Nanostructuring metal-carbon composites for electrochemical biosensors

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**NANOSTRUCTURING METAL-CARBON COMPOSITES
FOR ELECTROCHEMICAL BIOSENSORS**

TING SIONG LUONG

SCHOOL OF CHEMICAL AND BIOMEDICAL ENGINEERING

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List of Abbreviations

AC	Alternating Current
NH ₄ Cl	Ammonium Chloride
A	Ampere
ASV	Anodic Stripping Voltammetry
AA	Ascorbic Acid
CNT	Carbon Nanotubes
R _{ct}	Charge transfer resistance
<i>k</i>	Chemical reaction rate
CHI	Chitosan
CV	Cyclic Voltammetry
DI	Deionized
DPV	Differential pulse voltammetry
D	Diffusion coefficient
C _d	Double layer capacitance
<i>E</i>	Electrochemical cell potential
ERGO	Electrochemically reduced graphene oxide
EDRF	Endothelium-derived relaxing factor
EDX	Energy Dispersive X-ray Spectroscopy
EDC	Ethyl(dimethylaminopropyl) carbodiimide
EDTA	Ethylenediaminetetraacetic acid
<i>F</i>	Faraday's Constant
FAD	Flavin adenine dinucleotide
<i>J</i>	Flux
FTIR	Fourier Transform Infra-Red Spectroscopy
R	Gas constant

GCE	Glassy Carbon Electrode
GOD	Glucose Oxidase
AuNP	Gold Nanoparticle
GO	Graphene Oxide
GQD	Graphene Quantum Dots
Hb	Haemoglobin
HUVECs	Human Umbilical Vein Endothelial Cells
HCl	Hydrochloric Acid
H ₂ O ₂	Hydrogen Peroxide
<i>Im</i>	Imaginary
LA	Lactic Acid
MnO ₂	Manganese Dioxide
Hg	Mercury
MWCNT	Multiwalled Carbon Nanotube
Mb	Myoglobin
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
∅	Phase shift
PBS	Phosphate Buffer Saline
PDMS	Polydimethyl siloxane
KMnO ₄	Potassium Permanganate
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
<i>Re</i>	Real
SCE	Saturated Calomel Electrode
SEM	Scanning electron microscope
S/N	Signal to noise ratio
Ag	Silver

AgCl	Silver Chloride
ssDNA	single stranded DNA
NaBH ₃	Sodium Borohydride
KCl	Sodium Chloride
Re	Solution resistance
SWV	Square wave voltammetry
E_e^o	Standard Electrode Potential
H ₂ SO ₄	Sulphuric Acid
T	Temperature in Kelvin
3D	Three dimensional
UV-Vis	Ultra-Violet - Visible Light
UA	Uric Acid
E _{pa}	Anodic Peak Potential
E _{pc}	Cathodic Peak Potential

Abstract

High performance electrochemical sensors are capable of detecting various biosignals and molecules from biological samples. Particularly, certain analytes with very low concentration within body fluids such as hydrogen peroxide, nitric oxide or heavy metal ions are difficult to be detected. To fabricate electrochemical sensors, novel materials with excellent electrochemical properties are required. Nanostructured carbon-metal composite materials have high performance for electrochemical sensing. While some metal or metal oxide nanoparticles are good at catalysis of analytes, carbon based materials such as graphene and nanotubes are excellent electron-conducting template. Nanocomposites can synergize the properties of various individual nanomaterials, giving rise to materials with enhanced performance and interesting properties.

Nanostructuring refers to methods to form small nanoscale particles into high surface area three dimensional networks. Various forms of nanostructuring of these composite materials are explored in this thesis, with carbon based materials as starting template followed by nanostructuring using metal or metal oxides for electrochemical sensors. Namely, electrochemical deposition of gold nanoparticles onto reduced graphene oxide sheets, chemical functionalization between graphene nanosheets and gold nanoparticles, hydrothermal growth of metal oxides onto carbon nanotubes. Through these explorations, a flexible electrode formed by carbon nanotubes is fabricated and gold nanoparticles

grown onto the electrode *in situ* for enzymatic immobilization and detection of glucose.

In brief, this research project covers nanostructured metal and carbon composite materials' synthesis and characterization, utilizing them for electrochemical sensor. By combining these different materials, it is aimed to achieve higher sensitivity and performance, as well as allowing the better understanding on the fundamentals of electron transfer and redox reactions on electrochemical biosensing.

1. Introduction

1.1. Background

Electrochemical sensing plays vital roles in healthcare and environmental monitoring. For this, nanomaterial research is crucial in improving the electrochemical sensing performance such as sensitivities, detection limits and selectivities. These materials when used to modify common electrodes such as commonly used glassy carbon electrodes, gives rise to novel sensors capable of sensing various kinds of biochemicals.

Many methods have been explored to further increase the sensitivity of electrochemical detection. Recent works focused on the explorations of nanomaterials with various structures and properties for electrochemical sensors. Modifications of electrodes for electrochemical detection can involves films conductive polymer such as polyaniline [1] and polypyrrole [2], carbon materials such as graphene and carbon nanotubes [3], metal nanoparticles [4], metal oxide nanostructures [5] and even biomolecules such as myoglobin [6]. These materials are either incorporated with enzyme to achieve direct electron transfer effect, or increase the overall conductive surface area of the electrode surface in order to increase the sensitivity of the sensor.

To allow better selectivity in electrochemical detections, chemical modifications or catalytic reagents are required. Catalytic reagents such as enzymes ensures only target analytes are detected. Multiple layering of

nanomaterials can give a porous and high surface area. Nanomaterials such as metals can be composited with other material for certain function. For example, when metal nanoparticles are deposited with a polymer onto the surface of an electrode, it can give better immobilization due to polymeric properties, and also a unique structure of nanocomposite between the polymer and the metal nanoparticles.

1.2. Motivations

Although there are many electrochemical sensors in the literature to date, there is still a need to further enhance biosensor sensitivity and nanomaterial synthesis method. There are many fields in which an electrochemical sensor can be improved such as the fabrication cost, miniaturization, sensitivity, selectivity, multiplexed detection and stability. The motivation for this thesis is to improve on the sensor performance through material nanostructuring, forming nanocomposite by arranging its nanostructures. By doing so one could modify and possibly enhance the performance based on sensitivity, selectivity, stability and also possibly its cost.

1.3. Objective and Specific Aims

The objective of this thesis focuses on identifying various methods to fabricate nanostructure carbon and metallic nanomaterials in order to fulfill its roles as an electrochemical sensor. Nanocompositing is chosen as the approach

to enhance electron transfer and improve on the sensitivity of sensor materials. Carbon and metals have long been used as electrode materials for electrochemistry. The advantage of using this approach is not only to obtain synergy between different nanomaterials but also to improve on the surface area enhancement available.

The combination of carbon and metal can prove to be an excellent choice of nanocomposite materials. Metals can be easily deposited onto substrate through adsorption, covalent bonding and electrodeposition. Carbon materials on the other hands are easily deposited onto electrode through drop-coating or electrophoretic deposition. Both metal and carbon materials have proven to be capable of certain catalytic properties, good electron transfer with high surface area, the combination of these two classes of materials when done properly can enhance the performance of the sensor device.

In this thesis, three different methods will be investigated. Namely, layer by layer electrodeposition as described in chapter 3, chemical functionalization between carbon materials as described in chapter 4, and hydrothermal growth of nanocomposite as described in chapter 5. Each method will be used for fabrication of a sensor described in each chapter.

Through this thesis, it is aimed to investigate the underlying mechanism for enhancement of electron transfer through nanostructuring materials. This is done with focus on materials used for electrochemical sensing. Finally, it is also aimed to design and fabricate a novel flexible sensor using nanostructuring

methods studied earlier in order to obtain high electrochemical sensing performance.

2. Literature Review

2.1. Electrochemical Detection Principles

Electrochemical detection is a broad field which involves multidisciplinary knowledge of chemistry, material science, electrical engineering, device fabrication and possibly biology. The history of electrochemical sensors began when glass electrode was developed by Cremer in 1906 [7] and used by Haber *et. al.* for analysis [8]. Most electrochemical applications include environment, health and industrial analysis of substances. Electrochemical sensing refers generally to a device capable of transforming chemical signals into electrical signals through electro-chemical phenomena such as a redox reaction or an electrical property change at sensor surface.

Biosensing is the detection of chemicals found specifically in the biological samples such as blood or other bodily fluids. This aspect is particularly challenging due to biological sample's suitability as the electrolyte as well as the presence of many other interfering analyte species which may not be the analyte which the sensor intended to target. In this instance of general glucose sensing from blood, the sensor could possibly be interfered by other chemicals such as ascorbic acid or uric acid in blood depending on the materials of the sensor [9].

Other than electrochemical methods, there are many other methods of analysing biological samples such as through fluorescent imaging [10], mass

spectroscopy [11], liquid chromatography [12], x-ray photoelectron spectroscopy [13]. These methods provide accurate information from actual samples but are typically slow in acquisition time or require complicated chemistry.

Electrochemical method is fast and also capable of providing a real-time profile on the substance targeted. Another advantage of electrochemical sensors is that it can be miniaturable and integratable into modern electronic device. It senses analytes based on principles of electron transfers between the analyte and sensor surface using amperometry, voltammetry or impedance. In this section, basic detection principles based on electrochemistry and its mechanism of action will be discussed.

2.1.1. Electrochemical Cell

A basic electrochemical cell consists of the three electrodes and the electrolyte solution. The three electrodes are the working electrode, counter electrode and the reference electrode.

Figure 2.1 A three electrode electrochemical system

Choices of working electrode depend on the type of electrochemical experiments that is performed. In most electrochemical sensing, vitreous carbon or gold is commonly used. For the purpose of testing certain materials as sensor materials, they can be coated onto the surface of an inert electrode prior to the electrochemical experiment

The counter electrode serves to provide the opposing current of the equivalent magnitude. It is generally desirable for counter electrode surface area to be larger than the working electrode. The geometry of the counter electrode also has to be in a way such that it provides an even potential distribution to the working electrode surface. There should also be no interfering reaction on the counter electrode to the electrochemistry occurring on the working electrode.

The reference electrode can be saturated calomel electrode, silver/silver chloride (Ag/AgCl) electrode, mercury sulfate or mercury oxide electrodes. The first two are more commonly seen in a general electrochemical setup. The

purpose of the reference electrode is to serve as the reference point, since the standard hydrogen electrode is not readily available for most of the experiment setups due to inconvenience. Each types of reference electrode will have different potential as compared to the standard hydrogen electrode. In the case of a two electrode cell, one of the electrodes will have the combined function of a reference and counter electrode.

To study a reaction in an electrochemical cell, it is generally considered to be done in a cell with inert working electrode with low concentration of reaction species, with high concentration of inert electrolyte which serves to provide conductivity.

2.1.2. Electron Transfer and Mass Transport

In a typical electron transfer reaction:

The Nernst equation calculates the equilibrium potential of the specific reaction in the electrochemical cell. It is written as:

$$E_e = E_e^O + \frac{2.3RT}{nF} \log \frac{C_O}{C_R} \quad (2.2)$$

In this equation, E_e^O is the standard electrode potential, R is the gas constant, T is temperature in Kelvin, F is Faraday's constant, n is the number of electron transfer, C_O and C_R is the concentration of oxidized and reduced species in the

unit of Molarity. This standard equation is a simplified form when the activity of the reaction species is unity. This equation is generally used as guideline as comparisons to actual experimental electrochemical cell potentials.

Electron transfer occurs at the surface of the electrode as shown in the picture, where regions between bulk solution and electrode surface regions separated due to the properties of electrified surfaces. The pathway of a general electrode reaction often involves the mass transfer of reactants and products towards or away from the electrode surface, the chemical reaction, followed by adsorption of ionic species to the electrode surface and electron transfer. Mass transport may play an important role in controlling the rate of reaction at the electrode surface as it limits the transfer of reactants and products between bulk solution and electrode surface region.

Figure 2.2 A general mass transport flow for an oxidation process on an electrode

Mass transport can occur in three different modes namely diffusion, convection and migration. Diffusion refers to the movement of ions from higher concentration to lower concentration in order to reduce the concentration difference within a fluid. Convection is the hydrodynamic movement of the fluid due to external forces such as stirring. Migration is the movement of the dissolved ions due to electrical current and potential difference. The effect of migration in an electrochemical system can be avoided using an inert electrolyte. Therefore, general mass transport regime in an electrochemical system can be either as diffusion only, or through convection which is called convective-diffusion as diffusion would still occur but not as the dominant form. The latter form would occur in a rotating disc electrode which is not used in our experiments covered in this thesis.

Representation of diffusion can be followed using Fick's first law of diffusion, where J is flux of the particles, D is diffusion coefficient, C is concentration and x is position. This is in a model where the diffusion is occurring through a plane parallel to the surface of a flat electrode. Minus sign describes the diffusion of higher concentration to a lower concentration.

$$J = -D \frac{dC}{dx} \quad (2.3)$$

This representation when considered with respect to time becomes Fick's second law.

$$\frac{\delta C}{\delta t} = D \frac{\delta^2 C}{\delta x^2} \quad (2.4)$$

In this case the concentration becomes a function of time and distance. In an electrochemical experiment under a potential step, concentration at the surface of the electrode is modified due to reaction. As time increases, the diffusion reduces due to increase of the diffusion layer and smaller concentration difference. Therefore, different rate of diffusion thus can be carried out by utilizing the time of the experiment. By carrying out electrochemical experiment in short time, such as through increasing experiment time, scan rate or frequency, diffusion can be increased for the study of electron transfer.

In addition, it is assumed that diffusion occurs in a perpendicular angle to the electrode surface. Therefore, the mass transfer on the edge of the electrode is always greater than the center of the electrode, which is termed as the “edge effect”. In the case of a circular electrode surface which is mostly used as standard electrodes, the smaller the electrode area will have a larger contribution of edge effect due to the larger edge to surface area ratio. Nanomaterials with structures having more edges thus have the advantage of having increased electron transfer.

2.1.3. Methodologies and Detection Methods

Electrochemical characterizations are crucial to understanding the performance of the electrode material. There are several methods employed in this thesis to measure the performance of the sensors fabricated. This includes voltammetries, amperometries and impedance measurements. These methods

can be done using an electrochemical workstation. For this thesis, the workstation that was used was CHI760D (CH Instruments, China), which will be capable of carrying out various electrochemical experiments mentioned as above.

Voltammetric technique such as cyclic voltammetry is a method in which the potential is changed over time and the resulting current is measured. A resulting graph showing the change of current in the y-axis over the potentials in the x-axis is called a voltammogram. The scanning rate of the voltammetry refers to the rate at which the potential is changed over time, which is normally in the range of millivolt per second. For the case of cyclic voltammetry, the potential is cyclically ramped between two points of maximum potential and minimum potential. In this thesis, other methods such as differential pulse voltammetry and square wave voltammetry are also used. Each of which has different forms of ramping of potential. Comparisons of such methods can be demonstrated in a diagram shown below.

Figure 2.3 Comparisons between cyclic, differential pulse and square wave voltammetric methods and their general results

Amperometry is a technique in which the current at the working electrode is measured by applying a constant potential over time. It is also considered as a potential step from a zero faradaic current. This method has been used in many amperometric sensors which utilize the resulting current results from the redox reaction occurring at the electrodes at the applied potential. In the case of single potential step amperometry, the faradaic current should decrease over time as the active redox species is depleted at the surface of the electrode and only replenished by mass transport from bulk solution. Therefore, some form of stirring is required for replenishment of target analyte to the surface of the electrode in amperometric experiment.

Figure 2.4 The method of potential application in amperometry and its general result obtained.

Impedance spectroscopy or alternating current (AC) impedance is the measurement of impedance of a material through applying a sinusoidal potential in different frequencies. A typical electrochemical impedance spectroscopy for characterizing sensor materials depends on the diffusion process of the reactants. Any electrochemical system can be modelled in a circuit as shown below. It includes solution resistance R_e , polarization resistance R_{ct} , interface or double layer capacitance C_d , and the Warburg impedance.

Figure 2.5 Equivalent circuit for a redox reaction

Since impedance is complex and requires representation in real and imaginary parts, the resulting impedance can be formulated as follows, where $\lambda = \frac{k}{\sqrt{D}}$, and k is the chemical reaction rate and D is the diffusion coefficient.

$$Re(Z) = R_e + R_{ct} \left(1 + \frac{\lambda}{\sqrt{2\omega}} \right) \quad (2.5)$$

$$Im(Z) = \frac{R_{ct}\lambda}{\sqrt{2\omega}} \quad (2.6)$$

The data representation is generally in bode plot or nyquist plot. In nyquist plot, a semicircle is generally observed at high frequency region, while the diameter is given by R_{ct} . In high frequency region, the electron transfer is reaction rate limited, while in lower frequency, it is diffusion limited, which is represented by a straight line.

Figure 2.6 Comparison between Nyquist plot and Bode plot presentations in impedance spectroscopy

This measurement is also used in measurement of device impedance in solid state devices such as fuel cells, batteries or solar cells.

2.2. Electrochemical Sensing in Biosystems

Detection in biosystems in general has been challenging due to the reactive nature and the small amount of these analyte species. Therefore, it is vital that the detection strategy possesses fast response time to achieve rapid detection of these reactive species. Electrochemical detection methods have been proven to be useful in the detection of various biochemical analytes as compared to other methods. Compared to detection methods such as fluorescence imaging methods [14, 15] and liquid chromatography [16], electrochemical detection offers many advantages such as fast response time and allows real time quantitative monitoring and have superior sensitivity, selectivity and detection limits. The former methods are either too time consuming, complicated or require expensive reagents to work with and are unable to provide real time in situ detection. Currently, ROS and RNS detection kits are available on the market [17] but they also rely fluorescence detection and require fluorescence microscopy and flow cytometry instruments. Despite the large amount of research on biosensors, the exact mechanisms of production and function of such ROS/RNS remain poorly understood.

Therefore, electrochemical method is not only simple but economically feasible to be adapted into modern electronic circuit devices for biosensing functions. Current challenges to electrochemical sensing would involve sensitivity and selectivity, multiplexed detection and the miniaturization of devices.

2.2.1. Reactive Oxygen Species

Cells can produce and release a variety of ROS/RNS. Nitric oxide, superoxide and hydrogen peroxide are common ROS/RNS released from cells, and can react with other chemicals present within the body to produce the other kinds of reactive species such as peroxynitrite and nitrogen dioxide (Figure 2.7). These reactive species are vital in maintaining various cellular activities in physiological environment. In this thesis proposal, we will be focusing on two specific reactive species, nitric oxide (NO), a RNS, and hydrogen peroxide (H_2O_2), a ROS. These key ROS/RNS species are precursors to other reactive species. The electrochemical detection of each of these two species individually has been well established. However, the *in situ* and multiplexed detection of this two species has not been comprehensively researched.

Figure 2.7 The release of reactive species from cells, their diffusion distance and interactions with each other to produce the respective final reactive species.

[18]

Nitric oxide as a substance responsible for vascular relaxation, named endothelium-derived relaxing factor (EDRF), was first discovered by Dr Robert Furchgott in the 1970s [19-21]. Since then, research on the roles that nitric oxide plays in biological systems has been fervent. Apart from functioning as EDRF in the vascular system, nitric oxide as a signaling biomolecule also plays roles in nervous and immune system, such as in neurotransmission [22], anti-inflammatory [23] and anti-coagulant [24]. The excess or deficiency of nitric oxide has also been attributed to several pathological phenomena such as tumor angiogenesis [25], atherosclerosis [26], Parkinson's diseases [27] and diabetes [28, 29]. Therefore it is of great importance to quantify the concentration of nitric oxide in order to identify abnormality in biological samples.

Nitric oxide occurs naturally as a diatomic radical. It is a colorless gas but reacts rapidly in air to give brown colored nitrogen oxides (NO_x) due to the presence of oxygen and superoxides in physiological conditions [30]. This causes nitric oxide to have a short lifetime and poses a challenge for its rapid detection. Nitric oxide is synthesized intracellularly by nitric oxide synthases (NOS). There are three kinds of NOS, namely inducible (iNOS), endothelial (eNOS) and neuronal (nNOS) nitric oxide synthases,

Hydrogen peroxide is a colorless liquid with the chemical formula of H_2O_2 . It is acidic with an acid dissociation constant of 1.5×10^{-12} . Physiologically, hydrogen peroxide also acts as a signaling biomolecule and participates in signal transduction and plays a role in the immune system to regulate cellular processes such as activation of signaling pathway for cell proliferation, differentiation, migration and apoptosis [31]. However, hydrogen peroxide can be harmful as it is highly oxidizing which induces large biological damage to cells. The intermediate products of superoxides and hydrogen peroxides are highly damaging to cells and intracellular organelles as it is capable of oxidizing cell components such as proteins, lipids and DNA. Therefore since hydrogen peroxide level can cause both positive and negative effects, knowing the level of ROS in cells can provide indication of cytotoxicity level and disorders.

Abnormalities in ROS and RNS level are evident in the pathogenesis of cancer cells. Such enhanced oxidative and nitrosative stress level have been

studied in oral cancer squamous cell carcinoma [32] and cervical cancer [33]. Recent studies have showed that ROS stress is increased in cancerous cells due to stimulation of oncogenes, hyperactive metabolism of cancer cells and malfunction in the mitochondria of the cells. This increase in ROS level can cause further mutation, cell proliferation, genetic instability and drug resistance [34]. Most cancer cells have an overproduction of ROS, thus they are more susceptible to exogenous ROS levels as they have high ROS level already present within their system. This is exploited by some therapies targeting cancer cells as they are highly vulnerable to exogenously supplied ROS. ROS has also been identified to play roles in carcinogenesis, metastasis and tumor angiogenesis. Several cancer treatments use high level of ROS to kill cancer cell through the oxidative nature of hydrogen peroxide. However, if the ROS level does not reach lethality, it will have an adverse effect of inducing further mutation within the cell's gene, stimulating downstream expression of biomolecules that aid cancer growth [35]. Therefore ROS release within a cell and the ideal ROS concentration monitoring in cancer cell culture is imperative to understanding the nature of ROS and its effect on cancer development.

Nitric oxide is found to have both promoting and inhibitory effects on cancer development. The level of nitric oxide in cells is controlled by the expression of nitric oxide synthases as mentioned earlier. Studies have shown that all three isoforms of NOS have been detected in cancer cells [36]. There have been studies on the NOS activities in cancer but their specific roles remains ambiguous. Their dual effects have been proposed in literature, where high

level of activity of the synthases can cause cancer cell death while lower activity level has the reverse effect of promoting cell proliferation, an effect similar to that found in reactive oxygen species [37]. In certain cancers, the amount of nitric oxide is elevated, and it is also found that glucose transport is also affected by nitric oxide in different cells [38]. RNS is also named as the cause of pathogenesis [39] and it is possible use to determine stages of cancer based on its levels [40]. Other than cancer, nitric oxide deficiency also plays roles in other diseases such as hypertension, hyperglycemia, atherosclerosis, Parkinson's disease and Alzheimer's disease [41].

Therefore, in monitoring the reactive species of cancer cell, we could possibly identify unique release profile to that of a cancer cell and compare it to a normal cell, with possibility of using this as a form of identification for the metabolism of cancer cells, as well as useful in drug development process where study of cellular response is possible to use such devices.

In terms of detecting reactive oxygen species, most catalytic agents with high selectivity are of enzymatic nature such as cytochrome c and horse radish peroxidase (HRP) for hydrogen peroxide, due to the presence of the active sites. Enzymatic methods have their shortcomings from their intended lifetime, immobilization on electrode surface, sometimes being expensive and unstable [42]. Other than enzymatic method, certain metals or chemical complexes are also selective and workable as sensor materials. For example for nitric oxides, phthalocyanines and porphyrin has been well researched in the literature. For

the case of hydrogen peroxide, the challenge lies in providing chemical structures that are directly oxidizing or reducing hydrogen peroxides while remains reversible such as Prussian blue analogues [43, 44] and silver-DNA hybrid films [45, 46]. In terms of non-enzymatic methods, selectivity arises from the potential applied using amperometric methods as certain chemical only undergoes redox reaction at different oxidation or reduction potential applied. Nitric oxide has the oxidation potential at around 0.8V. Hydrogen peroxide on the other hand can be oxidized or reduced.

2.2.2. Heavy Metals

Heavy metals play a vital role in public health and environmental pollutions. The use of pesticides, heavy industrialization and human activity has caused increasing level of environmental pollution over the years. Heavy metal ions are highly toxic to living organism. Once these heavy metals enter the food chain such as through ingestion by marine plants and animals, they will bioaccumulate and this is highly dangerous and poses a threat to public health.

“Heavy metal” was coined as a term by Leopold Gmelin for the identification of elements within the period table [47]. To identify these metals, scientists have quantified a set of criterias in order to be called as heavy metals. These metals include those having atomic weight greater than that of sodium. It is mentioned also that these metals are of group III to group 16 within periods higher than 4 [48]. Amongst these metals, it was found that presence of some

of them in drinking water poses a health threat. Lead, cadmium, mercury, arsenic, chromium, copper, selenium, nickel, silver and zinc are some of the frequently found heavy metals in the environment [49]. Heavy metals are normally removed from the environment using methods such as ion exchange, biosorption, precipitation or oxidation.

Heavy metal poisoning can be categorized into acute exposure or chronic exposure. The former refers to the exposure to high level of heavy metals within a short period of time, while the latter refers to the exposure to a lower level but over a long period of time. Exposure to heavy metal can disrupt the metabolic pathways within an organism. They can either accumulate in vital organs and glands and thus disrupting their normal function, or displaces other standard minerals from their biological structure which results in their malfunction [49]. Mercury, cadmium and lead all have a strong affinity for ligands [50]. These ligands include purines, pteridines, porphyrins, and proteins with phosphate, cysteinyl and histidyl side chains. Therefore, they can easily bind and inhibits binded enzymes of their ordinary function. They could also affect nucleic acid conformation and disrupt oxidative phosphorylation pathways [51]. These biochemical effects of heavy metals ultimately lead to a variety of diseases. For the case of mercury, it could include kidney problems or neurotic disorder such as neurasthenia.

The general treatment of heavy metal poisoning is through the administration of chelating agents, such as calcium-sodium EDTA, Dimercaprol, succimer,

Prussian blue or Penicillamine [52]. The general detection methods of heavy metals poisoning level involves measuring heavy metal ions removed through the urines. In the case of environmental heavy metals ions such as water samples, they can be measured directly. Methods such as fluorescence, surface plasmon resonance [53, 54], laser-induced breakdown spectroscopy [55] and electrochemical detection has been used for identifying heavy metal ion level. Electrochemical sensors are highly sought after in heavy metal detection due to its simplicity and reliability towards heavy metal sensing. These device can be easily miniaturized and mass produced to get highly portable point of care devices to cope environmental problems posed by heavy metal ions or toxic chemicals even in rural places where high tech equipment are not readily available.

Anodic stripping voltammetry has been used as a general electrochemical method used for heavy metal detection. This method is powerful due to its ability to detect multiple metals at the same time at very low concentration [56]. It involves two steps which includes preconcentration and stripping. Preconcentration refers to the deposition of the metal onto the surface of the electrode. This is where the metal ions are attracted to the surface of the electrode and reduced using a certain voltage. The second step is to sweep towards a positive potential using voltammetric methods. This will reoxidize the metal on the electrode, providing a current readout that is proportional to the concentration of the metals present.

2.2.3. Glucose

Glucose sensing has been an important diagnosis for diseases such as diabetes mellitus in the health industry. Glucose is a hexose sugar and their level of presence in blood could indicate health conditions of the patients. Inability of the body to control blood glucose level is caused by either insufficient or inability of the body to produce insulin. Insulin controls the blood glucose level in the blood by converting glucose to glycogen. Without proper control of glucose level, patients could suffer from heart attack, stroke, kidney malfunction, blindness or limb amputations [57].

Typical glucose level test is done using a small electrochemical device or a colorimetric readout system, detecting from the blood obtained from a finger prick. Most of the electrochemical devices are enzymatic systems. In glucose sensing, the history can be divided into four different generations [58]. In the first generation, an enzyme such as glucose oxidase is used on the electrode. The enzyme glucose oxidase will catalyze the reaction of glucose and oxygen to produce products such as gluconolactone and hydrogen peroxide. By detecting the level of hydrogen peroxide, one can indirectly know the concentration of glucose. First generation sensors depend heavily on the presence of oxygen. Without sufficient oxygen in the system, the measured level of hydrogen peroxide will be limited and results in inaccurate measurements.

Figure 2.8 Comparisons of various kinds of glucose sensors and their mechanism of action. (*MO refers to metal oxide*)

In the second generation glucose sensing, a mediator is used to replace the use of oxygen in the sensing process. The mediator will act as the electron transporter to the enzyme in place of oxygen. These artificial mediators were of ferrocene derivatives or ferricyanide. This was able to remove the need for oxygen level. In the third generation glucose sensing, scientists tries to remove the need for the artificial mediator in the sensing system. This can be done using direct electron transfer from the electrode to the enzyme. Nanomaterials with high surface area can enhance the electron transfer between immobilized enzyme and the electrode surface materials [59]. These materials could include carbon nanomaterials such as graphene or metal oxide nanoparticles. Nanostructured materials brought a major change and improvement in electrochemical glucose sensing technique.

The last type of glucose sensing is done using non-enzymatic method. Certain metals and metal oxides have also shown to be catalytic towards

glucose oxidation. It is proposed that these materials form a hydrous oxide layer on the material surface, which is capable of mediating the reaction of glucose oxidation and regenerate to its original material [60].

2.3. Nanomaterials for Sensing

In this section, properties and synthesis methods of nanomaterials used by this thesis such as graphene, carbon nanotubes, gold nanoparticles and manganese oxide are discussed.

2.3.1. Carbon Nanomaterials

Carbon is the fourth most abundant element in the universe after hydrogen, helium, and oxygen [61]. Carbon nanomaterials can be categorized based on their shapes and structures, such as carbon nanotubes and graphene. These carbon nanomaterials have been utilized for various purposes such as electrochemical applications in energy and sensing devices, or commercial household applications such as optoelectronics.

Graphene is a sheet like single layer sp^2 hybridized carbon atoms which has a hexagonal structure. It is a zero band gap semiconductor. It has excellent charge carrying property and exhibit very high mobility of $10000\text{ cm}^2\text{v}^{-1}\text{s}^{-1}$ in room temperature [62]. In terms of physical properties, these graphene sheets are not only light in weight, they can withstand very high temperature up to

3825 degree Celsius and can have theoretical tensile strength of 130 gigapascal based on their carbon bonds. It was discovered by Brodie in 1859 by exfoliation of graphitic oxide [63]. Since then, many other methods has been developed for the synthesis of graphene oxide sheets such as Hummers method [64]. Later in 2004, it was successfully isolated from graphite using scotch tape technique by Andre and Konstantin which earned them nobel prize in 2010 [62], resulting in increased interest in graphene research. Graphene formation from exfoliated graphene oxide is an attractive experimental approach due to graphene oxide being soluble in water. Following which reduction methods has also been research in order to produce graphene sheets with variable amount of oxygen functional groups, as oxygen group has been studied to be affecting their electron transfer rate or useful for other molecules and enzyme adsorptions [65, 66]. Graphene can also be synthesized by chemical vapour deposition method which is favourable for synthesis in the industrial scale. With exceptional electrochemical properties such as large potential window, minimal charge transfer resistance and high electron transfer rate [67], graphene has been used in many electrochemical sensing applications. Graphene by itself has also been demonstrated to have electrocatalytic properties towards chemicals such as hydrogen peroxide and nitric oxide [68, 69]. It has also been used in enzyme sensors for direct electron transfer in enzymatic sensors [70].

Figure 2.9 Structures of graphene and carbon nanotube

Another class of carbon nanomaterial utilized in this thesis is carbon nanotube. Carbon nanotube is also of sp^2 hybridization but has a tube like structure. Earlier than graphene, it was discovered in 1952 by Radushkevich with images of nanometer sized carbon tubes [71]. Later in 1991, graphitic carbon hollow tube was synthesized using arc discharged by Iijima [72]. Although carbon nanotube does not have superior electroconductivity compared to graphene, it is still a popular choice of carbon nanomaterials due to its wide commercial availability and well documented literature. It can be classified into two categories of carbon nanotubes namely the single walled carbon nanotubes or multi-walled carbon nanotubes. Carbon nanotubes are highly ordered with high aspect ratio, high mechanical strength and conductivity similar to that of graphene due to sp^2 hybridization of carbon molecules. Though insoluble in water, it can be chemically functionalized to improve its dispersibility in water. Its catalytic properties gives rise to many applications in electroanalytical applications such as sensing of hydrogen peroxide [73], dopamine [74], uric acid [75] and ascorbic acid [76]. Carbon nanotube ends also attribute to

increased edge plane sites which promote electron transfer in electrochemical reactions [77].

Carbon nanotubes can be produced using many methods. The most common commercialized method is the arc discharge method. In arc discharge method [78], two carbon rods are placed in an inert gas with pressure around 700 mbar. They are then applied with a current of 100 amperes with 20V which causes a high temperature, resulting in evaporation of the anode and deposition of nanotubes on the cathode. Metal catalyst such as Co and Mo are also used in order to achieve nanotubes of 0.6 and 1.2nm size. Due to the synthesis method, nanotubes can contain large amount of impurities such as amorphous carbon and metals. Therefore, it is crucial to be purified prior to use through methods such as oxidations in air, acid reflux and annealing [79].

2.3.2. Metal and Metal Oxides

Metals and metal oxide nanostructures have been studied due to their increasing importance in sensing. They have been utilized in variety of sensing techniques such as electrochemistry, magnetic and optical methods [80]. In electrochemical detection, they have been used for small molecules and gas sensing due to their inherent electrocatalytic properties. Many precious metals often show high catalytic properties for many redox reactions. This high catalysis is often attributed to high surface area to volume ratio in these metal nanoparticles. For example, platinum nanoparticles have been utilized for

methanol oxidation [81], while iron nanoparticles showed efficient catalysis of hydrogen peroxide reduction [82]. As mentioned in a previous section, metal oxides are also used in non-enzymatic glucose sensing with a unique mechanism.

Metallic nanoparticles have also been used in enzyme-based sensing. Direct electron transfer was observed in many instances where metal nanoparticles such as gold, manganese dioxide, silicon dioxide were immobilized with an enzyme. In most cases, gold nanoparticles have been most successful in incorporation into enzymatic sensors [83, 84]. These enzymes may include haemoglobin, myoglobin, horseradish peroxidase or glucose oxidase. In these instances, good electrical contact or protein/enzyme alignment is required in order to achieve electron transfer between the metal and the protein molecules. Gold nanoparticles have shown to work as the electron conducting pathway between enzyme bodies' active center and the electrode substrate [85]. With good electrical contact between enzymes and metal nanoparticles, direct electron transfer becomes possible and removes the need for mediators and allows direct sensing of the enzyme's target analytes.

Lastly, metal nanoparticles have also been used to amplify signals as electrochemical labels in genosensors such as gold and silver nanoparticles [86, 87]. These metals are chosen because biological activities of the molecules such as nucleic acids' complementary pairing are still retained after the attachment of metal nanoparticles. In these cases where metal nanoparticles are labelled onto

the nucleic acids, once they have been attached onto target complementary strands, metal ions can still be released for detection using anodic stripping, or the metal nanoparticles themselves are also carrying other electroactive labels. This is particularly useful in immunoassays. The magnetic property of metal nanoparticles has also caught the attention in fields of research recently due to the ease of separation and integrable biospecific binding [88].

Metal nanoparticles can be synthesized by chemical methods such as reduction of metal ions with control over the aggregation using various surfactants or stabilizing agents. Chemical method allows better control of nanoparticles uniformity and able to achieve smaller sizes. Gold nanoparticles in particular, have been commonly synthesized using citrate reduction method. In this method trisodium citrate is used as the reducing agent. In addition, citrate will also control the aggregation by forming citrate caps around the gold nanoparticles. The size of the nanoparticles can be controlled by the ratio between sodium citrate and trichloroauric acid. This method was discovered by Turkevich in 1951 [89]. Though other reducing agent may also be used such as amine, EDTA or sodium borohydride [90-92], citrate method remains the most widely used in the literature.

Metal oxides, like metals, are also capable of forming unique nanostructures especially in the form of nanowires and nanotubes [93]. Metal oxides can be synthesized through a variety of methods such as hydrothermal [94], electrochemical synthesis/anodizing [95], or template sol-gel [96]. For example,

manganese oxides can be synthesized through hydrothermal method. Hydrothermal essentially refers to the growth of crystal structures under high pressure. This method typically involves a metal salt and an oxidant or hydroxide in a high temperature and pressure environment, which is conducive for precipitation of the precursors. Sometimes, a morphology directing agent is added in order to produce certain nanostructures. For example, MnO₂ nanotubes were produced in PVP, MnSO₄ and NaClO₃, where the latter two are the salt and oxidants, and the former directs nanotube formation [97].

2.3.3. Current Progress in Nanocomposite Materials

There has been an increase recently in research regarding materials composites to date that utilizes different material classes for increased performance in sensing. This is mostly due it being able to build on the research that had already been done regarding individual materials. Most of these literature focuses on using a catalytic material on another material template such as polymers and carbon. Carbon black was grafted with polymer such as polystyrene using nitroxide radicals in order to function as a gas sensor based on resistance change [98]. Recently, a molecularly imprinted polymer was also utilized to form using trinitrotoluene as the template material and copolymerization technique with gold nanoparticles for the detection of nitro-aromatic explosives as low as 0.04 fM [99].

Material such as graphene is also highly popular due to it being known as the miracle material to enhance sensing capabilities as mentioned. For example, graphene based polyaniline nanocomposite was grown using graphene as the templating material in order to sense DNA [100]. It is found that nanocomposites formed with this can achieve higher surface area for DNA polymerization and hybridization. An ultralow detection limit of 2.08×10^{-16} M was achieved.

Composites between metallic compounds have also been investigated. Titania nanotube array decorated with nickel nanoparticles has been fabricated through anodization of titanium foil and electrochemical deposition technique [101]. The resulting composite is used as highly efficient glucose sensor with detection limit of 2 μ M. The template titania array functioned as fast electron transfer substrate for electrocatalytic nickel nanoparticles.

These recent literatures demonstrated the versatility achieved using composite materials as compared to individual materials. This key concept allows us to further demonstrate the advantages and mechanism of nanocomposites for sensing in this thesis.

3. Gold Nanoparticles Decorated Reduced Graphene Oxide for Detecting the Presence and Cellular Release of Nitric Oxide^a

3.1. Abstract

Nitric oxide (NO) is an important biological signaling molecule playing important roles in vascular, nervous and immune system regulations. Nevertheless sensitive detection of nitric oxide molecule remains a challenge due to its low physiological concentration and short lifetime. In this work, we report the preparation of a unique nanocomposite consisting of gold nanoparticles (AuNPs) electrochemically deposited on electrochemically reduced graphene oxide (ERGO), and its use for sensitive detection of nitric oxide. ERGO network provides highly conductive pathways for electron conduction and a large surface area for catalyst support, while AuNPs act as efficient electrocatalysts towards the oxidation of nitric oxide. The synergistic integration of ERGP and AuNP realizes the electrochemical detection of nitric oxide (NO) with high sensitivity ($5.38 \mu\text{A}/\mu\text{M}/\text{cm}^2$), low detection limit (133 nM with a $S/N = \sim 5.5$), and a fast response time (3 s). Furthermore, we demonstrate the use of the AuNP-ERGO hybrid electrode to detect the dynamic release of NO from live human umbilical vein endothelial cells (HUVECs).

^a Reproduced in part from [Ting SL, Guo CX, Leong KC, Kim DH, Li CM, Chen P. *Electrochimica Acta*, 2013, 111: 441-446]. Copyright (2014) with permission from Elsevier

3.2. Introduction

Nitric oxide (NO) was first identified as an endothelium-derived relaxing factor (EDRF) in 1970s [102]. Since then, the research on the regulatory roles of NO in biological systems has been fervent. The studies have revealed that NO regulates many physiological functions including blood vessel dilation [103], anti-coagulation [24], neurotransmission [22], and anti-inflammation [104]. The excess or deficiency of NO can result in various pathological conditions such as tumor angiogenesis [105], atherosclerosis [26], Parkinson's diseases [27] and diabetes [28, 29]. Therefore it is of great importance to accurately quantify the nitric oxide level for study of cell functions and diagnosis. However, this is challenged by the low physiological concentration of NO and its short life time (~ 5 s) due to its rapid conversion to NO_x by oxygen and superoxides present in biofluids.

Compared to the detection methods based on fluorescence measurement [14, 15] and liquid chromatography [16], electrochemical detection allows simple, fast, real-time and quantitative detection with high sensitivity. Recently, efforts have been made to increase the sensitivity for electrochemical detection of NO by engineering the electrode with functional nanomaterials [106]. Among them, graphene (a monolayer of carbon atoms two-dimensionally arranged in a honeycomb structure) is of particular interest, due to its high conductivity, large surface area, wide electrochemical detection window, and chemical inertness [107].

But its lack of intrinsic catalytic activity makes it impossible to use bare graphene materials for NO detection. Gold nanoparticles (AuNPs) exhibit excellent biocompatibility and catalytic property towards NO oxidation. Therefore, AuNPs have been composited with various nanomaterials for the development of NO sensors [108-114]. However, there is still room to improve the performance of the current methods, particularly for the purpose of physiological measurements.

In this work, we report a hybrid film of electrochemically reduced graphene oxides (ERGO) and gold nanoparticles (AuNPs) simply made by electrophoretic deposition of graphene oxide sheets followed by *in-situ* electrochemical reduction and subsequent *in-situ* electrochemical growth of AuNPs. And we demonstrate the use of such hybrid electrochemical electrode for sensitive detection of NO and its dynamic release from live cells.

3.3. Experimental Section

3.3.1. Materials

Sulfuric acid (H_2SO_4), nitric acid (HNO_3), sodium nitrite (NaNO_2), potassium hydroxide (KOH), potassium chloride (KCl) and acetylcholine were purchased from Sigma-Aldrich. Phosphate buffer solution (PBS) (pH 7.4) was used as the electrolyte solution for all experiments. All solutions were prepared with deionized water purified from Millipore Milli-Q system.

3.3.2. Equipment

The morphology and X-ray energy dispersive spectrometry of the samples were examined by a field emission scanning microscope (JEOL JSM-6700F). UV-vis characterization was performed using a UV-vis spectrophotometer (Shimadzu UV-2400PC). Electrochemical characterizations and measurements were conducted with a CHI-660D electrochemical workstation (CH Instruments, China) using a three-electrode system consisting of a platinum counter electrode, a standard calomel electrode (SCE) as the reference, and a glassy carbon working electrode (GCE) without or with coating of AuNPs-ERGO thin film.

3.3.3. Preparation of Nitric Oxide Solution

PBS with saturated nitric oxide was prepared as previously reported [115]. In brief, all glasswares and PBS solution were purged with nitrogen gas prior to preparation. Then, 2 M of sulfuric acid was added drop-wise to a saturated sodium nitrite solution, leading to the production of nitrogen oxide gas through disproportionation reaction of sodium nitrite in the acidic solution. The gas produced was bubbled through two KOH solutions of decreasing concentration (0.1 g/mL and 0.025 g/mL) in order to remove other forms of nitrogen oxides. Finally, NO gas was collected in PBS solution and stored in nitrogen-protected

environment. The saturated NO solution at room temperature is reported to be 1.8 mM [115].

3.3.4. Fabrication of AuNP-ERGO Thin-film Coated Electrode

A polished glassy carbon electrode (GCE) was immersed consecutively in 100% ethanol and deionized water with sonication, followed by blown-drying with nitrogen gas. Graphite oxide was prepared using modified Hummer's method [116], and sonicated for two hours to exfoliate graphene oxide (GO) sheets. GO solution is diluted to 0.25 mg/mL and used for electrophoretic deposition. To electrophoretically deposit GO film on GCE, the electrode was immersed in the GO solution (0.25 mg/mL) with 2 V applied by an electrochemical station for 75 seconds. After the GO deposited electrode being dried in vacuum, GO film was electrochemically reduced to form ERGO film by cyclic voltammetry scanning in 1M KCl solution (0 to -1.2 V at 50 mV/s scan rate, for 20 cycles) [117]. Finally, AuNPs were in-situ synthesized onto ERGO film by applying -50 mV for 90s to the electrode immersed in a solution containing 1 mM HAuCl₄ and 0.5 M sulfuric acid. For comparison, the same protocol was used to fabricate GCE electrode coated with ERGO film only or AuNPs only.

3.3.5. Experiments on HUVEC Cells

Human umbilical vein endothelial cells (HUVECs, cell line obtained from Lonza) were cultured in MCDB131 medium supplemented with fetal bovine serum (10% v/v) and bovine brain extracts (0.2% v/v). After reaching confluence, the cells were harvested and suspended in PBS (6.60×10^5 cells per mL) for experiments. Using an AuNP-ERGO working electrode, the release from these cells (200 μ L cell suspension) was amperometrically determined, without or with stimulation by acetylcholine.

3.4. Results and Discussion

3.4.1. The AuNP-ERGO Hybrid Electrode

Usually, graphene modified electrodes are made by drop-casting GO dispersion onto the supporting electrode followed by chemical reduction of GO [118, 119]. Here, we deposit GO sheets onto a positively-biased glassy carbon electrode *via* electrophoresis because GO nanosheets bear abundant negatively charged hydroxyl, ketone carbonyls, epoxide, and carboxyl groups [120]. As compared to drop-casting and other coating methods, electrophoretic deposition can easily and reproducibly make uniform and continuous film with thickness controllable simply by timing [121, 122]. Subsequently, the deposited GO is electrochemically reduced to ERGO by cyclic voltammogram scanning [118,

123]. In comparison with the usual chemical reduction of GO using harsh or hazardous chemicals (most notably, hydrazine), electrochemical reduction is environmental friendly and fast [123].

As revealed by scanning electron microscopy (SEM) (Figure 3.1A), the resulting ERGO film is a continuous network of micron-sized individual sheets with numerous wrinkles and edges which are believed to facilitate electron transfer [107]. Finally, gold nanoparticles (AuNPs) were decorated onto the ERGO film by in-situ electrochemical deposition [124]. As shown in Figure 3.1B, AuNPs with an average size of ~50 nm and their small clusters uniformly disperse on the ERGO film. The identity of AuNP is also confirmed by energy-dispersive X-ray spectroscopy (EDX) (Figure 3.1C) which shows the atomic ratio of C:Au of ~ 3.1:1 and UV-vis absorption spectrum (Figure 3.1D) which shows the peak adsorption at 615 nm attributable to the surface plasmon effect of AuNPs [125].

Figure 3.1 (A) SEM image of ERGO film. Inset shows an ERGO sheet. (B) SEM image of AuNP-ERGO film. Inset shows individual AuNPs with a high magnification. (C) EDX spectrum. (D) UV-vis absorbance spectra of AuNP-ERGO and ERGO film.

3.4.2. Comparison between the Different Electrodes by Cyclic Voltammetry

The cyclic voltammograms (CVs) of bare glassy carbon electrode (GCE) and GCEs modified with ERGO, AuNP or AuNP-ERGO hybrid film were measured in PBS containing 200 μ M nitric oxide. As shown in Figure 3.2 and

summarized in Table 3.1, nitric oxide oxidation (via the following reactions: $\text{NO} - \text{e}^- \rightarrow \text{NO}^+$; $\text{NO}^+ + \text{OH}^- \rightarrow \text{HNO}_2$; $\text{HNO}_2 \rightarrow \text{H}^+ + \text{NO}_2^-$ [115]) can be observed from all the electrodes but AuNP-ERGO electrode shows largest oxidative current and lower oxidation potential than that of GCE, GO, or ERGO electrode. The superior performance of the AuNP-ERGO hybrid electrode suggests the synergistic integration of ERGO network and AuNPs: the former provides large active surface area for charge transfer and interconnected conducting pathways while the latter provides high catalytic activity towards NO [125].

Figure 3.2 CVs of 0.2mM of NO in PBS solution using glassy carbon electrode (GCE) modified with AuNP, AuNP-ERGO, ERGO, GO and bare electrode.

Inset shows the CV response in PBS solutions only.

Table 3.1 Summary of CVs from different electrodes from the experiments in Figure. 3.2.

Modifications	Average Peak	Average Oxidation	Current Density
	Current (μA)	Potential (V)	(mA/cm^2)
GCE	88.9 $\pm$ 9.9	0.975 $\pm$ 0.015	1.253 $\pm$ 0.139
AuNP	128.3 $\pm$ 11.5	0.796 $\pm$ 0.001	1.806 $\pm$ 0.162
AuNP-ERGO	143.5 $\pm$ 11.7	0.821 $\pm$ 0.018	2.021 $\pm$ 0.164
ERGO	108.1 $\pm$ 15.2	0.845 $\pm$ 0.025	1.523 $\pm$ 0.214
GO	61.8 $\pm$ 0.6	1.08 $\pm$ 0.056	0.871 $\pm$ 0.008

**data is mean +/- standard deviation obtained from 3 independent experiments*

Figure 3.3 CV scans with increasing concentration of NO from 25 μM to 0.2 mM (arrow indicates the direction of increasing concentration). Inset shows the peak CV current *vs.* NO concentration fitted by a line with a slope of 0.921 $\mu\text{A}/\mu\text{M}$.

3.4.3. NO Detection

CVs of the AuNP-ERGO electrode obtained at different NO concentrations (0 to 200 μM) show that the oxidation current scales linearly with the NO concentration (Figure 3.3). In comparison to CV based measurement, amperometric measurement is able to offer sensitive and real-time detection. Figure 3.4 shows the amperometric current response (at holding voltage = 0.8 V) to successive addition of NO to reach various final concentrations. As shown, the amperometric response to 133 nM NO can be clearly resolved with a signal-

to-noise-ratio (S/N) of ~ 5.5 . And the response time (the time taken to reach 95% of the steady state current) is ~ 3 s, which is short than the physiological lifetime (~ 5 s) of nitric oxide.

Figure 3.4 (A) Amperometric response of AuNP-ERGO electrode (biased at 0.8 V) to successive addition of NO to various concentrations. The response to 1.2 μM NO is displayed in the inset in an enlarged view. (B) Amperometric current response vs. NO concentration averaged from 3 independent experiments (the error bars indicate the standard deviations). The fitted line in

the linear response range has a slope of $0.382 \mu\text{A}/\mu\text{M}$. (C) Amperometric responses to $10 \mu\text{M}$ NO, oxalate, glucose (Glu), uric acid (UA), sodium chloride, or ascorbic acid (AA).

The dose response curve (steady-state amperometric response vs. NO concentration) is plotted Figure 3.4B. As seen the detection range is wide with a lower detection limit of $\sim 133 \text{ nM}$, and the linear response is up to $\sim 3.38 \mu\text{M}$ with a sensitivity of $\sim 5.38 \mu\text{A}/\mu\text{M}/\text{cm}^2$. Such performance is superior to the previously reported electrodes modified with RGO [118], AuNP array [111], AuNP-polyelectrolyte hybrid film [112], or AuNP-CNT-polyelectrolyte composite [108]. Furthermore, we show in Figure 3.4C that NO detection is insensitive to the common physiological interferences including oxalate, glucose, uric acid, sodium ions, and ascorbic acid.

3.4.4. Detection of Dynamic Release of NO from HUVEC Cells Upon Stimulation

The emerging nanoengineered electrodes or devices have brought new possibilities to resolve dynamic cell functions [126, 127]. Here, we demonstrate the use of our AuNP-ERGO hybrid electrode for real-time electrochemical detection of NO release from human umbilical vein endothelial cells (HUVECs) in response to acetylcholine stimulation. It is known that acetylcholine can

acutely stimulate NO production and secretion from endothelial cells via activation of Ca^{2+} -calmodulin dependent signaling cascade [128].

Using the experimental setup as shown in Figure 3.5 A, injection of 2 mM acetylcholine into the HUVEC cell suspension in PBS (6.60×10^5 cells / mL) causes almost instantaneous increase of NO concentration (within 1 s) which is followed by a slower rise and subsequent decay after a few seconds (Figure 3.5B). The peak current response ($1.67 \mu\text{A}$) corresponds to $3.99 \mu\text{M}$ increase of NO as the result of 2 mM acetylcholine stimulation is estimated based on the dose response curve shown in Figure 3.4 B; subsequently, peak current response (201 nA) corresponds to 149 nM of NO as result of 0.5 mM acetylcholine stimulation. Apparently, acetylcholine stimulated NO release from HUVECs is an acute and potent process. In comparison, the response triggered by a lower dose of acetylcholine (0.5 M) is significantly reduced and the electrode is not responsive to the addition of PBS solution (Figure 3.5B).

Figure 3.5 (A) Photo and illustration of the experimental setup for cell experiments. (B) Amperometric response from HUVECs stimulated with acetylcholine of various concentrations. Arrow indicates the injection of stimulation solution. Inset shows the cells grown in the culture flask.

3.5. Conclusion

In summary, we present a method to electrochemically prepare a hybrid thin-film electrode made of electrochemically reduced graphene oxide (ERGO) and gold nanoparticles (AuNPs). Comparing to the commonly used methods which usually involve functionalization of graphene oxides (GOs), drop-casting GOs onto the supporting electrode, and reduction of GOs using harsh chemicals [118,

119, 129], the herein reported method is simple, fast, environmental friendly, and able to reproducibly fabricate uniform thin film.

ERGO network provides highly conductive pathways for electron conduction and a large surface area for catalyst support, while AuNPs act as efficient electrocatalysts towards the oxidation of nitric oxide. The synergistic integration of ERGP and AuNP realizes the electrochemical detection of nitric oxide (NO) with high sensitivity ($5.38 \mu\text{A}/\mu\text{M}/\text{cm}^2$), low detection limit (133 nM with a $S/N = \sim 5.5$), and a fast response time (3 s). We further demonstrate that the AuNP-ERGO hybrid electrode can be used to electrochemically detect dynamic release of NO in response to acetylcholine stimulation from live human umbilical vein endothelial cells with high temporal resolution, suggesting its potential as tool to study the fundamental NO signaling processes.

4. Graphene Quantum Dots Functionalized Gold Nanoparticles for Electrochemical Detection of Heavy Metal Ions^a

4.1. Abstract

Graphene quantum dots are nano-sized graphene sheets that retains unique electrical properties of graphene. In this paper, a novel nanocomposite material is synthesized and utilized for detection of heavy metal using graphene quantum dots. Positively charged gold nanoparticles stabilized by cysteamine are chemically functionalized with graphene quantum dots using ethyl(dimethylaminopropyl) carbodiimide (EDC) for covalent bonding. Resulting nanocomposite is coated onto glassy carbon electrode surface. The sensor shows nanomolar detection with the detection limit of 0.02 nM and sensitivity of 2.47 $\mu\text{A/nM}$.

^aThis chapter has been accepted by Elsevier as a full paper pending for publication [Ting SL, Ee SJ, Arundithi A, Leong KC, Chen P, *Electrochimica Acta*, 2015].

Figure 4.1 Overall mechanism of action for the anodic stripping of GQD-AuNP sensor

4.2. Introduction

Heavy metal detection plays an important role in public health such as safe drinking water or as a pollution control. Heavy metal such as mercury is deemed the third most dangerous heavy metal to arsenic and lead by the Agency of toxic substances and disease registry [130]. Once it enters the food chain, bioaccumulation of mercury poses many lethal health risks such as renal and neural problems [131, 132]. It is a great challenge to design sensor for detecting very small concentration of heavy metals quickly and accurately. There is a need to develop highly sensitive detection of heavy metals as low as 10 nanomolar in concentration. According to Environment Protection Agency

(EPA), the limit of mercury in water is around 10nM to prevent potential health problems [133]. Electrochemical sensing provides a fast and direct detection of heavy metals directly from the water samples. It is also possible for electrochemical sensors to be integrated into microelectronics for miniaturization [134].

Graphene is a highly conductive two dimensional monolayer carbon network. Graphene has extraordinary electrical, thermal and mechanical property. Therefore it has been called the “miracle material” for many applications. Graphene quantum dot is a material that has the characteristics of graphene but with additional quantum confinement and edge effects [135]. Recently, Graphene quantum dots have attracted attention due to its fluorescent and biocompatible nature [136]. There has been many research into GQD’s various synthesis and applications, particularly in optical imaging and sensing [137]. Graphene quantum dots have been used to detect heavy metals by fluorescence quenching mechanism through nonradiative electron hole recombination annihilation [138] and induced aggregation [139]. Other than fluorescent properties, graphene quantum dots can be highly functionalized with carboxylate groups, which is the result of oxidative synthesis route [140]. Negatively charged oxygen functional groups are electrostatically be attracted to positively charged heavy metals to facilitate cation binding [141]. It has been demonstrated that graphene quantum dots can be used as electrochemical sensor for ssDNA via pi-pi stacking or for enzyme immobilization, mostly utilizing graphene-like electrically property as well as high surface area of GQD [136].

AuNPs has been used to electrochemically detect heavy metals such as mercury using ASV methods in the literature due to its high affinity to mercury [142]. GQD functionalized gold nanoparticles provides larger surface area for attachment through electrostatic attraction. Gold nanoparticles has been known to be synthesised through the citrate route, yielding negatively charged gold nanoparticles of controllable sizes via nucleation and growth processes [143]. It is also known that gold nanoparticles can be stabilized in cysteamine, forming positively charged nanoparticles [144]. Positively charged gold nanoparticles are due to the amine groups of cysteamine on the outer surface of the nanoparticles. This act as an excellent template for the graphene quantum dots to attach to via chemical bonding.

In this work, graphene quantum dot is functionalized onto cysteamine capped gold nanoparticles for the electrochemical detection of mercury ions. Functional groups present on the GQD surface allow chelation of mercury ions through electrostatic attraction and facilitates the detection of mercury through anodic stripping. It is to our knowledge the first time such nanocomposite is synthesized for the function of heavy metal detection. The sensor is capable of high sensitivity 2.47 $\mu\text{A/nM}$ with very low detection limit of 20 pM with signal to noise ratio of 6.25.

4.3. Experimental

4.3.1. Reagents and Apparatus

All chemicals are purchased from Sigma Aldrich unless mentioned otherwise. Electrochemical characterizations are done using CHI 660D electrochemical workstation (CH Instruments Inc., Texas, USA) using three electrode system with platinum counter electrode and standard calomel electrode (SCE) as reference electrode. The working electrode used is glassy carbon electrodes (GCE) of apparent surface area 0.071 cm^2 . Electrode surface morphology images and X-ray energy dispersive spectrometry are done using field emission scanning microscope (JEOL JSM-6700F). UV-vis characterization of film is done using UV-vis spectrophotometer (Shimadzu UV-2400PC). Fourier Transform Infrared Spectroscopy (FTIR) was done using PerkinElmer Spectrum GX FTIR system.

4.3.2. Synthesis of Graphene Quantum Dots

Graphene quantum dots were synthesized according to literature [140]. Briefly, CX-72 carbon black is refluxed in concentrated nitric acid for 24 hours. The resulting solution is cooled to room temperature and centrifuged a few times to obtain the supernatant. Supernatant is then heated to be fully dried to

measure its weight, and the redissolved in deionized water with known concentration.

4.3.3. Synthesis of GQD-Gold Nanoparticles.

Gold nanoparticles are synthesized via chemical reduction of gold chloride solution [144]. In brief, 40 mL of 1.42 mM of HAuCl_4 and 400 μL of 213 mM of cysteamine are stirred for 20 minutes in dark condition. This produces a brown solution and slowly turns red. 10 μL of NaBH_3 is added and continuously stirred in the dark for 10 minutes. The cysteamine capped gold nanoparticles is red wine in colour and stored in the fridge at 4 degree before use.

Following the synthesis of gold nanoparticles, 5 mL of as prepared gold nanoparticles are added with 5 mL of 2 mg/mL graphene quantum dots synthesized earlier and shaken lightly. 40 mg of ethyl(dimethylaminopropyl) carbodiimide (EDC) is added to the solution and shaken continuously for 1 hour. The solution will turn from red wine colour into purplish in colour. After shaking, the solution is then centrifuged at 10k rpm for 10 minutes to obtain the sediment. This process is repeated for 5 times to wash the functionalized particles. Finally the sediment is redispersed into 1 mL of deionized water.

4.3.4. Preparation of Modified Electrode

The glassy carbon electrode is first polished using alumina powder until mirror like finish. Electrode is washed with ethanol followed by deionized water while sonicated to remove remaining alumina powder. Electrode is blown dry using nitrogen gas. 3 μ L of prepared GQD-AuNPs solution is dropcasted onto the surface of the electrode and left to dry for half an hour. To ensure complete dryness, it is further dried in vacuum for another 10 minutes. Following this, 3 μ L of 5% nafion solution is drop-coated onto the modified electrode as a proton exchange membrane.

4.3.5. Anodic Stripping Procedures

Preconcentration of the electrode was done by immersing the electrode in various concentration of target analyte with 0.1 M HCl as the electrolyte, followed by application of -0.2 V for 120 s. Anodic stripping was carried out using square wave voltammetry at frequency of 40 Hz, amplitude of 20 mV and potential increment of 4 mV. Successive scanings were used until the deposited metal peak disappears.

4.4. Results and Discussions

4.4.1. Physical Characterizations

Figure 4.2 Scanning electron micrographs of drop-coated AuNP (A) and GQD-AuNPs (B) on glassy carbon electrode.

Scanning electron microscope results are shown in Figure 4.2. As shown in the Figure 4.2A, clear spherical gold nanoparticles are shown in the size of around 50 nm. The size of the gold nanoparticles are further confirmed by UV-vis spectroscopy results shown in Figure 4.3A with peaks of gold nanoparticles absorption at 533 nm, which corresponds to nanoparticles of around 50 nm in diameter as shown in Figure 4.2A. Gold nanoparticles in Figure 4.2B are functionalized with graphene quantum dots. It is observed that coagulation in plate-like appearance occurs more heavily after functionalization. Coagulation formation is due to the charge neutralization between positively charged gold nanoparticles and negatively charged graphene quantum dots. The UV-vis

absorbance after functionalization shifted towards longer wavelength at around 540 nm and 630 nm due to coagulations as seen in Figure 4.3A [145]. The wavelength corresponds to 60 nm sized gold nanoparticles and micrometer-sized large plate-like aggregations as seen in Figure 4.2B.

Figure 4.3 Physical characterization using UV-Vis spectroscopy (A) and energy dispersive spectroscopy (B) (Inset shows appearances of three solutions synthesized; from left to right GQD, AuNPs and GQD-AuNPs)

The visual comparisons in terms of colour of the solutions are shown in the inset in Figure 4.3A. The color of the gold nanoparticles turns from reddish in colour into purplish after chemical reaction signifying some degree of coagulations, while the graphene quantum dots solution appears to be brownish

yellow in colour. The as-produced functionalized gold nanoparticles remain miscible and stable in aqueous solution form when kept in the 4 degree fridge for a few days.

Due to extremely small size of graphene quantum dots, visual presence of graphene dots is not observable in scanning electron micrographs. The presence of graphene quantum dots functionalization onto gold nanoparticles is verified through energy dispersive x-ray (EDX) as shown in Figure 4.3B. Presence of carbon at 20.2%, oxygen at 8.9%, silicon 19.5% and gold at 51.3% is detected quantitatively. The presence of silicon is due to the silicon substrate.

Figure 4.4 FTIR results from samples of GQD (A) and GQD-AuNP (B).

Additionally, FTIR is done to show the chemical functionalization and bonding structure of the reaction product. Gold nanoparticles coated with

cysteamine contains NH_3 groups on the outer surface of the gold nanoparticles. GQDs presented in Figure 4.4B shows the presence of COOH functionalization as seen from the wide O-H peak. By reacting these two components with EDC, amide bond is formed between the amine and carboxylic groups. The presence of C-N, N-H and C=O peaks in Figure 4.4B shows the successful functionalization of GQD onto cysteamine coated gold nanoparticles. The samples are spinned and dried to remove excess GQD and interference from water before doing FTIR spectroscopy.

4.4.2. Electrochemical Characterization

Figure 4.5 Anodic stripping carried out using square wave voltammetry (A) and cyclic voltammetry (B) results from various modified electrodes in 0.1 μM of Hg^{2+} .

Electrodes coated with GQD-AuNPs are compared with electrodes without the coating electrochemically. As shown in Figure 4.5A, ASV results shows increased current response at with peak observed at 0.42 V for GQD-AuNPs electrode, while AuNPs only electrode has peak response at 0.38 V. Cyclic voltammetry comparison between various modified electrodes was also done in Figure 4.5B. Oxidation peak of mercury is observed to be at 0.33 V for AuNPs and 0.4V for GQD-AuNPs. It is shown that in the presence of 1 μM of Mercury, the current increases in GQD-AuNPs electrodes are much higher compared to that of AuNPs only electrode. This is due to the graphene-like heterogenous electron transfer properties present in the higher amount of edge planes in graphene quantum dots [146]. Increased current could also be attributed to the increased electroactive surface area with the addition of gold nanoparticles and graphene quantum dots to the bare electrode. Both ASV and CV results show the increased performance for GQD-AuNPs electrode when compared to bare GCE or gold nanoparticles only electrode.

Figure 4.6 (A) Anodic stripping voltammetry results based on different concentration of mercury (B) Sensitivity curved derived from three independent experiments, error bar shows standard deviation based on three independent results.

Figure 4.6A shows the anodic stripping response done in square wave voltammetry mode. The results shows increase in current when additional mercury concentration is added into the testing solution. The obtained sensitivity in Figure 4.6B is $2.47 \mu\text{A/nM}$ with linear range from 0.02 nM to 2 nM . The detection limit was 20 pM with signal to noise ratio of 6.25 . When

concentration is further increased, there seems to be a plateau effect which could be due to saturation of functional groups involved and surface area, with the sensitivity decreased to 0.75 $\mu\text{A/nM}$.

The mechanism behind the increased heavy metal molecule attachment is attributed to the surface functionalization remaining on the graphene quantum dots. Not all of the carboxylic groups on graphene quantum dots are functionalized to form amide bond with amine groups on the gold nanoparticles. The carboxylic groups on the outer surface of the composites which form an unstable ester with reaction in excess EDC will react with water to regenerate the carboxyl group if no amine groups are present. These regenerated carboxyl groups and inherent hydroxyl groups present on GQD surface act as excellent positive charged attractant [147]. It has also been theorized that carboxylic groups is capable to form chelation with mercury ions in the form of $\text{R-COO-(Hg}^{2+}\text{)-OOC-R}$ chelates to facilitate preconcentration of mercury ion on electrode surface [148]. When coupled with additional property of gold's high affinity towards mercury, mercury molecules can be easily deposited onto the surface of the electrode during the deposition phase [142].

Figure 4.7 (A) Anodic stripping voltammetry results for using different concentrations of Cu^{2+} , (B) Comparisons oxidation potential positions of 0.5 μM response between copper and mercury anodic stripping voltammetry.

Selectivity for heavy metal sensors using anodic stripping method is generally good due to the different oxidation potential for different metals. Other metals such as copper(II), lead(II) and iron(II) solutions are tested for GQD-AuNPs electrode. Copper metal detection has anodic stripping potential at around 0V. It is noted that copper with oxidation potential at around 0V was also detectable, but shows poorer and exhibits a non-linear sensitivity as

compared to mercury. Bubbling is also easily observed when negative potential is applied. For lead and iron metal ions on the other hand shows no observable response. Comparisons to various electrochemical detection of heavy metal using anodic stripping voltammetry methods are summarized in Table 4.1, showing the superior performance of our GQD-AuNP electrode.

Table 4.1 Comparisons of performances between various heavy metal ion sensors

Modifications	Detection limit	Sensitivity	References
Cysteamine-graphene oxide	Hg ²⁺ : 5 nM	Hg ²⁺ : 11.4nA/nM	Zhou <i>et. al.</i> [149]
Heated graphite nanoparticle screen printed electrode (SPE)	Hg ²⁺ : 3.68 nM	Hg ²⁺ : 12.7 nA/nM	Aragay <i>et. al.</i> [150]
Au-NPs/CNTs	Hg ²⁺ : 0.3 nM	Hg ²⁺ : 0.118 μ A/nM	Xu <i>et. al.</i> [151]
GQD-AuNP	Hg ²⁺ : 0.02 nM	Hg ²⁺ : 2.47 μ A/nM	This Work

4.5. Conclusion

In conclusion, an electrochemical heavy metal sensor based on nanocomposite of graphene quantum dot and gold nanoparticles was developed using simple chemical reaction. The synergistic properties between gold nanoparticles and graphene quantum dots enhance mercury molecules attachment onto the surface of the sensors, allowing increased current response using anodic stripping voltammetry when compared to bare glassy carbon

electrodes or gold nanoparticles only electrodes. This advantage allowed the sensor to reach very low detect limit with high sensitivity. The excellent performance of such sensor provides future prospect for graphene quantum dots utilization for electrochemical sensing applications.

5. Manganese Dioxide-Carbon Nanotube Nanocomposite for Highly Sensitive Non-enzymatic Detection of Hydrogen Peroxide

5.1. Abstract

Hydrogen peroxide sensing is crucial in various biomedical applications as it is closely related to metabolism and play vital role in oncogenesis. Non-enzymatic sensors provide better stability and simplicity as compared to most enzymatic sensors. In this chapter, a unique nanocomposite with long manganese dioxide (MnO_2) nanowire is synthesized in the presence of multi-walled carbon nanotube (MWCNT) and coated onto glassy carbon electrode with the help of chitosan as the binder. The nanocomposite was used for hydrogen peroxide sensing and achieved the performance of 8.3 mA M^{-1} in sensitivity and $15 \text{ }\mu\text{M}$ in detection limit.

5.2. Introduction

Hydrogen peroxide (H_2O_2) is an important component of living cells, having roles of host defence and oxidative biosynthetic reactions. At low concentration levels, increasing evidence shows that H_2O_2 is used as a signaling molecule in higher organisms. All aerobic organisms, from prokaryotes to humans, are observed to regulate similar intracellular levels of H_2O_2 concentrations [152].

In the human body, H_2O_2 is closely linked with human metabolism and excessive levels are able to damage cells through base modifications and strand breakage in genomic DNA [153], damage to lysosomal membranes [154], and induction of apoptosis [155]. Additionally, H_2O_2 is also seen to play a role in tumour incidence [156]. As a result, the development of a low cost, high sensitivity and good biocompatibility H_2O_2 sensor is of practical importance.

In electrochemistry, H_2O_2 can be directly reduced or oxidised on electrodes. However, the use of this process analytically is limited due to slow electrode kinetics, high overpotential which will degrade sensing performance and interference due to other electroactive species in real samples such as ascorbate, urate, bilirubin, *etc* [157]. Current research on H_2O_2 sensors is focused on electrode modifications in order to decrease the overpotential and increase electron transfer kinetics. Electrochemical sensors utilising immobilised biomolecules, such as, horse radish peroxidase [158], haemoglobin [159], have been developed. However, the use of biomaterials has been limited due to the disadvantages of high cost, instability and critical demand on the environmental condition. Research has been directed to the development and manufacturing of a new-style non-enzymatic sensor.

To assess a H_2O_2 sensor, the concentration levels of H_2O_2 that the sensor is able to respond to is of great importance. Intracellular H_2O_2 levels vary from 1 nM up to a maximum of 700nM in routine signalling [160-162]. It is observed

that intracellular levels above 1 μ M are toxic to cells, initiating cell death responses [160]

MnO₂ acts as a catalyst to aid the decomposition of H₂O₂ to O₂ [163-166]. J.Wang has shown that a low detection limit of 80 pg (about 1.5 x 10⁻⁷M) can be achieved using a glassy carbon electrode (GCE) modified with a film of MnO₂ operating in a strongly alkaline MnCl₂/NaOH solution [167].

Chitosan (Chi) is the deacetylated derivative of chitin and contains 2-acetamido-2-deoxy- β -D-glucopuranose and 2-amino-2-deoxy- β -D-glucopuranose residues. It has been of interest in use as a biocompatible polymeric matrix based on its excellent film-forming ability, high water permeability and susceptibility to chemical modifications [4].

Research on H₂O₂ detection is focused electrode modification to reduce applied overpotential and enhance electron transfer. As a result, a large variety of materials have been used to conduct electrocatalytic H₂O₂ detection. Nanomaterials have garnered a large amount of research interest in recent years due to desirable chemical, physical, and electronic properties that differ from bulk materials. Additionally, nanomaterials can be modified in terms of size and material structure to design a novel sensor with improved performance [168].

Prussian blue (PB) (also known as ferric hexacyanoferrate) is known as an artificial peroxidase because when it is reduced into Prussian white, it is able to

catalyze the reduction of H_2O_2 at small potentials (-50mV (vs. Ag/AgCl)) like peroxidases [169, 170]. The structure of PB in the form of polycrystals also allows the access of smaller molecules into its lattice structure. Bigger molecules such as ascorbic acid, uric acid, and *para*-acetylaminophenol are blocked out, giving it good catalytic specificity to H_2O_2 [171]. PB is used in H_2O_2 sensing by electro-chemically coating a layer of PB on the surface of the working electrode. After which, additional layers of materials are coated in order to stabilising PB, improving selectivity and improve loading of the enzyme. The nanostructure of PB can be modified as well for sensor construction [172]. The disadvantage of PB based sensor is its instability in solutions of higher pH value. Prussian white easily soluble in alkaline condition in the presence of hydroxides [173]. Other metal hexacyanoferrates have also been utilized for hydrogen peroxide sensing, such as copper [2, 174-177], nickel [178-180], cobalt [181-183], chromium [184, 185], vanadium [186], ruthenium [187, 188] and manganese [189] hexacyanoferrates. These metal sensors have weaker performance in the electrocatalytic reduction of hydrogen peroxide as compared to PB sensors. However, they have a greater structural stability in a wider pH range [172, 174].

Heme proteins are metalloproteins with an iron centred porphyrin. There are various kinds of heme proteins such as haemoglobin (Hb), myoglobin (Mb), catalase (CAT) , microperoxidase (MP) , horseradish peroxidase (HRP) and cytochrome c (Cyt c) [157]. The iron present in the structure is able to be oxidized or reduced easily. This redox reaction can occur over a large range of

overpotentials depending on its protein environment [190, 191]. This allows them to be utilized in many kinds of bioelectrochemical applications.

The use of CNTs are of high interest in chemical and biological sensing applications due to their large surface area, low resistance, reduced surface fouling, easy functionalization on its surface and having some form of catalytic activity [9, 192]. Compared to CNTs, graphene has various advantages such as, low manufacturing cost, mass producible and posing low risk to users [67]. It is also good for application in electrochemistry because it does not contain transition metals impurities which are commonly found in CNTs due to its manufacturing.

Transition metals and their compounds have different oxidation states. This allows other materials to adsorb onto their surface while activating them, making them good catalysts. Nano-sized materials have the advantage of increase mass transport kinetics, larger surface to volume ratio, improved electrical, chemical and optical properties due to their size which allows better utilisation of high cost materials [193]. Therefore, nanoparticles formed from transition metals can have very good catalytic capability due to high proportion of free valence containing surface atoms in comparison to the total atoms [194, 195]. The range of transition metals that have been successfully used for electrocatalysed H_2O_2 detection include, platinum (Pt), iridium (Ir), palladium (Pd), rhodium (Rh), iron (Fe) and copper (Cu). Carbon materials such as CNTs are usually used as substrates because of their larger surface area and

conductivity, which allows them to have high loading of nanomaterials giving them an overall increase in catalytic performance [157].

Metal oxides using transition metals include iridium oxide, manganese oxide, titanium dioxide, cobalt oxide and copper oxide. These materials have also been reported to be electrochemically catalytic towards hydrogen peroxide [157]. Though, many of them had high potentials applied to the working electrode to achieve electrocatalytic oxidation. Some of operating potentials were too high and thus not suitable for biological samples from an application point of view [157]. Methods to lower these operating potentials are still needed to be researched to allow applications in these fields.

In this chapter, a unique nanocomposite fabricated using combination of MnO_2 , carbon nanotube and chitosan, through the method of hydrothermal growth. The purpose of this chapter was to investigate the sensing capability, mechanism and physical property of the nanocomposite formed in this manner. To investigate this nanocomposite's properties, it is used for the detection of H_2O_2 . It is found to achieve the performance of 8.3 mA M^{-1} in sensitivity and $15 \text{ }\mu\text{M}$ in detection limit.

5.3. Experimental

5.3.1. Materials and Equipment

The materials, chemicals and equipment used that were used in the preparation of the MnO_2 -MWCNT composite are as follows: MWCNT (NANOAMOR), sonicator (S80H Elmasonic), vacuum oven (Cole-Parmer Model 281A), autoclave (Hirayama HV-50), NH_4Cl (Sigma), KMnO_4 (Sigma) and centrifuge (Sigma 3K30).

The materials, chemicals and equipment used that were used in the electrode characterisation are as follows: CHI760D (CH Instruments, China), Glassy carbon electrode, KCl saturated calomel electrode and platinum wire counter electrode.

5.3.2. Preparation of MnO_2 -MWCNT Composite

The MWCNT was placed in distilled water and sonicated for an hour. They were then collected by centrifugation and dried at at 50 °C in a vacuum oven overnight.

Synthesis of the MnO_2 -MWCNT was then carried out by mixing 25mg of MWCNTs with 0.2 mmol of NH_4Cl and 0.2 mmol of KMnO_4 . The 10mL mixture was sonicated for 1 hour at room temperature, and then transferred to a 25mL Teflon-lined stainless steel autoclave and maintained at 150 °C for 24 hours. The resulting black solids are then centrifuged for collection and then dried at 50 °C in a vacuum oven.

Fabrication by this method allowed the MWCNTs obtain to a purity of >95%, with an outer diameter of 10-30 μm , inner diameter of 5-10 μm , and a length of 5-15 μm . The obtained MnO_2 -MWCNT composite will then be used to prepare the electrode and will be further discussed.

5.3.3. Preparation of Modified Electrode

The MnO_2 -MWCNT composite is further modified with the addition of Chitosan, as a binder due to its excellent film-forming ability. Chitosan was added to the MnO_2 -MWCNT composite in a 1:2 ratio. The new suspension was then sonicated for 10mins to obtain a homogenous black suspension.

The MnO_2 -MWCNT-Chi suspension is then drop-coated onto the GCE in preparation for testing. Prior to use, the GCE was polished with 0.5 μm alumina powder until a mirror shiny surface appeared, and then rinsed successively with ethanol and double distilled water respectively. After that, 3 μL of the suspension was dropped onto the surface of the pretreated GCE and left to dry for 3hours.

5.4. Results and Discussion

5.4.1. Physical Characterization

Figure 5.1 SEM image of MnO₂ nanowire-MWNT-chitosan matrix

Figure 5.2 SEM image of MnO₂ nanowire-MWNT-chitosan

From Figure 5.1 and 5.2, it is observed that MnO_2 nanowires are long and needle like. The average length is around $2\mu\text{m}$ and diameter around $0.1\mu\text{m}$. CNT are seen to be interwoven around the MnO_2 network. The binding property of chitosan allows the formation of fairly even surface. The 3D structure of the film allows increased surface area in contact with the analyte.

5.4.2. Electrochemical Characterization

The MnO_2 -MWCNT-Chitosan modified electrode was compared to a bare GCE and CNT-Chitosan electrodes acting as controls. The 3 electrodes were utilized as the working electrode in the 3 electrode system to undergo the tests of cyclic voltammetry in H_2O_2 , cyclic voltammetry in ferricyanide solution and amperometry.

Figure 5.3 CVs of bare GCE, CNT-Chi-GCE and MnO₂-CNT-Chi-GCE in 5mM H₂O₂ solution. Scan rate: 50mVs⁻¹

To investigate into the electron transfer behaviour, cyclic voltammetry (CV) was conducted on the bare GCE, CNT-Chitosan modified electrode and MnO₂-MWCNT-Chitosan modified electrode. A solution of 5mM H₂O₂ was prepared, and each electrode was utilised as the working electrode in a 3 electrode system configuration. The scan rate was kept at a constant 50mVs⁻¹. The CVs obtained from the bare GCE, CNT-Chi-GCE and MnO₂-CNT-Chi-GCE are represented in Figure 5.3. The redox peaks on the MnO₂-CNT-Chi-GCE are far greater than the values of the peaks on the bare GCE and CNT-Chi-GCE proving that it is acting as an effective catalyst. On the CV of the MnO₂-CNT-Chi-GCE, a pair of defined redox peaks is observed at the potentials of 0.7280 V (Anodic peak potential, E_{pa}) and -0.6429 V (Cathodic peak potential, E_{pc}). The oxidation peak is attributed to the oxidation of manganese dioxide nanowires with mechanism as follows [5, 196]:

Figure 5.4 CV of MnO₂-CNT-Chi-GCE at different scan rates done in 5 mM ferricyanide with 1M KCl electrolyte solution

Figure 5.5 Plots of scan rate versus peak current

Figure 5.6 Square root of scan rate versus peak current

Ferricyanide experiments to investigate into the effect of the scan rate on the electrochemical response of the modified electrode was done in a solution of 5mM ferricyanide prepared with potassium ferricyanide and 1M of potassium chloride as the electrolyte. The results in Figure 5.4 show that the current behaves in an increasing trend up to 100 mVs^{-1} . At all scan rates, currents behave linearly with the square root of the scan rate as shown in Figure 5.6. This indicates the reaction kinetics to be a solution diffusion-limited process.

Figure 5.7 Amperometric response of MnO₂-CNT-Chi-GCE to successive addition of H₂O₂ at a fixed potential of 0.3V in PBS solution

Figure 5.8 The calibration curve (current versus H₂O₂ concentration) of the biosensor obtained from amperometric response

To investigate into the biosensing performance of the MnO_2 -MWCNT-Chi modified electrode, amperometry was conducted. The initial solution consisted of 10mL PBS, and H_2O_2 was dropped in at even intervals of 50s, starting at 600s. The drops contained 10 μL of H_2O_2 , and the first 5 drops were of concentration 1mM, and the subsequent 25 drops were of 10mM concentration. The amperometric measurements were performed under a stirring condition with the use of a magnetic stirrer, with increasing H_2O_2 concentration. The sensitivity of the MnO_2 -CNT-Chi-GCE is obtained from Figure 5.8 at 0.0083 AM^{-1} . The limit of detection is 15 μM which is the smallest concentration detectable with minimum signal to noise ratio of 3, which is calculated from Figure 5.7 at 0.025 μA . The obtained linear range according to Figure 5.8 is from 0.99 μM to 247 μM .

5.5. Conclusion

The direct electrochemical oxidation of H_2O_2 was achieved on the modified glassy carbon electrode with a drop coating of MnO_2 , CNT and Chitosan, which demonstrated presence of catalytic activity. Compared with bare GCE and a CNT-Chi modified electrode, the higher redox peak values of the MnO_2 -CNT-Chi-GCE were more advantageous, facilitating the diffusion and electrochemical reduction of H_2O_2 .

Comparing the performance to the other hydrogen peroxide sensors; the developed sensor has a lower performance. The comparison table is shown in

Table 5.1. Nevertheless, the sensor has exhibited the good characteristics of fast response times, satisfying stability and reproducibility. It is demonstrated that hydrothermal method is viable for possible for other electrochemical sensing.

Table 5.1 Summary of performance comparison with other hydrogen peroxide sensors

Modifications	Detection limit	Sensitivity	References
Polytetra fluoroethylene/Rod-MnO ₂	0.1 μM	62.9 $\mu\text{AmM}^{-1}\text{cm}^{-2}$	[197]
MnO ₂ /graphene oxide	0.8 μM	38.2 $\mu\text{AmM}^{-1}\text{cm}^{-2}$	[198]
MnO ₂ nanotubes/RGO	1.29 μM	194.5 $\mu\text{AmM}^{-1}\text{cm}^{-2}$	[199]
AgNP/rGO/GCE	35 μM	9.17 $\mu\text{AmM}^{-1}\text{cm}^{-2}$	[200]
MnO ₂ /Graphene paper	10 μM	59 $\mu\text{AmM}^{-1}\text{cm}^{-2}$	[201]
MnO ₂ -CNT-Chi-GCE	15 μM	117.4 $\mu\text{AmM}^{-1}\text{cm}^{-2}$	This work

As shown, the material of ultralong MnO₂ nanowires composited with carbon nanotube in this method is not ideal for electrochemical sensing of hydrogen peroxide. Improvement on the performance of the sensor still needs to be done, which includes improvement on the detection limit through other fabrication methods and materials explored in this thesis. Following which, the sensor can be tested inside a biological environment, to investigate if there are any changes to the sensing effectiveness in the presence of possible interferents. Tests on biocompatibility can also be carried out and investigation into the possibility of the addition of enzymes to the drop-coat as the CNT serves to

immobilise the enzyme and chitosan to stabilise, to make the sensor to be capable of detecting other molecules such as glucose.

6. Flexible Printable Electrode for Non-invasive Glucose Detection from Sweat using Enzymatic Carbon Nanotube/Gold Composite

6.1. Abstract

Traditional glucose level monitoring has been done mostly by finger pricking which poses inconvenience for diabetic patients. In this section, a non-invasive method is proposed to detect the presence of glucose on skin from sweats. A flexible electrode fashioned from nitrocellulose filter paper and carbon nanotube is decorated with gold nanoparticles. The composite electrode is then immobilized with glucose oxidase. Three-electrode system is easily fabricated using a simple masked filtration method. Direct electron transfer is observed from the glucose sensing. Using voltammetric technique, a sensitivity of $4.12 \mu\text{A}/\text{mM}$ is achieved for glucose sensing.

Figure 6.1 Using nanomaterial dispersed solution and filtration technique, filter paper can be patterned into an electrode (left) and further modified into a sensor using surface modification of gold nanoparticles and glucose oxidase (right).

6.2. Introduction

Materials and methods to achieve flexibility in electrode have attracted attention recently due to its compatibility in many applications and integration in flexibility electronics [202]. It is particularly useful in biomedical application where non-uniform surfaces limit the application of rigid electronics. Current methods of producing flexible electronics can be done through screen printing, or pattern transferring of materials onto flexible substrates. There is still need of improvement for the fabrication of such electrodes such as to have lower cost of production. Materials such as conductive polymers [1], graphene [203] and carbon nanotubes [3] has all been utilized to achieve flexibility. Flexible electronics has also achieve transparency and for optical applications as a waveguide and pressure sensor using materials such as polydimethyl siloxane (PDMS) [204]. In electrochemical applications, cheap and simple flexible electrodes are required for mass production. Smaller features can also allow more features to be drawn and mobility in the final device.

Carbon nanotubes are sheets of carbon atoms of sp^2 configuration that forms a tube like structure. Carbon nanotube has been known to be used as a popular material to fabricate flexible conductive materials such as bucky-paper [205]. Carbon nanotube as a material has high aspect ratio which gives it a good surface to volume ratio [206]. These nanoporous surface give a greater effective surface area which is excellent for an electrode material. Carbon nanotubes also have high tensile strength [207], thermal stability [208] and electrical

conductivity [209]. These material properties allow carbon nanotube to be an excellent candidate for flexible electrode.

Gold nanoparticles have been known to achieve direct electron transfer in glucose sensing. Gold nanoparticles can be synthesized easily using methods such as citrate method with excellent size uniformity [89]. Catalytic properties of gold nanoparticles also used for various sensor applications such as nitric oxide [112], hydrogen peroxide [210] and glucose [211]. Other than electrochemistry, gold nanoparticles are also capable of sensing through other mechanism such as fluorescence, surface plasmon resonance and surface enhanced raman scattering [212]. It has been deposited onto various surface such as graphene [213, 214], graphene oxide [215], carbon nanotubes [216] and glassy carbon surface [217]. This also gives the electrode a greater surface area due to the nanostructures of gold nanoparticles.

In this chapter, an alternative method to achieve easy patterning and highly flexible composite electrode is described. Its application in glucose sensing by using gold nanoparticles and glucose oxidase enzyme is achieved. Compositing with gold nanoparticles is done by simple electrodeposition step as done in Chapter 3. Flexible electrode is chosen due to its suitability in uneven skin surfaces and its possibility for integration into electronics. Sensing of glucose from sweat provides an alternate method of blood glucose detection from finger pricking. Pricking not only induce pain and discomfort, and is particularly irritable in small children. The significant correlation between sweat glucose

(SG) and blood glucose (BG) is found with minimum value of the ratio (SG:BG) at around 0.01:1 in diabetic subjects according to literature [218]. Normal human have blood glucose concentration around 5 mM. For diabetic patients the blood glucose concentration is greater than 7 mM. Therefore, sweat based glucose sensors are required to be capable of detecting 0.1 mM glucose. Other than glucose, it also opens to possibility of detecting other analytes from sweat for information on patient health.

6.3. Experimentals

6.3.1. Equipments and Materials

All chemicals are purchased from sigma Aldrich unless otherwise mentioned. Multi-walled nanotube is obtained from Carbon Solutions Inc. Silver paste is obtained from Aik Moh paint. Silicone rubber coating is obtained from Dow Corning (3140 RTV). Silicone rubber sheets used in masking is of 0.3mm thickness and obtained from Professional Plastics (Singapore). Laser cutting of the silicone rubber sheet is done using Universal M-300 Laser Platform (Universal Laser Systems Inc.). Filter paper is 0.22uM pore sized nitrocellulose and filtration system is obtained from Millipore. Sonication is done using S80H Elmasonic. Electrochemical characterizations are done using CHI 660D electrochemical workstation (CH Instruments Inc., Texas, USA). Electrode

surface morphology images are done using field emission scanning microscope (JEOL JSM-6700F).

6.3.2. Flexible Electrode Fabrication

Flexible electrode is fabricated from multi-walled carbon nanotube. Carbon nanotube is first treated with mild oxidation using 3M nitric acid and 35% hydrogen peroxide solution consecutively in sonication under room temperature. After oxidation, carbon nanotubes are filtered through nitrocellulose paper and washed using deionized water. Carbon nanotubes are then scraped off the paper surface and resuspended homogenously in deionized water due to its mildly oxidized surface. Finally, the resuspended solution is filtered again through the nitrocellulose filter paper with a patterned mask place above the filter paper. The thin PDMS mask is patterned from laser cutter with dimensions of the three electrodes drawn as shown in Figure 6.2. After filtration is completed, the mask is removed from the paper. The carbon nanotubes remains adhered onto the paper surface which forms the flexible electrode. The electrode was left to dry in air. This may be similar to bucky-paper synthesis method, but the filter paper was not removed in order for the three electrode system to remain in required configuration. This method is capable to achieve small structures up to 0.5mm in size. Other structures achieved using this method is shown in Figure 6.2B.

Figure 6.2 (A) Three electrodes structure fabricated from filtration method and the actual picture during detection in solution samples; (B) Other electrode structures fabricated from the filtration method.

6.3.3. Electrode Modifications

Three electrodes are fashioned by the filter mask technique on the filter paper, namely counter electrode, reference electrode and the working electrode. The area which is unintended to be in contact with testing solutions is covered using a thin layer of silicone rubber. The counter electrode is unmodified oxidized multiwalled carbon nanotube base material. It is circular in shape and encircled the working electrode in the center.

The reference electrode is made by drop-coating 2uL of silver paste onto an area of 1mm diameter. Following which chloridizing using electrochemical method is done by applying 0.04mA for 60 seconds in 3M KCl solution. The colour of the electrode surface should turn from silver to grayish in colour.

The working electrode is composited with gold nanoparticles by electrodeposition technique similar to method with graphene from literature [214]. In brief, a potential of -0.05mV is applied for 30 seconds in solutions of 0.05mM of HAuCl_4 in 0.5M H_2SO_4 as electrolyte. After deposition, electrode is rinsed in deionized water and dried in air.

After compositing carbon nanotubes with gold nanoparticles, electrode drop-coated with glucose oxidase solution, rinsed and dried in air within minutes. Finally, a thin layer of 0.5% Nafion is coated onto the working electrode. The paper electrode is kept in phosphate buffer saline before experiments.

6.4. Results and Discussion

6.4.1. Physical Characterization of Flexible Electrode

Figure 6.3 Scanning electron micrographs of flexible electrode surface (A), cross section of the electrode (B), gold nanoparticles decorated surface (C), and zoomed gold nanoparticles decorated nanotube electrode surface (D).

The filtered paper surface is studied using scanning electron microscope. From Figure 6.3A, it is observed that the paper surface is a highly interwoven network of carbon nanotube, with very even distribution of carbon nanotube. Figure 6.3B shows the cross section of the carbon nanotube paper with the filter paper removed. The carbon nanotubes thickness appears to be $35\mu\text{m}$. After composited with gold nanoparticles using electrodeposition, the electrode surface is also studied. Figure 6.3C shows gold nanoparticles with different sizes distributed evenly on the surface of the carbon nanotube network. Figure 6.3D shows a zoomed image of the electrodeposited gold nanoparticles. The particles are sized between 20nm to 80nm. The growth of gold nanoparticles is attributed to the mildly oxidized surface on the carbon nanotubes. These oxygen groups can be the positions of nucleation points for the gold nanoparticles [213].

6.4.2. Electrochemistry of GOD Modified Flexible Electrode

Figure 6.4 (A) Cyclic voltammetry responses from 0, 0.05, 5, 50mM of glucose in saturated air phosphate buffer solution, arrow showing increasing concentration. (B) Cyclic voltammetry responses from 0, 1, 2, 3, 4, 5mM of glucose in saturated oxygen phosphate buffer (Inset shows the current response

versus concentration graph), (C) Comparisons of voltammetry response between saturated oxygen (a), saturated air (b), and nitrogen purged (c) phosphate buffer.

Cyclic voltammetry was used to access the electrochemical performance of the sensor. Experiment was done using phosphate buffer of pH 7.5 using a scan rate of 50mV/s. In Figure 6.4C under saturated oxygen and air condition, an alternative irreversible peak is observed at -0.3V. It is also found that the peak at -0.3V is reduced at room oxygen level and completely disappears at nitrogen bubbled buffer solution. This peak is attributed to the oxygen reduction peak. The good catalysis of oxygen reduction could be due to the electrocatalytic properties of gold nanoparticles present in the electrode [219]. A pair of reversible redox peak observed using this electrode is found to be at -0.7V. The peak to peak separation is found to be around 90mV. Under the absence of oxygen with nitrogen gas bubbling, the reversible peak at -0.7V is still visible, showing the direct electron transfer without oxygen. This is characteristics of the reversible electron transfer of GOD's redox center, Flavin adenine dinucleotide (FAD) [70]. It can be concluded that this redox peak is that of FAD/FADH₂. The reversible reaction of the FAD/FADH₂ redox couple is as follows [220]:

FAD redox centers is known to be embedded within deep protein structures which increases the difficulty to achieve electron transfer between electrode and redox center. Gold nanoparticles aids in facilitating direct electron transfer between the redox center of enzyme and carbon nanotube electrode [221].

Under different concentration of glucose in phosphate buffer, cyclic voltammetry was also performed. In Figure 6.4A and B, it is shown that the peak shifts upward with increasing glucose concentration. The cathodic peak current reaches maximum under the absence of glucose, while it decreases with increasing glucose solution. On the other hand, the anodic peak current increases with increasing glucose concentration. With increasing glucose concentration, it is also observed that oxygen reduction is reduced significantly. This is due to consumption of oxygen in the catalytic regeneration of redox center [70]. It is likely that glucose oxidation occurs in the following reactions [222], where second and third equation is probably a competitive process with reference to competition between oxygen involvement and direct electron transfer regeneration of FAD;

Figure 6.4B inset shows that using voltammetric method, the sensor was able to achieve a sensitivity of 4.12 $\mu\text{A}/\text{mM}$. Under amperometry method, a more

unstable result is obtained as shown in Figure 6.5. A possible cause for poor amperometric curve could be due to this particular paper electrode not being well suited to be used in a solution under constant stirring with surface instability of enzyme attachment or having reduced integrity of nanomaterials under constant disturbance. Thus, voltammetric technique should be employed for measurement of glucose for this particular electrode setup. According to our results, CV method is capable of detecting 0.05 mM glucose in phosphate buffer solution, which is theoretically sufficient for detection of glucose level change in sweat samples, which is around 0.1 mM glucose or higher for diabetic patients.

Figure 6.5 Amperometric experiment with current measurement with indicated injection points of glucose

6.5. Improvements and Conclusion

It is important to work with real samples of sweats from human subjects to further validate the working principles of this sensor. However with real samples, it poses further complications with other issues such as interference and conductivity of the body fluids. One major challenge is in the volume of sweat obtainable. It is possible to obtain sweat samples from patients from simple exercises. Drugs such as pilocarpine have also been used for stimulation of sweat through iontophoresis [223]. Iontophoresis requires the application of electric current is used to drive the delivery of drug into skin [224]. This method has been used for obtaining sweat samples from patient to test for cystic fibrosis through finding out concentrations of sodium and chlorides. The cost of iontophoresis devices is not cheap, therefore, an alternative mean to stimulate the skin may be through the delivery of drug with microneedle rollers [225]. Microneedle rollers though slightly invasive but it does not causes much irritation to skin as shown in Figure 6.6. In this setup, microneedle rollers of needle length around 0.25 mm was rolled onto the subject's inner forearm for around five times. Following which, around 50 μ L of 0.5% pilocarpine was applied onto the area and rolling was continued until solution is absorbed or dried. A dry piece of tissue was secured onto the area to observe the effect of the drug. This could be one of the possible protocols to be used in conjunction of flexible sweat sensor in the future.

Figure 6.6 A demonstration of pilocarpine drug used for sweat stimulation within a period of 30 minutes through microneedle delivery.

In conclusion, a flexible sensor is achieved using carbon nanotube, gold nanoparticles and glucose oxidase enzyme. Multiwalled carbon nanotube was patterned using a simple filtration method to form a three electrode flexible sensor device. With further modifications, the device is capable of detecting glucose concentration via direct electrochemistry of glucose oxidase. This is particularly useful for future development of non-invasive sensors for external body fluids.

7. Overall Conclusion and Perspectives

7.1. Conclusion

In this dissertation, the electrochemical performance of metal-carbon nanocomposites for sensing is studied. It is aimed that sensors with excellent performance can be fabricated from nanocomposites between metallic and carbon materials. These two materials serve to synergize with each other on catalysis and surface area enhancement properties which are vital in electrochemical sensing. Four works on these composite materials are presented based on various nanostructuring methods and sensing targets. The goal of studying underlying mechanism of action is also achieved based on each individual sensor.

In the first nanocompositing method, gold nanoparticles are grown on electrochemically reduced graphene oxide with the aid of oxygen groups acting as nucleation spots that is present on graphene oxide. Electrocatalytic property of gold nanoparticles allows the oxidation of nitric oxide, while ERGO network provides highly conductive pathways for electron conduction and a large surface area for catalyst support. Additionally, these materials are shown to be biocompatible for cell works based on the result obtained from study done in culture medium and live cells. The fast response time of the sensor allows future researchers to study cellular metabolism kinetics. This work has

demonstrated new method of cell study and simple nanostructuring method useful for others working in the field of cell based sensing.

In the second nanocompositing method, chemical functionalization is used to form metal-carbon composite material of AuNP-GQDs. Heavy metal sensing for mercury ions through anodic stripping voltammetry is achieved in this sensor. The principle of attraction of heavy metals towards the surface of the electrode is also due to the highly charged surface of functionalized materials with hydroxyl functional group. These hydroxyl groups are also proposed to form chelation with mercury ions. These in combinations of the high affinity of gold towards mercury ions allow easy deposition of mercury ions. High sensitivity could also be attributed to the increased surface area of the nanostructured surface area with high edge effect facilitating heterogenous electron transfers. This work shows the potential of electrochemical performance in graphene quantum dots which is usually utilized for fluorescence study.

In the third nanocompositing method, carbon nanotubes are used as templates for the hydrothermal growth of long manganese oxide nanowires three dimensional network for the sensing of hydrogen peroxide. Manganese oxide nanowire itself is catalytic towards hydrogen peroxide oxidation. Carbon nanotubes which serve as conductive pathways for the electrons synergize well with electrocatalytic manganese oxide nanowires, allowing the composite to work as a sensor. In terms of performance, it is slightly less than what literature

has already reach, but still it is still suitable for certain applications of cell study. This chapter also presented a new nanocomposite method which can be utilized for other sensors.

The final work presented utilizes electrodeposition method studied earlier for application in flexible electrode based sensor. The carbon nanotubes used is mildly oxidized in order to increase nucleation sites for growth of gold nanoparticles. The use of gold in this work is for enzyme adsorption on electrode surface in this case and allows detection of glucose through glucose oxidase. Therefore, both metal and carbon material plays important roles in this sensor. Filtration patterning here is a novel approach to pattern flexible electrodes. This flexible and disposable sensor with ultimate goal of sensing from sweat sample provides a new concept and open up possibility in the area of glucose sensing.

Overall, this dissertation has demonstrated the adaptability of metal-carbon composite materials for electrochemical biosensors through the use of various nanostructuring strategies. Each strategy is suitable for its own application and there is no one-size-fit-all method for nanocompositing metal and carbon materials for every kind of biosensor. In this dissertation, sensors for nitric oxide, hydrogen peroxide, glucose and mercury ions with good performances have been accomplished. These materials have also been found useful in cell culture studies and flexible electronics. The synergizing property between metal and carbon has proven to be useful in forming a high performance

electrochemical sensor. These nanostructuring strategies and electrode fabrication method can be useful for future biosensors development.

7.2. Proposed Future Works

Although the biosensors have overall achieved a good performance, there are still improvements to be done for future works. Additionally, some limitations are also present in the sensors. Much of these sensors are studied on glassy carbon substrate. More works should be done on the physical properties of these composites such as flexibility for stand-alone material in order to improve the sensors' performance. Certain materials such as metallic oxide when composited with strong carbon nanotubes are still fragile and cracks easily when forming electrode films and thus not used as standalone electrodes. Additional studies into how to maintain carbon nanotube electrode's tensile strength even after compositing with metal oxide materials needs to be done.

In the future works, *in vivo* experiments or real samples should be used for further testing for the sensors. In the fourth work, external body fluids such as sweat, tears or saliva could be possibly used for sensing due to the ease of obtaining such fluid. Each body fluid will has different composition, therefore pathological differences with a healthy body fluid needs to be studied thoroughly prior to real experiments. Problems such as interference in each type of body fluids used should also be addressed either by using anti-interference coatings such as nafion or through purification of real samples. Additionally,

biomarkers present in these body fluids can also be studied albeit by a different kind of sensing mechanism.

With the focus on biological study using such sensors, future works should also focus tackling biocompatibility issues on such electrode material and the safety of using such composite materials on live culture or human testing. Issue such as biofouling of electrode surface in cell culture mediums is also an important aspect of such kind of studies.

It will also be interesting if such metal-carbon composite be integrated into microfabrication process either as a stand-alone electrodes or lab on chip devices. Multiplexed sensing from such devices can produced more meaningful results for biological studies such as the observation of real time kinetics of the metabolic analytes. This is highly possible since various kinds of reactive oxygen species and metabolites have been sensed using metal carbon nanocomposites.

References

1. Milind, V.K., et al., *Processing and formulation of inkjet printable conducting polyaniline based ink for low cost, flexible humidity sensors using untreated polymeric substrate*. Smart Materials and Structures, 2012. **21**(3): p. 035023.
2. Zhang, T., et al., *A novel nonenzymatic hydrogen peroxide sensor based on a polypyrrole nanowire-copper nanocomposite modified gold electrode*. Sensors, 2008. **8**(8): p. 5141-5152.
3. Yi, F., et al., *Flexible UHF Resistive Humidity Sensors Based on Carbon Nanotubes*. Sensors Journal, IEEE, 2012. **12**(9): p. 2844-2850.
4. Jia, N., et al., *A simple non-enzymatic hydrogen peroxide sensor using gold nanoparticles-graphene-chitosan modified electrode*. Sensors and Actuators B-Chemical, 2014. **195**: p. 165-170.
5. Li, L., et al., *A novel nonenzymatic hydrogen peroxide sensor based on MnO₂/graphene oxide nanocomposite*. Talanta, 2010. **82**(5): p. 1637-1641.
6. Gilles-Gonzalez, M.-A. and G. Gonzalez, *Heme-based sensors: defining characteristics, recent developments, and regulatory hypotheses*. Journal of Inorganic Biochemistry, 2005. **99**(1): p. 1-22.
7. Cremer, M., *Über die Ursache der elektromotorischen Eigenschaften der Gewebe, zugleich ein Beitrag zur Lehre von Polyphasischen Elektrolytketten*. Z. Biol., 1906. **47**(56).
8. Klemensiewicz, F.H.a.Z., *Über elektrische Phasengrenzkräfte*. Zeitschrift für Physikalische Chemie. Leipzig. Z. physik. Chem., 1909. **67**(385).
9. Zhu, Z., et al., *A Critical Review of Glucose Biosensors Based on Carbon Nanomaterials: Carbon Nanotubes and Graphene*. Sensors, 2012. **12**(5): p. 5996-6022.
10. Ligler, F.S., et al., *Array biosensor for detection of toxins*. Analytical and Bioanalytical Chemistry, 2003. **377**(3): p. 469-477.
11. Urban, P.L., et al., *High-density micro-arrays for mass spectrometry*. Lab on a Chip, 2010. **10**(23): p. 3206-3209.
12. Liden, H., et al., *Rapid alcohol determination in plasma and urine by column liquid chromatography with biosensor detection*. Journal of Pharmaceutical and Biomedical Analysis, 1998. **17**(6-7): p. 1111-1128.
13. Rickert, D., et al., *Biocompatibility testing of novel multifunctional polymeric biomaterials for tissue engineering applications in head and neck surgery: an overview*. European Archives of Oto-Rhino-Laryngology, 2006. **263**(3): p. 215-222.
14. Wu, P.Y., et al., *Luminescent Metal-Organic Frameworks for Selectively Sensing Nitric Oxide in an Aqueous Solution and in Living Cells*. Advanced Functional Materials, 2012. **22**(8): p. 1698-1703.
15. Zhang, X., et al., *Development of a novel fluorescent probe for nitric oxide detection: 8-(3',4'-diaminophenyl)-difluoroboradiaza-S-indacene*. Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy, 2004. **60**(13): p. 3129-3134.
16. Huang, K.J., et al., *Sensitive determination of ultra-trace nitric oxide in blood using derivatization-polymer monolith microextraction coupled with reversed-phase high-performance liquid chromatography*. Analytica Chimica Acta, 2007. **591**(1): p. 116-122.

17. Sciences, E.L. *ROS/RNS detection kit for microscopy*. Available from: <http://www.enzolifesciences.com/ENZ-51001-200/ros-rns-detection-kit-for-microscopy/>.
18. Borgmann, S., *Electrochemical quantification of reactive oxygen and nitrogen: challenges and opportunities*. Analytical and Bioanalytical Chemistry, 2009. **394**(1): p. 95-105.
19. F, F.R., *Endothelium-derived relaxing factor: discovery, early studies, and identification as nitric oxide*. In Les Prix Nobel, 1999. **1998**: p. 226-243
20. Furchgott, R.F. and D. Jothianandan, *Endothelium-dependent and endothelium-independent vasodilation involving cyclic-GMP - relaxation induced by nitric-oxide, carbon-monoxide and light*. Blood Vessels, 1991. **28**(1-3): p. 52-61.
21. Furchgott R F, Z.J.V., *The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine*. Nature, 1980. **288**: p. 373-376.
22. Southam, E. and J. Garthwaite, *Intercellular action of nitric-oxide in adult rat cerebellar slices*. Neuroreport, 1991. **2**(11): p. 658-660.
23. Granger, D.N. and P. Kubes, *Nitric oxide as antiinflammatory agent*. Nitric Oxide, Pt B, 1996. **269**: p. 434-442.
24. Radomski, M.W., R.M.J. Palmer, and S. Moncada, *An L-arginine nitric-oxide pathway present in human platelets regulates aggregation*. Proceedings of the National Academy of Sciences of the United States of America, 1990. **87**(13): p. 5193-5197.
25. P. Thejass, G.K., *Allyl isothiocyanate (AITC) and phenyl isothiocyanate (PITC) inhibit tumour-specific angiogenesis by downregulating nitric oxide (NO) and tumour necrosis factor- α (TNF- α) production*. Nitric Oxide, 2007. **16**(2): p. 247.
26. Napoli, C. and L.J. Ignarro, *Nitric oxide and atherosclerosis*. Nitric Oxide-Biology and Chemistry, 2001. **5**(2): p. 88-97.
27. Kavya, R., et al., *Nitric oxide synthase regulation and diversity: Implications in Parkinson's disease*. Nitric Oxide-Biology and Chemistry, 2006. **15**(4): p. 280-294.
28. Lin, K.Y., et al., *Impaired nitric oxide synthase pathway in diabetes mellitus - Role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase*. Circulation, 2002. **106**(8): p. 987-992.
29. Traub, O. and R. Vanbibber, *Role of nitric oxide in insulin dependent diabetes mellitus related vascular complications*. Western Journal of Medicine, 1995. **162**(5): p. 439-445.
30. Ciszewski, A. and G. Milczarek, *A new Nafion-free bipolymeric sensor for selective and sensitive detection of nitric oxide*. Electroanalysis, 1998. **10**(11): p. 791-793.
31. Veal, E.A., A.M. Day, and B.A. Morgan, *Hydrogen peroxide sensing and signaling*. Molecular Cell, 2007. **26**(1): p. 1-14.
32. Beevi, S.S.S., A.M.H. Rasheed, and A. Geetha, *Evaluation of oxidative stress and nitric oxide levels in patients with oral cavity cancer*. Japanese Journal of Clinical Oncology, 2004. **34**(7): p. 379-385.
33. Beevi, S.S., M.H. Rasheed, and A. Geetha, *Evidence of oxidative and nitrosative stress in patients with cervical squamous cell carcinoma*. Clinica Chimica Acta, 2007. **375**(1-2): p. 119-123.

34. Pelicano, H., D. Carney, and P. Huang, *ROS stress in cancer cells and therapeutic implications*. Drug Resistance Updates, 2004. **7**(2): p. 97-110.
35. Nishikawa, M., *Reactive oxygen species in tumor metastasis*. Cancer Letters, 2008. **266**(1): p. 53-59.
36. Thomsen, L.L., et al., *NITRIC-OXIDE SYNTHASE ACTIVITY IN HUMAN GYNECOLOGICAL CANCER*. Cancer Research, 1994. **54**(5): p. 1352-1354.
37. Xu, W.M., et al., *The role of nitric oxide in cancer*. Cell Research, 2002. **12**(5-6): p. 311-320.
38. Barnes, M.B. and J.L. Beverly, *Nitric oxide's role in glucose homeostasis*. American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 2007. **293**(2): p. R590-R591.
39. Korde, S., et al., *Nitric oxide and oral cancer: A review*. Oral Oncology, 2012. **48**(6): p. 475-483.
40. Azad, N., Y. Rojanasakul, and V. Vallyathan, *Inflammation and lung cancer: Roles of reactive oxygen/nitrogen species*. Journal of Toxicology and Environmental Health-Part B-Critical Reviews, 2008. **11**(1): p. 1-15.
41. Khanna, V.K., *Nitric oxide electrochemical sensors*, in *Nanosensors: Physical, Chemical, and Biological* 2012, CRC press.
42. Alves, W.A., et al., *A Chloro-Bridged Linear Chain Imine-Copper(II) Complex and Its Application as an Enzyme-Free Amperometric Biosensor for Hydrogen Peroxide*. European Journal of Inorganic Chemistry, 2009(15): p. 2219-2228.
43. Bustos, E., et al., *Preparation, characterization, and electrocatalytic activity of surface anchored, Prussian blue containing Starburst PAMAM dendrimers on gold electrodes*. Langmuir, 2005. **21**(7): p. 3013-3021.
44. Wu, S.G., et al., *Improvement of selectivity and stability of amperometric detection of hydrogen peroxide using Prussian Blue-PAMAM supramolecular complex membrane as a catalytic layer*. Electroanalysis, 2007. **19**(6): p. 659-667.
45. Wu, S., et al., *Electrodeposition of silver-DNA hybrid nanoparticles for electrochemical sensing of hydrogen peroxide and glucose*. Electrochemistry Communications, 2006. **8**(8): p. 1197-1203.
46. Liu, Y., L.M. Hu, and S.Q. Yang, *Amplification of bioelectrocatalytic signalling based on silver nanoparticles and DNA-derived horseradish peroxidase biosensors*. Microchimica Acta, 2008. **160**(3): p. 357-365.
47. F., H., *Gmelin and his Handbuch*. Bulletin for the History of Chemistry, 2009. **34**(1): p. 30-1.
48. SJ, H., *'What is a "Heavy Metal"?* Journal of Chemical Education, 1997. **74**(11): p. 1374.
49. Singh, R., et al., *Heavy metals and living systems: An overview*. Indian Journal of Pharmacology, 2011. **43**(3): p. 246-253.
50. Vallee, B.L. and D.D. Ulmer, *Biochemical effects of mercury, cadmium, and lead*. Annual Review of Biochemistry, 1972. **41**: p. 91-&.
51. Bertin, G. and D. Averbeck, *Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review)*. Biochimie, 2006. **88**(11): p. 1549-1559.
52. Jang, D.H. and R.S. Hoffman, *Heavy Metal Chelation in Neurotoxic Exposures*. Neurologic Clinics, 2011. **29**(3): p. 607-+.

53. Wang, S., E.S. Forzani, and N. Tao, *Detection of Heavy Metal Ions in Water by High-Resolution Surface Plasmon Resonance Spectroscopy Combined with Anodic Stripping Voltammetry*. Analytical Chemistry, 2007. **79**(12): p. 4427-4432.
54. Fen, Y.W. and W.M.M. Yunus, *Surface plasmon resonance spectroscopy as an alternative for sensing heavy metal ions: a review*. Sensor Review, 2013. **33**(4): p. 305-314.
55. Zhao, F., et al., *Ultra-sensitive detection of heavy metal ions in tap water by laser-induced breakdown spectroscopy with the assistance of electrical-deposition*. Analytical Methods, 2010. **2**(4): p. 408-414.
56. J Baron-Jaimez, M.R.J., J Barba-Ortega, *Anodic stripping voltammetry - ASV for determination of heavy metals*. Journal of Physics: Conference Series, 2013. **466**(012023).
57. Nichols, S.P., et al., *Biocompatible Materials for Continuous Glucose Monitoring Devices*. Chemical Reviews, 2013. **113**(4): p. 2528-2549.
58. Tian, K., M. Prestgard, and A. Tiwari, *A review of recent advances in nonenzymatic glucose sensors*. Materials Science & Engineering C-Materials for Biological Applications, 2014. **41**: p. 100-118.
59. Park, S., H. Boo, and T.D. Chung, *Electrochemical non-enzymatic glucose sensors*. Analytica Chimica Acta, 2006. **556**(1): p. 46-57.
60. Burke, L.D., *Premonolayer oxidation and its role in electrocatalysis*. Electrochimica Acta, 1994. **39**(11-12): p. 1841-1848.
61. Ambrosi, A., et al., *Electrochemistry of Graphene and Related Materials*. Chemical Reviews, 2014. **114**(14): p. 7150-7188.
62. Novoselov, K.S., et al., *Electric field effect in atomically thin carbon films*. Science, 2004. **306**(5696): p. 666-669.
63. Brodie, B.C., *On the Atomic Weight of Graphite*. Phil. Trans. R. Soc. Lond. , 1859. **149**: p. 249-259.
64. Hummers, W.S. and R.E. Offeman, *Preparation of Graphitic Oxide*. Journal of the American Chemical Society, 1958. **80**(6): p. 1339-1339.
65. Brownson, D.A.C. and C.E. Banks, *Graphene electrochemistry: an overview of potential applications*. Analyst, 2010. **135**(11): p. 2768-2778.
66. Shao, Y., et al., *Graphene Based Electrochemical Sensors and Biosensors: A Review*. Electroanalysis, 2010. **22**(10): p. 1027-1036.
67. Pumera, M., et al., *Graphene for electrochemical sensing and biosensing*. TrAC Trends in Analytical Chemistry, 2010. **29**(9): p. 954-965.
68. Fang, Y., et al., *Self-Assembly of Cationic Polyelectrolyte-Functionalized Graphene Nanosheets and Gold Nanoparticles: A Two-Dimensional Heterostructure for Hydrogen Peroxide Sensing*. Langmuir, 2010. **26**(13): p. 11277-11282.
69. Guo, C.X., et al., *RGD-Peptide Functionalized Graphene Biomimetic Live-Cell Sensor for Real-Time Detection of Nitric Oxide Molecules*. ACS Nano, 2012. **6**(8): p. 6944-6951.
70. Shan, C., et al., *Direct Electrochemistry of Glucose Oxidase and Biosensing for Glucose Based on Graphene*. Analytical Chemistry, 2009. **81**(6): p. 2378-2382.
71. LV, R., *About the structure of carbon formed by thermal decomposition of carbon monoxide on iron substrate*. Soviet Journal of Physical Chemistry, 1952. **26**: p. 88-95.

72. Iijima, S., *Helical Microtubules of graphitic carbon*. Nature, 1991. **354**(6348): p. 56-58.
73. Wang, J., M. Musameh, and Y. Lin, *Solubilization of Carbon Nanotubes by Nafion toward the Preparation of Amperometric Biosensors*. Journal of the American Chemical Society, 2003. **125**(9): p. 2408-2409.
74. Britto, P.J., K.S.V. Santhanam, and P.M. Ajayan, *Carbon nanotube electrode for oxidation of dopamine*. Bioelectrochemistry and Bioenergetics, 1996. **41**(1): p. 121-125.
75. Ye, J.S., et al., *Selective voltammetric detection of uric acid in the presence of ascorbic acid at well-aligned carbon nanotube electrode*. Electroanalysis, 2003. **15**(21): p. 1693-1698.
76. Wang, Z., et al., *Carbon nanotube-modified electrodes for the simultaneous determination of dopamine and ascorbic acid*. Analyst, 2002. **127**(5): p. 653-658.
77. Liu, G., et al., *Sensitive electrochemical detection of enzymatically generated thiocholine at carbon nanotube modified glassy carbon electrode*. Electrochemistry Communications, 2005. **7**(11): p. 1163-1169.
78. Yamaguchi, T., S. Bandow, and S. Iijima, *Synthesis of carbon nanohorn particles by simple pulsed arc discharge ignited between pre-heated carbon rods*. Chemical Physics Letters, 2004. **389**(1-3): p. 181-185.
79. Hou, P.X., et al., *Multi-step purification of carbon nanotubes*. Carbon, 2002. **40**(1): p. 81-85.
80. Wang, F. and S. Hu, *Electrochemical sensors based on metal and semiconductor nanoparticles*. Microchimica Acta, 2009. **165**(1-2): p. 1-22.
81. Gloaguen, F., J.M. Le Ger, and C. Lamy, *Electrocatalytic oxidation of methanol on platinum nanoparticles electrodeposited onto porous carbon substrates*. Journal of Applied Electrochemistry, 1997. **27**(9): p. 1052-1060.
82. Guo, L., et al., *Iron nanoparticles: Synthesis and applications in surface enhanced Raman scattering and electrocatalysis*. Physical Chemistry Chemical Physics, 2001. **3**(9): p. 1661-1665.
83. Hernández-Santos, D., M.B. González-García, and A.C. García, *Metal-Nanoparticles Based Electroanalysis*. Electroanalysis, 2002. **14**(18): p. 1225-1235.
84. Ahirwal, G.K. and C.K. Mitra, *Direct Electrochemistry of Horseradish Peroxidase-Gold Nanoparticles Conjugate*. Sensors, 2009. **9**(2): p. 881-894.
85. Zhao, J., et al., *Direct electron transfer at horseradish peroxidase—colloidal gold modified electrodes*. Journal of Electroanalytical Chemistry, 1992. **327**(1-2): p. 109-119.
86. Karadeniz, H., et al., *Electrochemical sensing of silver tags labelled DNA immobilized onto disposable graphite electrodes*. Electrochemistry Communications, 2007. **9**(9): p. 2167-2173.
87. Dequaire, M., C. Degrand, and B. Limoges, *An electrochemical metalloimmunoassay based on a colloidal gold label*. Analytical Chemistry, 2000. **72**(22): p. 5521-5528.
88. Wang, J., et al., *Magnetic bead-based label-free electrochemical detection of DNA hybridization*. Analyst, 2001. **126**(11): p. 2020-2024.
89. Turkevich, J., P.C. Stevenson, and J. Hillier, *A study of the nucleation and growth processes in the synthesis of colloidal gold*. Discussions of the Faraday Society, 1951(11): p. 55-&.

90. Newman, J.D.S. and G.J. Blanchard, *Formation of Gold Nanoparticles Using Amine Reducing Agents*. Langmuir, 2006. **22**(13): p. 5882-5887.
91. Dozol, H., et al., *On the Synthesis of Au Nanoparticles Using EDTA as a Reducing Agent*. The Journal of Physical Chemistry C, 2013. **117**(40): p. 20958-20966.
92. Polte, J.r., et al., *New insights of the nucleation and growth process of gold nanoparticles via in situ coupling of SAXS and XANES*. Journal of Physics: Conference Series, 2010. **247**(1): p. 012051.
93. Liu, A., *Towards development of chemosensors and biosensors with metal-oxide-based nanowires or nanotubes*. Biosensors & Bioelectronics, 2008. **24**(2): p. 167-177.
94. Hoyer, P., *Formation of a Titanium Dioxide Nanotube Array*. Langmuir, 1996. **12**(6): p. 1411-1413.
95. Lee, W.-J. and W.H. Smyrl, *Oxide nanotube arrays fabricated by anodizing processes for advanced material application*. Current Applied Physics, 2008. **8**(6): p. 818-821.
96. Wang, G.X., et al., *Synthesis and high gas sensitivity of tin oxide nanotubes*. Sensors and Actuators B-Chemical, 2008. **131**(1): p. 313-317.
97. Zheng, D., et al., *One-Step Preparation of Single-Crystalline β -MnO₂ Nanotubes*. The Journal of Physical Chemistry B, 2005. **109**(34): p. 16439-16443.
98. Wang, H., et al., *Composites of carbon black functionalized with polymers as candidates for the detection of methanol vapor*. Reactive and Functional Polymers, 2007. **67**(10): p. 977-985.
99. Guo, Z., et al., *1,3,5-Trinitrotoluene detection by a molecularly imprinted polymer sensor based on electropolymerization of a microporous-metal-organic framework*. Sensors and Actuators B: Chemical, 2015. **207**, Part B(0): p. 960-966.
100. Yang, T., et al., *Graphene-Based Polyaniline Arrays for Deoxyribonucleic Acid Electrochemical Sensor: Effect of Nanostructure on Sensitivity*. ACS Applied Materials & Interfaces, 2014. **6**(21): p. 19050-19056.
101. Yu, S., et al., *Ni nanoparticles decorated titania nanotube arrays as efficient nonenzymatic glucose sensor*. Electrochimica Acta, 2012. **76**(0): p. 512-517.
102. Furchgott, R.F., *Endothelium-derived relaxing factor: discovery, early studies, and identification as nitric oxide*. Les Prix Nobel, 1999. **1998**: p. 226-243.
103. Paslawska, U., et al., *Role of nitric oxide in the regulation of the cardiovascular function*. Medycyna Weterynaryjna, 2012. **68**(6): p. 349-352.
104. Granger, D.N. and P. Kubes, *Nitric oxide as antiinflammatory agent*. Methods Enzymol., 1996. **269**: p. 434-442.
105. Thejass, P. and G. Kuttan, *Allyl isothiocyanate (AITC) and phenyl isothiocyanate (PITC) inhibit tumour-specific angiogenesis by downregulating nitric oxide (NO) and tumour necrosis factor- α (TNF- α) production*. Nitric Oxide-Biol. Chem., 2007. **16**(2): p. 247.
106. Wen, W., et al., *A highly sensitive nitric oxide biosensor based on hemoglobin-chitosan/graphene-hexadecyltrimethylammonium bromide nanomatrix*. Sensors and Actuators B-Chemical, 2012. **166**: p. 444-450.
107. Liu, Y.X., X.C. Dong, and P. Chen, *Biological and chemical sensors based on graphene materials*. Chemical Society Reviews, 2012. **41**(6): p. 2283-2307.

108. Yu, A., et al., *Preparation and electrochemical properties of gold nanoparticles containing carbon nanotubes-polyelectrolyte multilayer thin films*. *Electrochimica Acta*, 2011. **56**(25): p. 9015-9019.
109. Kannan, P. and S.A. John, *Highly sensitive electrochemical determination of nitric oxide using fused spherical gold nanoparticles modified ITO electrode*. *Electrochimica Acta*, 2010. **55**(10): p. 3497-3503.
110. Subramani Thangavel, R.R., *Polymer membrane stabilized gold nanostructures modified electrode and its application in nitric oxide detection*. *J. Phys. Chem. C*, 2008. **112**(50): p. 19825-19830.
111. Zhang, J. and M. Oyama, *Gold nanoparticle arrays directly grown on nanostructured indium tin oxide electrodes: Characterization and electroanalytical application*. *Analytica Chimica Acta*, 2005. **540**(2): p. 299-306.
112. Yu, A., et al., *Nanostructured Electrochemical Sensor Based on Dense Gold Nanoparticle Films*. *Nano Letters*, 2003. **3**(9): p. 1203-1207.
113. Zhu, M., et al., *Novel nitric oxide microsensor and its application to the study of smooth muscle cells*. *Analytica Chimica Acta*, 2002. **455**(2): p. 199-206.
114. Zan, X., et al., *Freestanding graphene paper decorated with 2D-assembly of Au@Pt nanoparticles as flexible biosensors to monitor live cell secretion of nitric oxide*. *Biosensors and Bioelectronics*, 2013. **49**(0): p. 71-78.
115. Li, C.M., et al., *Electrochemical detection of nitric oxide on a SWCNT/RTIL composite gel microelectrode*. *Electroanalysis*, 2006. **18**(7): p. 713-718.
116. Kovtyukhova, N.I., et al., *Layer-by-layer assembly of ultrathin composite films from micron-sized graphite oxide sheets and polycations*. *Chemistry of Materials*, 1999. **11**(3): p. 771-778.
117. Guo, C.X., et al., *Layered Graphene/Quantum Dots for Photovoltaic Devices*. *Angewandte Chemie-International Edition*, 2010. **49**(17): p. 3014-3017.
118. Wang, Y.-L. and G.-C. Zhao, *Electrochemical sensing of nitric oxide on electrochemically reduced graphene modified electrode*. *International Journal of Electrochemistry*, 2011. **2012**: p. 482780.
119. Kim, Y.-R., et al., *Electrochemical detection of dopamine in the presence of ascorbic acid using graphene modified electrodes*. *Biosensors and Bioelectronics*, 2010. **25**(10): p. 2366-2369.
120. Marcano, D.C., et al., *Improved Synthesis of Graphene Oxide*. *ACS Nano*, 2010. **4**(8): p. 4806-4814.
121. Wu, M.-S., C.-J. Lin, and C.-L. Ho, *Multilayered architecture of graphene nanosheets and MnO₂ nanowires as an electrode material for high-performance supercapacitors*. *Electrochimica Acta*, 2012. **81**(0): p. 44-48.
122. Liu, S., et al., *A simple two-step electrochemical synthesis of graphene sheets film on the ITO electrode as supercapacitors*. *Journal of Applied Electrochemistry*, 2011. **41**(7): p. 881-884.
123. Liu, C.B., et al., *Direct Electrodeposition of Graphene Enabling the One-Step Synthesis of Graphene-Metal Nanocomposite Films*. *Small*, 2011. **7**(9): p. 1203-1206.
124. Dong, X.C., W. Huang, and P. Chen, *In Situ Synthesis of Reduced Graphene Oxide and Gold Nanocomposites for Nanoelectronics and Biosensing*. *Nanoscale Research Letters*, 2011. **6**.

125. Daniel, M.C. and D. Astruc, *Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology*. Chemical Reviews, 2004. **104**(1): p. 293-346.
126. Huang, Y.X., et al., *Label-free detection of ATP release from living astrocytes with high temporal resolution using carbon nanotube network*. Biosensors & Bioelectronics, 2009. **24**(8): p. 2716-2720.
127. Huang, Y.X., D. Cai, and P. Chen, *Micro- and Nanotechnologies for Study of Cell Secretion*. Analytical Chemistry, 2011. **83**(12): p. 4393-4406.
128. Cohen, R.A. and P.M. Vanhoutte, *ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION - BEYOND NITRIC-OXIDE AND CYCLIC-GMP*. Circulation, 1995. **92**(11): p. 3337-3349.
129. Chen, Y., et al., *Fabrication of gold nanoparticles on bilayer graphene for glucose electrochemical biosensing*. Journal of Materials Chemistry, 2011. **21**(21): p. 7604-7611.
130. Risher, J.F., et al., *Summary report for the expert panel review of the toxicological profile for mercury*. Toxicol Ind Health, 1999. **15**(5): p. 483-516.
131. Rice, K.M., et al., *Environmental mercury and its toxic effects*. J Prev Med Public Health, 2014. **47**(2): p. 74-83.
132. RONG Li-Ping, X.Y.-Y., JIANG Xiao-Yun, *Heavy metal poisoning and renal injury in children*. CJCP, 2014. **16**(4): p. 325-329.
133. Agency, U.S.E.P. *National Primary Drinking Water Regulations*. 2009; Available from: <http://water.epa.gov/drink/contaminants/upload/mcl-2.pdf>.
134. Nagy, G. and L. Nagy, *Electrochemical Sensors Developed for Gathering Microscale Chemical Information*. Analytical Letters, 2007. **40**(1): p. 3-38.
135. Li, L., et al., *Focusing on luminescent graphene quantum dots: current status and future perspectives*. Nanoscale, 2013. **5**(10): p. 4015-4039.
136. Sun, H., et al., *Recent advances in graphene quantum dots for sensing*. Materials Today, 2013. **16**(11): p. 433-442.
137. Prasad, K.P., et al., *Fluorescent quantum dots derived from PEDOT and their applications in optical imaging and sensing*. Materials Horizons, 2014.
138. Lu, W., et al., *Economical, Green Synthesis of Fluorescent Carbon Nanoparticles and Their Use as Probes for Sensitive and Selective Detection of Mercury(II) Ions*. Analytical Chemistry, 2012. **84**(12): p. 5351-5357.
139. Ananthanarayanan, A., et al., *Facile Synthesis of Graphene Quantum Dots from 3D Graphene and their Application for Fe³⁺ Sensing*. Advanced Functional Materials, 2014. **24**(20): p. 3021-3026.
140. Dong, Y., et al., *One-step and high yield simultaneous preparation of single- and multi-layer graphene quantum dots from CX-72 carbon black*. Journal of Materials Chemistry, 2012. **22**(18): p. 8764-8766.
141. Rajawat, D., N. Kumar, and S. Satsangee, *Trace determination of cadmium in water using anodic stripping voltammetry at a carbon paste electrode modified with coconut shell powder*. Journal of Analytical Science and Technology, 2014. **5**(1): p. 19.
142. Abollino, O., et al., *Determination of Mercury by Anodic Stripping Voltammetry with a Gold Nanoparticle-Modified Glassy Carbon Electrode*. Electroanalysis, 2008. **20**(1): p. 75-83.
143. Polte, J., et al., *Mechanism of Gold Nanoparticle Formation in the Classical Citrate Synthesis Method Derived from Coupled In Situ XANES and SAXS*

- Evaluation*. Journal of the American Chemical Society, 2010. **132**(4): p. 1296-1301.
144. Lee, S.H., et al., *Amine-functionalized gold nanoparticles as non-cytotoxic and efficient intracellular siRNA delivery carriers*. International Journal of Pharmaceutics, 2008. **364**(1): p. 94-101.
 145. Yang, Y., et al., *Controlling the aggregation behavior of gold nanoparticles*. Materials Science and Engineering: B, 2007. **140**(3): p. 172-176.
 146. Ambrosi, A., et al., *Electrochemistry of Graphene and Related Materials*. Chemical Reviews, 2014.
 147. Yu, C., et al., *Ultrasensitive and selective sensing of heavy metal ions with modified graphene*. Chemical Communications, 2013. **49**(58): p. 6492-6494.
 148. Chen, K., et al., *Hg(II) Ion Detection Using Thermally Reduced Graphene Oxide Decorated with Functionalized Gold Nanoparticles*. Analytical Chemistry, 2012. **84**(9): p. 4057-4062.
 149. Zhou, H., et al., *Sensitive and selective voltammetric measurement of Hg²⁺ by rational covalent functionalization of graphene oxide with cysteamine*. Analyst, 2012. **137**(2): p. 305-308.
 150. Aragay, G., J. Pons, and A. Merkoci, *Enhanced electrochemical detection of heavy metals at heated graphite nanoparticle-based screen-printed electrodes*. Journal of Materials Chemistry, 2011. **21**(12): p. 4326-4331.
 151. Xu, H., et al., *Microwave-radiated synthesis of gold nanoparticles/carbon nanotubes composites and its application to voltammetric detection of trace mercury(II)*. Electrochemistry Communications, 2008. **10**(12): p. 1839-1843.
 152. James R. Stone, S.Y., *Hydrogen Peroxide: A Signaling Messenger*. Antioxidants & Redox Signaling., 2006. **8**(3-4): p. 243-270.
 153. Roninson, I.B., *Tumor cell senescence in cancer treatment*. Cancer Research, 2003. **63**(11): p. 2705-2715.
 154. Antunes, F., E. Cadenas, and U.T. Brunk, *Apoptosis induced by exposure to a low steady-state concentration of H₂O₂ is a consequence of lysosomal rupture*. Biochemical Journal, 2001. **356**: p. 549-555.
 155. Geiser, T., et al., *H₂O₂ inhibits alveolar epithelial wound repair in vitro by induction of apoptosis*. American Journal of Physiology-Lung Cellular and Molecular Physiology, 2004. **287**(2): p. L448-L453.
 156. Valko, M., et al., *Role of oxygen radicals in DNA damage and cancer incidence*. Molecular and Cellular Biochemistry, 2004. **266**(1-2): p. 37-56.
 157. Chen, W., et al., *Recent advances in electrochemical sensing for hydrogen peroxide: a review*. Analyst, 2012. **137**(1): p. 49-58.
 158. Wang, L. and E.K. Wang, *A novel hydrogen peroxide sensor based on horseradish peroxidase immobilized on colloidal Au modified ITO electrode*. Electrochemistry Communications, 2004. **6**(2): p. 225-229.
 159. Chen, S., et al., *Amperometric third-generation hydrogen peroxide biosensor based on the immobilization of hemoglobin on multiwall carbon nanotubes and gold colloidal nanoparticles*. Biosensors and Bioelectronics, 2007. **22**(7): p. 1268-1274.
 160. Jonsson, G. and L. Gorton, *An electrochemical sensor for hydrogen-peroxide based on peroxidase adsorbed on a spectrographic graphite electrode*. Electroanalysis, 1989. **1**(5): p. 465-468.

161. Lvovich, V. and A. Scheeline, *Amperometric sensors for simultaneous superoxide and hydrogen peroxide detection*. Analytical Chemistry, 1997. **69**(3): p. 454-462.
162. Wu, S., et al., *Electrodeposition of silver-DNA hybrid nanoparticles for electrochemical sensing of hydrogen peroxide and glucose*. Electrochemistry Communications, 2006. **8**(8): p. 1197-1203.
163. Schachl, K., et al., *Amperometric Determination of Hydrogen Peroxide With a Manganese Dioxide-modified Carbon Paste Electrode Using Flow Injection Analysis*. Analyst, 1997. **122**(9): p. 985-989.
164. Turkusic, E., et al., *Amperometric determination of bonded glucose with an MnO₂ and glucose oxidase bulk-modified screen-printed electrode using flow-injection analysis*. Talanta, 2005. **65**(2): p. 559-564.
165. Turkusic, E., et al., *Amperometric determination of glucose with an MnO₂ and glucose oxidase bulk-modified screen-printed carbon ink biosensor*. Analytical Letters, 2001. **34**(15): p. 2633-2647.
166. Hocevar, S.B., et al., *Glucose Microbiosensor Based on MnO₂ and Glucose Oxidase Modified Carbon Fiber Microelectrode*. Electroanalysis, 2004. **16**(20): p. 1711-1716.
167. Taha, Z. and J. Wang, *Electrocatalysis and flow detection at a glassy carbon electrode modified with a thin film of oxymanganese species*. Electroanalysis, 1991. **3**(3): p. 215-219.
168. Wang, J., *Nanomaterial-based electrochemical biosensors*. Analyst, 2005. **130**(4): p. 421-426.
169. Karyakin, A.A., *Prussian Blue and Its Analogues: Electrochemistry and Analytical Applications*. Electroanalysis, 2001. **13**(10): p. 813-819.
170. Karyakin, A.A., E.E. Karyakina, and L. Gorton, *Amperometric Biosensor for Glutamate Using Prussian Blue-Based "Artificial Peroxidase" as a Transducer for Hydrogen Peroxide*. Analytical Chemistry, 2000. **72**(7): p. 1720-1723.
171. Karyakin, A.A., E.E. Karyakina, and L. Gorton, *Prussian-Blue-based amperometric biosensors in flow-injection analysis*. Talanta, 1996. **43**(9): p. 1597-1606.
172. Liu, S.-Q., J.-J. Xu, and H.-Y. Chen, *Electrochemical behavior of nanosized Prussian blue self-assembled on Au electrode surface*. Electrochemistry Communications, 2002. **4**(5): p. 421-425.
173. Scharf, U. and E.W. Grabner, *Electrocatalytic oxidation of hydrazine at a Prussian Blue-modified glassy carbon electrode*. Electrochimica Acta, 1996. **41**(2): p. 233-239.
174. Garjonyte, R. and A. Malinauskas, *Operational stability of amperometric hydrogen peroxide sensors, based on ferrous and copper hexacyanoferrates*. Sensors and Actuators B: Chemical, 1999. **56**(1-2): p. 93-97.
175. Schwake, A., B. Ross, and K. Cammann, *Chrono amperometric determination of hydrogen peroxide in swimming pool water using an ultramicroelectrode array*. Sensors and Actuators B: Chemical, 1998. **46**(3): p. 242-248.
176. Wang, J., X. Zhang, and M. Prakash, *Glucose microsensors based on carbon paste enzyme electrodes modified with cupric hexacyanoferrate*. Analytica Chimica Acta, 1999. **395**(1-2): p. 11-16.
177. de Mattos, I.L., et al., *Development of biosensors based on hexacyanoferrates*. Talanta, 2000. **52**(5): p. 791-799.

178. Pournaghi-Azar, M.H. and H. Razmi-Nerbin, *Electroless Preparation and Electrochemistry of Nickel-Pentacyanonitrosylferrate Film Modified Aluminum Electrode*. *Electroanalysis*, 2000. **12**(3): p. 209-215.
179. Cai, C.-X., et al., *Amperometric biosensor for ethanol based on immobilization of alcohol dehydrogenase on a nickel hexacyanoferrate modified microband gold electrode*. *Talanta*, 1997. **44**(3): p. 339-347.
180. Fiorito, P.A. and S.I. Córdoba de Torresi, *Hybrid nickel hexacyanoferrate/polypyrrole composite as mediator for hydrogen peroxide detection and its application in oxidase-based biosensors*. *Journal of Electroanalytical Chemistry*, 2005. **581**(1): p. 31-37.
181. Lin, M.S., Y.C. Wu, and B.I. Jan, *Mixed-valence compound-based biosensor*. *Biotechnology and Bioengineering*, 1999. **62**(1): p. 56-61.
182. Lin, M.S. and B.I. Jan, *Determination of hydrogen peroxide by utilizing a cobalt(II)hexacyanoferrate-modified glassy carbon electrode as a chemical sensor*. *Electroanalysis*, 1997. **9**(4): p. 340-344.
183. Malinauskas, A., et al., *Evaluation of operational stability of Prussian blue- and cobalt hexacyanoferrate-based amperometric hydrogen peroxide sensors for biosensing application*. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*, 2004. **24**(4): p. 513-519.
184. Lin, M.S., T.F. Tseng, and W.C. Shih, *Chromium(III) hexacyanoferrate(II)-based chemical sensor for the cathodic determination of hydrogen peroxide*. *Analyst*, 1998. **123**(1): p. 159-163.
185. Zhang, W., et al., *Amperometric Detection of Hydrogen Peroxide Using Glassy Carbon Electrodes Modified with Chromium Hexacyanoferrate/Single-Walled Carbon Nanotube Nanocomposites*. *Electroanalysis*, 2009. **21**(2): p. 179-183.
186. Tsiafoulis, C.G., P.N. Trikalitis, and M.I. Prodromidis, *Synthesis, characterization and performance of vanadium hexacyanoferrate as electrocatalyst of H₂O₂*. *Electrochemistry Communications*, 2005. **7**(12): p. 1398-1404.
187. Pauliukaite, R., et al., *Novel electrochemical microsensor for hydrogen peroxide based on iron-ruthenium hexacyanoferrate modified carbon fiber electrode*. *Electroanalysis*, 2008. **20**(1): p. 47-53.
188. Pena, R.C., et al., *Flow injection amperometric determination of hydrogen peroxide in household commercial products with a ruthenium oxide hexacyanoferrate modified electrode*. *Microchimica Acta*, 2009. **166**(3-4): p. 277-281.
189. Eftekhari, A., *Aluminum electrode modified with manganese hexacyanoferrate as a chemical sensor for hydrogen peroxide*. *Talanta*, 2001. **55**(2): p. 395-402.
190. Zhao, G., et al., *Direct electrochemistry and electrocatalysis of heme proteins immobilized on self-assembled ZrO₂ film*. *Electrochemistry Communications*, 2005. **7**(7): p. 724-729.
191. Liu, X.J., et al., *Electrochemical characteristics of heme proteins in hydroxyethylcellulose film*. *Sensors and Actuators B-Chemical*, 2006. **113**(1): p. 106-111.
192. Varghese, S.H., et al., *Sensors Based On Carbon Nanotubes and Their Applications: A Review*. *Current Nanoscience*, 2010. **6**(4): p. 331-346.
193. Su, S., et al., *Nanomaterials-based sensors for applications in environmental monitoring*. *Journal of Materials Chemistry*, 2012. **22**(35): p. 18101-18110.

194. Beer, P.D. and J. Cadman, *Electrochemical and optical sensing of anions by transition metal based receptors*. Coordination Chemistry Reviews, 2000. **205**: p. 131-155.
195. Yam, V.W.W. and K.K.W. Lo, *Recent advances in utilization of transition metal complexes and lanthanides as diagnostic tools*. Coordination Chemistry Reviews, 1999. **184**: p. 157-240.
196. Xu, B., et al., *A highly sensitive hydrogen peroxide amperometric sensor based on MnO₂-modified vertically aligned multiwalled carbon nanotubes*. Analytica Chimica Acta, 2010. **674**(1): p. 20-26.
197. Babu, K.J., et al., *The influences of shape and structure of MnO₂ nanomaterials over the non-enzymatic sensing ability of hydrogen peroxide*. Journal of Nanoparticle Research, 2014. **16**(2).
198. Li, L., et al., *A novel nonenzymatic hydrogen peroxide sensor based on MnO₂/graphene oxide nanocomposite*. Talanta, 2010. **82**(5): p. 1637-41.
199. Mahmoudian, M.R., et al., *Facile preparation of MnO₂ nanotubes/reduced graphene oxide nanocomposite for electrochemical sensing of hydrogen peroxide*. Sensors and Actuators B: Chemical, 2014. **201**: p. 526-534.
200. Liu, X., et al., *Synthesis of graphene nanosheets with incorporated silver nanoparticles for enzymeless hydrogen peroxide detection*. Analytical Methods, 2013. **5**(9): p. 2298-2304.
201. Dong, S., et al., *High loading MnO₂ nanowires on graphene paper: Facile electrochemical synthesis and use as flexible electrode for tracking hydrogen peroxide secretion in live cells*. Analytica Chimica Acta, 2015. **853**(0): p. 200-206.
202. Kim, D.-H., et al., *Epidermal Electronics*. Science, 2011. **333**(6044): p. 838-843.
203. Lee, C., et al., *Graphene-based flexible NO₂ chemical sensors*. Thin Solid Films, 2012. **520**(16): p. 5459-5462.
204. Ramuz, M., et al., *Transparent, Optical, Pressure-Sensitive Artificial Skin for Large-Area Stretchable Electronics*. Advanced Materials, 2012. **24**(24): p. 3223-3227.
205. Che, J., P. Chen, and M.B. Chan-Park, *High-strength carbon nanotube buckypaper composites as applied to free-standing electrodes for supercapacitors*. Journal of Materials Chemistry A, 2013. **1**(12): p. 4057-4066.
206. Kauffman, D.R. and A. Star, *Carbon Nanotube Gas and Vapor Sensors*. Angewandte Chemie International Edition, 2008. **47**(35): p. 6550-6570.
207. Kai, L., et al., *Carbon nanotube yarns with high tensile strength made by a twisting and shrinking method*. Nanotechnology, 2010. **21**(4): p. 045708.
208. Kim, Y.A., et al., *Thermal stability and structural changes of double-walled carbon nanotubes by heat treatment*. Chemical Physics Letters, 2004. **398**(1-3): p. 87-92.
209. Ebbesen, T.W., et al., *Electrical conductivity of individual carbon nanotubes*. Nature, 1996. **382**(6586): p. 54-56.
210. Jv, Y., B. Li, and R. Cao, *Positively-charged gold nanoparticles as peroxidase mimic and their application in hydrogen peroxide and glucose detection*. Chemical Communications, 2010. **46**(42): p. 8017-8019.
211. Zhou, Y.-G., et al., *Gold nanoparticles integrated in a nanotube array for electrochemical detection of glucose*. Electrochemistry Communications, 2009. **11**(1): p. 216-219.

212. Saha, K., et al., *Gold Nanoparticles in Chemical and Biological Sensing*. Chemical Reviews, 2012. **112**(5): p. 2739-2779.
213. Goncalves, G., et al., *Surface Modification of Graphene Nanosheets with Gold Nanoparticles: The Role of Oxygen Moieties at Graphene Surface on Gold Nucleation and Growth*. Chemistry of Materials, 2009. **21**(20): p. 4796-4802.
214. Ting, S.L., et al., *Gold nanoparticles decorated reduced graphene oxide for detecting the presence and cellular release of nitric oxide*. Electrochimica Acta, 2013. **111**(0): p. 441-446.
215. Zhang, N., et al., *Fabrication of gold nanoparticle/graphene oxide nanocomposites and their excellent catalytic performance*. Journal of Materials Chemistry, 2011. **21**(30): p. 11080-11083.
216. Hou, X., et al., *Coating multiwalled carbon nanotubes with gold nanoparticles derived from gold salt precursors*. Diamond and Related Materials, 2011. **20**(10): p. 1329-1332.
217. Hezard, T., et al., *Gold nanoparticles electrodeposited on glassy carbon using cyclic voltammetry: Application to Hg(II) trace analysis*. Journal of Electroanalytical Chemistry, 2012. **664**(0): p. 46-52.
218. Moyer, J., et al., *Correlation Between Sweat Glucose and Blood Glucose in Subjects with Diabetes*. Diabetes Technology & Therapeutics, 2012. **14**(5): p. 398-402.
219. El-Deab, M.S. and T. Ohsaka, *Electrocatalysis by nanoparticles: oxygen reduction on gold nanoparticles-electrodeposited platinum electrodes*. Journal of Electroanalytical Chemistry, 2003. **553**(0): p. 107-115.
220. Xiao, Y., et al., *"Plugging into Enzymes": Nanowiring of Redox Enzymes by a Gold Nanoparticle*. Science, 2003. **299**(5614): p. 1877-1881.
221. Zhao, S., et al., *Glucose oxidase/colloidal gold nanoparticles immobilized in Nafion film on glassy carbon electrode: Direct electron transfer and electrocatalysis*. Bioelectrochemistry, 2006. **69**(2): p. 158-163.
222. Liu, J., et al., *Carbon-decorated ZnO nanowire array: A novel platform for direct electrochemistry of enzymes and biosensing applications*. Electrochemistry Communications, 2009. **11**(1): p. 202-205.
223. Hjortskov, N., et al., *Pilocarpine iontophoresis test - An index of physiological sweat secretion*. Clinical Physiology, 1995. **15**(4): p. 409-414.
224. Gibson, L.E. and R.E. Cooke, *A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis*. Pediatrics, 1959. **23**(3): p. 545-549.
225. Wing, D., M.R. Prausnitz, and M.J. Buono, *Skin pretreatment with microneedles prior to pilocarpine iontophoresis increases sweat production*. Clinical Physiology and Functional Imaging, 2013. **33**(6): p. 436-440.

Publications

1. **Ting SL**, Ee SJ, Arundithi A, Leong KC, Chen P. Graphene quantum dots functionalized gold nanoparticles for sensitive electrochemical detection of heavy metal ions. *Electrochimica Acta*, **2015** (Available online: doi:10.1016/j.electacta.2015.01.026)
2. **Ting SL**, Guo CX, Leong KC, Kim DH, Li CM, Chen P. *Gold nanoparticles decorated reduced graphene oxide for detecting the presence and cellular release of nitric oxide*. *Electrochimica Acta*, **2013**, 111: 441-446.
3. Ee SJ, Pang HC, Mani U, Yan QY, **Ting SL**, Chen P. *An Interwoven Network of MnO₂ Nanowires and Carbon Nanotubes as the Anode for Bendable Lithium-Ion Batteries*. *Chem Phys Chem*, **2014**, 15: 2445–244.
4. Pang HC, Dong YQ, **Ting SL**, Lu JL, Li CM, Kim DW, Chen P. *2D single- or double-layered vanadium oxide nanosheet assembled 3D microflowers: controlled synthesis, growth mechanism, and applications*. *Nanoscale*, **2013**, 5(17): 7790-7794.
5. Teh JJ, **Ting SL**, Leong KC, Li J, Chen P. *Gallium-doped tin oxide nanocuboids for improved dye sensitized solar cell*. *ACS Applied Materials & Interfaces*, **2013**, 5: 11377-11382.