meanwhile the reference and auxiliary electrodes have been put into the reference solution. In such way, the electrical current flow between the working and auxiliary

electrode was provided by addition of analytes in sensing solution to react with PB at the membrane electrode together with the reaction react at counter electrode inside the reference solution. This strategy is different from the common analyte detections using nanoporous membranes [8–10] in one or two-compartment cells to drive the sensing reactions by the external applied electrical potential [11–15].

Hydrogen peroxide (H_2O_2) was chosen as the analyte because of its significant waste in ground water, cell metabolism, signal transduction, as stress indicators of living cells, as clinical markers for diseases [16–21]. In addition, H_2O_2 is one of the products of plenty of oxidases enzymes, thus it is widely used in enzyme based biosensors [22]. Therefore, it is desirable to develop an accurate, specific, rapid and low cost detection method. PB, as a well-established electrocatalyst for reduction of H_2O_2 , can be applied in nanoporous platinum membranes to form nanotubes structures by using potential cycling method, thus PB modified electrodes have been frequently utilized in the electrochemical sensors and biosensors [23–25]. Herein, an amperometric sensor employing hydrogen peroxide as analyte, which was constructed from Prussian blue nanotubes coupled to a Galvanic cell giving the sensing signal derived from the galvanic current flow. A cell reaction with a theoretical driving force of 0.55 V would be afforded by combination of two half-cell reactions (reduction of H_2O_2 and oxidative of water) occurred in working electrode and counter electrode.

Scheme 2.1 Construction of the porous PB-*nt* membrane electrode by (A) sputter coating an 50 nm thick platinum layer on one side of a 60 μm thick nanoporous alumina membrane with 20 nm nominal pore size, followed by (B) electrochemical deposition of PB onto the porous Pt membrane electrode. (C) The optimized sensor design comprises the nanoporous PB-*nt* membrane which separates two solutions of a 2-compartment sensor cell. Analyte H₂O₂ oxidizes PB-*nt* in the sensing solution, followed by Galvanic current flow between the porous PB-*nt* membrane electrode in the sensing solution and auxiliary electrode in the reference solution, which gives the sensing signal.

2.2 Materials and methods

2.2.1 Chemicals

Nanoporous alumina membranes with diameter of 13 mm and pore size of 20 nm (AnodiscTM) were purchased from Whatman (Maidstone, Kent, U.K.). Hydrogen chloride with concentration of 37% was bought from P. P. Chemicals. 30% H₂O₂ and KCl were purchased from Scharlau. The potassium hexacyanoferrate(III), D(+)-glucose anhydrous and Nafion[®] perfluorinated resin solution were obtained from Sigma-Aldrich. The anhydrous ferric chloride was required from Merck, and tris(hydroxymethyl)-aminomethane from Bio-Rad Laboratories. Glucose oxidase enzyme (GOx) from *Aspergillusniger* (EC 1.1.3.4, ~200 units/mg), was obtained as lyophilized powder from Sigma and stored at -20 °C. Ultrapure water purified with the Sartorius Ultrapure Water System was used to prepare the solution.

2.2.2 Instrumentation

An auto Fine Coater (JEOL JFC-1600) has been used to sputter coating platinum layer onto the mesoporous alumina membrane. Electrochemical workstation of CHI7750D has been used to perform the cyclic voltammetric experiments. A CHI1220B potentiostat has been used for measuring the open-circuit potential. The e-corder 401 (eDAQ) and a potentiostat (eDAQ EA161) have been connected to do measurements of close-circuit current. A two-compartment cell as shown in Scheme 2.1 and the three-electrode system by using porous PB-nanotubes (PB-*nt*) membrane electrode as the working electrode, a Ag/AgCl (1 M KCl) as the reference electrode and a platinum mesh as the counter electrode has been applied for performing all of measurements.

2.2.3 Sensor fabrication

In order to enhance the electrochemical activity of the porous Pt-coated membrane towards hydrogen peroxide, the nanoporous platinum membrane was coated with PB by using potential cycling method which forms nanotubes structures embedded within the membrane [26]. A thin layer of platinum was firstly sputtering coated onto one side of alumina membrane (AAO) with pore size of 20 nm. Then the conductive membrane was subsequently performed potential cycling from -0.5 to +0.6 V at 50 mV s⁻¹ for 15 cycles in a mixture of 5.0 mM K₃Fe^{III}(CN)₆, 5.0 mM FeCl₃, 0.1 M KCl and 0.01 M HCl for 2 times. After finish the potential sweep, a blue coloured membrane has been obtained, which indicated the formation of PB-nanotubes onto the membrane. After rinsing with ultrapure water, the porous PB-*nt* membrane was left to dry overnight at room temperature.

2.2.4 OCV and closed-circuit current measurements

The voltage difference between the PB-*nt* membrane working electrode and the platinum mesh counter electrode under open circuit condition has been measured over time using a CHI1220B potentiostat with the OCP measurement function. The amperometric current between the PB-*nt* membrane working and platinum mesh counter electrodes under the closed circuit has been measured over time using a potentiostat mode (eDAQ EA161) connected to a 4-channel data acquisition unit (eDAQ e-corder 401), as shown in Scheme 2.1. All amperometric signals were collected using 1 Hz low-pass filter to remove significant background noise.

2.2.5 Determination of H₂O₂ and glucose

The stock hydrogen peroxide solutions were prepared from H₂O₂ with 30% wt in H₂O. PB-*nt* membrane as working electrode has directly faced to the sensing solution, while both reference and counter electrodes have been placed into the reference solution. The stocked glucose solution in 1 M Tris has been added successively into a 0.1 M KCl in 1 M Tris solution containing 4.0 mg mL⁻¹ glucose oxidase for glucose sensing. During the amperometric detection of H₂O₂ and glucose, no external electrical potential has been applied.

2.3 Results and discussion

2.3.1 Preparation of PB-*nt* membrane electrode

The typical cyclic voltammograms as shown in Figure 2.1A demonstrates the voltammetric deposition of PB onto Pt-coated alumina membrane in a solution containing 5.0 mM K₃Fe(CN)₆, 5.0 mM FeCl₃, 0.1 M KCl and 0.01 M HCl. The first cycle describes the oxidation and reduction peaks of the Fe(CN)₆⁴⁻ and Fe(CN)₆³⁻ ions. While the potential cycling continues, the cathodic peak current corresponding to the intercalation of K⁺ within PB increases owing to the growth of ultrathin PB film within the membrane. After 30 cycles of voltammetric deposition, the blue coloured porous PB-*nt* membrane has been subsequently transferred into a supporting electrolyte (0.5 M KCl) and electrochemically activated by cycling voltammetry. The cyclic voltammogram of the PB-*nt* membrane in supporting electrolyte solution is shown in Figure 2.1B. The redox peaks are found out around 0.2 V which is corresponding to the typical electrochemical reaction of high spin Fe³⁺/Fe²⁺ ions.

Figure 2.1 Cyclic voltammograms of (A) Pt-coated membrane in a solution containing 5.0 mM $K_3Fe^{III}(CN)_6$, 5.0 mM $FeCl_3$, 0.1 M KCl and 0.01 M HCl and (B) PB-*nt* membrane in 0.5 M KCl solution.

2.3.2 Oxygen and hydrogen peroxide reduction at porous PB-*nt* and Platinum membrane electrode

Liner sweep voltammetry has been conducted to investigate the electrochemical behavior of the PB-*nt* membrane electrode towards H_2O_2 reduction. The current–potential

curves for H_2O_2 reduction as shown in Figure 2.2 A has been obtained in a de-aerated solution compared to oxygen reduction under ambient and open air conditions. To achieve the steady-state condition, the voltammogram of PB-*nt* membrane was carried out at a slow potential sweep rate of 1 mV S^{-1} . The typical voltammetric waves of Pt-coated membrane with absence of PB are shown in Figure 2.1B. The reduction potential of H_2O_2 as shown in Figure 2.2A commences at $\sim +0.05 \text{ V}$ (vs Ag/AgCl, 1 M KCl) at PB-*nt* membrane electrode, a $\sim 200 \text{ mV}$ overpotential compared to a control voltammetric experiment which was performed by using porous platinum (Pt) membrane electrode as shown in figure 2.2B. It shows the linear sweep voltammogram of H_2O_2 reduction on the Pt membrane. In addition, comparing with reduction current towards H_2O_2 on PB-*nt* membrane electrode, the Pt-coated membrane shows lower reduction current (Figure 2.3A and B). These observations are consistent with the more rapid electrochemical rate constant for H_2O_2 reduction at PB-*nt* membrane electrode compared to Pt-coated membrane electrode [23]. Furthermore, there is an oxidation peak at potentials positive of -0.1 V (vs. Ag/AgCl, 1 M KCl) on the Pt-coated membrane electrode, which is ascribed to chloride reaction [27]. In contrast, it shows negligible oxidation current at the PB-*nt* membrane electrode indicating an obvious reduced platinum activity when the platinum membrane is coated with PB (Figure 2.2A and B). Figure 2.2B shows a reduction current of oxygen observed at the Pt-coated membrane electrode at $\sim 0 \text{ V}$ vs. Ag/AgCl under ambient condition. However, for the PB-*nt* membrane, the electrical current of oxygen reduction can be negligible compared to H_2O_2 reduction as shown in Figure 2.2A. Overall and important for this work, the membrane electrode with coating a layer of PB-*nt* has not been interfered obviously by the underlying Platinum layer and reduction of solution oxygen under ambient condition.

Figure 2.2 Linear sweep voltammetric curves for reduction of solution oxygen under ambient condition, oxygen reduction in air-saturated solution , and hydrogen peroxide (8 mM) reduction in nitrogen-saturated solution at (A) porous PB-*nt* membrane (or porous Pt membrane coated with PB); (B) porous Pt membrane. Conditions: 1 mV S^{-1} scan rate; 0.5 M KCl solutions.

2.3.3 Effect of adding H₂O₂ on the open-circuit and closed-circuit potentials of PB-*nt* membrane electrode

The open-circuit potential (OCP) between the PB-*nt* membrane electrode and the auxiliary electrode which have been arranged into the two-compartment cell towards H₂O₂ has been measured using the OCP function of a potentiostat. The changes of potential difference between the PB-*nt* membrane electrode and the auxiliary electrode towards increasingly adding H₂O₂ into the sensing solution under open-circuit condition have been recorded as shown in Figure 2.3A. Under the open-circuit condition, owing to no current flow between the auxiliary and working electrodes, a S-shaped titration curve has been obtained which ought to be attribute to the fully depletion of ES as H₂O₂ concentration increased. Different with the OCP changes, the potential curve with closed-circuit condition as shown in Figure 2.3B, which presents the driving force in voltage between the PB-*nt* membrane and auxiliary electrodes, displays an incremental trend when aliquots of H₂O₂ are dropped into the sensing solution. The closed-circuit means there is current flow between the auxiliary electrode and working electrode. In overall, the incremental increasing of the driving force in voltage under the close-circuit condition gives a powerful evidence that the reaction between PB and ES at the PB-*nt* membrane electrode can be interconverted even in excess amount of H₂O₂.

As shown in the figure 2.3B, the driving force of this two-compartment cell sensor towards each addition of H₂O₂ solution can reach a steady-state in a rapid time of ~30–60 s when the sensor is treated with 10 μ M to 5 mM H₂O₂ solution. These steady-state driving force in volt have been plotted vs. the logarithm of H₂O₂ concentration as shown in Figure 2.4C which shows an average slope close to 59/2 mV. This is in confirmity with the steady-state model derived from the Nernst relation: Eq. (6), thus means steady-state

condition for the H₂O₂-powered sensor could be achieved to some extent with a particular concentration range. However, due to the poor correlation between the experimental and fitted data, the steady-state potential values measured under current discharge condition are not analytically useful.

Herein, it is necessary to mention that the highest power density of the sensor using slow scan linear sweep voltammetry [28] is $\sim 30 \mu\text{W cm}^{-2}$ at cell voltage of 0.105 V (vs. auxiliary electrode). Owing to the capability of the two-compartment self-powered sensor with trace amount of analyte which is different from the conventional fuel and biofuel cells, this small power output is going to be promising in the practical uses.

Figure 2.3 (A) Effect of addition of H_2O_2 on the open-circuit voltage between PB-*nt* membrane electrode and counter electrode. (B) Effect of addition of H_2O_2 on the driving force measured from the potential difference between PB-*nt* membrane electrode and

auxiliary electrode under closed-circuit condition. (C) Plot of steady-state values of the driving force obtained from B vs the logarithm of H_2O_2 . Inset: magnified view of Figure 4B shows the driving force reaches steady-state values in ~30–60 s, upon addition of each aliquot of H_2O_2 .

2.3.4 Effect of K^+ ions concentration

The typical amperometric response of the sensor towards H_2O_2 , measured from the steady-state current between the PB-*nt* membrane electrode and the auxiliary electrode, shows enhanced steady-state current responding to the increasing H_2O_2 concentration. Three different permutations of cell solution were arranged for studying the influence over the intercalation of K^+ ion on the steady-state current of the H_2O_2 -powered sensor: (A) $\text{Pt}|\text{xM KCl}||\text{H}_2\text{O}_2, \text{xM KCl}| \text{PB}$; (B) $\text{Pt}|1 \text{ M Tris}||\text{H}_2\text{O}_2, \text{xM KCl}| \text{PB}$ and (C) $\text{Pt}|\text{xM KCl}||\text{H}_2\text{O}_2, 1 \text{ M Tris}| \text{PB}$. $\text{Pt}|\text{KCl}$ or $\text{Pt}|\text{Tris}$ refer to the auxiliary electrode placed in KCl or Tris reference solution while $\text{H}_2\text{O}_2, \text{KCl}| \text{PB}$ or $\text{H}_2\text{O}_2, 1 \text{ M Tris}| \text{PB}$ refer to PB-*nt* membrane electrode placed in the H_2O_2 sensing solution with KCl or Tris as background electrolyte. A control experiment was carried out by using 1 M Tris buffer with the absence of KCl as both reference and sensing solutions, and the results show lower currents compared to the above cell arrangements. From the figure 2.4, it clearly shows that the sensor gives the lowest sensitive response towards H_2O_2 without K^+ . Meanwhile, the sensor sensitivity (response slope) has been largely improved with increasing the concentration of K^+ . This sensitivity attenuation caused by K^+ is attributed to the K^+ dependent interconversion between PB and ES which impacts on its electrochemical reduction and oxidation rate. In consideration of the dependence of the steady-state current signal on this electrochemical rate, coupled to the 2 e^- reduction of H_2O_2 , the

steady-state model in Eq. (8) shows perfect fits for the experimental data as shown in Figure 2.4.

The similar analytical signal responses are shown in Figure 2.4 with addition of K^+ into either the reference or sensing solution, however, the cell arrangement for high K^+ concentration in the reference solution is the more effective to avoid the interference toward the sample solution caused by the addition of K^+ ion into the sensing cell. This demonstration of a high sensitivity H_2O_2 sensor without relying on any external electrical potential will open up the development of analytical sensing system based on the two-compartment Galvanic cell design employing the mesoporous electrocatalytic membrane electrode as cathode and a counter electrode as anode.

Figure 2.4 Effect of K^+ with increasing concentrations in the reference or sensing solution on the sensing performance towards the addition of H_2O_2 to the sensing solution.

Three sets of cell arrangements as followed: (A) Pt|x M KCl||H₂O₂, x M KCl|PB; (B) Pt|1

M Tris||H₂O₂, x M KCl|PB; (C) Pt|x M KCl||H₂O₂, 1 M Tris|PB. The non-linear curves have been fitted using Equation (8).

2.3.5 Determination of H₂O₂ and glucose

Comparing with the thin PB film, PB-*nt* membrane affords a significant advantage of adequate PB nanotubes loading of $\sim 1 \times 10^{-7}$ mol cm⁻², detected by charge passed during potential cycling in 0.5 M KCl solution (figure 2.1B), which is 10–100 times higher than thin PB films [29]. Thus this demonstrates that higher amperometric signal is possible when applied in the self-powering mode.

Figure 2.5A shows the sensor's amperometric signal response towards H₂O₂ under the optimized conditions. It shows a rapid response time of 30 s, excellent linearity from low H₂O₂ concentrations to 80 μ M, a low detection limit of 0.1 μ M and sensitivity of 48 mA M⁻¹ cm⁻². The detection limit of this H₂O₂ sensor is comparable to the reported potential controlled PB sensors [25, 30, 31]. The experiments towards the response of reproducibility have been carried out by using three different sensors. By normalizing the sensors' signals against the amount of charges passed during charging of PB-*nt* upon closing the circuit in the absence of H₂O₂, a reasonable standard deviation of $\sim 20\%$ has been obtained. The typical standard errors of each individual sensor are in the range of 1–3% and the each one could be applied for four sets of 15 measurements.

Interestingly, this new concept of H₂O₂ sensor design can be extended to enzyme based biosensor. To demonstrate the general utility of this new sensor design as an enzyme biosensor, the reduction of H₂O₂ at PB-*nt* membrane coupled to the glucose oxidase (GOx) catalysis of glucose, selected for its well-studied reaction, high stability of

GOx and significance of glucose [32, 33]. The amperometric response of the enzyme based sensor during successive addition of glucose to an air-saturated 1 M Tris pH 7.0 buffer, 0.1 M KCl solution containing 4.0 mg mL⁻¹ GOx enzyme has been shown in Figure 2.5B. Apparent Michaelis K_m (glucose) value of 25(±3.4) mM derived from non-linear curve fitting of the biosensor response is similar to reported value (33 mM) of the free enzyme, thus indicates the sensor response is controlled by homogeneous enzyme kinetics [34]. With linear working range from 1 to 25 mM and rapid response time of ~60 s, the sensor offered a typical homogeneous GOx biosensor which could be applied for measurement of physiological concentration of glucose in blood [35].

Figure 2.5 Chronoamperometric responses of (A) H_2O_2 sensor with successive additions of H_2O_2 (indicated by arrows), and (B) glucose sensor with successive additions of glucose (indicated by arrows) into quiescent sensing solution. Insets: Calibration plots with the best curves fitting.

The proposed biosensor has been practically used to test against some redox species which were coexisting with glucose in blood samples. The amperometric response of the proposed biosensor toward glucose and the interference species such as uric acid and

ascorbic acid has been shown in Figure 2.6. As shown, negligible responses have been induced by uric acid (1 mM) and ascorbic acid (1 mM) while a significant change in the response of self-powering current has been observed for the 1 mM glucose. However, the physiological concentrations of uric acid and ascorbic acid in human blood samples are much lower than 1 mM, which suggests potential development of the low-power sensing devices for the monitoring of chemicals and biochemicals by incorporating appropriate oxidase enzymes [22].

Figure 2.6 Amperometric response of the self-powered glucose sensor toward 1 mM glucose (Glu) and interference species, 1 mM uric acid (UA) or 1 mM ascorbic acid (AA). Conditions: quiescent sensing solution contains 1 M Tris buffer (pH 7) with 4 mg mL⁻¹ glucose oxidase, reference solution contains 0.1 M KCl in 1 M Tris buffer (pH 7). The sensing solution has been stirred rapidly for ~3 s with each addition to make the sharp transient current peaks.

2.3.6 Steady-state model

The electrochemical inter-conversion of PB and ES in the presence of the intercalating K^+ is well described as following equation:

PB

ES

In the presence of H_2O_2 , the ES form of the PB is oxidized to the PB form as follows:

The rate of this catalyzed reaction can be described using a bimolecular rate constant k_1 ($M^{-1} s^{-1}$):

$$\text{Rate} = k_1[ES][H_2O_2] \quad (3)$$

The rate of the Galvanic cell reaction at the auxiliary electrode which regenerates the ES form under closed-circuit condition of the 2-compartment cell can be described by the electroreduction of PB:

$$\text{Rate} = k_2[PB][K^+] \quad (4)$$

At steady-state, the rates for H_2O_2 reduction (Eq. 3) and Galvanic cell reaction (Eq. 4) must be equal and opposite in direction:

$$k_1[ES][H_2O_2] = k_2[PB][K^+] \quad (5)$$

Combining Eq. (5) with the potential dependence logarithmic ratio of PB and K^+ ion to ES according to the Nernst relation $E = E^0 + \frac{RT}{nF} \ln \frac{[PB][K^+]}{[ES]}$, the steady-state potential of the PB-*nt* membrane is related to H_2O_2 concentration as follows:

$$E = E^0 + \frac{RT}{nF} \ln \left\{ \frac{k_1}{k_2} [H_2O_2] \right\} \quad (6)$$

Similarly, we can derive steady-state amperometric current response of the PB-*nt* membrane toward varying H_2O_2 concentrations. The measured current signal i is derived from the current passing from the auxiliary electrode to the PB-*nt* membrane which reduces PB to the ES form:

$$i = nFAk_2\Gamma_{PB}[K^+] \quad (7)$$

where the more appropriate surface coverage Γ_{PB} (mol cm⁻²) is used instead of concentration since the amperometric sensor response depends on the current flux flowing through the Galvanic cell. If Γ_{Σ} is the total loading of PB and ES, then the amount of ES can be expressed as $\Gamma_{\Sigma} - \Gamma_{PB}$. Expressing the steady-state amounts of PB and ES in term of total polymer and steady-state PB amounts, Γ_{Σ} and Γ_{PB} , and combining Eq. 5 and Eq. 7 gives the overall current flow through the sensor in terms of H_2O_2 concentration, total polymer loading and K^+ concentration as follows:

$$i = \frac{nFAk_2\Gamma_{\Sigma}[K^+][H_2O_2]}{\frac{k_2}{k_1}[K^+] + [H_2O_2]} \quad (8)$$

where $n = 2$ since two e^- are needed to reduce one H_2O_2 molecule.

At very low hydrogen peroxide concentrations, $\frac{k_2}{k_1} [K^+] \gg [H_2O_2]$, so Eq. 8 simplifies to a linear relation between current and H_2O_2 concentration which is analytically useful for low concentration measurement by the sensor:

$$i = 2FAk_1 \Gamma_{\Sigma} [H_2O_2] \quad (9)$$

For glucose sensing in the presence of glucose oxidase enzyme, the steady-state current i is assumed reasonably to obey Eq. 9 for the low amount of H_2O_2 generated from the equilibrium enzyme reaction. For example, at 20 mM glucose and 0.25 mM oxygen, the equilibrium H_2O_2 concentration derived from enzyme reaction is $\sim 30 \mu\text{M}$ which lies within the sensor's linear response region (main text Figure 2.5A inset). Following usual analytical expression for enzyme-based biosensor in which the electrode response is linear toward the enzyme product, the following Michaelis-Menten equation is used to describe the self-powering glucose biosensor response in Figure 2.5B inset:

$$i = \frac{k[\text{glucose}]}{K_M + [\text{glucose}]} \quad (10)$$

where K_M is the apparent Michaelis-Menten constant and k contains the enzyme turnover rate.

2.4 Conclusions

In summary, we have described a self-powered sensor and biosensor for the detection of hydrogen peroxide and glucose based on a porous PB-*nt* membrane electrode.

This unique self-powered sensor has been constructed based on a Galvanic cell without utilization of any external applied electrical potential. PB nanotubes have been characterized to present similar characteristics as the PB films through the experiments of potential voltammetric and measurement of the sensor electrochemical potential, which indicated the sensing reaction based on PB-*nt* proceeds via reduction of hydrogen peroxide. The spontaneous reaction of hydrogen peroxide reduction at the PB-*nt* membrane electrode in conjunction with the regeneration of Everitt's salt form of PB by the Galvanic cell reaction under steady-state conditions, have been demonstrated well through the excellent fitting curves of the potential and current data to the theoretical models. The self-powered hydrogen peroxide sensor has shown an outstanding analytical performance which was comparable to the potential-controlled PB sensors. Proof-of-concept glucose biosensor derived from the hydrogen peroxide sensor has been demonstrated in the presence of a homogeneous solution of glucose oxidase enzyme, indicated possibility for other oxidase enzyme-based biosensors.

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Chapter 3

**Fuel Cell Virus sensor Sensor using
Virus Capture within
Antibody-coated Membrane Nanochannels**

Chapter 3 Fuel Cell Virus Sensor using Virus Capture within Antibody-coated Membrane Nanochannels

3.1 Introduction

Inspired from the potential needs of the comprehensive and powerful monitoring methods to prevent and control disease epidemics, a fuel cell as sensing system that can respond specifically to viral diseases is of scientific and technological significance, and will be one of the most desirable and promising tool for the screening and detection of viral diseases [1–3]. Fuel cells as power sources can be applied in the sites ranging from aircraft, train, cruiseship cabins to the publicly accessed enclosed spaces including offices, schools and entertainment centers [4, 5]. Due to the continuous power production, fuel cells can be also deployed application in the implanted biomedical devices [6, 7]. In the past two decades, applications of biofuel cells in electrochemical sensors and biosensors have aroused great interest among researchers. Recently, a variety of biosensors and sensors employing the conversion of chemical-to-electricity as driving force have been widely studied [8–14]. To date, no report on applying the alternative energy sources for virus sensing has been presented, due to its difficult application of virus based on enzyme, cell or redox reactions at the biocatalytic anodes or cathodes. It is still desirable to develop a virus-specific enzyme and redox reactions, which could be applied in the fuel cells as a sensing system for virus detection.

In the previous chapter, we have developed a unique Prussian blue nanotubes membrane based sensor using a two-compartment cell derives the current signal from the chemical energy of the H_2O_2 analyte, in absence of electrical potentials. According to the previous research work, we have known PB-*nt* membrane, as cathode material with high surface area, showed high electrocatalytic activity towards reduction of H_2O_2 [15, 16] and

could provide sufficient current at microampere level at low concentration of H_2O_2 [17]. In addition, the complete coverage of the platinum layer by PB-*nt* avoids the interfering reaction of oxygen reduction in solution because PB is fairly inert toward oxygen [18]. Therefore, H_2O_2 fuel cell based on PB-*nt* membrane offers a great potential in sensing and biosensing system. Moreover, H_2O_2 is a carbon-free energy carrier which itself can be easily generated abundantly from other renewable energy sources [19, 20]. Owing to the similar dimension with protein size, membrane with nanopores has been widely applied in protein recognition and sensing system when coupled with electrochemical method [21–23]. To further extend the analytical utility of the PB-*nt* membrane based fuel cell sensor design, a H_2O_2 powered fuel cell virus sensor has been developed for direct recognition of unlabelled virus particles on the basis of antibody-virus complexes formation within the PB-*nt* membrane nanochannels.

The virus sensor has been constructed based on a two-compartment fuel cell design as shown in Scheme 3.1. PB-*nt* membrane employed both as cathodic electrode for reduction of H_2O_2 and sensing electrode has been placed into the sensing solution, while an auxiliary platinum electrode served as anode for oxidation of water has been placed in reference solution together with the reference electrode. An electrolyte solution within membrane nanochannels has been used to complete the circuit. In the fabrication of virus sensor, the membrane with pore diameter of 200 nm which is larger than the virus-antibody complex (~70 nm in size) has been utilized to capture virus by the nanochannel-adsorbed antibody molecules. During the operation of the fuel cell virus sensor, the ion conductivity of the membrane could be reduced, resulted from the formation of the virus-antibody complexes in the nanochannels, which led to observable reducing amperometric signal response. This strategy shows high efficiency for high sensitivity quantitative measurements [24].

Scheme 3.1 Schematic of the fuel cell virus sensor using a 2-compartment cell separated by a Prussian blue nanotubes membrane, showing the binding of virus to antibody molecules within the nanochannels.

Dengue is a mosquito-borne viral infection including four types of dengue virus (DENV): DENV 1, DENV 2, DENV 3 and DENV 4 which have been recognized in over 100 countries and territories cause 50 to 100 million infections in the worldwide per year [25, 26]. Therefore, it is desirable to develop a direct and reliable method for early diagnosis of dengue virus, especially in the outburst of epidemics. Herein, a fuel cell virus sensor which offers an efficient responding to dengue virus has been developed. To achieve high selective response of the virus sensor, monoclonal antibodies specific toward dengue virus were chosen. Two structurally similar dengue virus serotypes have been successfully distinguished within a short analysis time of ~5 min with high selectivity against each other.

3.2. Materials and methods

3.2.1 Materials and instrument

Materials: Nanoporous alumina membranes with diameter of 13 mm and pore size of 200 nm (AnodiscTM) were purchased from Whatman (Maidstone, Kent, U.K.). Hydrogen chloride with concentration of 37% was bought from P. P. Chemicals. 35% H₂O₂ was bought from Alfa Aesar. KCl were purchased from Sinopharm Chemical Reagent Co. Ltd. (SCRC). The potassium hexacyanoferrate(III) and Nafion[®] perfluorinated resin solution were obtained from Sigma-Aldrich. The anhydrous ferric chloride was required from Merck, and tris(hydroxymethyl)-aminomethane from Bio-Rad Laboratories. Mouse anti-dengue type II and type III virus monoclonal antibodies were purchased from Millipore and stored at 4 °C. Ultrapure water purified with the Sartorius Ultrapure Water System was used to prepare the solution.

Instrument: An auto Fine Coater (JEOL JFC-1600) has been used to sputter coating platinum layer onto the mesoporous alumina membrane. A CHI1220B potentiostat has been used for monitoring the open-circuit potential. The e-corder 401 (eDAQ) and a potentiostat (eDAQ EA161) have been connected to do measurements of close-circuit current. A two-compartment cell as shown in Scheme 3.1 and three-electrode system with porous PB-nanotubes (PB-*nt*) membrane electrode as the working electrode, a Ag/AgCl (1 M KCl) reference electrode and a platinum mesh as the counter electrode have been applied for performing all of measurements. The Autolab PGSTAT128N (Ecochemie, The Netherlands) has been used for impedance study. The field-emission scanning electron microscope (SEM, JEOL JSM-6340F) has been used for characterization of morphology of PB-*nt* membrane.

3.2.2 Fabrication of PB-*nt* membrane and immobilization of antibody

The preparation of Prussian blue nanotubes membrane has been discussed in previous chapter.

Immobilization of antibody within the nanochannels of PB-*nt* membranes before use as virus sensor used the physical adsorption as follows: the stocks of monoclonal antibodies of Mouse anti-dengue virus type II or type III were diluted to $0.2 \mu\text{g mL}^{-1}$ in 1 M TRIS buffer with pH 7 and were stored in 4 °C before used. 50 μL of the $0.2 \mu\text{g mL}^{-1}$ antibody solution was directly applied onto the PB-*nt* side of the membrane and incubated at 4 °C for 45 min and subsequently taking the membrane at room temperature for incubation with further 15 min before use.

3.2.3 Procedure for open-circuit voltage (OCV) and closed-circuit current measurements

The measurements of OCV and closed-circuit current were similar to the procedure described in the previous chapter.

In open-circuit voltage (OCV) measurement, the sensing solution of the fuel cell virus sensor employed 1mM H_2O_2 . The voltage changes between working electrode of PB-*nt* membrane and counter electrode of Platinum mesh has been measured over time with addition of dengue virus serotype-2 (DENV-2), by OCP function of the CHI1220B potentiostat.

3.2.4 Virus culturing

Firstly, *Aedes* mosquito cells (C6/36) have been infected with dengue serotype 2 viruses at a Multiplicity of Infection (MOI) of 0.01 for 1 hour at 28 °C. Following the cell

infection, the medium has been removed and fresh growth medium (RPMI, 5% heat-inactivated fetal bovine serum (FBS) and 5 mM 2-mercaptoethanol) has been added. The cell culture has been incubated at 28 °C for 5 days. After 5 days, the medium has been collected and a plaque assay was conducted to determine the concentration of the dengue virus in terms of plaque forming unit (pfu mL⁻¹). The virus sample has been subsequently heat inactivated at 56 °C for 30 min. All experiments have been performed in a biosafety level 1 laboratory since Dengue virus is not an air-borne virus. As an additional safety precaution, chlorine tablet has been added before the Dengue virus solution has been discarded.

3.2.5 Procedure for virus sensing

Before sensing to virus, 14 µL of 0.1 M H₂O₂ has been initially added into the 1.4 mL 1 M TRIS buffer sensing solution so the H₂O₂ concentration was 1 mM. After the background of H₂O₂ current has reached at steady-state condition, the stock virus solution with 600 plaque forming unit per mL (pfu mL⁻¹) has been successively added into the H₂O₂ solution for sensing.

3.2.6 Integrated membrane fuel cell virus sensor

The method for preparation of PB-*nt* membrane has been improved compared to the previous method. A thin layer of platinum was firstly sputtering coated onto both sides of alumina membrane (AAO) with pore size of 200 nm. Then the conductive membrane with one side employed as working electrode and another side as counter electrode was subsequently performed potential cycling from -0.5 to +0.6 V at 50 mV s⁻¹ for 15 cycles in a mixture of 5.0 mM K₃Fe^{III}(CN)₆, 5.0 mM FeCl₃, 0.1 M KCl and 0.01 M HCl for 2 times. After finish the potential sweep, a blue coloured membrane has been obtained, which indicated the formation of PB-nanotubes onto the membrane. After drying at room

temperature for overnight, 15 μL of nafion solution was dropped onto the side without PB-*nt* of the membrane to penetrate into the nanochannels of the membrane.

In the fabrication of integrate membrane fuel cell virus sensor, the immobilization of antibodies is same with the previous one. The PB-*nt* membrane side has been used as anode and the opposite of PB-*nt* membrane with Pt coating as cathode exposed to surrounding air, which resulted a simple two-electrode cell arrangement. During virus sensing, 80 μL of 0.5 mM H_2O_2 was firstly dropped onto the PB-*nt* coated side of the dry membrane probe under closed-circuit condition and allowed to generate steady-state current values after ~ 7 min. After that, aliquots (2 μL) of 1000 pfu mL^{-1} virus stock solution were successively added into the H_2O_2 solution.

3.3 Results and discussion

3.3.1 Morphology of Prussian blue nanotubes membrane

The morphologies of the Pt-coated membrane have been scanned by SEM, which presents the states before and after the deposition of PB (Figure 3.1). In Figure 3.1A, the SEM image has outlined the PT-coated membrane before deposition of PB, which displays a highly ordered pore arrangement with diameter around 150 nm. This SEM image demonstrates ~ 50 nm thick coating of platinum along the nanopore and nanochannels of the alumina membrane. The image scanned after the deposition of PB is shown in Figure 3.1B, on which a clear layer of PB can be observed. Moreover, the image of the cross sectional view as shown in inset of figure 3.1B reveals that the PB has been successfully deposited into the nanochannels of membrane, and that the PB deposited membrane still remains a good mesoporous structure. The depositon of Prussian blue along

within the nanochannels can be up to 5 μm using the one-step potential cycling method [27].

Figure 3.1 Scanning electronic micrograms of platinum-coated membrane and PB deposited membrane. Top view images of platinum-coated membrane (A) and PB deposited membrane (B), cross sectional view images platinum-coated membrane (inset of A) and PB deposited membrane (inset of B).

3.3.2 Prussian blue nanotubes membrane as cathode

The cyclic voltammograms of a PB- *nt* membrane electrode as shown in Figure 3.2A have been obtained with 100 cycles from 0 to 0.5 V. It presents the PB-*nt* membrane as cathode [28] considered to be stable in its reaction with hydrogen peroxide, where its anodic and cathodic peak currents decrease by only 25% after 100 potential cycles. This indicates the PB-*nt* membrane can be re-used for many times without significant loss in its electroactivity. During amperometric sensing towards H_2O_2 under closed-circuit condition using the two-compartments fuel cell setup, it is important that the H_2O_2 current hasn't change significantly throughout the measurement so that any changes of current due to the virus analyte can be normalized against the initial background of H_2O_2 current. Figure 3.2B shows that the steady-state amperometric current responding towards 1 mM H_2O_2 under closed-circuit condition gives high stability where the amperometric current changes within 5% error over 50 min.

Figure 3.2 (A) Cyclic voltammogram of a PB-*nt* membrane electrode with a scan rate at 50 mV s^{-1} . (B) Amperometric response of the PB-*nt* membrane toward 1 mM H_2O_2 using the 2-compartment cell setup. Conditions: PB-*nt* membrane working electrode is immersed in sensing solution, while counter platinum mesh and Ag/AgCl reference electrodes are placed in the reference solution according to Scheme 3.1. Sensing solution contains 1M TRIS buffer (pH 7) and reference solution contains 0.5 M KCl in 1 M TRIS buffer (pH 7). Amperometric current is monitored under closed circuit condition with time using a galvanostat. No external potential is applied to the electrodes.

With a geometric area of 0.12 cm^2 calculated from the radius of the electrode area, the electrode directly contacts with the sensing solution in the 2-compartment cell. The effective surface area of Prussian blue in contact with the solution includes the membrane surface as well as the internal nanochannel walls. For nanotubes with $\sim 5\text{ }\mu\text{m}$ in length, the effective surface area of Prussian blue in contact with electrolyte solution is $\sim 3.0\text{ cm}^2$ i.e. ~ 25 times larger than the membrane geometric area. The hydrogen peroxide current density of the porous membrane supported PB is $208\text{ }\mu\text{A mM}^{-1}\text{ cm}^{-2}$ which is about 3 folds larger than those record ($\sim 60\text{ mA M}^{-1}\text{ cm}^{-2}$) using PB coated solid planar electrodes [29].

3.3.3 Characterization of the two-compartment PB-*nt* membrane cell

The voltammetry of the PB-*nt* membrane using the membrane cell setup in Scheme 3.1 has afforded a typical electrocatalytic response. To compare the relative changes in the voltammetric current, all of the voltammetric current signals have been normalized to the anodic peak current of each voltammogram as the potential cycling may cause some dissolution of PB. As shown in Figure 3.3A, it gives a typical electrocatalytic response where the normalized cathodic peak current is enhanced compared to the normalized anodic peak current with presence of H_2O_2 . When with presence of virus, the normalized peak current decreases slightly. In the other hand, the normalized limiting currents (plateau shape) at high and low potentials are clearly lowered in the presence of virus, which suggests a limiting process that is sensitive toward virus concentration as shown in figure 3.3B.

Figure 3.3 Cyclic voltammogram of (A) a PB- *nt* membrane electrode catalysis of H_2O_2 and (B) response of a PB- *nt* membrane electrode immobilized with anti-dengue type II monoclonal antibody towards DENV-2. Condition: scan rate = 50 mV s^{-1} ; working PB-*nt* membrane electrode, counter platinum mesh and Ag/AgCl reference electrodes are arranged in a 2-compartment cell according to Scheme 1B. Sensing solution contains 1M TRIS buffer (pH 7); reference solution contains 0.5 M KCl in 1 M TRIS buffer (pH 7).

3.3.4 Performance of the two-compartment PB-*nt* membrane fuel cell

The curve of OCV response of the PB-*nt* membrane based fuel cell toward the addition of an excess amount of H_2O_2 is displayed in Figure 3.4A. A typical sigmoidal titration curve has been observed under open-circuit voltage, which reached a plateau value and became steady upon further addition of H_2O_2 . The steady state of the figure could be explained by total oxidation of PB-*nt* to its PB form. In contrast, the closed-circuit current response of a PB-*nt* membrane as shown in Figure 3.4B continues to increase at high 60 mM H_2O_2 concentration. This is because under closed-circuit condition, the cathodic current which reduces the PB-*nt* electrode increases as more PB-*nt* becomes oxidized by H_2O_2 in the presence of increasing H_2O_2 concentration. It is clear from Figure 3.4B that the magnitude of the closed-circuit current response of the PB-*nt* membrane is strongly influenced by the H_2O_2 concentration.

The power density curve of the PB-*nt* sensor as shown in Figure 3.4C has been obtained from steady-state linear sweep voltammetry (at 1 mV s^{-1} scan rate) in the presence of 5 mM H_2O_2 . Initially in the absence of H_2O_2 , the PB-*nt* based virus sensor gives insignificant power output. While addition of 5 mM H_2O_2 , the power density increased ~50 times to a maximum power density value of $12.7 \mu\text{W cm}^{-2}$. Another control experiment with virus added to the membrane cell, resulted a lower power density of H_2O_2 powered fuel cell sensor. A notably high power density has been observed with 60mM H_2O_2 , under the steady state closed circuit current discharge condition. The typical closed-circuit current of a PB-*nt* membrane using a 60 μm thick, 200 nm pore size membrane template can reach 300 μA at a closed-circuit potential of ~0.3 V (measured using high impedance voltage measurement function of CHI1220B potentiostat). Thus, an impressive power output of $\sim 100 \text{ mW cm}^{-3}$ (or 0.625 mW cm^{-2}) can be obtained under

this optimal condition, which offers a great potential in low power applications. The power output of the fuel cell could be not only determined by H_2O_2 concentration, but also other current dependent parameters, such as the amount of deposited PB-*nt*, quality of the sputtered metal layer which influences its conductivity and membrane pore size. While incorporated with anti-virus antibody, the PB-*nt* membrane could also serve as a virus sensor. In the situation of virus detection using a single compartment arrangement, there is however, no change in the background H_2O_2 current during the addition of DENV 2 to the sensing solution (Figure 3.4D). This is in contrast to the amperometric current responses when the virus sensor uses the 2-compartment cell (Figure 3.5A). Therefore, experiments done as follow were all carried out with two-compartment cell arrangement.

Figure 3.4 Response of (A) Open-circuit voltage and (B) closed-circuit steady-state current of a 2-compartment PB-*nt* membrane cell toward H_2O_2 . (C) Polarization power curves of the PB-*nt* membrane sensor obtained in the absence and presence of 5 mM H_2O_2 and 20 pfu mL^{-1} virus. (D) Closed-circuit current responses of a single compartment PB-*nt* membrane cell toward DENV 2.

3.3.5 Analytical performances of the fuel cell virus sensor

It is urgent necessity for development of low cost virus sensor based on fuel cell design which are highly specific and can quickly distinguish between viruses with similar

disease symptoms for rapid therapeutic response at points of care. Generally, fuel cell sensors relying on redox reactions cannot be used to perform the detection of unlabeled viruses. Therefore, specific recognition which incorporated the antibody molecules into the nanochannels of the PB-*nt* membrane has been applied to capture the target virus particles specifically. In Figure 3.5A, it shows that the closed-circuit current of the fuel cell virus sensor responds and decreases within an extreme short time of ~5 min to each addition of dengue serotype 2 virus (DENV-2), using an anti-dengue virus serotype 2 antibody-incorporated PB-*nt* membrane. Initially, a low concentration of 1 mM H₂O₂ has been applied in the virus sensor to produce a current output which can be achieved a steady-state value a short time of 7-12 min. Under this situation, the open-circuit voltage and closed-circuit current outputs are in the range of 0.25 V and 25 μ A, so the virus sensor is operated with power of ca. 8.2 to 1.7 mW cm⁻³ over the concentration range from 3 to 45 pfu mL⁻¹.

Figure 3.5 (A) Typical closed-circuit steady-state current response of a fuel cell sensor toward DENV-2virus and (B) its calibration plot of normalized closed-circuit steady-state current versus virus concentration; line is non-linear best fitted data; error bars are

standard deviation of experiment data (points). (C) Selective and non-selective responses of the fuel cell virus sensor toward DENV-2 and DENV-3, added sequentially to the sensing solution.

As shown in Figure 3.5B, the calibration curve of the fuel cell sensor has been plotted with the closed-circuit current with presence of virus, I_{virus} , which normalized to its initial steady-state closed-circuit current after the addition of 1 mM H_2O_2 but with absence of virus, $I_{\text{virus}=0}$. With normalization of the current response, a low standard error of 2–6% has been obtained from three different membrane sensors. However, without normalization, the uncertainties could be up to ~20% calculated from 3 different membrane probes, since it is difficult to achieve identical thicknesses for each membrane and loading amounts of the nanochannel or surface adsorbed antibody molecules. A low detection limit of 0.04 pfu mL^{-1} could be achieved using this unique virus capture approach to impede the fuel cell sensor current. The detection limit of the fuel cell virus sensor has been determined from the minimum DENV-2 concentration that gave a decreasing current equivalent to three times the standard deviation of background current in the absence of DENV-2. This was calculated from the fitting equation $y=1-(x/A)^{(1/n)}$ (where $A=66.97$, $1/n=0.573$), which derived from the membrane resistance model in eqn (2) below. In case of LOD at 0.04 pfu mL^{-1} , the method is comparable to state-of-the-art real-time reverse transcriptase PCR (LODs: 0.6 pfu mL^{-1} , 0.4 pfu mL^{-1} , 0.01 pfu mL^{-1}) [30–32]. One critical merit of this virus sensor is its selectivity to distinguish closely similar virus targets with highly conserved amino acid residue sequences. The fuel cell virus sensor which incorporated the anti-dengue type II monoclonal antibody could differentiate between DENV-2 and DENV-3, in case of adding sequentially to the sensing solution of the two-compartment sensor cell (Figure 3.4C). At 6 pfu mL^{-1} virus

concentration, the changed in current for DENV-2 is significant. In contrast, the current for DENV-3 did not change after the initial perturbed transient current relaxes back to the background current value after ~4 min (Figure 3.5C). The same transient current due to solution mixing is observed for a PB-*nt* membrane without immobilized antibody in its response towards DENV-2 and PB-*nt* membrane sensors with immobilized antibody towards non-analytes (Figure 3.7). This outperforms has outlined methods to examine the dengue virus serotypes in which the cross-reactivities of dengue serotypes due to common epitopes still are considered to a great challenge [33–34].

Thus, the fuel cell virus sensor which embedded with anti-dengue 2 3H5 antibody with PB-*nt* membrane serves as a highly specific, selective and sensitive sensor in analysing *unlabeled* dengue serotype 2 virus. Different with typical electrochemical and optical antibody-based virus sensors, this fuel cell virus sensor has no requirement of any voltage power input rather relies on a very small amount of 1 mM H₂O₂ for each series of measurements. H₂O₂ is the common signalling reagent consumed or generated *in-situ* during enzyme linked immunosorbent assay (ELISA) for the direct detection of virus particles, virus protein antigens and antibodies associated with viral infections [35–36]. Therefore, H₂O₂ is an ideal reagent for powering the electroactive Prussian blue nanotubes in the presence of immune reagents. In addition, the fuel cell virus sensor showed an impressive outperformance with responding to the virus in a short analysing time of ~5 min, which exceeded all other immune-based and PCR-based methods. The linear range is less than 100 pfu mL⁻¹ which is highly relevant for early diagnosis of the dengue disease in human patients and is superior to the ‘gold standard’ plaque assay which requires incubation time of at least one week [37]. Notably, the detection limit of 0.04 pfu mL⁻¹ for dengue virus is comparable to existing state-of-the-art high sensitivity electrically powered multiplex reverse transcriptase PCR [30–32].

3.3.6 Membrane resistances of virus sensor

The membrane resistances of the fuel cell virus sensor have been measured by electrochemical impedance spectroscopy (EIS), with and without virus. Figure 3.6A reveals that the immobilization of ~15 nm size antibody molecules increased the membrane resistances of 200 nm pore size membrane as expected. It shows a small increase of only ~1 ohm in the ohmic cell resistance after incubation in the antibody solution. Subsequent addition of 8 pfu mL⁻¹ virus particles further increased the ohmic cell resistance by 4 ohm, ~3% of ohmic cell resistance (Figure 3.6A) due to the capture of virus particles (~50 nm) within its 200 nm nanochannels. According to the calibration plot in Figure 3.5B, the corresponding larger decrease in the steady-state sensor current of ~20%, indicates most of the resistance change occurs at the membrane instead of the solution or the porous Pt electrode. To confirm if the driving force of the fuel cell virus sensor is also affected by the virus, we measure its open-circuit voltage in increasing virus concentration against its Pt mesh counter electrode (Figure 3.6B). It is clear that the virus does not influence the sensor's OCV, unlike the sigmoidal-shaped OCV-time plot of the PB-*nt* membrane cell when titrated with increasing H₂O₂ concentrations [17]. Thus the steady-state current response of the fuel cell virus sensor toward the virus does not arise from thermodynamic change. Therefore, the driving force of many energy conversion devices can deteriorate over time due to cathode or anode 'poisoning' by solution species, resulted in major loss in power outputs and efficiencies over time [38].

Figure 3.6 (A) Nyquist plot showing the electrochemical impedance response of a 200 nm pore size PB-*nt* embedded membrane in the presence of antibody and its specific virus target. Inset: Magnified view of Nyquist plot to reveal ohmic cell resistance.(B) Unchanged potential of a 200 nm pore size membrane sensor (immobilized with anti-dengue 2 antibody) in the presence of increasing virus concentration. Potentials were measured against the counter electrode under open-circuit condition. Inset: Magnified view of figure 3.6A.

Control experiments were carried out using non-specific analytes, silica particles (100 nm) and BSA protein (~15 nm), which can enter the large 200 nm pores. Figure 3.7 demonstrates the signal responses of the PB-*nt* membrane biofuel cell sensor with immobilized anti-virus antibody toward (A) BSA and (B) silica nanoparticles and the signal response of a PB membrane without immobilized antibody toward DENV-2. While proceeding with non-virus analytes, the current response of the virus sensor stayed unchanged. This phenomenon could be explained as antibody would not affect the internal membrane resistance, if not capture the virus particles by membrane nanochannels. To respond with virus analyte, the pore size of membrane in virus sensor should be designed to be larger than the virus dimension.

Figure 3.7 Signal responses of the PB-nt membrane biofuel cell sensor with immobilized anti-virus antibody toward (A) BSA and (B) silica nanoparticles. (C) Signal response of a PB membrane without immobilized antibody toward DENV-2.

3.3.7 Model of sensor response

Electrolyte resistance is a key parameter that controls performance of fuel cells. In the fuel cell virus sensor, the membrane resistance R_{mem} is related with cross-section area of membrane nanochannels, A , resistivity of electrolyte solution in the nanochannels ρ , and membrane thickness l as follows [39]:

$$R_{\text{mem}} = \frac{\rho l}{A} \quad (1)$$

Changes in the fuel cell virus sensor current response in the presence of virus concentration, $[\text{virus}]$, are attributed to the ‘plugging’ of the nanochannels due to virus-antibody binding. The cross-section pore area has a reciprocal relation to the membrane resistance. Therefore, the binding between virus and antibody in the nanochannels is assumed to obey the simple Langmuir relation. Following the same manner, fractional coverage θ of the bound virus in the nanochannels has an inverse ratio with the total cross-section area of the unblocked membrane nanochannels, A_0 , to $(1 - \theta)A_0$. The correlation between the virus sensor’s closed-circuit current and virus concentration could be demonstrated following the Langmuir-Freundlich model:

$$\theta = \frac{[\text{virus}]^{\frac{1}{n}}}{[\text{virus}]^{\frac{1}{n}} + K} \quad (2)$$

K (M) in the model is the dissociation constant of the antibody-virus complex. The equation is attributed by the initial binding of virus within a single antibody-coated nanochannel, in which channel resistance varies in a largest range in comparison with subsequent capture of the virus with the same nanochannel. Under optimized sensing conditions, the virus concentration ([virus]) is generally in a few pfu mL⁻¹ and 1 pfu is assumed to contain of ca. 100,000 virus particles [40]. Therefore, the virus concentration is $\sim 10^{-14}$ M. Since the value of K (in range of 10^{-9} to 10^{-7} M) is much larger than [virus], the fractional coverage can be simplified as Eq. (3):

$$\theta = \frac{[\text{virus}]^{\frac{1}{n}}}{K} \quad (3)$$

According to Ohm's Law ($V=RI$), the closed-circuit current of fuel cell sensor in the presence of virus, I_{virus} , is in direct proportion to membrane resistance R_{mem} . When equation (1) is combined with Ohm's Law and the simplified θ expression, a direct relation between I_{virus} and virus concentration [virus] is derived as follows:

$$I_{\text{virus}=0} = \frac{A_0 V}{\rho l} - \frac{A_0 V [\text{virus}]^{\frac{1}{n}}}{K \rho l} \quad (4)$$

Non-linear curve fitted results using equation (2) normalized to the initial closed-circuit current in the absence of virus, shows excellent agreement with experiment (Figure 3B). The calculated dissociation constant K derived from non-linear curve fitting 1.1×10^{-8} M

and using the pfu-to-virus particle ratio of 1:100,000, is within the reported range (10^{-9} - 10^{-7} M) of observed DENV2-IgG interactions [40].

3.3.8 Integrated Nafion[®] incorporated membrane probe

Although the two-compartment fuel cell virus sensor offered an efficient response and differentiation toward the two structurally similar dengue virus serotypes, it brought some inconvenience for operation under field conditions. In order to further simplify the 2-compartment cell and three-electrode system, an integrated two-electrode fuel cell setup based on a standalone PB-nt membrane has been developed as shown in Scheme 3.2. Different with fabrication membrane-based two-compartment fuel cell, the integrated membrane fuel cell virus sensor has been constructed with incorporation of Nafion[®] perfluorinated resin into the membrane to promote the ionic conduction between the two electrodes [41]. While operation of the membrane virus sensor, one side of Pt-coated membrane serves as reference/counter electrodes and the other side of PB-*nt* coated membrane serves as working electrode for sensing reaction.

Scheme 3.2 Photograph and schematic of the standalone Nafion-filled membrane probe using the nanometer thick metal layer of the membrane at the air-electrolyte interface as the reference and counter electrode.

The closed-circuit current responses of the integrated membrane probe immobilized with anti-dengue type II monoclonal antibody toward DENV2 and DENV 3 have been shown in Figure 3.8. It shows a rapid specific response toward DENV-2 and dull-response toward DENV-3 virus by initial addition of 80 μL of 0.5 mM H_2O_2 solution, and subsequent addition of 2 μL virus solution to the PB-*nt* membrane side of the sensor. This demonstrates the unique utility of the nanochannel-based antibody-virus binding fuel cell virus sensor-under ambient dry condition employing single step analysis without electrical power input.

Figure 3.8 Closed-circuit current response of a dry membrane probe immobilized with anti-dengue type II monoclonal antibody toward (A) DENV-2 and (B) DENV-3. Background slope correction is carried out on the data presented at (B) to compensate for background drift and clearly shows the negligible effect of DENV-3 on the fuel cell virus sensor response.

3.4 Conclusion

Comparing with traditional sensors with an external energy devices, the fuel cell virus biosensor based on a PB nanotubes modified membrane with immobilization of antibody in nanochannels is more practical with significantly low cost. To prevent spreading epidemic diseases, tremendous efforts have been usually made to pursue more human sources and economy support. But the efforts are not always readily available in developing countries where many infectious diseases are of great concern. In addition, portable reagents and miniaturized membranes used in the virus-based fuel cells which would function conveniently under instructions could be accessed on the grounds of the feasibility and practicality in terms of design, suitable for deployment.

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Chapter 4

Hand-held Fuel Cell Sensor Device Based on Prussian Blue Nanotubes Membrane

Chapter 4 Hand-held Fuel Cell Sensor Device Based on Prussian Blue Nanotubes

Membrane

4.1 Introduction

The novel concept of developing biosensor devices based on chemical-to-electrochemical energy transformation in biofuel cells offers high possibility for portability of sensor device [1–3]. Among different fuel cells, due to nontoxicity, highly efficient energy production, easy transport and handling in aqueous phase, H_2O_2 fuel cells have been constantly attractive [4–6]. Current reported H_2O_2 fuel cells commonly require a strong basic or acidic electrolyte and a concentrated H_2O_2 solution [7–11], which limit their application as power source into the analytical sensors, especially in the biosensors which always require a neutral or weak base buffer.

PB has been presented as an efficient electron transfer mediator for reduction of H_2O_2 in the direct borohydride-peroxide fuel cell systems [12, 13]. Besides the advantage of low cost, PB electrodes can minimize the H_2O_2 decomposition [14]. In addition, PB electrodes offer a fast H_2O_2 reduction kinetics which is comparable with Pt electrode and lower overpotential [15, 16]. Therefore, it is very desirable to develop the employing PB as cathode in H_2O_2 fuel cells.

Previously, we have successfully developed a H_2O_2 fuel cell sensor and H_2O_2 power fuel cell virus sensor based on PB nanotube membrane which have operated in the sensing solution with pH 7 [17, 18]. Due to the low power output, the sophisticated electrochemical instrument has been still required to transduce and analyze the responding signals. In this chapter, we described a real hand-held sensor device based on H_2O_2 fuel cell, which would get rid of the cumbersome instrument and just need a micro-current meter to read the responding signals. This H_2O_2 fuel cell sensor was constructed

using four standalone nafion-filled PB-*nt* membranes connected in parallel as shown in scheme 4.1, which employed the PB-*nt* membrane as both electrodes and fuel reservoir. The PB-*nt* membrane side was applied as cathode for reduction of H₂O₂ and reservoir cell, and the opposite of the PB- *nt* membrane coated by platinum was served as anode for oxidation of water to oxygen. The two-electrode system was adopted for the performance tests. This hand-held H₂O₂ fuel cell sensor provided an OCP of 0.54 V with a maximum power density of 0.7 mW cm⁻² at a low concentration of H₂O₂ solution (10 mM). Additionally, the volume of the solution required for the whole measurements could be limited to a few hundreds microliters. To investigate its analytical performance, a micro-current meter was applied to record the response signals of the fuel cell towards varying concentrations of H₂O₂. This low power fuel cell sensing design offers a promising potential for realization of the low cost and portable sensor devices.

The electrochemical reaction taking place on the cathode and anode of the fuel cells were the electro-reduction of H₂O₂ and oxidation of water to oxygen.

Combining with these two half-cell reactions, it gave a theoretical driving force of 0.55 V.

Scheme 4.1 Scheme of H_2O_2 fuel cell sensor based on PB-nt membranes connected in parallel. PB-nt side of PB-nt membrane was employed both as cathodic electrode and fuel reservoir, the opposite of the PB-nt membrane sputtering coated by platinum was served as anode. The two-electrode system was adopted for the performance tests.

4.2 Materials and methods

4.2.1 Chemicals

Nanoporous alumina membranes with diameter of 13 mm and pore size of 200 nm (AnodiscTM) were purchased from Whatman (Maidstone, Kent, U.K.). Hydrogen chloride with concentration of 37% was bought from P. P. Chemicals. 35% H_2O_2 was obtained from Alfa Aesar, and KCl was from Sinopharm Chemical Reagent Co. Ltd. (SCRC). The potassium hexacyanoferrate(III) and Nafion[®] perfluorinated resin solution were purchased

from Sigma-Aldrich. The anhydrous ferric chloride was required from Merck, and tris(hydroxymethyl)-aminomethane from Bio-Rad Laboratories. Ultrapure water purified with the Sartorius Ultrapure Water System was used to prepare the solution.

4.2.2 Instrumentation

An auto Fine Coater (JEOL JFC-1600) has been used to sputter coating platinum layer onto the mesoporous alumina membrane. EDAQ EA161 and eDAQ e-corder 401 have been used for preparation of PB-*nt* membrane and evaluation of the performance of the fuel cell sensor. Electrochemical workstation (760E) has been used for monitoring the open-circuit potential of the fuel cell setup.

4.2.3 Fabrication of the fuel cell sensor

In the fabrication of the hand-hold H₂O₂ fuel cell sensor, the method for preparation of PB-*nt* membrane has been improved compared to the previous method [19]. A thin layer of platinum was firstly sputtering coated onto both sides of alumina membrane (AAO) with pore size of 200 nm. Then the conductive membrane with one side employed as working electrode and another side as counter electrode was subsequently performed potential cycling from -0.5 to +0.6 V at 50 mV s⁻¹ for 15 cycles in a mixture of 5.0 mM K₃Fe^{III}(CN)₆, 5.0 mM FeCl₃, 0.1 M KCl and 0.01 M HCl for 2 times. After the finish the potential sweep, a blue coloured membrane has been obtained, which indicated the formation of PB-nanotubes onto the membrane. After drying at room temperature for overnight, 15 µL of nafion solution was dropped onto the side without PB-*nt* of the membrane to penetrate into the nanochannels of the membrane. After that, four pieces of PB-*nt* membrane were connected in parallel using the conductive aluminium tape to set up a fuel cell sensor device as shown in figure 1. Due to the difficulty in operation of the

three-electrode system under field conditions and in practical applications, two-electrode system was adopted during the examinations and measurements.

4.3 Results and discussion

4.3.1 Electrochemistry of PB-*nt* membrane electrode with H₂O₂

Since PB is not stable in alkaline solution, the applications and electrocatalytic performance of PB are always operated under acidic conditions [20, 21]. However, there are few reports regarding to the electrocatalytic properties of PB under neutral condition [22, 23]. In order to demonstrate the electrocatalytic activity of PB in a neutral solution, cyclic voltammetry has been carried out to examine the electrochemical catalytic reduction of H₂O₂ over PB-*nt* membrane within a solution containing 0.5 M KCl and 5 mM H₂O₂. Figure 4.1 shows the typical cyclic voltammograms (CVs) of a platinum-coated membrane and a PB-*nt* membrane in the presence of H₂O₂ at a scan rate 50 mV S⁻¹. There was no redox peaks on the Pt-coated membrane electrode even when the concentration of H₂O₂ was increased to 10 mM, meanwhile the violent bubbles were observed, which indicated that the H₂O₂ on Pt-coated membrane was decomposed into water and oxygen rather than reduced into water. As shown in Figure 4.1B, it is obvious that the normalized cathodic peak current is significantly enhanced comparing with the normalized anodic peak current in the presence of H₂O₂. The enhancement of the cathodic current demonstrates the reduced PB form of Prussian white (PW) can be re-oxidized to the PB form at the cathode in presence of H₂O₂, thus the interconversion of PB and PW at cathode afford high electrocatalytic activity towards H₂O₂.

Figure 4.1 Cyclic voltammograms of (A) a PB-*nt* membrane (black) and Pt-coated membrane (red) in the presence of H_2O_2 , and (B) a PB-*nt* membrane in absence and presence of H_2O_2 . The measurements were performed in a solution of 0.5 M KCl or a solution of 0.5 M KCl with 5mM H_2O_2 with scan rate at 50 mV S^{-1} . The current signals were normalized to the anodic peak current of the voltammograms of PB-*nt* membrane in absence and presence of H_2O_2 in figure (B).

4.3.2 Open-circuit potential

The OCP of the H_2O_2 fuel cell sensor has been monitored for 300 seconds before running the polarization curves. Figure 4.2 gives open-circuit potential of the H_2O_2 fuel cell sensors based on single PB-*nt* membrane and 4 pieces of PB-*nt* membrane. The fuel cell based on single PB-*nt* membrane produced an OCP of 0.43 V, whereas the fuel cell sensor based on the 4 pieces of PB-*nt* membrane could provide an OCP of 0.54 V which was comparable to the theoretical driving force of the H_2O_2 fuel cell (refer to cell reactions).

Figure 4.2 Open-circuit potential of the H_2O_2 fuel cell sensors based on single PB-*nt* membrane and 4 pieces of PB-*nt* membrane.

4.3.3 Performance

Linear potential sweep voltammetry has been used to examine the performances of the H_2O_2 fuel cell sensors based on PB-*nt* membrane. The steady-state current-potential

curves have been obtained in a solution containing 0.5 M KCl and 10 mM H₂O₂ under saturated nitrogen condition. Figure 3 presents the power density and the polarizations curves of the fuel cell sensors based on single PB-*nt* membrane and four pieces of PB-*nt* membrane. The current output of the fuel cell sensor based on four pieces of PB-*nt* membrane connected in parallel increases ~3 times comparing to current output of the fuel cell sensor based on single PB-*nt* membrane, while the open-circuit potential is just increased by 0.1 V. In the presence of 10 mM H₂O₂, the power density of the fuel cell sensor based on four PB-*nt* membranes in parallel can reach to a maximum power output value of 0.7 mW cm⁻² which is ~3 times of the power density output of the single PB-*nt* membrane fuel cell sensor.

Figure 4.3 Current-potential (*I-V*) and current-power (*I-P*) curves of the H₂O₂ fuel cell sensors based on four pieces of PB-*nt* membrane connected in parallel. Performance test was carried out within 400 μ L of 0.5 M KCl solution containing 10 mM H₂O₂. Currents and powers were normalized by a geometric surface area of electrode.

To further evaluate the performance of the fuel cell based on the PB-*nt* membrane connected in parallel system, the fuel cell constructed by using two pieces of PB-*nt*

membrane has also been examined by linear sweep voltammetry as shown in Figure 4.4. It gave a maximum power density of $\sim 0.45 \text{ mW cm}^{-2}$ at the cell voltage of 0.22 V.

Figure 4.4 Current-potential (I - V) and current-power (I - P) curves of the H_2O_2 fuel cell sensors based on two pieces of PB-*nt* membrane.

4.3.4 Analytical performance

The analytical performance of fuel cell sensor based on four PB-*nt* membranes has been monitored by recording the amperometric response towards varying concentrations of H_2O_2 solution using a micro-current meter. Figure 4 shows the amperometric signal response of the fuel cell towards different concentrations of H_2O_2 solution and presents an excellent linear relation between the current signals and low concentrations of H_2O_2 solution. However, the linear relationship was not applicable for high concentration of H_2O_2 solution.

Figure 4.5 Amperometric signals of four pieces of PB-*nt* membrane fabricated fuel cell sensor towards various concentration of H_2O_2 .

The standard deviation of reproducible responses obtained from three different fuel cells was not satisfying (shown in the Figure 4.5). The reason is the current signal is strictly affected by the amount of the total PB loading onto the membrane. Before electrodeposition of PB onto the alumina membrane, a thin layer of platinum nanoparticles was sputtering coated onto the membrane surface and nanochannels. However the sputtering coating of the platinum did not offer a smooth layer but just sputtering nanoparticles onto the membrane surface and channels which resulted in the poor conductivity of the membrane [17]. As a result, the PB coating onto each membrane cannot be identical to each other. Therefore, each PB-*nt* membrane towards H_2O_2 may not give same response. However, our PB-*nt* membrane fuel cell can be reused after a thorough washing, and the typical standard error is just ~8 % when reusing the same fuel cell which is quite useful for analytical sensors.

4.4 Conclusions

A high performance H₂O₂ fuel cell sensor based on four pieces of PB-*nt* membrane has been successfully fabricated, in which PB-*nt* membrane was applied both as electrodes and reservoir cell in a two-electrode system as an effort to simplify the fuel cell sensor. It gave an OCP of 0.54 V and a maximum power density of 0.7 mW cm⁻² at 10 mM H₂O₂ in a neutral solution. Additionally, such fuel cell sensor presents an ideal analytical performance responding to the low concentrations of H₂O₂ solution. This small energy output fuel cell design offers a dramatic potential for realisation of the portable sensor devices since it just requires a small amount of solution for measurements and a multi-meter to transduce the responding signals.

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Chapter 5

Summary

Chapter 5 Summary

This thesis has presented the research studies on the exploration of Prussian blue nanotubes membrane electrode for development of fuel cell sensors and biosensors. This kind of fuel cell sensors using PB-*nt* membrane as cathode and chemical as power source to drive the redox reaction, exhibits significantly low cost advantage over current state-of-the-art energy storage devices and solar powered devices, and offers high possibility for portable sensor device.

In chapter 2, we have described a self-powering amperometric sensor and biosensor based on two-compartment Galvanic cell design for detection of H_2O_2 and glucose. This unique fuel cell sensor has not been applied any electrical potential to drive the redox reaction between analyte and electrode, mediator or electrocatalytic species to produce the electrochemical responding signal. Through analysis of the responding signals and fitting curves, it was observed this fuel cell based sensor gave an outstanding analytical performance towards H_2O_2 . Coupled with glucose oxidase, the fuel cell sensor can be extended for design a glucose biosensor. The proof-of-concept glucose biosensor derived from the hydrogen peroxide sensor is demonstrated in the presence of a homogeneous solution of glucose oxidase enzyme, indicates possibility for other oxidase enzyme-based biosensors.

Based on the research studies in chapter 2, we demonstrated a two-compartment fuel cell virus biosensor powered by H_2O_2 for the direct detection of *unlabeled* virus particles based on the formation of antibody-virus complexes within the biosensor's membrane nanochannels in chapter 3. This fuel cell virus sensor powered by chemical of H_2O_2 offers significantly low cost advantage over current state-of-the-art energy storage and fuel cell sensors.

To simplify the sensor device, the integrated PB-*nt* membrane when filled with Nafion demonstrates powerful utilization as a standalone virus probe without input of electrical power, which offends the outstanding promise of a sustainable, low cost and rapid H₂O₂-powered virus detection tool.

In chapter 4, we reported a hand-held H₂O₂ fuel cell sensor based on four pieces of standalone Nafion-filled PB-*nt* membrane connected by parallel. This H₂O₂ fuel cell sensor was constructed using four standalone nafion-filled Prussian blue nanotubes membranes connected in parallel, which employed the PB-*nt* membrane as both electrodes and fuel reservoir. An open-circuit potential of 0.54 V with a maximum power density of 0.7 mW cm⁻² has been achieved at a low concentration of 10 mM H₂O₂ solution. The volume of the solution required for the whole measurements can be limited to a few hundreds microliters. The analytical performance of the fuel cell sensor was directly monitored using a micro-current meter to record the responding signal towards varying H₂O₂ concentrations, which offered portable sensor device.

Publications

1. Wei, Y. Y.; Lei, Q.; Lee, J. Y. Q.; Ba, S.; Zhang, H.; Li, T. H.; Toh, C. S. Hand-hold fuel cell sensor device based on Prussian Blue nanotubes membrane, to be submitted.
2. Wei, Y. Y.; Wong, L. P.; Toh, C. S. Fuel cell virus sensor using virus capture within antibody-coated nanochannels, *Anal. Chem.*, **2013**, 85, 1350-1357.
3. Wong, L. P.; Wei, Y. Y.; Toh, C. S. Self-powered amperometric sensor and biosensor, *J. Electroanal. Chem.*, **2012**, 671, 80-84.

Conference paper

1. Wei, Y. Y.; Wong, L. P.; Toh, C. S. Self-Powering Amperometric Sensor and Biosensor, 18th Australian Electrochemistry Symposium, 15th April 2012, Perth Australia.
2. Wei, Y. Y.; Wong, L. P.; Toh, C. S. Self-powered virus sensor capture within antibody-coated nanochannels, Singapore International Conference on Dengue and Emerging Infections, 21-23 November 2012, Singapore.