Performance-enhanced biosensors constructed using one-dimensional nanostructured materials

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**PERFORMANCE-ENHANCED BIOSENSORS
CONSTRUCTED USING ONE-DIMENSIONAL
NANOSTRUCTURED MATERIALS**

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Summary

One-dimensional (1D) nanostructured materials possess unique electronic, optical and mechanical properties owing to their nanoscale dimension and high aspect ratio nature. At nanoscale, the direct interaction between the size-comparable transducer elements and the individual target biomolecules produces great opportunities for the construction of performance-enhanced biosensors. Although various nanomaterials-based biosensors have been developed, it remains a great challenge to synthesize 1D nanostructured materials with specific properties and to tailor the 1D nanostructure-based biosensors for multiple detections with rapid response, high sensitivity, and good stability. Therefore, two main objectives were set in this thesis: (1) to synthesize 1D nanostructures of conductive polymers (CPs) with special properties; (2) to construct high performance biosensors by employing 1D nanostructured materials and building novel architectures through nanoengineering approaches.

To fulfil the goals, several strategies were employed in this study: (1) to electrochemically synthesize 1D nanostructured CPs with novel chemical and physical properties by developing a template-free synthetic approach, construct biosensors by using the synthesized 1D nanostructured CPs, and further apply the constructed sensors to detect glucose; (2) to investigate the mechanism of the formation of 1D nanostructured CPs; (3) to enhance the performance of 1D nanostructure-based biosensors by developing novel nanoengineering approaches;

and (4) to design and fabricate economic arrayed biosensor chips for the multiple detections of lactate and glucose.

In this thesis, a new template-free electrochemical synthetic route has been developed to fabricate 1D nanofiber structures of conductive polypyrrole (PPy) and its formation mechanism was proposed with significance in materials science. The non-covalent interactions are the driving forces in the formation of 1D nanostructure, which also lead to the successful growth of a well-aligned cone-shaped nanostructure of PPy. The 1D nanostructures used in this work for biosensor applications include both home-made and commercially available 1D nanomaterials such as PPy nanofiber and carbon nanotube (CNT). These materials were employed to construct high performance biosensing devices by nanoengineering approaches. The PPy nanofiber is superhydrophilic so that it targets biomolecules like glucoses, which exhibits fast diffusion rates in the nanofiber-incorporated sensing layer. Meanwhile PPy nanofiber contains high specific surface, which results in high electrocatalytic sensitivity. Thus, a very fast response glucose sensor is developed. On the other hand, CNT increases the sensitivity and stability of the designed biosensors. The specific surface area of powder microelectrode is greatly increased when the electrode is incorporated with CNT, thus the sensitivity of CNT-based nitric oxide sensor is significantly enhanced. Furthermore, printed circuit board-based electrode array is modified with CNT-integrated sensing layer, and is utilized to realize the highly sensitive, simultaneous detection of glucose and lactate.

In summary, this work not only investigates nanoengineering approaches to synthesize novel nanostructure materials and architectures for performance enhanced biosensors, but also explores the mechanism of nanostructure formation and electrocatalytic behaviors with great significance in materials and catalysis science. The constructed biosensors may have potential applications in diagnostics, life science, environmental science and food analysis.

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Chapter 1. Introduction

1.1 Characteristic Structures and Properties of Nanomaterials

1.1.1 Overview

Advances in the capability of manipulating and measuring matter on the nanometer level have paved the way to an exciting era in the discovery of nanostructured materials with novel properties and further applications of nanomaterials in industry. In the past decades numerous research activities have been carried out in this challenging area.^[1-3] Different sorts of nanomaterials have been discovered, such as fullerenes,^[4, 5] carbon nanotubes (CNTs)^[6] and nanoparticles.^[7, 8] Nanoscience and nanotechnology have developed into fast-growing fields.^[9, 10] In fact, today's state-of-the-art nanotechnology plays a key role in a variety of modern industrial processes, including nanoelectronics,^[11, 12] nanocatalysis,^[13, 14] nanomedicine,^[15, 16] and so on.

The rapid growth of nanotechnology is attributed to the superior characteristics of nanomaterials. In principle, scaling down the size of matter to nanometer range allows revolutionary changes of the basic properties of matter.^[17, 18] In general, these unique properties include high specific surface area, enhanced chemical reactivity and diffusion capability. A wealth of new interesting phenomena have been revealed to be associated with nanomaterials, including size quantization (i.e. blue-shift in optical spectra), single-electron charging, single-charge effects, depletion of charge, ballistic transport, luminescence from gold nanoparticles and metal-insulator transitions.^[19] For example, CNTs have the advantages of high

mechanical strength, excellent heat conduction, high chemical stability and other interesting electrical and electronic properties.^[20, 21]

1.1.2 Classification of Nanostructures

Principally, nanostructured materials are materials with structural elements - clusters, crystallites or molecules - confined in the 1-100 nm range at least in one dimension. By comparing the lengths of the nanostructures in three orthogonal dimensions, i.e. L_x , L_y , L_z , with the critical nanoscale length L_o , nanostructures are usually classified into zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D) and three-dimensional (3D) subgroups (Table 1.1). Nanoparticles, such as quantum dots which exhibit quantum effects, are typical examples of 0D nanostructures. 1D nanostructures include nanotubes, nanowires and nanofibers. 2D nanostructures include nanoplates and thin films. 3D structures can be considered as nanostructures if 0D, 1D or 2D nanostructures are involved.^[22] In this thesis I focus on the synthesis and application of 1D nanostructured materials. Here I give a brief introduction to 0D and 2D nanostructures and the 1D nanostructured materials will be discussed in more detail in the next section.

Zero-D Nanostructures

Recognized as 0D nanostructures, nanoparticles are of great scientific interest as they exhibit distinctly different physical and chemical properties from those bulk materials and atomic or molecular structures. The edge of the valance and conduction bands split into discrete, quantized, electronic levels when the size of crystals reaches nanoscale.^[23] The size quantization is because the size of a nanoparticle is comparable to the de Broglie wavelength of its charge carriers, i.e.

electrons and holes. The physical properties of a bulk material usually keep constant regardless of the size of the material. On the contrary, nanomaterials exhibit unique size-dependent properties. Examples of such size-dependent properties include melting point, electronic absorption spectra, quantum effect in semiconductor particles, surface plasmon resonance in metal particles and superparamagnetism in magnetic materials.^[17] Metal nanoparticles of 1 to 2 nm diameter exhibit unexpected catalytic activity, which has great potential application in industry.

Table 1.1 Examples of reduced-dimensional material geometries, definitions of the dimensionality of nanomaterials and their associated types of confinement.

Lengths	Confinement	Dimensionality	Type	Illustration
$L_{x,y,z} > L_0$	None	No Nanostructures	Bulk Material
$L_x > L_0 > L_z$	1D	2D Nanostructures	Layer or Film
$L_x > L_0 > L_{y,z}$	2D	1D Nanostructures	Nanowire or Nanotube
$L_0 > L_{x,y,z}$	3D	0D Nanostructures	Nanoparticle

There are several methods to produce nanoparticles. Attrition and pyrolysis are two common methods.^[24, 25] Macro or micro scale particles are ground in a ball mill or a planetary ball mill to nanoparticles in attrition method. In pyrolysis,

nanoparticles were achieved when an organic precursor (liquid or gas) is forced to pass an orifice at high pressure and burned. Nanoparticles have been synthesized by homogeneous nucleation from liquid or vapour, or by heterogeneous nucleation on substrates.^[26] Quantum dots can be prepared by phase segregation through annealing solid materials at elevated temperatures. Nanoparticles can be synthesized by confining chemical reactions, nucleation and growth processes in a small space such as micelles. In general, the synthesis methods can be grouped into two categories: thermodynamic equilibrium approach and kinetic approach. In the thermodynamic approach, synthesis process consists of three steps: generation of supersaturation, nucleation, and subsequent growth. The kinetic approaches include microemulsion, aerosol pyrolysis and template-based deposition.^[26]

Two-D Nanostructures

Two-D nanostructures possess a nanoscale dimension only in the *z*-axis and infinite length in the *x-y* plane, including monolayer and thin film. The 2D nanomaterials have been utilized in area such as electronics and catalysis.^[27-30] In particular, recent developments of 2D nanosheet crystals, such as stable graphene and transition-metal chalcogenides, have sparked new interest in condensed-matter physics and electronics.^[31] Further miniaturization of these 2D structures by lateral confinements is potential in modulating electron-transport phenomena and enhancing their 2D host capabilities, which arise from the enlarged surface area and improved diffusion processes upon the intercalation of guest molecules.^[32]

Deposition of thin films has been intensively studied for almost a century.^[33, 34]

Various methods have been developed and many of them have been widely

employed in industries,^[35] which in turn becomes a great driving force for the further development and improvement of the deposition techniques. Film growth methods can be categorized into two groups: vapour-phase deposition and liquid-based growth. The former approach includes evaporation, molecular beam epitaxial sputtering, chemical vapour deposition, and atomic layer deposition. Examples of the latter category are electrochemical deposition, chemical solution deposition, Langmuir-Blodgett films and self-assembled monolayer. In general, the thin film deposition involves predominantly heterogeneous processes including heterogeneous chemical reactions, evaporation, adsorption, and desorption on growth surfaces, heterogeneous nucleation and surface growth.

1.2 One-D Nanostructures

One-D nanostructures have recently become the focus of intensive research as they are ideal building blocks for the nanoscale electronic, photonic electromechanical, and electrochemical devices.^[36] Many 1D nanostructures exist, including nanowires, nanofibers, and nanotubes of metals (Au, Pt, Ni), semiconductors (Si, InP, GaN, ZnO, SnO₂, CPs, etc), and insulators (SiO₂, TiO₂). 1D nanostructured materials provide a good system for the investigation of the dependence of electrical and thermal transport or mechanical properties on material dimensionality and size reduction. This is because electrons in 1D nanostructure are confined laterally and thus occupy energy levels that are different from the traditional continuum of energy levels of bands found in bulk materials.

The interface between 1D nanostructure and biological and therapeutic sciences is expected to bring the most promising impact to biosensors and bioelectronics. However, the applications of 1D nanostructured materials were hindered by the difficulties in synthesis of 1D nanomaterials.^[36] A variety of techniques have recently emerged in the synthesis of 1D nanostructured materials with special properties. In the book written by Cao *et al.* these approaches were grouped into four categories: (1) Spontaneous growth, including evaporation (or dissolution)-condensation, vapor (or solution)-liquid-solid (VLS or SLS) growth, stress-induced recrystallization; (2) Template-based synthesis, including electroplating and electrophoretic deposition, colloid dispersion, melt, or solution filling, and conversion with chemical reaction; (3) Electrospinning; (4) Lithography.^[26] Spontaneous growth, template-based synthesis and electrospinning are considered as bottom-up approaches, whereas lithography is a top-down technique. Spontaneous growth commonly forms single crystalline nanowires or nanorods along a preferential crystal growth direction, depending on the crystal structures and surface properties of the nanowire materials. In contrast, template-based synthesis produces polycrystalline or even amorphous products, which are usually less conductive compared to 1D nanostructures of metals and conductive polymers (CPs). In this thesis I developed a novel method in synthesis of 1D nanostructures of CPs, hence I will give a more detailed review on the synthesis of 1D nanostructure of CPs in Chapter 2.

1.3 Introduction to Biosensors

1.3.1 Biosensors Configuration

A biosensor is an analytical device that responds to a target analyte and interprets its concentration as an electronic/optical signal by incorporating a biological recognition system and a physicochemical transducer. Since the first glucose sensor was developed by Clark and Lyons in 1962 ^[37], biosensors have been extensively employed to a wide variety of applications in clinic, environment, and food analysis.

Biosensors essentially are comprised of two key components: a bioreceptor and a transducer. As shown in Scheme 1.1, the bioreceptor interacts with the analyte and responds in the manner that can be detected by a transducer. The bioreceptor is a crucial component and its function is to impart selectivity so that the sensor responds only to a particular analyte such as the biomolecules of interest, and interferences from other substances are avoided. The transducer is the other key component of the biosensor, which transfers the biorecognition event into an electrical signal. Common transducers include electrochemical, optical, and piezoelectric platforms.^[38] In this thesis our objective was to develop nanomaterial-based electrochemical biosensors which target on glucose, lactate and nitric oxide. Hence in the following sections I will introduce the background of each sensor type separately.

Scheme 1.1 Schematic illustration of a typical biorecognition process.

1.3.2 General Requirements and Bench Marks for Sensor Designs

- Fast response: The detection of the target molecules should be as fast as possible, like within 5 mins;
- Accuracy: The detection of analytes must be with minimal errors at the presence of interfering species (usually deviation should within 5%);
- Sensitivity: It should have a large signal to noise ratio, and a detectable signal must result from small changes in lactate and glucose concentrations ($0.3\text{-}3\text{ nA mM}^{-1}$)
- Range: All glucose concentrations in physiological and pathophysiological range from 1 to 30 mM and lactate from 0.1 to 10 mM is preferable for clinic application. The detection of nitric oxide must be in the range of 1-50 μM . However, for the application in laboratory, food and environmental industry, the range will be varied depending to the specific application.

- **Stability:** The signal to analytes should be stable (within $\pm 5\%$) for its average value during the operational time of the measuring instrument.

1.3.3 Background of Glucose, Lactate and Nitric Oxide Sensors

Diabetes mellitus is characterized by disordered metabolism and abnormally high hyperglycemia due to deficient levels of the hormone insulin. The most characteristic symptom is the deviation of the blood glucose concentration from the normal range of 4.4 -6.6 mM.^[39] Thus a sensitive and accurate glucose sensor is of fundamental importance in clinical diagnostics. Blood glucose sensing for diabetics is by far the largest biosensor market. The reason is that no other disease or illness both afflicts such a large proportion of the population ($\sim 1\%$) and required for frequent (on average 2 to 4 times per day) recording of clinical data (i.e. blood glucose concentration). Glucose sensor thus plays a central role in stimulating biosensor research and results in successful commercialization.

Although the determination of lactate is not so frequently conducted compared to the detection of glucose, it has attracted increasing attention in clinical chemistry, sports medicine and food analysis. For example, the monitoring of serum lactate levels in infants has been found to be useful in the prevention of a mother-to-child transmission of HIV.^[40] The recent report has confirmed that high lactate levels are an accurate predictor of a poor outcome in fulminant hepatic failure, a sudden and severe shutdown of the liver.^[41] The monitoring of lactate has become popular to assess athletes' fitness and training, particularly in endurance sports such as

swimming, rowing, cycling, speed skating and weight lifting. In food industry, fermentation or preservation requires precise determination of the lactate levels to improve the quality management.

Nitric oxide (NO) is another target molecule to detect in this thesis. NO is released by many cells in mammalian systems; it plays a number of essential biological roles such as neurotransmitter, cytostatic agent and blood pressure regulator. It has also been implicated in the pathogenesis of several diseases, due to deficient or excessive concentration of NO.^[42-44] From a biochemical as well as a medical perspective, it is important to quantify the generation of NO in abnormal and normal tissues, including *in vivo* measurements. However, NO reacts rapidly with hemoglobin, oxygen and other biological species *in vivo* or *in vitro*. In the presence of superoxide, NO is rapidly converted to peroxynitrite. Consequently, NO has a half-life period of two to six seconds *in vivo*. Thus, the detection of NO is still a challenge.

1.3.4 Electrochemical Biosensors

More than half of the biosensors in the detection of pathogens and viruses are based on electrochemical transducers.^[45] Electrochemical transduction possesses several appealing advantages, such as low cost (compared to spectroscopic method), high sensitivity, and ease of fabrication. In addition, electrochemical sensors can be easily miniaturized, which suits well to today's advanced microfabrication technology. Besides, electrochemical detection requires relatively simple instrumentation and thus less energy consumption. These characteristics are highly attractive for the commercialization and practical applications of biosensors.

Several electrochemical approaches, e.g. amperometric, potentiometric and impedance measurements, have been employed to monitor the signal changes that reflect the concentration changes of the target analyte during detection. The guideline on which approach to choose is based on the type of signals, such as redox, ionic and conductivity, which originate from the properties of the analyte and matrix. In this thesis, I focus on amperometric biosensors which are based on redox enzymes. A more detailed review on electrochemical biosensors will be given in Chapter 2.

1.3.5 Nanotechnology-Based Biosensors

The convergence of nanotechnology and biological events generates breakthrough opportunities in medical diagnosis, drug delivery and environmental industry. For example, Lieber's group has achieved highly sensitive, label-free, multiplexed electrical detection of cancer markers using silicon-nanowire field-effect devices in which distinct nanowires and surface receptors are incorporated into arrays.^[46] The nanowire arrays allowed highly selective and sensitive multiplexed detection of prostate specific antigen (PSA), PSA- α 1-antichymotrypsin, carcinoembryonic antigen and mucin-1. In addition, nucleic acid receptors enabled real-time assays of the binding, activity and small-molecule inhibition of telomerase using unamplified extracts from as few as ten tumor cells.^[46] Dai's group prepared a solution of SWCNTs wrapped in poly(ethylene glycol) (PEG) with a tumour-targeting cyclic arginine-glycine-aspartic acid peptide to the end of the PEG chains.^[47] This solution was injected into mice bearing tumours and it was observed that the targeted SWCNTs accumulated in tumours. Thus potential drug delivery applications have been achieved and demonstrated with efficient *in vivo*

accumulation of SWCNTs in mice tumours.^[47] Nanotechnology can also contribute to environmental protection by reducing the consumption of raw materials, limiting the emission of pollutants, and cleaning up contaminants in soil and water.^[48] In all, the combination of nanomaterials with biosensors opens an exciting area with great potential applications in complex diseases diagnosis, drug delivery and environmental protection.

1.4 Motivation

Compared with other nanostructures like nanoparticles and nanosheets, the advancement of 1D nanostructure was slow until very recently. The progression was originally hindered by the difficulties associated with the synthesis and control of the nanostructure with well-controlled dimensions, morphology, composition and chem.-physical properties. 1D nanostructures can now be fabricated by using a number of advanced nanolithography techniques, such as electron-beam^[49] or focused-ion-beam writing,^[50] proximal-probe patterning,^[51] and X-ray and extreme-UV lithography.^[52] However, the further development of the practical routes to efficiently produce 1D nanostructures with specific chemical and physical properties for biosensor application at reasonably low cost, still requires great ingenuity.

As introduced in section 1.2, 1D-nanostructured materials possess unique electronic, electrocatalytic and mechanical properties, which provide great opportunities for the construction of performance-enhanced biosensors. Although great progresses have been achieved in fabrication and applications of the 1D nanomaterial in biosensors, many challenges and technical barriers remain. The

biocompatibility and bioconjugation of 1D nanomaterials need to be further investigated for safety, reliability and feasibility in biosensing applications.^[53] It remains a great challenge to tailor the 1D nanostructure-based biosensors for multiple detection with rapid response, high sensitivity, and good stability. Unconventional methods based on electrochemical synthesis may provide an alternative intriguing strategy for generating 1D nanostructures that matches the requirements of material diversity, low cost and high efficiency. Compared with other methods, the advantage of electrochemical synthesis is that the production process is relatively simple, i.e. well-controlled 1D nanostructure can be formed on a microelectrode surface through direct electrochemical deposition. In short, electrochemical synthesis opens up a new opportunity for designing nanomaterial-based devices. Therefore, in this thesis I set out to synthesize 1D nanostructured materials by using electrochemical methods.

The key point to detection is the transduction of a signal associated with the selective recognition of species of interest. A few approaches have been reported for this target, such as enzyme-linked immune Sorbent Assay (ELISAs)^[54] and surface plasmon resonance.^[55] Each of these approaches has its own advantages. However, none of them has been demonstrated to match every requirement of rapid, highly sensitive and multiple detections of biomolecules. The 1D nanostructures used in this work, including both home-made and commercially available 1D nanomaterials such as PPy nanofiber and CNT, were employed to construct high performance biosensing devices by nanoengineering approaches.

The functionalities of PPy nanofiber and CNT and their correlation with the enhanced performance of the biosensors constructed here are described here. For PPy nanofiber, its superhydrophilic network formed by PPy nanofiber (in Chapter 4) provides an ideal platform for the high performance biosensor construction. The small diameter of the nanofiber, high-density hydrophilic function groups, and homogeneous distribution of the nanofiber in micro scale, make the diffusion of the substrate very fast during measured, and enhanced chemical reactivity, which leads to a fast response time and a high sensitivity of the biosensor constructed using PPy nanofiber fabricated in the thesis. There are three prominent properties corresponding for the CNT in the construction of CNT based biosensors. First, CNT can bring in the good electrocatalysis toward electrooxidation of substrate (like nitric oxide in Chapter 5) and electron mediator (like PVI-Os in Chapter 6 and 7). Second, its excellent conductivity makes it a desirable electron transport channel in nanoscale in biosensing layer (in Chapter 5, 6 and 7), effectively shuttling the electrons between enzymes and macroelectrode. Third, the good mechanical properties of the CNT make the sensing layer stable during operation. These nanostructures represent excellent primary transducers for producing signals that ultimately interface to macroscopic instruments. The unique and interesting properties introduced by these 1D nanostructures give us great opportunities to construct a variety of performance-enhanced biosensors, namely glucose sensor, lactate sensor and nitric oxide sensor.

1.5 Objectives

Two main objectives were set in this thesis: (1) to synthesize 1D nanostructure of CPs with special properties; (2) to construct high performance biosensors with 1D

nanostructured materials and novel architectures through nanoengineering approaches.

To fulfil the goals several strategies are set in this study: (i) to template-free electrochemically synthesize 1D nanostructured CPs with novel chemical and physical properties, to construct biosensors based on the synthesized 1D nanostructured CPs and to further apply the sensors to detect glucose; (ii) to investigate the mechanism of the formation of 1D nanostructured CPs; (iii) to enhance the performance of 1D nanostructure-based biosensors by developing novel nanoengineering approaches; iv) to design and fabricate economic arrayed biosensor chips for the multiple detection of lactate and glucose.

In summary, this thesis will investigate the synthesis of 1D nanostructured materials, i.e. the conductive polypyrrole (PPy), which exhibits superior chemical and physical properties. I systematically investigate its formation mechanism, which could be extended to the synthesis of other 1D nanostructures. I further investigate the biosensing performance of 1D nanostructured PPy, i.e. to make glucose sensor. The detection of glucose is of fundamental importance in the diagnosis of diabetic diseases and food industries. Therefore, by performing these approaches, I hope to develop an economic route to construct glucose sensor with fast response and high sensitivity. On the other hand, I also set out to examine the biosensing performance of the other 1D nanomaterial, CNT. I design various CNT-based biosensors by nanoengineering to detect NO, glucose and lactate. In all, this thesis was to construct high performance biosensors based on the two types of 1D nanomaterials, i.e. 1D CPs and CNT.

1.6 Major Contributions of the Thesis

The contributions of the thesis are summarized as follows:

- (1) A new template-free electrochemical synthetic approach is developed to form a homogenous PPy nanofiber network with superhydrophilic property. I also propose a new template-free formation mechanism with significance in materials science, in which the non-covalent interactions are the driving forces in the formation of 1D nanostructure. This approach could be extended to the synthesis of other 1D nanostructures.
- (2) A new well-aligned cone-shaped nanostructure of PPy is formed based on the above template-free electrochemical synthetic method. The formation of 1D nanostructure in the array architecture can be interpreted by the above template-free formation mechanism. The formation of well-aligned architecture is attributed to the steric hindrance effect, which was reported to form well-aligned CNT.
- (3) The PPy nanofiber is superhydrophilic to target biomolecules like glucose with fast diffusion rates in the nanofiber-incorporated sensing layer, while its high specific surface provides high electrocatalytic performance for high sensitivity. Thus, a very fast response glucose sensor is developed.
- (4) CNT incorporated with powder microelectrode greatly increases the specific surface area of the electrode, thus the sensitivity of CNT-based nitric oxide sensor is significantly enhanced. The electrochemical behaviour of the nanocomposite microelectrode is systematically investigated and the sensing mechanism is analyzed.

- (5) A novel nanocomposite-based lactate sensor is constructed by engineering a networked nanostructure with CNT, selected matched material components at an optimized composition ratio. CNT is employed to increase the sensitivity and stability of the designed lactate biosensor. The novel nanoengineering approach could be extended to other enzyme biosensors.
- (6) The printed circuited board-based electrode array is modified with CNT-integrated sensing layer, and utilized to realize the highly sensitive, simultaneous detection of glucose and lactate in mouse serum. Due to its low cost, chemical-tolerant and interference free, the application of the presented system is potential to extend to other biosensors for simultaneous measurements.

1.7 Organization of the Thesis

This thesis is devoted to investigate the synthesis of 1D nanostructure of CPs, the constructions of high performance biosensors using 1D nanostructured materials and novel architectures through nanoengineering approach, and to explore the scientific fundamentals for biosensing enhancement. Chapter 1 gives a general introduction to nanostructured materials and biosensors. The motivation, objectives, and major contribution of this study are also introduced in this chapter. Chapter 2 describes a literature review on the synthetic approaches of 1D nanostructured CPs. The basic theory of biosensor and progress of nanomaterials-based biosensors are also described. Chapter 3 gives the general description of the experimental setup, chemicals and instrumentations. Chapter 4 describes a one step, template-free electrochemical polymerization method to develop 1D nanostructures of PPy. A

template-free formation mechanism is proposed to interpret the formation process. The 1D nanostructures of PPy were characterized and used to build a fast-response glucose sensor. The well-aligned 1D PPy nanostructures are also synthesized by a one-step, template-free electrochemical polymerization method based on the above mechanism. The template-free formation mechanism for the aligned PPy nanostructure is also discussed. Chapter 5 describes the application of CNTs in a nitric oxide sensor. CNTs were incorporated in ionic liquid at room temperature to build a high sensitive nitric oxide sensor. Chapter 6 gives an engineering method which employs CNTs together with a polymerized electron mediator to construct a sensitive lactate sensor. Chapter 7 describes the developed engineering method is further used to build a glucose sensor. The developed lactate sensor and glucose sensor are integrated into a printed circulated board based array biosensors to detect the corresponding substrates in mouse serum. Chapter 8 summarizes the works done in this thesis and concludes the results, and some suggestions are made on the future research.

Chapter 2. Literature Survey

2.1 Synthesis of 1D Nanostructure of CPs

The central of 1D nanostructure formation is about crystallization for inorganic NWs or polymerization for CPs, a process that has already been investigated for hundreds years. The formation of a 1D nanostructure usually involves two fundamental steps: nucleation and growth. As the concentration of the building blocks (atoms, ions, or molecules) becomes sufficiently high, they aggregate into nuclei through homogeneous nucleation. With a continuous supply of the building blocks, these nuclei can serve as seeds for further growth to form larger structures. However, it is still a great challenge to quantitatively describe the process. Neither is to achieve a complete control over this process.

Figure 2.1 The chemical structures of common CPs. (1) poly-(*para*-phenylene), (2) poly(phenylenevinylene), (3) polypyrrole, (4) polythiophene, (5) polyfuran, (6) poly(heteroaromatic vinylenes), $Y=NH$, NR , S , O , (7) polyaniline. Taken from reference ^[57].

CP is defined as an organic polymer that possesses the electrical, electronic, magnetic and optical properties which are similar to that of a metal, while retains the common properties of conventional polymers, such as mechanical properties

and processibility.^[56] CP is an intrinsically conducting polymer and is commonly known as a synthetic metal. Fig. 2.1 shows the chemical structures of common CPs. Those polymers undergo either p- or n-redox doping through electrochemical or other chemical processes; during these processes, the number of electrons associated with the polymer backbone changes.

Generally, the synthetic approaches to form 1D nanostructured CPs can be categorized into hard template synthesis, soft template synthesis and template-free synthesis.^[58] In this section I will introduce the two template-based synthetic methods. The template-free methods will be discussed in more details in a separate section, as the synthetic method I developed in this thesis belongs to the template-free category.

2.1.1 Hard Template Synthesis

Hard template synthesis is one of the most widely used approaches to prepare freestanding, non-oriented and oriented 1D nanostructures of various materials, such as polyaniline and polypyrrole. These materials can be prepared by confining the polymerization in the channels of various templates, such as zeolites, anodized alumina membrane, and track-etched polymeric membranes.^[59-61] Other commonly used membranes include nanochannel array on glass, radiation track-etched mica, mesoporous materials, porous silicon, and CNTs.^[62] Martin and his coworkers are pioneers in this method.^[59] The typical way of hard template synthesis is to coat a metal film onto one surface of the membrane and use this film to electrochemically synthesize the desired polymer within the pores on the membrane. Chemical hard

template synthesis is accomplished by simply immersing the membrane into a solution of the desired monomer and its oxidizing agent.^[63]

During the synthesis of polymers in hard templates, an interesting phenomenon was observed: the polymer preferentially nucleates and grows on the pore walls. Therefore tubular structures are easy to form on the pore walls when the polymerization time is reduced.^[59] The solvophobic interaction between the polymer and the pore wall is the major factor that causes the polymer nucleates and grows preferably on the pore walls. Besides, there is also a contribution from the electrostatic interaction between the cationic polymer and anionic sites on the pore walls.^[59] The two non-covalent interactions, i.e. the solvophobic and electrostatic interactions, are also the major driving forces in our template-free synthesis of 1D nanostructured CPs. I will discuss them in more detail in Chapter 4 and 5.

One advantage of the hard template method is that it effectively produces 1D nanostructured materials with controllable diameter, length and orientation via hard template. Furthermore, enhanced conductivity of CPs prepared by this method was also reported, owing to the enhanced molecular and supermolecular order as polymer chains align along the pore walls.^[64] As mentioned above, the alignment of the polymer chains is induced by the non-covalent interactions in hard template synthesis. This understanding enlightened us to design a method for the template-free synthesis of nanostructures of PPy, as I will discuss in detail in Chapter 4 and 5. Another advantage for the hard template method is that it allows the formation of multilayers with different types of materials by multi-step processes.^[65]

The hard template method has several drawbacks. The preparation of the template is not always easy. The post procedure to remove the template usually needs strong acidic or basic solution, which could possibly damage the final product, and impurities could also be introduced during the treatment process. Besides, the structure of products depends on the structure of the pores. This also limits the variety of the products.

2.1.2 Soft Template Synthesis

Another interesting route to synthesize 1D nanostructured CPs is the soft template method. It employs structure-directing molecules as a soft template or molecule template, such as surfactants,^[66-69] organic acid with surfactant functionalities,^[70] DNA,^[71] polyelectrolyte,^[72] sulfonated porphyrin,^[73] liquid crystalline,^[74] and ethanol^[75]. The commonly used surfactants include sodium dodecylbenzenesulfonate (SDBS), hexadecyltrimethylammonium bromide (CTAB), camphorsulfonic acid (CSA), naphthalenesulfonic acid (NSA), azobenzenesulfonic acid (ABSA), etc. In a typical surfactants based procedure, monomers of desired polymers and surfactants with different molar ratios are mixed in certain amount of solvents. A transparent solution of monomer/surfactant salt is formed and then cooled down to a specific temperature. A solution of oxidant, usually ammonium peroxydisulfate (APS), is pre-chilled to the same temperature and then added to the transparent solution to initiate the polymerization. After a predetermined period of reaction time, the mixture is filtered, washed, and dried to obtain the 1D nanostructures of desired polymer.^[58] The proposed mechanism for the formation of the nanostructures is that the surfactants self-assemble into nano- or micro-structured intermediates with

monomers, owing to the hydrophobic property of monomers and the hydrophilic property of the surfactants. The diameters and the lengths of the 1D nanostructures are dependent on several factors, such as the materials of the soft templates, ratio of the surfactant to monomers, reaction temperature, and reaction time. Since no hard template is involved in the soft template method, no post treatment is required. However, this method introduces the materials of soft template into the product, which may affect the properties of the final product.

2.2 Template-Free Synthesis of 1D Nanostructure of CPs

2.2.1 Electrospinning Synthesis

Electrospinning synthesis is one of the most widely used template-free methods. It is an old polymer processing technique which was reported in a patent in 1934, when Formalas disclosed an apparatus for producing polymer filaments by taking advantage of electrostatic repulsions between surface charges.^[76] Recently electrospinning has been restored because it provides a simple and versatile way for creating nano- to micro-fibers from a rich variety of materials, such as conductive and non-conductive polymers, composites and ceramics.^[77, 78] Figure 2.2 describes the experimental setup of the electrospinning technique.^[79] A syringe contains the target polymer solution with its metallic needle fixed at a certain distance from the metal cathode.^[56] A power supply with high variable voltages is attached between the metal needle of the syringe and the metal collector sheet. When the voltage is applied, ca. 14 000 V at a ca. 20 cm separation, the charge overcomes the surface tension of the deformed drop of the polymer solution and an electrified jet is produced. The jet is then elongated and repelled each other due to

the same positive charge the polymer molecules bear. Evaporation of solvent allows the stretch of this jet, which in turn forms nano to micron-diameter fibers.^[78] In total, electrospinning technique is a simple, rapid, inexpensive, electrostatic and nonmechanical method.

Figure 2.2 Schematic illustration of electrospinning process for polymer nanofiber production. Taken from reference ^[79].

Nanofibers prepared by electrospinning exhibit a range of unique features and properties that distinguish themselves from other 1D nanostructures fabricated by other techniques. For example, electrospinning process can produce extremely long nanofibers as several kilometers.^[80] Compared to 1D nanostructures fabricated by conventional mechanical techniques, electrospun nanofibers are much smaller in diameter and hence they possess much higher surface-to-volume ratio. Another advantage of electrospun nanofiber is that they possess high order of alignment on the molecular level. The polymer chains trend to align along the fiber axis because they are subjected to a strong shear force during the rapid electrospinning process, as proved by Foster and co-workers.^[81]

However, there are some limitations to use the electrospinning technique to synthesize polymer nanofibers. Firstly, the nanofibers with diameters below 100 nm are still not easy to be electrospun at the current stage of development. Secondly, the application of electrospinning to conjugated polymers is limited by their molecular weights and solubility.^[78]

2.2.2 Other Template-Free Synthetic Methods

Recently, polyaniline nanofibers have been templatelessly synthesized through either dilute-chemical^[82] polymerizations or interfacial polymerizations.^[83] Here I introduce the formation of 1D nanostructured polyaniline by interfacial polymerization method. Kaner and Huang studied the morphological evolution in a conventional oxidation polymerization process by using TEM, and their results indicate that nanofibers form in early stages.^[84] During continuous polymerization, the nanofibers become scaffolds for the secondary growth of polyaniline, and finally irregular-shaped polymers are formed. Based on the above understandings, interfacial polymerization method was developed to suppress the secondary growth. In a typical interfacial polymerization process, the aniline monomer and the oxidation agents, usually ammonium persulfate (APS), are separated by the boundary between the aqueous and the organic phase. Polymerization occurs only at the interface where all the necessary components come together.^[83-85] The newly formed nanofibers are not soluble in either solvent, and thus they rapidly move away from the interface and the polymerization stops. The nanofibers continuously form and withdraw from the reaction interface, therefore the secondary growth is

avoided and new nanofibers are allowed grow at the interface. The disadvantage of this method is that the production yield is relatively low.

2.3 Nanomaterials-Based Biosensors

The target of this thesis was to apply 1D nanostructured materials to enhance the performance of biosensors. Hence I will give a brief introduction on biosensors, including their basic principles, recent progresses, and current levels.

2.3.1 Theory of Electrochemical Biosensors

To understand the theoretical basis of the sensing mechanism can help in the design of biosensors. Electrochemical enzymatic biosensors are usually categorized into three groups based on the sensing mechanism. The first group is relied on oxygen electrode or hydrogen peroxide electrode. A thin layer of enzyme is entrapped with a semi-permeable dialysis membrane on an oxygen electrode. Determination of the analyte is realized by monitoring the oxygen consumed by the enzyme catalyzed reaction. The signal is generated by the electrochemical reduction of oxygen at a cathodically polarized electrode, usually a Pt electrode, at a potential range of $-0.6 \sim -0.9$ V vs. Ag/AgCl, as shown in Eq (2.1). Another idea on the design of the first group biosensor is based on the hydrogen peroxide electrode. Hydrogen peroxide can be oxidized at the anodically polarization electrode at a potential from $+0.6 \sim +0.8$ V vs. Ag/AgCl, as shown in Eq (2.2). However, the method based upon oxygen reduction at an oxygen electrode is one of the simplest but suffers from several disadvantages, including slow response characteristics, low accuracy and reproducibility. Measurements based on hydrogen peroxide oxidation can overcome the problems and indeed represents by

far the most popular approach. Both methods are based on the detection of co-substrate oxygen concentration, which is sufficient compared with the concentration of target substrates we analyze.

The second group of electrochemical enzymatic biosensors is based on the utility of artificial electron mediator as signal transducer. It is well-known that most redox enzymatic reactions are carried out together with their cofactors at the active center. The cofactors are usually deeply buried inside the steric structure of the enzyme; therefore insulate the electrochemical reaction from the bulk electrode. However, molecular oxygen diffuses into the shell and reacts with the enzyme. In such a process molecular oxygen functions as an electron acceptor or “electron mediator”, i.e. to shuttle electrons between the redox center of the enzyme and the bulk electrode. This is the mechanism for the above-mentioned oxygen or hydrogen peroxide electrode. Based on this mechanism, artificial electron mediators were developed, which function as the molecular oxygen. Several types of mediators have been developed to diffusively shuttle electrons between the electrode and enzymes: soluble mediators, immobilized mediators, and mediators incorporated into porous matrices. A vast number of compounds can be employed as enzyme mediators, such as ferrocene and its derivatives, quinines, ferrocyanide, phthalocyanines, organic dye, Osmium complex, Ruthenium complex, Rhodium complex and other metal complexes. Metal complexes are the most popular one among all the mediators.

The third group of electrochemical enzymatic biosensors is based on the direct electron transfer between redox enzymes and electrodes. Direct electron transfer was first discovered between the electrode of conducting organic salts like N-methylphenazinium- 7,7,8,8-tetracyanoquinodimethanide (NMP)⁺(TCNQ)⁻ and glucose oxidase.^[86] It is believed that the reduction form of the redox enzyme is directly regenerated at the electrode surface instead of being oxidized by molecular oxygen or an artificial mediator. Although the mechanism is still under discussion, researches on the sensors based on this concept are becoming increasingly popular. However, the performance of the direct electron transfer based biosensors is still not practical and a great deal of efforts is needed before its commercialization.

2.3.2 Nanomaterials-Based Biosensors

Nanomaterials, such as nanoparticles and nanowires, offer new opportunities to develop novel biosensors. The diameters of these nanostructures are comparable to that of biological and chemical species being sensed. Therefore, they represent excellent primary transducers for producing signals that ultimately interface to macroscopic instruments.

Nanoparticles have been widely used in recent years as versatile and sensitive tracers for different signal transductions, including electronic, optical and microgravimetric signal transduction. Such signal transductions were utilized to detect different biomolecules such as DNA and proteins.^[87-89] The enormous signal enhancement associated with the formation of nanoparticle-biomolecule assemblies provides the basis for ultrasensitive optical and electrochemical

transduction.^[90] Gold nanoparticles, for example, were found to greatly facilitate electron transfer across self-assembled monolayer.^[91]

One-D nanomaterials have a high aspect ratio, which makes them exhibit extreme sensitivity and superior response toward biosensor application. The successful realization of 1D nanomaterials-based biosensors requires proper control of their chemical and physical properties, as well as their functionalization and enzyme immobilization. For example, due to the high aspect ratio nature of the Si nanorods, dye molecules attached to the Si nanorods was reported to produce an enhanced fluorescence upon capture and detection of Salmonella.^[92] An ultrasensitive electrochemical detection of cytokeratin-7 can be achieved with Au nanowires.^[93] Cytokeratin-7 is a protein expressed in epithelial tissue and is used to differentiate between types of cancers. The functionalized Au nanowires were used to enhance the sensitivity and selectivity of the cancer biomarkers detection. A strong dependence of the anodic peak current with the concentrations of CK-7 resulted in detection down to 10 ng mL^{-1} concentration. The hetero-structured silicon/gold nanorod array was functionalized with anti-Salmonella antibodies and organic dye molecules. Feng et al also reported a highly sensitive detection of DNA hybridization by the functionalized nanotubes.^[94] The nanotubes were produced by depositing three types of graded-band gap quantum dots of $\text{Zn}_x\text{Cd}_{1-x}\text{Se}$ alloy into ordered porous alumina membranes. An electrochemical nucleic acid biosensor with high sensitivity was developed by Chang et al., using the conducting polyaniline nanotube array as the signal enhancement element.^[95] With the immobilization of glucose oxidase and Nafion, nanocomposite of conductive

polyaniline nanofibers and Au nanoparticles was used to construct a sensitive and selective glucose biosensor.^[96]

The unique chemical and physical properties of CNTs have paved the way to new and improved sensing devices, especially electrochemical biosensors. CNT-based electrochemical transducers offer substantial improvements in the performance of amperometric enzyme electrodes, immunosensors and nucleic-acid sensing devices. One problem associated with CNT-based biosensing application is the insolubility of CNT in most solvents. Many polyelectrolytes, such as Nafion and ionic liquid, have been used to solubilise CNT, which provides a good opportunity for preparing CNT-based electrode transducers for the sensing application. The excellent electrochemical behavior of the biosensor prepared by the composite of CNT/polyelectrolytes arises from not only the CNT itself, but also from the proper interactions between the polyelectrolytes and CNT.^[97] The successful growth of vertically aligned CNTs can act as molecular wires to allow direct electron communication between the enzyme and electrode surface. This is particularly interesting for reagentless sensing application.^[98]

The typical performance of the glucose sensors, lactate sensors and nitric oxide sensors that have been reported in the literature is shown in Table 2.1, Table 2.2 and Table 2.3, respectively.

Table 2.1 The performance of the nanostructured glucose sensors in literature

Glucose sensor (Transducer Design)	Linear Range (mM)	Sensitivity (mA M ⁻¹ cm ⁻²)	Detection limit (μM)	Response Time (Sec)	Reference
Au NPs / PAiN NF	0.01- 0.8	2.3 (mA M ⁻¹)	0.5		[96]
MWCNT- Polymer	0.02 - 15		1	4	[99]
MWCNT	0.02-2.2		10		[100]
Chit-SWNT			25		[101]
SWCNT/ nile blue A	0.1-5.0			5	[102]
PB NPs / CNT / Polymer	0.01 - 2.5		5	5	[103]
Pt NPs / ACNT	0.01 - 7			5	[104]
Pt NPs / MC	0.04– 12.2		1	15	[105]
TiO ₂ NTs	0.5 -14	0.24		5	[106]
SWCNT / FTMA	0.04– 0.38	1.7 (mA M ⁻¹)			[107]
XGnP	0 – 6	14.2	10	5	[108]

Table 2.2 The performance of nanostructured lactate sensors in literature

Lactate sensor (Transducer design)	Linear range (mM)	Sensitivity (mA M ⁻¹ cm ⁻²)	Detection limit (μM)	Response time (Sec)	Reference
CL	0.05 - 4		9.2	120	[109]
SiO ₂ sol-gel	0.002– 0.03		0.8		[110]
Optical Fiber			446	100	[111]
Au NPs / LDH	0 – 0.8	446 (mA M ⁻¹)	0.1		[112]
SiO ₂ / LDH		1.5 (mA M ⁻¹)	1.5		[113]
PAA / LDH	~ 0.01		0.2		[114]
SPE / sol-gel / LDH	0.125-2.48	260 (mA M ⁻¹)		30	[115]
SPE / polysulfone / LDH	0.001 – 0.012	80 (mA M ⁻¹)	0.87	30	[115]
HMF / LOD	0 - 0.3	0.77	10		[116]

Table 2.3 The performance of nitric oxide sensors in literature

Nitric oxide sensor (Transducer design)	Linear range (μM)	Sensitivity ($\text{A M}^{-1} \text{cm}^{-2}$)	Detection limit (nM)	Reference
He NT / Chit	0.05 – 1.25	1.3×10^5	50	[117]
Au NPs	0.05 - 10	2.0×10^4	27	[118]
Cr / Polymer	10 - 40		500	[119]
Hb / Polymer	0.1 - 300		100	[120]
Pt	0.01 - 1000	4.0×10^3	50	[121]
CMF ($\varnothing$ 8 μm)	0.04 – 0.2	2.9×10^4	27	[122]
CMF ($\varnothing$ 0.5 μm)	~ 300	1.1×10^3	10	[123]
Glassy Carbon	1 - 100	39	1000	[124]

The full expressions of the abbreviations of the word in the three tables are listed as below:

NPs: Nanoparticles

PAiN: Polyaniline

NF: Nanofiber

Chit: Chitson

PB: Prussian Blue

ACNT: Aligned CNT

MC: Mesoporous carbon

NT: Nanotube

FTMA: 11-(ferrocenyl)-undecyltrimethylammonium bromide

XGnP: Exfoliated graphite nanoplates

CL: Chemilumiscence

LDH: L-lactate dehydrogenase

LBL: Layer-by-layer

PAA: Polyacrylic acid

SPE: Screen-printed electrodes

HMF: Hydroxymethylferrocene

He: Hematite

CMF: Carbon microfiber

Chapter 3. Experimental Approaches

3.1 Sample Preparation, Treatment and Electrochemical Techniques

3.1.1 Electrochemical Synthesis of PPy Nanofiber

PPy can be synthesized by either chemical or electrochemical methods. A typical procedure of chemical synthesis of PPy is that a diluted pyrrole monomer solution is mixed with an oxidized reagent in a solution with electrolytes so that the monomers can be oxidized to form polymer. This method is suitable for large scale production. The electrochemical synthesis of PPy is easy to control and can deposit thin film of product on complex surface. A typical electrochemical synthesis of PPy is that the pyrrole monomers in a solution with electrolytes are oxidized on the electrode surface under oxidation potential. The electrochemical polymerization can be carried out by several electrochemical techniques, namely cyclic voltammetry, chronoamperometry, chronocoulometry, and sometime pulse voltammetry. The chronoamperometry is chosen for all the electrochemical synthesis in this thesis. The experimental setup of electrochemical synthesis usually employs a three-electrode system with one working electrode, one counter electrode and one reference electrode. The working electrode I used is gold, platinum, glassy carbon or other materials prepared based on these substrates. The counter electrode is either platinum wire or platinum sheet. The reference electrode is Ag/AgCl in saturated KCl solution.

3.1.2 Synthesis of Electron Mediator Polyvinylimidazole-Os (PVI-Os)

PVI-Os is one of the most powerful electron mediators which can effectively shuttle the electrons between the macro electrode and the deeply buried redox centers in redox protein. PVI-Os is a redox polymer in hydrogel form. It was synthesized according to the reported method.^[125] During preparation, Os-(4,4'-dimethylbpy)₂Cl₂ and poly(1-vinylimidazole) (PVI) were made first with the procedure in the literature.^[126] Then Os-(4, 4'-dimethylbpy)₂Cl₂ (132 mg, 0.21 mM) was refluxed with PVI (200 mg, 2.1 mM) under N₂ in 200 mL ethanol for 3 days. After filtering, the solution was added to 1.5 L diethyl ether with vigorously stirring. The product was precipitated and separated by decanting.

3.1.3 Chemical Treatment of CNT

Multiwall CNTs and single-wall CNTs were purchased from Shenzhen Nanotech Co. Ltd.. The samples usually contaminated with amorphous carbon and metal particles.^[127] In our experiments, the CNTs were purified with an oxidative treatment to remove amorphous carbon and an acid treatment to remove metal particles. CNTs used in the thesis were first purified by refluxing in 3 M HNO₃ for 3 days. The long time treatment with acid created defects in the wall of CNTs and carboxyl group at the tips of CNTs. CNTs with a bunch of carboxyl groups make them negatively charged and easy to be dissolved in aqueous solution. After precipitation and centrifugation, the CNTs were washed with deionized (DI) water from a Millipore Milli-Q-System for three times at least until a neutral pH value reached. Finally, the solid sample was vacuum-dried for about 4 hours. CNTs were then solved to prepare a 3.0 mg/ml aqueous solution for further use.

3.1.4 Electrochemical Techniques

There are several electrochemical techniques used in this thesis, including cyclic voltammetry (CV), amperometric i-t, differential pulse voltammetry (DPV), and AC impedance. The brief descriptions of these techniques are given below:

Cyclic Voltammetry

CV is a type of potentiodynamic electrochemical technique, which is widely used to study the electrochemical properties of an analytes in a solution.^[128] In a CV, potential is ramped linearly versus time. This ramping is known as the experiments' scan rate (V/s). The potential is scanned from an initial potential toward either a high potential or a low potential. The potential will then scan back. The current is recorded as the function of potential. The analyte studied by CV is usually redox active within the experimental potential window.

Amperometric i-t Curve

In amperometric i-t Curve (i-t), a constant potential is applied and the current is recorded as the function of time. The applied potential is usually an oxidation overpotential or a reduction overpotential of a redox reaction. The current change due to the concentration change of the analyte can be monitored, so that an amperometric biosensor can be constructed.

Differential Pulse Voltammetry

In differential pulse voltammetry (DPV), the base potential is incremented from an initial potential toward a final potential. At the same time a potential pulse is applied. The current before the potential pulse and at the end of the potential pulse are sampled. The difference of these two current samples is recorded as the

function of potential. The DPV is very sensitive to the current change near a redox potential.

AC Impedance

In AC Impedance, the base potential is held constant at an initial potential. A sine waveform is superimposed to the base potential. The subsequent current is also sinusoidal with a value $\Delta i \sin(\omega t + \phi)$. The harmonics of this current (2ω , 3ω ... etc.) will also flow. The relationship between the applied potential and the current flow is known as the impedance. The frequency is scanned from high frequency to low frequency with 12 components per decade frequency. The current and the potential are sampled and analyzed to obtain the real and imaginary impedance. The impedance (Z) has a magnitude ($\Delta E/\Delta i$) and phase (ϕ) and is thus a vector quantity. Usually, an electrochemical cell can be treated as a network of capacitors and resistors, which is known as an equivalent circuit. From an impedance spectrum (plotted in the real/imaginary plane), it is possible to obtain the equivalent circuit and determine the significance of the different components.

3.2 Chemicals

All chemicals were of reagent grade and all solutions were prepared in deionized water (milliQ). Pyrrole was distilled under reduced pressure. A series of phosphate buffer solutions (PBSs) with various pH values were prepared by dilution of 1 M PBS with deionized water (DI) to adjust the molar ratio of $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$. Lactate Oxidase (LOD), from *Pediococcus* sp. lyophilized powder, $\sim 41 \text{ U.mg}^{-1}$, and Glucose Oxidase (GOD), $\sim 200 \text{ U.mg}^{-1}$, from *Aspergillus niger* were used as received. L-lactate, β -D(+)-glucose, L-ascorbic acid, was purchased from Sigma.

Lactate and glucose stock solution was obtained by dissolving Sodium L-lactate and β -D(+)-glucose powder into 0.01 M PH 7.4 phosphate buffer solution (PBS).

Sodium dithionite, 1-Vinylimidazole, and 4,4'-dimethyl-2,2'-dipyridyl (4,4'-dmebpy) were purchased from Aldrich. K_2OsCl_6 and ethylene glycol were received from Acros. 2, 2'-azobis (isobutyronitrile) (AIBN) was from Polysciences and further purified by double recrystallization with methanol followed by storage at -20°C for use.

Specimen of 1-Hexyl-3-methylimidazolium hexafluorophosphate (HMIMPF₆) was purchased from Fluka. The chitason (medium molecular) solution of 1.0 wt% was obtained by dissolving chitason flacks into acetic acid. After the solution was shacked in shacking bed in water bath at room temperature overnight, a homogenous solution was formed. Its pH was adjusted to 5~6 using NaOH. N-(3-Dimethylaminopropyl)- N'-ethylacrbodiimide hydro chloride (EDC), acetonitrile and hydrochloric acid were purchased from Merck. K_2OsCl_6 and ethylene glycol were received from Acros. 2, 2'-azobis (isobutyronitrile) (AIBN) was from Polysciences and further purified by double recrystallization with methanol followed by storage at -20°C for use.

3.3 Instrumentation

- CH Instruments 660B and 760B potentiostat was used to perform most of the electrochemical experiments including electrochemical synthesis and measurement.
- Autolab (PGSTAT30, Eco Chemie B.V., Utrecht, The Netherlands) was used

to perform parts of the electrochemical experiments.

- 1260 Impedance Gain-Phase Analyzer and 1287 Electrochemical Interface system (Solartron Inc., Houston, Tex.) were used to perform impedance measurements.
- Field emission scanning electron microscopy (FESEM) (JOEL JSM-6700F, Japan) provided the morphologies of the products.
- FTA2000 (First Ten Angstroms, Inc., USA) was used to measure the contact angle at ambient temperature.
- UV-visible spectrophotometer (Shimadzu UV-2450, Japan) was used to measure the absorption spectra of PPy, with a resolution at 1 nm.
- Sputtering was performed by using Explorer 14 (Denton Vacuum, NJ, USA).
- Probe station Summit 11751B-6 system was used to characterize conductivity of the various films in air.
- X-ray diffraction (XRD) (Bruker AXS, Germany) provided the XRD pattern.

Chapter 4. Synthesis of PPy Nanofiber and its Biosensor

4.1 Introduction

PPy is one of the most important CPs because of its high stability, electronic conductivity, ion exchange capacity, and biocompatibility. The conductivities of oxidized PPy film are in the range of 10- 100 S cm⁻¹.^[129] Various nanostructures of PPy and its derivatives, nanoarrays and nanocomposites have been synthesized for promising applications.^[130-134] The synthetic approaches of 1D nanostructures of CPs have been reviewed in detail in Chapter 2. The 1D nanostructured PPy can also been synthesized by using “hard” templates and “soft” templates such as organic acids, surfactants and ionic liquid.^[64, 134-137] PPy with 1D nanostructures such as nanofibers, nanotubes and nanorods attracts particular interest because they can be used as molecular building blocks to construct nanoelectronic devices, field-effect transistors, sensors, and actuators.^[138-143]

However, either the existing approaches toward 1D nanostructures of PPy are still time-consuming or an organic solvent is needed during the interfacial synthesis, which is not environmentally friendly in a mass production. Further, the formation mechanism of 1D CPs remains unclear. Therefore, it would be of both scientific and technological interest to explore a simple, economic and reliable route for synthesis of nanostructured CPs in an aqueous solution with a controlled manner, which in turn can provide broad applications.

Nanomaterials with the hierarchical design of well-defined and highly oriented 3-dimensional (3D) arrays consisting of 1-dimensional (1D) nanostructures such as CNTs and nanowires of semiconductors, metals, and oxides have been under intense investigation recently.^[144-146] The 3D arrays demonstrate unique properties and advantages over randomly oriented 1D nanostructures in various applications such as chemical/biological sensing, electron emission, energy conversion and storage, microelectronics, and catalysis.^[147, 148] However, there was little success in alignment of 1D nanostructures of CP on a large area until the pioneer work was done by Martin, who used mesoporous templates to grow the aligned PPy.^[60] However, the approach uses a strong acidic or basic medium to dissolve the membranes, which not only damages the products but also brings out safety concerns and environmental hazards. Recently some methods such as nanoimprint and direct laser interference micro/nanopatterning^[149, 150] have been developed to produce conductive polymeric arrays. These methods involve either harsh conditions or complicated technologies, and thus suffer from environmental contaminations, limited substrate selection, or high cost. A practical route to manufacture thin film devices on complicated physical area on a large scale and at an affordable cost still requires great ingenuity. Thus, I am reporting here a unique and simple template-free, low-temperature electrodeposition approach to fabricate large orientally nanostructured CP arrays in an aqueous solution.^[151] The electrochemical deposition can be used to easily tailor 1D nanostructure on complicated surfaces (such as on a microelectrode surface) by simply varying current or potential, which is particularly essential in fabrications of microdevices for various applications.

In this chapter, a simple, economic and environmentally friendly approach is investigated to fabricate PPy nanofiber, a 1D nanostructure, by using phosphate buffer solution (PBS) without any “hard” or “soft” template. A large scale well-aligned cone-shaped nanostructure of PPy (WACNP) has also been developed based on the above template-free approach. Such an ordered PPy nanostructure has not been reported yet. The driving forces in formation of the PPy nanofiber assisted by a “soft” template are often attributed to hydrophobic interaction, electrostatic interaction and/or hydrogen bonding between the polymer molecule and the templates.^[60, 84] The templateless synthesis of the polyaniline nanofibers with using organic solvents is ascribed to the separation of nucleation and elongation processes or the suppression of the second growth of the polymer.^[83, 152] To explore the formation mechanism of 1D PPy nanofiber in an aqueous solution, without using the organic solvent, I systematically investigate the effects of various synthetic conditions including pH, oxidation potential and concentrations of PBS, pyrrole (Py) and dopant ClO_4^- on the produced PPy nanostructures. X-ray diffraction (XRD) and UV-vis absorption are used to examine the nanostructured PPy for the mechanism analysis. Based on the experimental results, a new mechanism is proposed for the template-free synthesis of 1D nanostructured PPy and WACNP structure in an aqueous solution. The hydrophilicity of the synthesized PPy nanofiber network, which is essential in their sensing and biomedical applications, is studied by water contact angle measurements. The glucose biosensor application based on the superhydrophilic nanofiber network has been developed in this chapter.

4.2 Results and Discussion

4.2.1 Preparation of PPy Nanofiber Network

The PPy nanofiber was potentiostatically polymerized at 0.85 V vs Ag/AgCl with CHI-660B electrochemical station (CH Instruments Inc. USA) in an aqueous solution with pH 6.8 containing 0.1 M Py, 0.07 M LiClO₄ and 0.2 M PBS, if no additional description. A 300 nm thin gold film deposited on silicon wafer with an intermediate layer of Ti was used as the working electrode, and a platinum sheet and Ag/AgCl were used as the counter and reference electrode, respectively. Irregular structured PPy was prepared with 0.1 M Py and 0.07 M LiClO₄.

As shown in Figure 4.1, a homogeneous PPy nanofiber network is potentiostatically synthesized at a large scale without assistance of any “hard” or “soft” template. The diameters of the nanofibers are in a range of 50–220 nm, and can be simply controlled by the reaction time from 2 min (Fig. 4.1c) to 1 h (Fig. 4.1d). The formation process of the PPy nanofiber structure was investigated from infant to its growth. Many small nanorods with diameters of 25–50 nm are formed within 1 s of reaction time (Fig. 4.2a), which grow larger and longer in 5 s (Fig. 4.2b). After 10 s of reaction time, the 1D PPy nanofibers are clearly formed (Fig. 4.2c) with heads perpendicular to the substrate surface. With further growth until 30 s, the nanofibers become even longer and larger with diameters in a range of 40–80 nm (Fig. 4.2d). A PPy nanofiber network is finally produced after 2 min (Fig. 4.1c). Compared to the original sizes of the nanorods at 1 s, the length of the nanofiber increases at least three orders of magnitude, while the radius of the fiber only enlarged one order of magnitude. This indicates that elongation growth rate is

much faster than that at the radial direction, thus resulting in a fiber instead of nanoparticles.

Figure 4.1 FESEM images of PPy nanofiber: (a) for low magnification; (b) for edge view; (c) and (d) for high magnification. The deposition duration is 2 minutes for c) and 1 hour for a), b) and d).

Figure 4.2 PPY nanofiber formed at different growth stages. The deposition time is (a) 1, (b) 5, (c) 10 and (d) 30 s.

4.2.2 Effect of Synthetic Parameters on PPY Morphologies

In order to understand the template-free formation mechanism of PPY nanofibers, I studied the effects of the synthetic conditions on the nanostructure of the PPY fibers. Five conditions including pH value, oxidation potential and concentrations of PBS, pyrrole and ClO_4^- were examined. In each experiment only one condition was the variable but the ratio of $[\text{HPO}_4^{2-}]/[\text{H}_2\text{PO}_4^-]$ was kept constant for pH 6.8 through change of total phosphate concentration, i.e. $[\text{PBS}] = [\text{HPO}_4^{2-}] + [\text{H}_2\text{PO}_4^-]$. Experimental results demonstrate that only irregular shaped PPY forms at a phosphate concentration smaller than 0.05 M (Fig. 4.3a). A microrod PPY with a diameter around 2 μm starts to occur after PBS is up to 0.1 M (Fig. 4.3b). When PBS is over 0.2 M, the PPY nanofiber is synthesized (Fig. 4.3c). Particularly, when

PBS was zero in the solution, only irregular shaped PPy generated, no matter how I change other parameters. The results here clearly reveal that PBS involves the reaction to form 1D nanostructure and a minimum PBS concentration is required.

Figure 4.3 The effect of the concentration of PBS on PPy morphologies: (a) 0.05 M, (b) 0.1 M, and (c) 0.3 M. Py 0.1 M, pH 6.8, LiClO₄ 0.07 M, E=0.85 V.

Figure 4.4 The effect of pH value on PPy morphologies: (a) 6.0, (b) 6.4, (c) 6.6, and (d) 7.4. Py 0.1 M, PBS 0.2 M, LiClO₄ 0.07 M, E=0.85 V. (e) PPy nanofiber forms when the solution contains only Py 0.1 M, Na₂HPO₄, LiClO₄ and at potential of 0.85 V.

The pH value was changed by adjusting the ratio of $[\text{HPO}_4^{2-}]/[\text{H}_2\text{PO}_4^-]$ while keeping the total concentration of phosphate constant (PBS 0.2 M). The irregular shaped PPy (Fig. 4.4a) and the microrod PPy with diameter of 1-2 μm (Fig. 4.4b and c) forms, respectively, when pH is 6.0 and 6.4-6.6. However, the 1D nanofiber PPy is produced when pH is above 6.8 (Fig. 4.4d). The result shows that a high pH promotes the nanofiber formation process. A control experiment was conducted and further confirmed that PPy nanofiber could be formed through HPO_4^{2-} in the solution free of H_2PO_4^- (Fig. 4.4e).

The effect of Py concentration on the PPy nanostructure (Fig. 4.5a and b) shows that irregular PPy forms at a Py concentration >0.1 M (Fig. 4.5a and b) while the nanofiber is produced at a Py concentration ≤ 0.1 M (Fig. 4.5c).

Figure 4.5 The effect of the concentration of Py on PPy morphologies: (a) 0.3 M, (b) 0.2 M, and (c) 0.05 M. PBS 0.2 M, pH 6.8, LiClO_4 0.07 M, $E=0.85$ V.

The concentration of ClO_4^- also has considerable effect on the produced PPy nanostructure. When ClO_4^- concentration is $\geq 0.2\text{M}$ and $\leq 0.07\text{M}$, irregular shaped PPy (Fig. 4.6a) and nanofiber PPy (Fig. 4.6c and d) forms, respectively. It is

observed that microrod PPy begins to form when ClO_4^- achieves 0.1 M (Fig. 4.6b). The existence of the dopant ClO_4^- was confirmed with EDX analysis.

The nature of the deposition potential is actually related to the electrochemical polymerization rate. A high polymerization potential would cause a fast deposition of PPy. Results show that the microrod PPy starts to form at an oxidation potential ≥ 0.95 V (Fig. 4.7a), but only nanofiber is produced when the potential range is over 0.65-0.85 V (Fig. 4.7b).

Figure 4.6 The effect of the concentration of LiClO_4 on PPy morphologies: (a) 0.2 M; (b) 0.1 M; (c) 0.05 M and (d) 0.02 M. Py 0.1 M, PBS 0.2 M, pH 6.8, $E=0.85$ V.

Figure 4.7 The effect of anodic potential on PPy morphologies: (a) 0.95 V; (b) 0.75 V. Py 0.1 M, PBS 0.2 M, PH 6.8, LiClO₄ 0.07 M.

In a brief, the experimental results above demonstrate that the presence of PBS is the most critical factor among all influential synthesis conditions to produce the PPy nanofiber structure, and a relatively high concentration of PBS (≥ 0.2 M), high pH (≥ 6.8), low concentration of Py (≤ 0.1 M), low concentration of dopant ClO₄⁻ (≤ 0.07 M) and low oxidation potential (≤ 0.85 V) favor the formation of 1D PPy nanofiber.

4.2.3 Formation Mechanism of Nanofiber

Based on the experimental observations above, a mechanism for the template-free formation of the PPy nanofiber in an aqueous solution is proposed as shown in Scheme 4.1. It is known that Py can form various oligomers and further randomly aggregate or orderly self-assemble, strongly depending on the nature of its interaction with the anion present and/or the surface of the electrode,^[153, 154] in which electrostatic interactions enhance the random aggregation, while hydrogen bonding promotes the oligomer orderly self-alignment.^[155-158] In the PBS/Py system presented here, electrostatic interactions occur between the electrooxidized, positively charged Py/oligomers and anions, while the hydrogen bonding is produced by the N-H group of the Py ring and the oxygen atoms of the phosphate anion. Thus, during the nucleation process, if the solution environment favors hydrogen bonding to complex Py and HPO₄²⁻/H₂PO₄⁻, the oligomers could self-align to form well-ordered bundle structure (Sch. 4.1A). Clearly, the ordered oligomer bundles can serve as seeds to result in further growth of the 1D PPy

nanostructures for the template-free formation process. That is why a minimum of PBS concentration is required to produce the nanofibers (Fig. 4.3c). The pH effect can be explained since a high pH environment dissociates H^+ from $HPO_4^{2-}/H_2PO_4^-$ to promote its complex with Py through hydrogen bonds (Fig. 4.4d). In contrast, if the electrostatic reaction is much stronger than the hydrogen bond, it would break the self-aligned Py oligomer for random aggregation, resulting in irregular shapes (Sch. 4.1D). A strong acid has a weak conjugate base including hydrogen bond. $HClO_4$ is a strong acid, and thus the role of ClO_4^- is mainly an anionic dopant through its strong ionic bonds rather than its weak hydrogen bonds in the PPy polymerization. Since electrostatic interactions enhance the random aggregation,²¹ a high concentration of ClO_4^- should produce irregular PPy nanostructure, which is well in agreement with the results illustrated above (Fig. 4.6a). However, when the random aggregation rate is comparable to the self-alignment, it possibly results in micro-rods with diameters in a micron scale (Sch. 4.1B). It is also discovered that, for all cases, the formation mechanism of PPy electrodeposition involves the simultaneous presence of adsorption and 3D nucleation, limited by diffusion of Py and/or different Py oligomer species in the processes.^[159] If the electrochemical reaction rate is much faster than the diffusion rate of the self-aligned Py oligomers or the self-alignment rate of Py oligomers (in solution or on electrode surface), only Py could diffuse onto the electrode surface and thus an irregular PPy structure could be produced as shown in Sch. 4.1C. The rate of an electrochemical reaction can be boosted by high polymerization potential and high reactant concentration. Thus, irregular nanostructure formation at a high oxidation potential or a high concentration Py (Fig. 4.5a and b; Fig. 4.7a and b) is in good agreement with the mechanism proposed in Scheme 4.1C.

Using the proposed mechanism I can also explain the formation of the nanofiber network. As shown in Scheme 4.1.A, HPO_4^{2-} is likely to play the major role in formation of the ordered-bundle structure and further developed network compared to H_2PO_4^- . In comparison to H_2PO_4^- , HPO_4^{2-} has a higher charge/volume ratio to form stronger hydrogen bonding. Increase of both phosphate concentration and pH value results in the increase of $[\text{HPO}_4^{2-}]$; therefore the conditions are favorable for ordered-bundle network structure formation and for further developed nanofiber network at the end.

Scheme 4.1 Schematic illustration of the proposed mechanism for the template-free formation of PPy nanofiber in an aqueous solution. (A) Nanofiber forms when experimental condition favors the self-alignment of the oligomers by hydrogen bonding; (B) micro-rod forms when the random aggregation process is comparable to the self-alignment process; (C) irregular shaped PPy forms when electrochemical reaction rate is much faster than diffusion rate of the self-aligned Py oligomers or the self-alignment rate of Py oligomers; (D) irregular shaped product forms when the random aggregation process dominates. The inset is hydrogen bonds formed between the oligomers and $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$.

4.2.4 Structure and Physical Properties of PPy Nanofiber

The existence of the well-ordered bundle structures of the polymer chains in PPy nanofibers is proved by the low angle XRD experiment (Fig. 4.8). An intense peak at $2\theta = 3.76^\circ$ is shown in the XRD pattern of nanofibers (Fig. 4.8a), which has not been observed in irregular shaped PPy (Fig. 4.8b). The corresponding Bragg spacing $d = 2.35$ nm is assigned as the periodic distance of the aligned PPy chains, indicating the existence of supermolecular order in the PPy nanofiber.^[64, 160, 161] In contrast, only a broad weak hump is observed in the XRD pattern of irregular shaped PPy (Fig. 4.8b). This shows that no short-range ordered structure of polymer chains is in the irregular shaped product.

Figure 4.8 XRD patterns of (a) PPy nanofiber and (b) irregular shaped PPy.

The UV-vis spectrum of PPy nanofiber indicates the existence of hydrogen bonds (Fig. 4.9). The absorption spectrum of PPy nanofiber (Fig. 4.9a) is given together with the spectrum of irregular PPy (Fig. 4.9b) for comparison. Two characteristic

peaks (at 1.52 eV and ~ 2.6 eV, respectively) in both absorption spectra indicate the presence of the polarons and bipolarons in the oxidized PPy. The peak at 1.52 eV is attributed to the transition from the bonding to the antibonding polaron state, which corresponds to the doped part of PPy.^[162] The peak at ~ 2.6 eV is associated with the transition from valence band to the antibonding bipolaron state (radical cation/dication level).^[163] The red shift of the absorption peak from 2.68 eV (absorption peak of irregular PPy) to 2.61 eV (absorption peak of PPy nanofiber) possibly indicates the existence of hydrogen bonds.^[164]

Figure 4.9 The UV-vis absorption spectra of a) PPy nanofibers, and b) irregular shaped PPy.

The physical properties for the synthesized PPy nanofibers were characterized by I-V and contact angle (CA) measurements. The electrical transportation property of a single PPy nanofiber was tested using a two-electrode device with a 4–5 μm gap, which was reported in our previous work.^[165] The I-V curve for the single PPy nanofiber at room temperature exhibits a reproducible and non-linear response, a typical signal in the formation of Schottky barriers at the PPy nanofiber/gold

electrode interfaces (Fig. 4.10).^[166] The conductivity of the single PPy nanofiber is about one to two orders of magnitude lower than the random PPy thin film. This can be attributed to several reasons. One reason is that the formation of Schottky barriers between the PPy nanofiber and gold electrode interface increase the resistance of the measured one. Another reason is that the introduction of the dopant ions of H_2PO_4^- detracts the conductivity of PPy.

Figure 4.10 I-V curve for a single PPy nanofiber bridging the gap of two gold electrodes; Insets: the SEM image (top left) and the schematic view (bottom right) of the single PPy nanofiber connecting two gold electrodes.

The surface wettability of PPy film is important for practical applications.^[167, 168]

In the water contact angle measurement the water droplet (1 μL) spread fast over the surface and soon was imbibed in the PPy nanofiber film. The film shows a superhydrophilic property with a contact angle close to zero. It is known that both topographic structure and chemical composition contribute to the wettability of a

solid surface.^[169] The surface of the PPy nanofiber network exhibits homogeneous nanostructure and roughness in the submicrometer scale, resulting in a great enhancement of hydrophilicity. In addition, the incorporation of $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ anions in PPy nanofiber film can also contribute to the hydrophilicity improvement through their hydrophilic functional groups.

4.3 Synthesis of Well-Aligned 1D PPy

4.3.1 Synthesis of WACNP

WACNP was potentiostatically electropolymerized at 0.85 V vs Ag/AgCl in an aqueous solution containing 0.6 M Py and 0.2 M PBS, pH 6.8. A 300 nm gold film deposited on silicon wafer with an intermediate layer of Ti was used as the working electrode. The FESEM images in Figure 4.11 clearly show that the produced PPy has a tubular, cone-shaped nanostructure with vertical orientation on the substrate. The outer and inner diameters of the PPy nanocone tip are 66 ± 26 and 20 ± 10 nm, respectively. The apex angle of the PPy nanocone is $22 \pm 5^\circ$, which provides a higher mechanical and thermal stability than a narrow tube or cylinder. The space between the tips of nanocones is spacious and much larger than the outer diameter of the cone, which is very convenient for further modification and electrochemical accessibility.

Our experimental results showed that the concentration of monomers has a significant effect on the morphologies of PPy. I fixed the concentration of PBS (0.2 M) and varied the concentration of Py. As shown in Figure 4.12A, the PPy nanofiber with diameter of 80-100 nm forms at a random form when Py

concentration is 0.4 M. The relative shorter nanofiber (compared to the PPy nanofiber network shown in Figure 4.1) ends with different directions. Neither network nor well-aligned array is formed at this condition. When the concentration of Py increases to extremely high such as 1.5 M, the irregular-shape PPy forms (Fig. 4.12B).

Figure 4.11 FESEM images of the well-aligned cone-shaped nanostructures of PPy at low magnification (A) and high magnification (B).

Figure 4.12 FESEM images of PPy prepared at different concentration of Py. (A) random PPy nanofiber at Py 0.4 M; and (B) irregular-shape PPy at Py 1.5 M.

4.3.2 Formation Mechanism of WACNP

In section 4.2, PPy nanofiber was template-free synthesized in an aqueous solution and a new formation mechanism was proposed. The nanofiber formation is driven by the hydrogen bonding between the N–H group of the Py ring and the oxygen atom of the phosphate anions, while being blocked by the electrostatic interactions between the positively charged Py oligomers and dopant ClO_4^- .^[170] In this work, when polymerization was conducted with a solution containing zero $[\text{ClO}_4^-]$ and a high concentration of Py, a new vertically well-aligned cone-shaped nanostructure of PPy was formed as shown in Figure 5.1, which is different from the 1D nanostructured PPy prepared in our previous work. As discussed in our previous work, the presence of PBS in the monomer solution only promotes formation of the 1D nanostructured PPy.

The vertical alignment of the PPy cone nanostructures is most likely ascribed to the high concentration of Py, which causes a steric hindrance effect as shown in Scheme 4.2. This is a model widely accepted to interpret the vertically growth aligned CNT.^[171, 172] In more detail, when Py reaches a certain high concentration, the density of nucleation sites on the substrate is greatly increased (Scheme 4.2, step A), resulting in prohibited growth of PPy nanostructures in directions other than vertical direction due to the steric hindrance from the adjacent PPy (Scheme 4.2, steps B and C). The assumption is confirmed by the results shown in Figure 4.12A, where 1D nanostructure of PPy forms randomly when Py concentration is relatively low. Apparently, the steric hindrance effect is getting stronger and stronger with the PPy growth progress. This can lead to the formation of cone-shaped product with larger diameter at its bottom and smaller diameter at its tip.

Scheme 4.2 Schematic drawing of the growth of WACNP. A) High density of nucleation sites; B) initial alignment of PPy due to steric hindrance effect; c) vertical alignment of PPy in the following growth.

The mechanism here is further supported by the result of low angle XRD of WACNP, which shows an intense peak centered at $2\theta = 4.84^\circ$ (Figure 4.13). The corresponding Bragg spacing $d = 1.83$ nm is assigned as the periodic distance of the aligned PPy chains, indicating the existence of short-range-ordered bundles in the product.^[64, 160, 161] The result is consistent with our previous XRD result of PPy nanofiber prepared in the presence of both PBS and ClO_4^- .

Figure 4.13 XRD patterns of WACNP at low angle region.

4.4 Biosensor Application

The electrochemical method to form Poly pyrrole propylic acid (PPa) and Poly (pyrrole-co-pyrrole propylic acid) PPy/PPa film has been developed in our lab.^[133]

Their application on immunosensor indicated that the introduction of huge carboxyl group by pyrrole propylic acid (Pa) greatly enhances the capability of the film to covalently immobilize the protein. Based on the understanding of the PPy nanofiber formation mechanism I proposed above, PPy/PPa nanofiber network was electrochemically synthesized in an aqueous solution containing 90 mM Py, 10 mM Pa, 0.2 M PBS, pH 7.4 and 0.07M LiClO₄, as shown in Figure 4.14. The diameter of the nanofiber is about 113 ± 17 nm. Such a film might provide a nanoscale porous structure with enough carboxyl group to the immobilize proteins for the application on biosensors. The PPy/PPa nanofiber film also shows a superhydrophilic property with a contact angle close to zero.

Figure 4.14 SEM image of PPy/PPa nanofiber network.

A glucose sensor based on the PPy/PPa nanofiber network was constructed. First, a potential of 0.7 V was hold for two hours to complete oxidize the PPy/PPa nanofiber film. The PPy/PPa nanofiber film was then immersed in the solution of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and N-hydroxysuccinimide (1 mg mL^{-1}) for 30 mins and GOD (10 mg mL^{-1}) for 1 hour, and washed with PBS. Finally, the film was covered by a layer of Nafion to hold the enzymes.

The calibration curve of the glucose sensor was obtained by an amperometric method, which is presented in Figure 4.15. The current response to the standard glucose stock solution prepared in PBS was recorded. The glucose standard stock solution was added sequentially into a stirred PBS (pH 7.4) and the dose responses to the glucose sensor were obtained. As shown in the inset of Figure 4.15, the results demonstrate that the glucose biosensor can have a response within 2

seconds. This is much faster than that in high performance glucose sensors, which have been shown in Table 2.1, usually 6-15 seconds.^[173] Such a fast response is attributed to the superhydrophilic property of the composite nanofiber network, which facilitates the diffusion of glucose in the sensing layer.

Figure 4.15 Amperometric response to glucose by the PPy/PPa nanofiber based glucose sensor. The inset is the expansion of the first response jump to show the response

Figure 4.16 Calibration plot of the nanocomposite based glucose sensor. The dashed line is a fit according to Michaelis-Menten equation.

Figure 4.16 shows the calibration curves for the nanocomposite based glucose sensor. The data points can be well fitted according to Michaelis-Menten equation, which will be discussed in detail as below. The sensitivity of the sensor (determined from the slope of the initial linear part of the calibration) was $1.37 \mu\text{A mM}^{-1} \text{cm}^{-2}$, which had a significant improvement than the newly reported glucose biosensor with a sensitivity of $0.097 \mu\text{A mM}^{-1} \text{cm}^{-2}$.^[174] The correction factor, R^2 , in linear range of the calibration was 0.98. Particularly, the linear range of the sensor is about 0-25 mM, which is large enough for most applications. As we know, the wide linear range will reduce the linear sensitivity according to Michaelis-Menten equation. Therefore, such a high sensitivity achieved in the wide linear range is attributed to the nanoporous network structure and a great deal of carboxyl group introduced by PPa in the nanocomposite.

Here I want to introduce a little bit of the Michaelis-Menten equation, which is used in above section and will be used in the following chapter to interpret the enzymatic reactions in an enzymatic biosensor. The Michaelis-Menten equation for an enzymatic reaction can be expressed as in Eq. 4.1, where the current density for the biosensor response, I_{Glu} is a measure of the overall enzyme catalyzed reaction rate, I_{max} is the maximum reaction rate when the enzyme is saturated in binding the substrate, $[\text{Glu}]$ is the glucose concentration, and K_M is the apparent Michaelis constant, reflecting the barriers to substrate/enzyme binding. In an enzymatic biosensor, K_M and J_{max} can be used to characterize performance and specification of the sensor since K_M represents the linear range ($\sim K_M/2$) and the sensitivity is equal to $\sim J_{\text{max}}/K_M$.^[175]

$$I_{\text{Glu}} = \frac{I_{\text{max}}}{1 + K_M / [\text{Glu}]} \quad (4.1)$$

4.5 Summary

In summary, I demonstrate a template-free electrochemical synthetic route to fabricate a superhydrophilic PPy nanofiber networked film and a well-aligned cone-shaped nanostructure of PPy, in an aqueous solution by using PBS. A mechanism for the nanofiber formation in an aqueous solution without assistance of any “hard” and “soft” template is proposed, in which the presence of the hydrogen bonding between phosphate and Py oligomers is essential to produce the 1D nanofiber structure, and the electrostatic interactions between ion dopant and Py oligomers lead to an irregular nanostructure. Additionally, the electrochemical reaction rate plays a significant role in the formation of the 1D ordered nanostructure. The proposed mechanism is not only in good agreement with the characterization of various PPy films formed with different synthetic conditions but also further confirmed by low angle XRD and UV-visible absorption spectra. The synthetic scheme was extended to form a superhydrophilic nanofiber of PPy/PPa nanocomposite, which was covalently immobilized the GOD for the development of a glucose sensor. The nanofiber based glucose sensor proved to be a high performance sensor, i.e., a high sensitivity, a large linear response range, and a very fast response time (<2 s). This is due to not only the nanoporous structure of the network, but also the superhydrophilic property of the film. The developed method opens a way to *in situ* synthesize PPy nanofiber network in an aqueous solution, and has potential to be developed as a universal method for

template-free synthesis of 1D nanostructures. Particularly, the superhydrophilic property of the conductive nanofiber network could enable its great potential applications in biosensors, energy systems and drug delivery devices.

Chapter 5. CNT Based Nitric Oxide Sensor

The unique chemical and physical properties of CNT make it an excellent candidate material for the sensor application, especially for electrochemical biosensors. CNT-based electrochemical transducers offer a substantial advantage in the application of direct oxidation of target molecular (in Chapter 5) and amperometric enzymatic sensor (in Chapter 6 and 7).

5.1 Introduction

NO detection has been reported with spectroscopic, chromatography and electrochemical methods.^[176-178] Mass spectroscopy and gas chromatography for NO detection are not sensitive. Reported spectroscopic methods also include UV-visible spectroscopy, electron spin resonance spectroscopy, and fluorescence emission spectroscopy or spin trapping. Electrochemical detections offer several prominent features that are not available for the spectroscopic analysis, which include the capability of employing microelectrodes *in situ* measurement of NO in single cells near the source of NO synthesis (nitric oxide synthase, NOS), the direct NO detection based on electron exchange between NO and an electrode, and a portable detection instrument.^[44, 179, 180]

Electrochemical detection of NO is based on its oxidation because its reduction reaction often interfered by oxygen. The main challenges to improve the performance of NO sensors are: (i) adopting the selective polymer membrane to exclude the interference from some biological species such as nitrite anions,

ascorbic acid, dopamine and L-arginine; (ii) significantly improving the sensitivity for low detection limit.^[181] Polymeric porphyrins as electrocatalysts have been reported to improve the sensitivity.^[179, 180, 182] Recently, nanoparticles and nanotubes as new electrode materials have been used in electrochemical NO biosensor.^[183, 184] However, there is still a need to further develop new types of NO sensors for performance improvement.

Some superior properties of room temperature ionic liquid (RTIL) such as high thermal stability, wide liquid range, high conductivity and negligible vapor pressure make it especially promising toward the sensor application. Ionic liquid based CNT composite demonstrated direct electrochemistry of redox proteins.^[185, 186] The previously reported electrodes made from conventional coating method, had poor stability due to the desquamation of the gel in solution. An upside-down technology based electrode was adopted to overcome the shortcoming, but it showed a rather large IR drop, which hampers to obtain the correct kinetic information.^[187] In this chapter a novel gel microelectrode based on the hydrophobic IL was employed to prevent the leaching problems and improve the stability of the NO sensor. Micro-hole electrode, the tip of which is etched with a cavity, is a powerful tool not only for the investigation of electrochemical processes but also the application for electrochemical sensors and devices. Various powder materials could be filled in the cavity to fabricate the powder microelectrodes, which demonstrated excellent performance in electrocatalysis and sensor applications.^[188-192] In this report, I filled the micro-hole with a conductive gel composite by SWCNTs and ionic liquid to fabricate the gel microelectrode.

The electrochemical properties of the gel microelectrode and its NO sensor application were investigated.

5.2 Preparation of NO and Fabrication of Electrode

5.2.1 Preparation of the Saturated NO Aqueous Solution

NO gas was generated by slowly dropping a 2 M H_2SO_4 into a glass flask containing saturated NaNO_2 solution, followed by passing the gas sequentially through a 5% (w/v) pyrogallol solution in saturated potassium hydroxide and 10% (w/v) potassium hydroxide to remove oxygen and other nitrogen oxides, respectively. Before addition of H_2SO_4 , all apparatus were degassed meticulously with nitrogen gas for 30 minutes to exclude O_2 , as the generated NO could rapidly react with O_2 . The NO saturated concentration in solution is 1.8 mM at 20 °C. To produce a saturated NO solution, 10 ml deoxygenated PBS (pH = 7.4) containing 0.1 M NaCl was bubbled with NO gas for 30 minutes and kept under NO atmosphere until use. Since NO gas is toxic at concentration higher than 100 ppm, all preparation and storage were carried out in a fume hood. NO standard solutions were prepared by making successive dilutions of a saturated NO solution. NO standard solutions were made fresh for experiments and were kept in a glass flask with a rubber and light-free septum with wrapped black foils.

Before experiment the phosphate buffer solution (PBS) with 0.1M NaCl was bubbled by nitrogen gas to eliminate the soluble oxygen. The electrochemical cell was kept in the nitrogen atmosphere during the experiments. All experiments were carried out at room temperature (20 °C).

5.2.2 Electrode Fabrication

The SWCNT was strongly supersonicated for 10 hours in 1:1 (v/v) concentrated H_2SO_4 and HNO_3 mixed solution. The treated suspension was centrifuged, washed with dilute NaHCO_3 solution and then washed with pure water for 3 times to reach a neutral pH value. The pre-treated SWCNT was dried in an oven and stored for further use. 30 mg SWCNTs mixed with 0.3 ml HMIMPF_6 was ground with an agate mortar for about 20 minutes, and then a black quasi-solid gel was formed.

The micro-hole electrode was prepared with an ultra fine platinum wire in a radius of 50 micrometers as reported in the literature.^[193] The flat disc microelectrode was etched in hot aqua regia to create a cavity with 10-50 micrometers deep at the tip of the electrode. Then the cavity was packed with SWCNT/RTIL gel described above by grinding the tip against small lumps of the material on a clean flat surface such as a glass plate. The schematic pictures of the electrode were depicted in Figure 5.1.

Scheme 5.1 The schematic drawing of microelectrodes, (a) a disk microelectrode, (b) a hole microelectrode, (c) a gel microelectrode, and (d) a Nafion coated gel microelectrode.

5.3 Results and Discussion

5.3.1 Electrochemical Behavior of the SWCNT/RTIL Microelectrode

The electrochemical behavior of the SWCNT/RTIL composite gel microelectrode was characterized by the redox couple $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$. The typical cyclic voltammograms obtained at the gel microelectrode in 10 mM $\text{K}_3\text{Fe}(\text{CN})_6$ + 100 mM NaCl + 10 mM PBS (pH=7.4) solution were presented in Figure 5.1. The relationship between the anodic peak currents, I_{pa} , and the scanning rates, ν , were shown in Figure 5.2. It could be observed that the linear relationship of $I_{pa}-\nu^{1/2}$ was limited in a rather narrow range of the low scanning rates, whereas, the linear relationship of $I_{pa}-\nu$ was in a rather wide range. It should also be noted that the interception of the latter case at current (I) axis was not zero.

The behavior of the gel microelectrode can be explained by considering it as a combination of a planar disk electrode formed by its outside surface and a thin-layer cell formed by the inner porous gel/SWCNT material filled with the electrolyte in the micro hole. Thus, the current response of the gel microelectrode could be calculated by the sum of the response I_D and I_T , where I_D and I_T refers the current of microdisk component and the thin-layer component, respectively. Thus, the cyclic voltametric response^[128, 188, 190-192] could be described by Eq (5.1),

Figure 5.1 The cyclic voltammograms of the gel microelectrode in 10 mM $K_3Fe(CN)_6$ + 100 mM NaCl + 10 mM PBS (pH=7.4) solution, the scanning rate is 1000, 800, 600, 400, 200 $mV.s^{-1}$ in (a), 100, 80, 60, 40, 20 $mV.s^{-1}$ in (b), and the expanded curve of 20 $mV.s^{-1}$ in (c), along the arrow in sequence.

Figure 5.2 The plot of $I_{pa}-v$ (hollow circle) and $I_{pa}-v^{1/2}$ (solid circle) of the gel microelectrode obtained from the CV results in Fig. 5.2.

$$I = I_D + I_T = K_D v^{1/2} + K_T v \Delta E_{p(D)} > \Delta E_p > \Delta E_{p(T)} \quad (5.1)$$

where K_D and K_T are constants independent of the scanning rate for the planar disk electrode and the thin-layer device, respectively; $\Delta E_{p(D)}$ and $\Delta E_{p(T)}$ are the difference between the anodic and the cathodic peak potentials for the microdisk and the thin-layer device, respectively. From the literature, I_D and I_T are proportional to $v^{1/2}$ and v , respectively; $\Delta E_{p(D)} = 59/n$ mV and $\Delta E_{p(T)} = 0$ mV, respectively. It is easy to understand that the diffusion layer is thicker at the low scanning rates and the reversible species can only react on the outside planar surface.^[128] That is why ΔE_p was about 59 mV and I_p was linear with $v^{1/2}$. The diffusion layer became thinner at the high scanning rates so that sufficient reactants penetrated into inner hole and formed a thin-layer cell. As shown in Figure 5.1 and 5.2, the anodic and cathodic current peaks were very symmetrical at high scan rates, and the plot of $I_{pa}-v$ was linear in the whole range. The results show that the thin-layer effect plays dominate role in the gel microelectrode and the behavior of

the planar electrode only occurred at a narrow range of very low scanning rates. It is because that the surface area of the planar electrode can be ignored compared with the very high surface area of the inner porous electrode.

When the scanning rate was sufficiently slow, the system would achieve a so-called steady-state, at which the current of the thin-layer device should be zero at a fixed potential. Therefore, the steady-state diffusion-limited current at the gel electrode is the same as that of the planar microdisk electrode, according to the Eq (5.1). That is why the interception of the linear curve of I_p vs. v in Figure 5.2 is not equal to zero. The intercepted current should abide by the following equation^[194] if the gel microelectrode behaved only as a microdisk:

$$I_{ss} = 4nFDc^br \quad (5.2)$$

where, I_{ss} is the steady-state current of the gel microelectrode, r is the geometric diameter of the outside surface of the gel microelectrode, c^b is the concentration of the bulk solution of $K_3Fe(CN)_6$. From the Eq (5.2) the diffusion coefficient of $[Fe(CN)_6]^{3-}$ was calculated as $6.7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, which is in agreement with the reported value. A same scheme for the micro pore electrodes was reported in, in which the micro pore electrodes were filled by only fine powder electrode materials.^[188]

5.3.2 NO Oxidation on the SWCNT/RTIL Microelectrode

Typical cyclic voltammograms of the gel microelectrode in the NO solution were shown in Figure 5.3. No significantly reductive current could be observed even at a relatively high scanning rate of 1 V s^{-1} . The NO oxidation apparently is an irreversible reaction. The NO oxidation possibly proceeds via an electrochemical

reaction followed by a chemical reaction (EC mechanism). The electrochemical reaction transfer one electron from the NO molecule to the electrode with a cation formation, the nitrosonium ion:

NO^+ is a relatively strong Lewis acid. It is converted into nitrite (NO_2^-) then:

Nitrite could be further oxidized into nitrate. Since the rate of the Lewis acid-base reaction Eq (5.4) is much faster, the whole electrochemical process is kinetically controlled by NO oxidation and appears as an irreversible process. Figure 5.4 shows the relationship of I_{pa-v} and $I_{pa-v}^{1/2}$, which were obtained from the CV measurement in NO solution. For the electrochemical oxidation of NO, the peak currents vs. scan rates showed linear in a certain range, and it would not reach zero when the scanning rate was slow enough. Since the interception of the fit plot I_{p-v} at I_p axis in Figure 5.4 was the steady-state limiting current, which was in good agreement with the data obtained in Figure 5.5. The good linear relationship of the plot of $E - \log((i_L - i)/i)$ in the inset of the Figure 5.5 showed the diffusion control for the gel microelectrode in the NO solution. The interception of the plot $I_{pa-v}^{1/2}$ for NO was zero as the same as the results in the previous section. Differences between the NO oxidation and the redox reaction of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ were observed. It showed that the thin-layer effect became weaker at the higher scanning rates whereas the anodic peak current negatively departed from the linear relationship at the high scanning rate. It was also observed that the anodic peak potential also positively shifted with the increase of the scanning rate, and showed a linear relationship with the $\ln v$, as shown in the inset of Figure 5.4. This may be caused by much slower reaction rate of the NO oxidation at the gel microelectrode

compared with that of the reversible reaction of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$. The slow irreversible NO oxidation could not consume all reactant molecules in the inner porous electrode cavity in a short time due to a strong thin-layer cell effect. All the above results showed that the oxidation of NO on the gel microelectrode is a one electron transfer process followed by an irreversible chemical reaction, which was in agreement with the results reported in the literatures.^[195-197]

Figure 5.3 The cyclic voltammograms of the gel microelectrode in 0.5 mM NO + 100 mM NaCl + 10 mM PBS (pH=7.4) solution, the scanning rate is 1000, 800, 600, 400, 200, 100, 80, 60, 40, 20 mV.s^{-1} , along the arrow in sequence.

Figure 5.4 The plots of I_{pa} - v (hollow circle) and I_{pa} - $v^{1/2}$ (solid circle) of the gel microelectrode obtained from CV results in Fig. 5.3. The inset is the plot of the $\ln v$ dependent of the anodic peak potential.

Figure 5.5 The steady-state voltammogram of the gel microelectrode in 0.5 mM NO + 100 mM NaCl + 10 mM PBS (pH=7.4) solution, the inset is the hemi-logarithm relationship between potential and the Faraday current in the kinetic region.

The electrochemical impedance spectroscopy was also employed to characterize the electrochemical behaviour of the gel microelectrode in NO solution. Figure 5.6 showed the different impedance measurements in the blank solution (Curve 1), the $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ solution (Curve 2), and the EC reactions of NO oxidation (Curve 3 for 1 mM NO and Curve 4 for 0.1 mM NO), respectively. The reaction rate is inversely proportional to the diameter of the semicircle in the Nyquist Plots as shown in Figure 5.6.^[128] It could be observed that the rate constant of NO reaction was one or two orders lower than that of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$, which further verified the rationality of the results obtained by cyclic voltammogram.

Figure 5.6 The electrochemical impedance spectroscopy measurements of the gel microelectrode in the solution of (1) 100 mM NaCl + 10 mM PBS (pH=7.4), (2) 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ + 100 mM NaCl + 10 mM PBS (pH=7.4), (3) 1 mM NO + 100 mM NaCl + 10 mM PBS (pH=7.4), and (4) 0.5 mM NO + 100 mM NaCl + 10 mM PBS (pH=7.4).

5.3.3 Detection of NO on the SWCNT/RTIL Microelectrode

The gel microelectrode was used as the NO sensor to detect NO in solutions. Since the concentration of nitrite anion is much higher than that of NO in the real system and their oxidation potential is very close, the interference from nitrite anion has to be considered. Nafion as a cation-exchange membrane was employed to exclude the interference of anions such as nitrite, ascorbic acid and so forth. Figure 5.7 showed the differential pulse voltammetric (DPV) responses for the gel microelectrode with or without Nafion membrane when the nitrite and NO coexisted. It could be observed that nitrite and NO were oxidized at the almost same potential at the electrode without Nafion membrane, whereas the interference of nitrite anion was excluded effectively at the Nafion membrane coated electrode. It could be concluded that the gel microelectrode is more sensitive to NO than nitrite anion. There was no significant change of the current response when 40-fold more nitrite anion than NO was added into the testing solution.

The DPV responses of NO oxidation at different concentrations obtained from the Nafion coated gel microelectrode were shown in Figure 5.8. The peak current vs. the concentrations of NO shows good linear relationship and a large dose response range with four orders of magnitudes, from 100 nM to 100 μ M. The sensitivity of the sensor was 43.6 μ A mM⁻¹. The coating of Nafion membrane not only enhanced the selectivity of the NO sensor, but also prevented the fouling of the SWCNT/RTIL coating and the gel desquamation on the electrode surface. The reliability of the NO sensor was continuously tested in a month and no significant changes of the dose responses were observed. I also found there was no significant decrease of the current response after the Nafion coating on the gel microelectrode.

The common ions in a biological solution such as Na^+ , K^+ , Mg^{2+} , Cl^- , NO_3^- , SO_4^{2-} , PO_4^{3-} , HPO_4^{3-} and $\text{H}_2\text{PO}_4^{3-}$, have also been tested for the interference. No interference effects on NO detection were observed.

Figure 5.7 DPVs of NO oxidation: (1) dotted curve for gel microelectrode in 20 mM NO_2^- + 0.5 mM NO + 100 mM NaCl + 10 mM PBS (pH=7.4) solution, (2) dashed curve for Nafion coated gel microelectrode in 20 mM NO_2^- + 0.5 mM NO + 100 mM NaCl + 10 mM PBS (pH=7.4) solution, (3) solid curve for Nafion coated gel microelectrode in 0.5 mM NO + 100 mM NaCl + 10 mM PBS (pH=7.4) solution.

Figure 5.8 (a) DPVs of NO oxidation at different concentrations on the Nafion coated gel microelectrode: 25, 12.5, 6.3, 3.2, 1.6 and 0.8 μM , along the arrow in sequence, (b) the linear relationship between the logarithm of peak DPV currents and the logarithm of NO concentrations.

5.4 Summary

SWCNT was incorporated into a RTIL to form a new nanocomposite SWCNT/RTIL gel. The gel nanocomposite was further used to fill into the hole of the microelectrode. The electrochemical behavior of the SWCNT/RTIL gel microelectrode was systematically investigated. The results demonstrated that the Faraday response was contributed from two electrochemical components: a microdisk electrode formed by outside surface and a thin-layer cell formed by inner porous electrode materials, where the thin-layer cell effect played the dominate role in oxidation of NO. An EC mechanism, electrochemical oxidation of NO followed by a chemical oxidation was responsible for the reaction of NO at the gel microelectrode. The Nafion coated gel microelectrode was tested as a NO sensor. The experimental results of its sensitivity, selectivity, dynamic range and reproducibility demonstrated that the gel microelectrode could have great potential for NO sensor application. Although the work indicated the interferences of the anions and negative charged, electrochemically active species in biological samples for NO oxidation could be eliminated by using negative charged Nafion-membrane, but some neutral biological species such as dopamines and acetaminophen still need to be further investigated for their interferences.

Chapter 6. CNT Based Lactate Sensor

A good performance CNT based nitric oxide sensor has been presented in the previous chapter. In this chapter, a new biosensor design will be presented, which combines the properties from both CNT and a polymer electron mediator.

6.1 Introduction

It is well known that lactate is related to several diseases,^[198] food quality,^[199] and fermentation. Thus, detection of lactate is important for clinical diagnostics, medicine validation, and bioscience research and food analysis. Lactate biosensors employ different methods to attach lactate oxidase on electrodes for its detection, which include covalent attachment, conducting polymer entrapment,^[200, 201] and confinement in sol-gel matrix.^[202-204] Mediator-based lactate sensors have been further developed to overcome the disadvantages of indirect detection by H_2O_2 and errors from fluctuation of O_2 concentration.^[205, 206] However, leaching of the mediators, efficiency of enzyme immobilization, reproducibility and sensitivity are still the main challenges for reliable, long life-time and reusable lactate biosensors.^[116]

In last decades, the biosensor performance has been significantly enhanced by introduction of nanomaterials in the sensing layer such as CNTs,^[207, 208] nanowires^[209] and nanoparticles.^[210] However, to construct a nanostructured composite with different components for high stability and good performance is a very challenging state-of-the-art, which needs to engineer the nanostructure for

required chemical and physical properties, functionalization and efficient surface immobilization.

In this chapter, I report a novel CNT-lactate-Au enzymatic network nanocomposite for the lactate sensor, in which chitosan, a positively charged biopolymer was selected to entrap the negatively charged lactate oxidase due to its good stability and biocompatibility, and PVI-Os was used as a leachablless electron mediator in the nanostructure because of its polymeric redox form.^[211-213] The positively charged PVI-Os could also enhance the entrapment capability for negatively charged enzymes.^[214-216] Negatively charged CNT was designed as a cross-linker to network chitosan and PVI for the CNT-PVIOs-chitosan-LOD nanocomposite. Although CNT has been used for electrochemical biosensor,^[217-222] the work here is the first time to explore how to engineer CNT and matched components to construct network nanostructure for a high performance biosensor.

6.2 Preparation of Enzyme Electrodes

L-lactate enzyme biosensors were prepared by the following procedure. A 1% chitosan stock solution was prepared by HCl and pH value was then adjusted to ~5.0 with concentrated NaOH. The chitosan/CNTs/LOD/PVI-Os solution was made by mixing 10 μL solution per each of 1% chitosan, 5 $\mu\text{g mL}^{-1}$ CNTs, 10 mg mL^{-1} LOD and 4 mg mL^{-1} PVI-Os. The gold electrode was immersed into 1 mM mercaptopropionic acid (MPA)/ethanol solution for at least 4 h for MPA immobilization through a self-assembly process. After thoroughly washing with ethanol and water, a 3 μL aliquot of the chitosan/CNTs/LOD/PVI-Os solution was

dropped on the MPA modified gold electrode surface, and then was dried in an incubator at 37 °C for 3 h. The prepared enzyme electrode was placed in stirring PBS for 2 hours to remove loosely bound enzyme and stored in air at 4 °C.

6.3 Results and discussion

6.3.1 CNT/chitosan/PVI-Os Composite

The morphologies of CNTs, chitosan/PVI-Os film and CNT/Chitosan/PVI-Os film prepared on the Au electrode surface were studied by FE-SEM and the results are shown in Figure 6.1. The purified CNTs were observed to uniformly dispersed with diameters of 20-40 nm (Fig. 6.1 A and B) on the electrode surface. Compared with chitosan/PVI-Os composite film (Fig. 6.1C and D), the addition of CNTs into the composite changed the composite structure significantly (Fig. 6.1 E and F), showing a network formed by ~200 nm fibers with uniform micropores. There was no plain CNT observed. This indicated that CNTs were totally coated by chitosan/PVI-Os polymers. In comparison to the dense structure of the Chitosan/PVI-Os composite, the more porous nanostructure of the former shown from the SEM results could apparently load much more enzyme molecules and also provide better venues for the lactate diffusion and reaction. The change of the nanostructure was obviously due to the role of CNTs. The purification of CNT described above made it negatively charged since carboxylic acid groups were produced on the surface by oxidation of HNO₃, which was proved by Raman spectroscopy and will be reported in a separate paper.^[223] Naturally, it should electrostatically attract the positively charged Chitosan and PVI-Os, and then

formed a porous, network nanostructure by self-assembly process. The CNT functioned as a cross-linker.

Figure 6.1 FESEM images of purified CNTs (A, B), Chitosan/PVI-Os/LOD (C, D) and CNTs /Chitosan/PVI-Os/LOD (E, F) composite on gold electrode surface. (B), (D) and (F) are images with high magnification for (A), (C) and (E), respectively.

Figure 6.2 Nyquist plots at Au electrodes modified with MPA (a), chitosan/PVI-Os/LOD/MPA (b), and CNTs/chitosan/PVI-Os/LOD (c), respectively. Supporting electrolyte, 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ in pH 7.4 PBS with 0.15 M NaCl.

6.3.2 Electrochemical Properties of Nanocomposites

The electrochemical properties of the films were investigated by AC impedance spectroscopy in $\text{Fe}(\text{CN})_6^{4-/3-}$ solution. As shown in Figure 6.2, the impedance spectra measured at MPA/Au, chitosan-PVI/Os/LOD/MPA/Au and CNT/chitosan/PVI-Os/LOD/MPA/Au electrode, respectively showed well-defined semicircles over a high frequency range followed by a straight line portion over a lower frequency region. The Randle circuit (inset in Fig. 6.2) was used to fit the kinetic parameters of the electrode process.^[224, 225] The diameter of the semicircle corresponded to the interfacial electron-transfer resistance (R_{ct}), and was found to be ca. 821, 1005, and 324 Ω for MPA/Au, chitosan-PVI-Os-LOD/MPA/Au and CNTs-chitosan-PVI-Os-LOD/MPA/Au, respectively. The highest R_{ct} at the chitosan-PVI-Os-LOD electrode (curve b) indicated that the coated film was not

very conductive and slowed down the redox reaction of $\text{Fe}(\text{CN})_6^{4-/3-}$. After addition of CNTs (curve c), the R_{ct} reduced drastically, even lower than that at MPA modified electrode (curve a), indicating that the CNT-networked film provided much better electron transfer paths to have higher electrocatalytic activity. These results were in agreement with the change of the nanostructure morphology observed in Figure 6.1 (C-F).

Figure 6.3 Cyclic voltammograms of composite modified electrodes in PBS (A) with different scan rate: 5, 10, 20, 50 and 100 mV/s. (B) CVs with the scan rate of 5 mVs^{-1} : no lactate (...), 8.8 mM lactate (—). pH 7.04.

Figure 6.3A shows the results of cyclic voltammetry (CV) conducted on the CNT-networked nanocomposite electrode in 0.1 M phosphate buffer (pH 7.04). In absence of lactate, the amperometric response should be ascribed to redox reaction of $\text{Os}^{2+}/\text{Os}^{3+}$. The peak current increased linearly with an increase of scan rate and the difference between the anodic and cathodic peak potentials was insignificant, showing a fast surface-immobilized redox reaction.^[226, 227] The loading value of the electron mediators, PVI-Os could be calculated from the area of peaks and was found out to be 2.25×10^{-9} mol/cm² for the apparent geometric electrode surface (diameter of electrode = 2 mm). The value was very comparable with the loading value of $\text{Os}(\text{bpy}_3)^{2+/3+}$, a much smaller redox species in a Nafion-coated thick film (1.9×10^{-9} mol/cm²). Considering PVI-Os was a much larger molecule than $\text{Os}(\text{bpy}_3)^{2+/3+}$, the loading in the CNT nanocomposite was excellent. As an electron mediator, PVI-Os is much better than $\text{Os}(\text{bpy}_3)^{2+/3+}$, since the latter is small and often suffers from leaching problem. The CNT based network nanostructure is very efficient to introduce a high concentration of active charge-transfer sites for the lactate sensor. In 8.8 mM lactate solution, a prominent oxidation current wave was observed and the reduction peak of the redox polymer disappeared (Fig. 6.3B). Since the half-wave potential ($E_{1/2}$) was the same as the redox potential of PVI-Os (~ 0.2 V, Fig. 6.3), the results demonstrated that the network nanocomposite provided superior catalysis for the lactate oxidation. The composition of the composite was optimized to reach the maximum electrocatalytic current. The calibration curves of nanocomposite with different amount of CNTs (0, 1, 5, 10, 100 ng on each enzyme electrode) and fixed amounts of PVI-Os (4 μg) and LOD (10 μg) were obtained by using amperometric method at 0.300 V in PBS with

sequential additions of a concentrated lactate solution into a stirred PBS (data not shown). The results showed that a ratio of 10 μg chitosan : 5 ng CNT : 4 μg PVI-Os : 10 μg LOD had the best reaction activity for the lactate oxidation. It was observed that the conductivity was much poorer with lower loading of CNTs than the optimized condition, while higher CNT loading than the value resulted in release of lactate oxidase from the composite due to the adsorption competition between both negatively charged CNT and lactate oxidase. Thus, engineering the nanostructure for an optimized ratio of the components was essential for its performance.

The amperometric response of the CNT networked nanocomposite sensor was tested by sequential additions of a concentrated lactate solution into a stirred PBS (pH 7.04) for dose responses. The results are shown in Figure 6.4, demonstrating that the lactate biosensor had a rapid response time (less than 7 s) and high sensitivity. The inset is the calibration curves for composites with (curve a) and without (curve b) CNTs. It was seen that the CNT introduction as the cross-linker not only enhanced the sensitivity, but also improved the reproducibility of the biosensor. After incorporation of CNTs into the composite, the sensitivity increased by 2 times and the standard deviation was reduced from 19.6% to 4.9%. This result apparently demonstrated that the network nanostructure was more stable and could significantly reduce the measurement variations from swelling and resistance change often occurred on a polymer film. Under the optimized condition, the detection limit was 5 μM with a signal to noise (S/N) ratio of 3. The sensitivity of the sensor was 19.7 $\mu\text{A mM}^{-1} \text{cm}^{-2}$, which had significant improvement than the newly reported lactate biosensor with a sensitivity of 0.15

$\mu\text{A mM}^{-1} \text{cm}^{-2}$ [228] and $3.85 \mu\text{A mM}^{-1} \text{cm}^{-2}$, [116] respectively. The good linear range of the sensor in this work is about 1mM, but it can be optimized to become larger by tailoring the ratio of chitosan: CNT: PVI-Os: LOD. In addition, more concentrated lactate in blood can be diluted for detection with a sensitive sensor. An arrayed chip has been further designed and fabricated to simultaneously detect lactate and glucose, which will be discussed detailed in next chapter. Optimization, pH and temperature effects and stability test have been conducted on the biochip and will be discussed.

Figure 6.4 Amperometric response of CNTs/chitosan/PVI-Os/LOD nanocomposite film to different concentrations of lactate in pH 7.04 PBS. Potential: 300 mV vs Ag/AgCl. Inset is calibration curve of amperometric response on the concentration of lactate for the composite with (a) and without (b) CNTs.

6.4 Summary

A novel nanocomposite lactate sensor was constructed by engineering a networked nanostructure with CNT, selected matched material components and an optimized composition ratio. In the composite, CNT was the cross linker to network the positively charged chitosan and PVI-Os as a matrix for enzyme immobilization and, a polymer electron mediator, respectively. The sensor greatly improved the electroactivity, stability, S/N ratio and sensitivity. The novel nanoengineering approach could be extended to other enzyme biosensors, and could have broad potential applications in diagnostics, life science and food analysis.

Chapter 7. CNT Based Array Biosensors

7.1 Introduction

The increasing demand for fast, effective and reliable detection of the lactate and glucose levels in clinics, food industry and environmental engineering has stimulated intensive research efforts on electrochemical biosensors.^[229, 230] However, a large portion of these efforts focused on the detection of only single analyte at a time in a sample.^[132, 189] Significant advantages, such as reliability and efficiency, can be expected if a biosensor array is introduced for parallel detection of several analytes in a sample.^[231-233] Various approaches have been developed to realize array-based biosensors to monitor multiple analytes, such as oxidase-encapsulated optical array biosensors,^[234] biosensor arrays based on photolithography,^[235] soft lithography,^[236] surface plasmon resonance imaging,^[237] planar waveguide array biosensors, and biosensor arrays based on pin printing protein-doped xerogels.^[238] The disadvantage of these techniques is that complicated processes are often involved during the detection procedure. Moreover, high cost is another crucial factor that hinders the commercial application of the former-mentioned techniques, as today most commercial biosensors are required to be disposable. In contrast, screen printed electrochemical sensors have been widely used in clinical diagnostics due to its low-cost and reproducibility.^[239-241] Another alternative approach is to use low-cost printed circuit boards (PCBs) rather than expensive silicon wafers as the platform to build sensor arrays.^[242]

Numerous research efforts have been devoted on the detection of lactate and glucose using biosensors or biosensor arrays, however, several issues still remain unresolved. Such issues include enzyme leakage, enzyme denaturation, electrode fouling and interference effects. On the other hand, chemical cross-talk between array members is another problem in the simultaneous detection of multiple compounds.^[243] When two electrodes are placed adjacently on one chip, the electroactive substances generated by enzymatic reactions on one electrode may diffuse to the other electrode and be oxidized, and in turn contribute to the detection signal from the analytes.

In this chapter, I demonstrate a simple, inexpensive and reliable approach to fabricate biosensor arrays for the simultaneous detection of lactate and glucose. The lactate and glucose sensors were assembled to PCBs with several complementary components, including redox polymer Polyvinylimidazole-Os (PVI-Os), enzyme immobilization matrix chitosan, counter charged lactate oxidases (LODs) or glucose oxidase (GODs) and cross-linker CNTs (CNTs). As demonstrated in our previous work,^[207] the negatively charged CNTs work as a backbone in the positively charged chitosan and redox polymer, and the negatively charged oxidase could be effectively entrapped in the chitosan and polymer. Redox gel polymer PVI-Os works as the electron mediator and it helps to prevent the deactivation of the mediator, which comes from the leakage of the mediator out of the transducer layer. PVI-Os also helps to lower the working potential for the sensor system. The biocompatible and physiologically harmless properties of chitosan together with its positively charged feature make it a good matrix for the immobilization of acidic enzymes.^[244] Our previous results have proved that the

incorporation of CNTs in the system can significantly improve the conductivity and stability of the composite layer, and lead to an enhanced sensitivity of the lactate sensor.^[207] Electrochemical characterizations of the array sensors as a function of temperature, pH, and concentration of substrates were systematically carried out to optimize the sensor performance. Further amperometric measurements were conducted to analyze the chemical cross-talk between neighbor electrodes, electrode redundancy and interference effects from L-ascorbic acid in the sample. The array system was further applied to simultaneously detect the lactate and glucose concentration in the mouse serum. Although CNTs have been used in several electrochemical biosensors,^[217-222] this work here is the first time to our knowledge to explore how to engineer CNTs and matched components to construct the network nanostructure for high performance biosensors on one array chip.

7.2 Multi-Biosensor Transducers Fabrication

PCB based electrode array chip was supplied by Motorola, USA. As shown in Figure 7.1, the chip was composed by 16 gold disk working electrodes (1 mm in diameter) in four groups, in which the gold rings were fabricated as reference electrodes (4 mm for outer ring diameter and 3 mm for inner ring diameter) and the larger disk electrodes were fabricated as counter electrodes (2 mm in diameter). All the electrodes were electrically connected with contact pads by copper leads, on which are covered by a mask for the insulation consideration. The ring Ag/AgCl reference electrodes were fabricated as reported.^[245] A thick silver layer of about 5 μ m was electrodeposited in the AgNO₃ solution containing sodium

nitrite. Subsequently they were galvanically chlorinated in 1 M HCl solution to form a condensed layer of AgCl.

Figure 7.1 Photograph of the PCB based electrode array chip used for multi-biosensor base.

The mixed solution was prepared by mixing 10 μL of 1% chitosan solution containing 5 $\mu\text{g mL}^{-1}$ CNTs, 10 μL of 10 mg mL^{-1} LOD solution (30 mg mL^{-1} GOD for glucose sensor fabrication) and 10 μL of 4 mg mL^{-1} PVI-Os solution. An aliquot of 3 μL of above mixture solution was dropped on the mercaptopropionic acid (MPA) modified gold electrode. This procedure resulted in a transfer of 10 μg chitosan, 5 ng CNTs, 4 μg PVI-Os and 0.2 U LOD (6 U GOD for glucose sensor) to the electrode surface. They were dried at a 37 $^{\circ}\text{C}$ incubator for 3 h. The resulting film was suspended in the stirred PBS for two hours to remove loosely bound enzyme and stored in air at 4 $^{\circ}\text{C}$.

7.3. Results and Discussion

7.3.1 CVs of Lactate and Glucose Sensing Layer

CV curves of the electrodes with lactate sensing layer in blank PBS without lactate (solid curve) and with lactate (dashed curve) are presented in Figure 7.2, respectively. In the absence of the substrate, a couple of well-defined redox peaks were clearly observed to be centered at the potential of 225 mV, which is resulted from the electrochemical oxidation/reduction from the redox polymer PVI-Os. In the presence of 30 mM lactate solution, a typical catalytic electrooxidation current wave was detected with an elimination of the reduction peak. This was a consequence of a one electron transfer process mediated by the redox couple $\text{Os}^{2+}/\text{Os}^{3+}$ in the redo polymer, which was incorporated with the enzyme in the sensing layer. The catalysis current reached its saturation at +0.3 V. Similar catalytic curves were observed for the electrode with glucose sensing layer. Therefore the potential of +0.3 V was chosen as the working potential for the lactate and glucose sensors in this work. Other parameters like temperature and pH were further optimized with more experiments.

Figure 7.2 Cyclic voltammograms for typical lactate sensor in PBS (solid) and 30 mM lactate (dash), scan rate 5 mV s⁻¹, at 37 °C, pH 7.4.

7.3.2 Temperature Effect

The temperature dependent amperometric current responses to 0.5 mM lactate by lactate sensor and to 5 mM glucose by glucose sensor were measured in pH 7.4 PBS, respectively, as shown in Figure 7.3. It was found that the current responses for both sensors were greatly temperature dependent. For the lactate sensor, the current increases with the increase of the temperature at first up to 37 °C and drops dramatically at the point after 45°C. While for the glucose sensor, the signal increases with increasing temperature and reaches a pseudo-plateau range from 36°C to 40°C. It increases continuously after that and reaches its maximum above 60 °C, then dramatically drops with further increase of the temperature. Considering the real situation and to optimize the sensors performance, 37 °C was chosen for all of the other experiments.

Figure 7.3 The temperature dependent current responses to 0.5 mM lactate for

lactate sensor and to 5 mM glucose for glucose sensor, respectively, were measured by chronoamperometric method, $n = 4$ times.

7.3.3 pH Effect

The amperometric current responses to 0.5 mM lactate by lactate sensor and to 5 mM glucose by glucose sensor as a function of pH were optimized in 37 °C PBS, respectively, as shown in Figure 7.4. The lactate response increases gradually from pH 5.8 up to 7.4 and decreases slightly for higher pH. The glucose sensor reaches its maximum response at pH 6.6, decreases gradually up to 7.4, and then drops rapidly at higher pH. For a compromise, the pH of the PBS, i.e. 7.4, was chosen for all of the other experiments.

Figure 7.4 The pH dependent current responses to 0.5 mM lactate for lactate sensor and to 5 mM glucose for glucose sensor, respectively, were measured by chronoamperometric method, $n = 4$ times, at 37 °C.

7.3.4 Simultaneous Detection of Lactate and Glucose

As shown in Figure 7.5, the representative calibration curves for the glucose sensors in the array chip were obtained by the amperometric measurement with a single electrode, and repeated by two electrodes which were simultaneously connected to the bi-potentiostat. It was found that the average increase of the current response from two electrodes is 2.08 (± 0.12) times of that from a single electrode. This indicates that the individual sensor in array chip can work either independently or together with other sensors, which is critical for the application on the sensor redundancy. Redundancy of the sensors can effectively enhance the reliability of the whole system by providing redundant information on the analytes rather than relying only on one single sensor.^[233]

Figure 7.5 Amperometric response of glucose sensor to glucose level in pH 7.4 PBS, at 37 °C. Current response from a single electrode (▲) was measured independently followed by the sum of the current response from simultaneous

measurement of two electrodes (■) in the array chip, measurement $n = 4$ times.

Under the same operation potential, two electrodes with lactate sensing layer and glucose sensing layer, were used to simultaneously monitor the current response to their substrates successively injection. After a series of enzyme reactions, the final electroactive substances (reduced PVI-Os) were oxidized on the electrodes and could not leak and diffuse to other electrodes, due to the design that they were incorporated with the enzymes and immobilized in the sensing layers. This design was proven to effectively eliminate the chemical cross talk effect, as shown in Figure 7.6.

Figure 7.6 Trace of the output of a two channel multi-biosensor operated in stirred 0.01 M pH 7.4 phosphate buffer at working potential of +0.3 V. (A) Signal record of lactate sensor with four successive addition of 0.1 mM lactate, (B) signal record of glucose sensor with four successive additions of 0.5 mM glucose. No cross talk between glucose and L-lactate was observed.

Figure 7.7 Trace of the output of a two channel multibiosensor operated in stirred 0.01 M pH 7.4 phosphate buffer at working potential of +0.3 V. Lactate sensor (A), Glucose sensor (B), and sensor layer without enzyme (C), with a successive addition of the mixed target solution with 1 mM glucose, 0.5 mM lactate and 25 μM L-ascorbic acid. No cross talk between glucose and lactate was observed.

In the course of real sample monitoring, the interference is always a problem for amperometric biosensors. Interference results from the species other than analytes, such as ascorbic acid or uric acid which are known in blood serum and fermentation products. Enzymes like LOD and GOD are highly specific to their substrates due to their special protein structures. However, the signals obtained from the sensor electrodes comprise both the enzyme catalytic current and the current from other species in the sample which are oxidizable at the working potential. The design of biosensor array chip in this work enables the construction of additional electrodes to detect the sensing signals, which contributed from the species other than analytes at the same potential. To cater for the purpose, the electrodes were fabricated with the same amount of sensing layer as that of the

lactate sensors and glucose sensors in the array chip and without the enzymes. The simultaneous measurements of lactate and glucose in the mixed solution in the presence of L-ascorbic acid are shown in Figure 7.7. Curve A presents the signals combined with lactate and L-ascorbic acid, curve B presents the signals contributed from glucose and L-ascorbic acid, and curve C presents the signals only from L-ascorbic acid. After subtracting the current from interference, the pure signals from substrate are derived.

7.3.5 Calibration Curves for the Array Sensors

The calibration curves for lactate sensors and glucose sensors in one array chip were obtained from independent steady state amperometric measurements (Figure 7.8). The relative standard deviation of the current response between the four array members was within 7.5% for lactate sensors and within 6.0% for glucose sensors. It was found that the sensitivity of the lactate sensor is about $25.46 \mu\text{A cm}^{-2} \text{mM}^{-1}$ in the range of 0 to 0.6 mM, with R^2 of 0.97 and $2.44 \mu\text{A cm}^{-2} \text{mM}^{-1}$ for glucose sensor in the range of 0~6 mM, with R^2 of 0.98. The detection limit calculated with 3δ is 0.6 μM for lactate sensor and 5 μM for glucose sensor. The superior detection limit and narrow linear ranges of the investigated array sensors were suitable for the analysis of diluted samples, e.g. athletic condition in blood samples and fermentation status in food industry.

Figure 7.8 Calibration curve for lactate sensor (solid circle ●, $R^2 = 0.99126$, $n=4$ electrodes), glucose sensor (Solid rectangular ■, $R^2 = 0.99354$, $n=4$ electrodes), at 37 °C, in PBS pH 7.4.

Table 7.1 Recoveries of lactate and glucose added to 10-fold diluted mouse serum

Analytes added	Read in PBS (μA)	Read in mouse serum (μA) ($n=4$)	RSD (%)	Recovery (%)
Lactate 0.4 mM	0.52	0.44 $\pm$ 0.05	11.4%	84.6
Glucose 4 mM	0.37	0.32 $\pm$ 0.04	12.5%	86.5

7.3.6 Application of Sensor System in Mouse Serum

To evaluate the applicability of the system to real sample analysis, the biosensor array system was used for determination of lactate and glucose concentrations in mouse serum. The mouse serum was diluted with 0.01 M PBS with 10 folds. The selected concentrations of lactate of 0.4 M and glucose of 4 M were added to the diluted mouse serum. Measurements were repeated four times for the sample. Table 7.1 illustrates the amperometric current responses to 0.4 mM lactate and 4 mM glucose in the 10-fold diluted mouse serum for the biosensor array system in

PBS and in mouse serum, respectively. The recovery values for 0.4 mM lactate and 4 mM glucose are 84.6% and 86.5%, showing the system is useful in evaluating the lactate and glucose level in serum sample.

7.4. Summary

The PCB based biosensor array system was developed to simultaneously determine the concentrations of lactate and glucose in mouse serum. The sensor layers were modified with the combination of redox polymer, enzyme immobilization matrix, oxidases and cross-linker CNTs. The negatively charged oxidases were effectively entrapped by gel formation with positively charged chitosan and redox polymers, in which CNTs work as a backbone. The redundancy electrodes in this array chip effectively enhanced the reliability of the sensor system. The additional electrodes with no enzymes but modified with the sensing layer eliminated the interference effect during real sample measurements. A good agreement was obtained for the simultaneous detection of lactate and glucose between in mouse serum and PBS. Due to its low-cost, chemical-free and interference free, the application of the presented system is potential to extend to other biosensors for simultaneous measurements.

Chapter 8. Summary, Conclusion, and Recommendations for Future Research

8.1 Summary and Conclusion

In order to construct biosensors with enhanced performance, a new method has been developed to synthesize 1D nanostructure of CPs with unique properties. The recent advancements in the synthesis of 1D nanostructured CPs were reviewed in Chapter 2. The understanding of the polymer chains alignment introduced by the non-covalent interactions either in hard template or soft template boosts us to develop a new method for template-free synthesis of 1D nanostructures of PPy. I systematically investigated the effects of various synthetic parameters on the PPy morphology and proposed a mechanism for the PPy nanofiber formation in an aqueous solution without assistance of any “hard” and “soft” template. The hydrogen bonding between phosphate and Py oligomers is essential to produce the 1D nanofiber structure, while the electrostatic interactions between ion dopant and Py oligomers leads to an irregular nanostructure. Additionally, the electrochemical reaction rate plays a significant role in formation of the ordered 1D nanostructures. Furthermore, I extended the synthetic scheme to form PPy/PPa nanofiber nanocomposite network, and to synthesize more ordered nanostructure, well-aligned cone-shaped nanostructure of PPy. The presence of the hydrogen bonding between phosphate and Py oligomers is essential to produce well-aligned nanostructure, while the steric hindrance effect produced from the high concentration of Py boosts its further vertical alignment.

In this thesis, I presented a new approach to study the morphology evolution of PPy from irregular shape, micro-rod, and nanofiber to well-aligned nanocone array. I also uncovered the underneath formation mechanism, which is a further development of the existed template-free synthesis mechanism. This approach has significance in materials science and could be extended to the synthesis of other 1D nanostructures.

The network of PPy nanofiber or PPy/PPa nanocomposite nanofiber was found to be a superhydrophilic film. The superhydrophilic PPy/PPa nanofiber network-based glucose sensor has been proved to be a high performance sensor with very fast response time. This is attributed to both the nanoporous structure of the network and the superhydrophilic property of the film.

Besides, the distinctive chemical and physical properties of CNTs make them potential in the applications of biosensors. SWCNT was incorporated into a polyelectrolyte to form a gel nanocomposite, which was further filled into the hole of the microelectrode to detect nitric oxide. The electrochemical measurement results demonstrated that the Faraday response was contributed from two electrochemical components: a microdisk electrode formed by outside surface and a thin-layer cell formed by inner porous electrode materials, where the thin-layer cell effect played the dominate role in oxidation of NO. The CNTs based gel microelectrode showed greatly enhanced sensitivity in the detection of NO.

Furthermore, the CNTs were engineered to form nanostructure networks with selected matched material components and an optimized composition ratio. In the

nanocomposite, CNT was the cross linker to network the positively charged polymers, and polymer electron mediator works as a matrix for enzyme immobilization. The nanocomposite greatly improved the electroactivity, stability, signal/noise ratio and sensitivity of both glucose sensor and lactate sensor. The novel biosensor nanoengineering approach provides a good opportunity to fabricate an array sensing system based on PCB to simultaneous detection of the concentrations of lactate and glucose in mouse serum. Due to its low cost, chemical-tolerant and interference free, the application of the presented system is potential to be extended to other biosensors for simultaneous measurements.

To conclude, I have successfully synthesized 1D nanostructured materials, i.e. the PPy nanofiber, with superior chemical and physical properties. I have further investigated its formation mechanism and its enhanced performance in biosensing application. In addition, I have also examined the biosensing performance enhancement with other 1D nanomaterials, i.e. CNTs. The enhanced performance of the biosensors fabricated in this thesis is summarized in Table 8.1, which has been compared with the existed clinical biosensors and those reported in literatures.

Table 8.1 Summary of the performance of the sensors fabricated in this thesis, and the comparison with current state-of-art for both market and literature

Sensor performance	Glucose sensor in Chapter 4	Glucose sensor in array in Chapter 7	Glucose sensor in current clinical application	Current nanomaterials based glucose sensor in literature
Linear range (mM)	0 – 25	0 - 6	2-25	Varied from 0 – 6 to 0 -30
Sensitivity (mA M ⁻¹ cm ⁻²)	1.37	2.24	0.01	0.24-10
Detection limit (μM)	10	10	100	0.1 – 10
Response time (Sec)	2	5	60 - 300	5 - 15

Sensor performance	Lactate sensor in Chapter 6	Lactate sensor in array in Chapter 7	Lactate sensor in current clinical application	Current nanomaterials based lactate sensor in literature
Linear range (mM)	0 – 1	0 – 0.6	1-10	Varied from 0 - 0.01 to 0 - 4
Sensitivity (mA M ⁻¹ cm ⁻²)	19.7	25.46	0.01	0.77
Detection limit (μM)	5	0.6	100	0.2 - 446

Sensor performance	Nitric oxide sensor in Chapter 5	Nitric oxide sensor in current clinical application	Current nanomaterials based nitric oxide sensor
Linear range (μM)	1 x 10 ⁻⁴ to 0.1	1 -1000	Varied from 0.04 - 0.2 to 0.01 - 1000
Sensitivity (mA M ⁻¹ cm ⁻²)	5.5 x 10 ⁵	10	39 – 1.3 x 10 ⁵
Detection limit (μM)	0.1	1	0.1 - 10

8.2 Recommendations for Future Research

Numerous exciting advances in the field of biosensors have been achieved recently. Particularly, great improvements in the sensitivity have been revealed in this thesis through the combination of the biological events with nanotechnologies. The advantages and applications of 1D nanostructure with tailored properties into the biosensors are so great that it remains to be a very active area of research. Accordingly, I will focus on three directions, i.e. development of new 1D nanostructures with tailored properties, functionalization of nanoparticles, and combination of microfluidic technologies.

In Chapter 4, the study of the template-free formation mechanism of the 1D nanostructures of PPy is of fundamental importance for us to understand the synthetic process and morphology evolution of CPs. The mechanism uncovered by this thesis is expected to be extendable to a wider scope of 1D materials synthesis. The investigation of the role of the non-covalent interactions in the process of the formation of other 1D nanostructures requires more research efforts.

Nanoparticles process special characteristics, such as exceptional catalysis effect from nanoparticles of Au, Pt, Ru, and Pd, magnetic properties from CrO₂, CoO, NiO₂ and Fe₂O₃ beads, or fluorescence effect from quantum dots. The integration of nanoparticles with these characteristics into the 1D nanostructure will greatly enhance the performance of biosensors.

In Chapter 4, a WACNP has been developed by modifying the template-free synthesis method for PPy nanofiber developed in Chapter 4. The modification of the WACNP, its application on biosensor and the comparative study between the aligned PPy and random PPy are interesting and need further efforts.

In Chapter 7, I investigated the simultaneously electrochemical detection of glucose and lactate in a biosensor array system. The advantages of microfluidic technologies include not only it is portable and suitable for small volume detection, but also it integrates sample preparation, injection, fluid, reactors and mixers, separation and detection processes into one microchip. Therefore, the combination of 1D nanostructure-based electrochemical biosensor array and microfluidic technology could advocate a great promising progress in biosensing field.

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