# ELECTROCHEMICAL APTAMER BIOSENSOR BASED ON REDUCED GRAPHENE OXIDE MODIFIED ELECTRODE FOR CORTISOL DETECTION

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MASTER OF SCIENCE
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**VAYITHISWARY KANNAN**

Thesis submitted to the Centre for Graduate Studies, Universiti Pertahanan Nasional Malaysia, in fulfilment of the requirements for the Degree of Master of Science (Biology)

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# ABSTRACT

Stress, a common issue faced by almost everyone, is usually associated with absenteeism, lack of motivation and performance. The frequent exposure to stress leads to chronic stress, elevating the risk of other psychological health problems such as anxiety and depression. Regular monitoring of stress level is crucial for health and wellbeing which calls for a need for a simple, convenient and portable stress biosensor. In this study, an electrochemical biosensor for the detection of cortisol as the stress biomarker was fabricated using reduced graphene oxide modified screen printed carbon electrode, rGO-SPCE. Graphene oxide was drop-casted onto the working electrode of the SPCE and electroreduced into rGO using cyclic voltammetry (CV). Amine modified cortisol aptamer was utilised for cortisol specific sensing molecule and immobilised onto the rGO surface using N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide (EDC-NHS) linker at a ratio of 1:1 (v/v). Activation of carboxylic group on rGO layer, COOH into $COO^-$ favoured the attachment of the amine ($NH_2$) group of the cortisol aptamer through amide bond interaction. Bovine serum albumin (1 mg/ml) was added to block non-specific binding of cortisol onto the modified layer. The self-assembled monolayer was then characterised using Field Emission Scanning Electron Microscope (FESEM), Fourier-Transform Infrared Spectroscopy (FTIR) and electrochemically using CV. Using 5 mM potassium ferricyanide ($K_3[Fe(CN)_6]$) in 0.1 M potassium chloride (KCl) solution as the redox electrolyte, the electrochemical performance of rGO-SPCE was optimised and analysed through differential pulse voltammetry (DPV). Interaction of cortisol with cortisol aptamer hindered the

$[Fe(CN)_6]^{3-/4-}$ flow across the sensing surface thus resulting in a decrease of current. At rGO concentration of 1.5 mg/ml and 0.1 μM aptamer, C-Apt/rGO-SPCE was able to detect cortisol through the highest peak current reduction obtained within 15 minutes of cortisol incubation time. The electrochemical response exhibited a linear dependence on the cortisol concentration ranging from 0.001 µg/ml to 10 µg/ml, with a detection limit of 1.9836 µg/ml. The highest peak current reduction recorded at 65% (1.54 μA) from the interference study proved that the fabricated biosensor was highly specific towards cortisol amongst other steroid based hormones. This proposed technique demonstrates its potential application in monitoring stress.

## ABSTRAK

Tekanan merupakan isu biasa yang dihadapi oleh hampir semua orang, yang biasanya dikaitkan dengan ketidakhadiran, kekurangan motivasi dan prestasi. Pendedahan yang kerap kepada tekanan boleh menjurus kepada tekanan kronik serta meningkatkan risiko masalah kesihatan psikologi lain seperti keresahan dan kemurungan. Pemantauan tahap tekanan yang berkala adalah penting untuk kesihatan. Hal ini menjurus kepada keperluan sebuah peranti penderiaan yang ringkas, mudah alih dan berpatutan. Dalam kajian ini, penderia bio elektrokimia telah dirangka dengan menggunakan lapisan grafin teroksida yang terturun (rGO) di atas permukaan elektrod karbon tercetak terubahsuai (SPCE) untuk mengesan kehadiran hormon kortisol, iaitu penanda bio bagi tekanan. Grafin oksida telah dititiskan pada permukaan elektrod SPCE dan diturunkan kepada bentuk rGO menggunakan teknik voltametri berkitar (CV). Aptamer kortisol yang terubahsuai dengan kumpulan amina digunakan sebagai molekul pengesan khusus untuk kortisol dan telah dilekatkan pada permukaan lapisan rGO menggunakan EDC-NHS (N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide) sebagai penghubung pada nisbah 1:1 (v/v). Pengaktifan kumpulan karboksil pada lapisan rGO, iaitu COOH kepada $COO^-$ membolehkan pengikatan kumpulan amino ($NH_2$) pada Aptamer kortisol melalui interaksi ikatan amide. 1 mg/ml BSA (bovine serum albumin) telah ditambahkan untuk menghalang pengikatan kortisol yang tidak spesifik di atas permukaan lapisan yang terubah suai. Lapisan tunggal penyusunan kendiri ini telah dicirikan dengan menggunakan FESEM (Field Emission Scanning Electron Microscope), FTIR (Fourier-Transform Infrared Spectroscopy) dan secara

elektrokimia melalui CV. Dengan menggunakan 5 mM kalium ferisianida ($K_3[Fe(CN)_6]$) dalam larutan 0.1 M kalium klorida (KCl) sebagai sumber elektrolit redoks, prestasi elektrokimia rGO-SPCE telah dioptimumkan dan dianalisis melalui voltametri denyutan berbeza (DPV). Tindak balas antara kortisol dan Aptamer kortisol menghalang aliran $[Fe(CN)_6]^{3-/4-}$ sekitar permukaan penderiaan dan mengakibatkan catatan penurunan arus. Pada kepekatan rGO sebanyak 1.5 mg/ml dan kepekatan Aptamer sebanyak 0.1 μM, C-Apt/rGO-SPCE dapat mengesan kortisol melalui penurunan puncak arus yang tertinggi yang diperoleh dalam tempoh pengeraman tindak balas kortisol selama 15 minit. Tindak balas elektrokimia mempamerkan kebergantungan linear pada kepekatan kortisol dari 0.001 μg/ml hingga 10 μg/ml, dengan had pengesanan sebanyak 1.9836 μg/ml dan pengurangan puncak arus yang tertinggi yang direkodkan pada 65% (1.54 μA) daripada kajian interferens membuktikan bahawa penderia bio yang telah difabrikasi ini adalah sangat spesifik terhadap kortisol dalam kalangan hormon berasaskan steroid yang lain. Teknik yang dicadangkan ini menunjukkan potensi aplikasi dalam pemantauan tekanan.

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# APPROVAL

The Examination Committee has met on **29 March 2022** to conduct the final examination of **Vayithiswary A/P Kannan** on her degree thesis entitled **Electrochemical Aptamer Biosensor Based On Reduced Graphene Oxide Modified Electrode For Cortisol Detection.**

The committee recommends that the student be awarded the of Master of Science (Biology).

Members of the Examination Committee were as follows.

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## APPROVAL

This thesis was submitted to the Senate of Universiti Pertahanan Nasional Malaysia and has been accepted as fulfilment of the requirements for the degree of **Master of Science (Biology)**. The members of the Supervisory Committee were as follows.


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**UNIVERSITI PERTAHANAN NASIONAL MALAYSIA**

**DECLARATION OF THESIS**

Student's full name : Vayithiswary A/P Kannan

Date of birth : 2 May 1995

Title : Electrochemical Aptamer Biosensor Based On Reduced Graphene Oxide Modified Electrode For Cortisol Detection

Academic session : 2019/2020

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| GO | - | Graphene Oxide |
| rGO | - | Reduced Graphene Oxide |
| SPE | - | Screen Printed Electrode |
| SPCE | - | Screen Printed Carbon Electrode |
| C-Apt | - | Cortisol Aptamer |
| C-Mab | - | Cortisol Monoclonal Antibody |
| CV | - | Cyclic Voltammetry |
| DPV | - | Differential Pulse Voltammetry |
| EDTA | | Ethylenediaminetetraacetic acid |
| TE | | Tris-EDTA |
| EDC | - | 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide |
| NHS | - | N-Hydroxysuccinimide |
| FESEM | - | Field Emission Scanning Electron Microscopy |
| FTIR | - | Fourier-Transform Infrared Spectroscopy |
| ELISA | - | Enzyme-Linked Immunosorbent Assay |
| HPLC | | High Performance Liquid Chromatography |
| GC-MS | | Gas Chromatography Mass Spectrometry |
| POCT | - | Point of Care Testing |
| LOC | - | Lab on Chip |
| SELEX | - | Systematic Evolution of Ligands by EXponential Enrichment |
| PCR | - | Polymerase Chain Reaction |
| DNA | - | Deoxyribonucleic Acid |
| PBS | - | Phosphate Buffered Saline |
| RSD | - | Relative Standard Deviation |
| LOD | - | Limit of Detection |

# LIST OF SYMBOLS

| | | |
|---|---|---|
| $\Delta E_p$ | - | Peak-to-peak potential separation |
| $E_{p(anodic)}$ | - | Anodic peak potential |
| $E_{p(cathodic)}$ | - | Cathodic peak potential |
| $Ip_a$ | - | Anodic peak current |
| $Ip_c$ | - | Cathodic peak current |
| $^oC$ | - | Degree celcius |
| $\mu$ | - | Micro |
| $\pi$ | - | pi |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Rapid globalisation has greatly influenced lifestyles in this modernised era. A fast-paced life along with a competitive nature increases exposure towards potential stressors that might put an individual in a stressed state. As a result, stress is a very common mental health issue faced by many people nowadays. Stress is known as the condition of the body responding to the pressure arising from a psychological or physical factor. Psychological stress has been increasing due to multiple reasons. Problems related to finances, family, health and wellbeing, workplace, relationship and education are known to be the cause of the elevating cases of psychological stress reported. Globally, stress related statistics have shown an increasing trend over the years accounting for various etiologies. In a recent global survey conducted by The Regus Group, stress levels in the workplace have been rising where 6 in 10 workers in major global economies are experiencing increased workplace stress, with China (86%) topping the list (Esmond, n.d). Over the past six years, approximately 44% of Americans reported that their stress levels have increased to a significantly, in which they are experiencing at least one symptom of stress (American

Psychological Association, 2015). Meanwhile in Malaysia, a research conducted among Malaysian students revealed the prevalence of moderate to extremely severe levels of stress (12.9% to 21.6%) from one out of 10 individuals in 2011 to one in five in 2016 (Manap *et al.* 2019). Apart from that, according to a survey done by AIA Vitality in 2018, 50.2% of employees in Malaysia face at least one dimension of work-related stress. Alarmingly, the prevalence of mental health disorders in Malaysia have been steadily increasing compared to the prevalence rate over the past decades (Raaj *et al.* 2021). The National Health Morbidity Survey in 2015 reported that the prevalence of mental health problems among adults and children were 29.2% and 12.1% respectively (Institute for Public Health 2015; Malaysian Healthcare Performance Unit 2016).

Stress is also known as the silent killer which is linked to a spectrum of mental health disorders such as anxiety and depression. The prolonged exposure towards a stressful lifestyle may result in chronic stress and altered physiological functions of the body (Melchior *et al.* 2007; Wagner *et al.* 2000). Moreover, in some cases, certain individuals do not realise that they are undergoing chronic stress as a result of constantly facing the stressors in their daily life. Example of professions that has a high risk of stress are the military service, healthcare professionals, prosecutors, aircraft engineers, pilots and many more.

A critical measure in ensuring that the mental wellbeing of an individual is maintained is through counselling, therapy, regular monitoring of health status and also coping mechanisms that ward off stress related diseases. The conventional methods of screening for stress usually involve a battery of questionnaires such as the Depression, Anxiety and Stress Scale (DASS-21) in which the stress scale is measured according to the DSM-IV from the Diagnostic and Statistical Manual of Mental Disorders (Carter, 2014) classification, Job Satisfaction Scale (JSS) and the Perceived Stress Scale (PSS), all of which put the subject through a long set of questions (Ng *et al.* 2007; Henry & Crawford, 2005; Cohen *et al.* 1983).

Apart from regulating various physiological processes such as balancing blood pressure and glucose levels, controls carbohydrate metabolism and electrolyte balance, cortisol, a type of steroid, also plays a huge role in the stress response of the body. Known as the stress biomarker, cortisol levels are raised in a stressed state and present in blood, urine, saliva, sweat, and even in nail and hair (Zea *et al.* 2020). The secretion of cortisol follows a circadian pattern of the body whereby its level is highest during daybreak (30 minutes after waking) and progressively lowers by the end of the day (Corbalan-Tutau *et al.* 2012; Nicholson, 2008). At a normal condition (without stressor), the cortisol level in the circulation system of a typical healthy adult is roughly around 5 – 25 µg/ml during the day and 2 µg/ml during midnight (Zea *et al.* 2020). The level of cortisol peaks during the onset of stress resulting from a stressor. Thus, apart from being used as a biomarker for mental disorders, the measurement of cortisol level in the body fluids has also recently been used in directly monitoring stress levels (Dziurkowska & Wesolowski, 2021). Conventional method of detecting cortisol level clinically involving biochemical tests such as enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC) and gas or liquid chromatography-tandem mass spectrometry (GC–MS, LC-MS/MS) offers a reliable and accurate detection of cortisol from human samples (Corbalan-Tutau *et al.* 2012; Ramamoorthy *et al.* 2016; Zea *et al.* 2020; Miller *et al.* 2013; Zheng *et al.* 2015). However, the downside of these tests is the utilisation of expensive reagents and bulky and high-maintenance instruments which requires operation by highly-trained laboratory personnel. Apart from that, the complex sample processing and testing steps are usually time consuming and have a high turnaround time (TAT) which makes these conventional methods laborious. Hence, a portable, inexpensive and real time detection of cortisol as a point of care (POC) is much needed.

Fortunately, along with the rapid development of the nanotechnology industry, portable devices are in high demand for their significant characteristics such as portability and convenience. In the medical technology sector, there is an increasing trend in the biosensor development and it is gaining the attention of many researchers as the integration of biochemical and mechanical properties of transducers with nanomaterials has been revolutionizing the chemical and biological analysis field (Malhotra *et al.* 2017). The development of a "gold standard" analytical device in assessing cortisol as a biomarker has been proven to be challenging within the biomedical sectors. This is because, various approaches are being used in creating a portable analytical device for cortisol detection nowadays in terms of sensing technology and transduction process and the challenge that comes along with it are firstly to improve its sensitivity, specificity, reproducibility, response time and detection limit. On the other hand, the challenges are also faced in miniaturisation of the biosensing device using micro and nano fabrication technologies and also efficient capturing of biorecognition signals (Naresh & Lee, 2021). This is due to the fact that different types of biosensor have different signal to noise ratio depending on the type of the sensing matrix used and also because biosensors requires minute sample volume which demands for an ultra-sensitive detection of the biomarkers at very low levels (Yoo *et al.* 2020). In recent times, electrochemical immunosensing has emerged as a promising technology for a simple, portable, cost-effective, and efficient detection of cortisol in bio-fluids. It measures the changes in the electrochemical current signal generated as a result of the interaction between cortisol present in the sample applied across the active sensing surface of the biosensor (Singh *et al.* 2014; Omar *et al.* 2017).

In improving the electrochemical performance of the biosensor, nanomaterials have been utilised to enhance sensitivity and selectivity in the detection of cortisol. Owing to its excellent electroconductivity, high surface to volume ratio, good electron mobility and excellent mechanical strength, reduced graphene oxide, a type of graphene derivative, is often utilised in this study. Due to its large surface area and ease of functionalising its surface (by manipulating the carboxyl and hydroxyl functional group) (Ray, 2015), it can accommodate highly sensitive capturing probes such as cortisol specific monoclonal antibodies and cortisol specific aptamer. Utilising self-assembly technique coupled with voltammetric electrochemical detection on a screen printed carbon electrode, this study poses the potential of measuring cortisol as a point of care testing in regular stress monitoring tests.