# NANO-DIAGNOSTICS-ON-CHIP: AN INNOVATIVE APPROACH OF QUANTIFIABLE MULTI-ANALYTE VIA TRIPARTITE POLYSILICON NANOGAP FOR PRENATAL CARE

by

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Ascorbic Acid |
| AC | Alternating Current |
| AFM | Atomic Force Microscopy |
| Ag/AgCl | Silver/Silver Chloride |
| AIDS | Acquired Immune Deficiency Syndrome |
| Al | Aluminum |
| APTES | 3-aminopropyl)triethoxysilane |
| Au | Gold |
| AuNP | Gold Nanoparticle |
| BOE | Buffered Oxide Etchant |
| BSA | Bovine Serum Albumin |
| CEA | Carcinoembryonic Antigen |
| $CHCl_3$ | Chloroform |
| CNT | Carbon Nanotube |
| COOH | Carboxyl |
| CPE | Constant Phase Element |
| DC | Direct Current |
| DEX | Dextrose Monohydrate |
| DI | Deionized |
| DNA | Deoxyribonucleic acid |
| EDAC | N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride |
| EDS | Energy Dispersive X-ray Spectroscopy |
| EDTA | Ethylenediaminetetraacetic acid |
| ELISA | Enzyme Linked Immuno-Sorbent Assay |

| | |
|---|---|
| FESEM | Field Emission Scanning Electron Microscope |
| $FeSO_4$ | Iron (II) Sulfate |
| FTIR | Fourier Transform Infrared Spectroscopy |
| GDM | Gestational Diabetes Mellitus |
| GLUC | Glucose |
| GOX | Glucose Oxidase |
| GPMS | Glycidoxypropyl(trimethoxy)silane |
| GTD | Gestational Trophoblastic Disease |
| $H_2O_2$ | Hydrogen Peroxide |
| $H_2SO_4$ | Sulfuric Acid |
| HB | Hemoglobin |
| hCG | Human Chorionic Gonadotropin |
| hCGab | Human Chorionic Gonadotropin antibody/ anti-hCG |
| hCGα | Human Chorionic Gonadotropin α-subunits |
| hCGβ | Human Chorionic Gonadotropin β-subunits |
| HCl | Hydrochloric Acid |
| HIV | Human Immunodeficiency Virus |
| $HNO_3$ | Nitric Acid |
| HPM | High Power Microscopy |
| IPA | Isopropyl alcohol |
| I-V | Current-Voltage |
| IVD | In Vitro Diagnostic |
| $K_3[Fe(CN)_6]$ | Potassium hexacyanoferrate III |
| $K_4[Fe(CN)_6]$ | Potassium hexacyanoferrate II |
| LOC | Lab-on-Chip |

| | |
|---|---|
| LOD | Limit of Detection |
| LPCVD | Low Pressure Chemical Vapour Deposition |
| LPM | Low Power Microscopy |
| MEMS | Microelectromechanical systems |
| MWCNT | Multi-Walled Carbon Nanotube |
| $NaHCO_3$ | Sodium bicarbonate |
| NG | Nanogap |
| $NH_4OH$ | Aqueous Ammonia |
| NHS | N-Hydroxysuccinimide |
| OH | Hydroxyl |
| PBS | Phosphate Buffered Saline |
| PDMS | Polydimethylsiloxane |
| PiH | Pregnancy-induced Hypertension |
| PL | Photoluminescense |
| POC | Point-of-Care |
| PS | Polysilicon |
| PSNG | Polysilicon Nanogap |
| P-V | Peak-Valley |
| PVD | Physical Vapor Deposition |
| RC | Resistor-Capacitor |
| RCA | Radio Corporation of America |
| RIA | Radioimmunoassay |
| RIE | Reactive Ion Etching |
| RMS | Root-Mean-Square |
| RSD | Relative Standard Deviation |

| | |
|---|---|
| SAL | Saliva |
| SD | Standard Deviation |
| SEM | Scanning Electron Microscopy |
| Si | Silicon |
| $SiH_4$ | Silane |
| $SiO_2$ | Silicon Dioxide |
| TB | Toluidine Blue |
| tBOC | Di-tert-butyl dicarbonate |
| TFA | Trifluoroacetic acid |
| Ti | Titanium |
| UA | Uric Acid |
| UV-Vis | Ultraviolet–Visible Spectroscopy |
| XRD | X-ray Diffraction Spectroscopy |

## LIST OF SYMBOLS

| | |
|---|---|
| $A_g$ | Grain Height |
| μΩ | Microohm |
| A | Area |
| $B_{max}$ | Maximum Number of Binding Sites per Antigen |
| C | Capacitance |
| $C_{apt}$ | Aptes Capacitance |
| $C_e$ | Electrode Capacitance |
| $C_{hcg}$ | hCG Capacitance |
| $C_{hCGab}$ | hCGab Capacitance |
| cm | Centimeter |
| $C_t$ | Total Capacitance |
| $d_e$ | Dielectric width of electrode |
| $d_{hcg}$ | Dielectric width of hCG |
| $d_{sm}$ | Dielectric width of total surface modification |
| ε’ | Dielectric Constant |
| ε” | Loss Factor |
| $\varepsilon_e$ | Permittivity of electrode |
| $\varepsilon_{hCG}$ | Permittivity of hCG |
| $\varepsilon_{hCGab}$ | Permittivity of hCGab |
| $\varepsilon_{sm}$ | Permittivity of total surface modification |
| $\varepsilon_T$ | Total permittivity |
| fM | femtoMolar |
| h | Hour |
| Hz | Hertz |

| | |
|---|---|
| I | Current |
| IU | International Unit |
| $K_d$ | Dissociation Constant |
| $l_e$ | Electrode Width |
| $l_g$ | Gap Width |
| $l_{hcg}$ | hCG Width |
| $l_{sm}$ | Width of total surface modification |
| mg | Milligram |
| ml | Milliliter |
| mM | Millimolar |
| mm | Millimeter |
| MΩ | Megaohm |
| nA | Nanoampere |
| nF | Nanofarad |
| nm | Nanometer |
| nM | Nanomolar |
| nS | Nanosiemens |
| $^oC$ | Celsius |
| pg | Picogram |
| R | Resistance |
| $R^2$ | Linear Regression |
| $R_{apt}$ | APTES Resistance |
| $R_e$ | Electrode Resistance |
| $R_g$ | Grain Resistance |
| $R_{gb}$ | Grain Boundary Resistance |

| | |
|---|---|
| $R_{hcg}$ | hCG Resistance |
| $R_{hCGab}$ | hCGab Resistance |
| $R_T$ | Total Resistance |
| s | Second |
| S/N | Signal-to-Noise Ratio |
| $S_a$ | Surface Roughness |
| t | Time |
| tan δ | Loss Tangent/Dissipation Factor |
| V | Voltage |
| $V_{rms}$ | Root-Mean-Square Voltage |
| Z | Impedance |
| Z’ | Real part of impedance |
| Z” | Imaginary part of impedance |
| $Z_T$ | Total Impedance |
| $\Delta\varepsilon_{hCG}$ | Change in hCG permittivity |
| $\Delta I_{hCG}$ | Changes in hCG current |
| $\Delta\rho_{hCG}$ | Changes in resistivity of the hCG |
| µA | Microampere |
| µF | Microfarad |
| µg | Microgram |
| µl | Microliter |
| µM | Micromolar |
| µm | Micrometer |
| ν | Applied Voltage |
| $\rho_{air}$ | Air Resistivity |

| | |
|---|---|
| $\rho_e$ | Electrode Resistivity |
| $\rho_{hCG}$ | hCG Resistivity |
| $\rho_{hCGab}$ | hCGab Resistivity |
| $\rho_{SM}$ | Resistivity of total surface modification |
| $\sigma$ | Conductivity |
| $\tau$ | Relaxation Time |
| $k_m^{app}$ | Apparent Michaelis–Menten Constant |

**Nanodiagnostik Berasaskan Cip: Satu Pendekatan Inovatif Bagi Pengkuantitian Pelbagai Biomolekul Melalui Tiga Segmen Jurang Nano Polisilikon Untuk Penjagaan Pranatal**

## ABSTRAK

"Perutku semakin membulat, lebih besar and aneh, dan saya disamping itu, tertanya-tanya akan apa yang engkau bakal berubah menjadi di dunia ini"– Carrie Fisher. Satu pepatah yang menggambarkan impian dan emosi setiap ibu hamil yang ingin memberikan kesihatan dan kehidupan yang baik untuk anaknya. Tesis ini didorong oleh kepentingan penyediaan pemeriksaan pada peringkat awal sebagai pencegahan serta penjagaan kesihatan untuk ibu dan bayi, dan ia juga merupakan salah satu matlamat utama yang ditumpu oleh Pertubuhan Bangsa-Bangsa Bersatu dalam Matlamat Pembangunan Milenium pada tahun 2015. Kepentingan utama diberikan pada kawasan-kawasan yang tidak mencukupi penjagaan kesihatan ibu, dimana peningkatan angka secara mendadak dilihat dalam kes kematian ibu dan janin semasa kehamilan. Penyediaan hasil ujian pantas berhampiran pesakit adalah perlu untuk melaksanakan langkah-langkah keselamatan dan juga mengurangkan kebimbangan pesakit semasa menunggu keputusan. Dalam ini, peranti nano-diagnostik yang diperkenalkan sebagai 'Penjagaan Pranatal berasaskan Cip' berprinsipkan pengesanan makmal atas cip yang didemonstrasikan menggunakan elektrod bernanostruktur polisilikon jurang nano, yang membantu penghantaran setitik cecair melalui saluran mikro tiga segmen untuk pemeriksaan pelbagai analit. Bahan pengarusan-separa, polisilikon dipilih sebagai elektrod untuk membentuk struktur jurang nano dan disesuaikan secara khas dengan mengubah tempoh pemendapan dan proses penyepuhlindapan untuk menyediakan permukaan electrod yang sepatutnya. Reka-bentuk dan fabrikasi cip ini dijalankan dalam makmal melalui litografi konvensional serta struktur elektrod jurang nano melalui teknik pengurangan dan pengembangan saiz jurang. Teknik litografi lembut telah diguna-pakai untuk pembentukkan saluran mikro melalui cara pengacuanan. Satu lagi aspek penting adalah pengubahsuaian kimia pada permukaan elektrod, yang sangat penting untuk pemilihan analit dan kebolehpercayaan cip. Pelbagai pengubahsuaian kimia telah diuji dalam kajian ini untuk menganalisis prestasi (kesesuaian, keserasian dan sensitiviti) reseptor biologi terhadap pengesanan pelbagai analit. Analit yang paling penting untuk dikesan dan yang mempunyai permintaan tinggi di pusat jagaan kesihatan seperti hormon kehamilan (Human Chorionic Gonadotropin) untuk penilaian kehamilan dan glukosa untuk kencing manis gestasi telah diperiksa bagi penjagaan pranatal. Kedua-dua sampel yang disediakan dan sampel air kencing dan air liur klinikal telah disiasat dan dianalisis melalui pengajian morfologi, optik, struktur dan elektrik bagi tujuan penilaian prestasi cip tersebut.

**Nano-Diagnostics-on-Chip: An Innovative Approach of Quantifiable Multi-Analyte via Tripartite Polysilicon Nanogap for Prenatal Care**

## ABSTRACT

"Everything grows rounder and wider and weirder, and I sit here in the middle of it all and wonder who in the world you will turn out to be"– Carrie Fisher. A quote portraying the dreams and emotions of every mother who wished to provide good health and life for the unborn. Current thesis has been motivated by the importance of providing early stage diagnosis and preventive healthcare to mother and child, which is one of the prime goals focused by the United Nation in Millennium Development Goals in 2015. Key importance has been given to the areas that have inadequate maternal healthcare, as rising numbers seen in maternal and fetal mortality and morbidity. Providing a rapid test result near patient is necessary to undertake safety precautions as well as reducing the patient's anxiety of waiting time for the results. Herein, a novel, point-of-care nano-diagnostic device based on lab-on-chip biosensing is demonstrated using nanostructured polysilicon nanogap electrode, which assists a single drop fluid delivery by tripartite microchannels for multi-analyte diagnosis called 'Prenatal-Care-on-Chip'. Polysilicon semiconducting material was chosen as the electrode for nanogap structure, and fine-tuned by varying deposition period and annealing process to facilitate a proper biosensing surface. The designing and fabrication of the lab-on-chip were performed in-house via conventional lithography and nanogap electrode structures by size reduction and expansion technique. Soft-lithography was employed to create microchannels by mold-casting. Another important aspect is the surface functionalization on the electrode, which is crucial for selectivity and reliability of the biosensor. Various chemical modifications were examined in this study to analyze the performance (suitability, compatibility and sensitivity) of bio-receptor towards multi-analyte detection. Most crucial and healthcare demanding targets (analytes) for prenatal care were examined, such as Human Chorionic Gonadotropin for pregnancy assessment and Glucose for Gestational Diabetes Mellitus. Both prepared and clinically tested urine and saliva samples were investigated and analyzed by morphological, optical, structural and electrical studies to evaluate performance of the biochip.

# CHAPTER 1

# INTRODUCTION

## 1.1. Research Background

Growing demand for Point-of-Care (POC) diagnostic devices in recent years with proliferation of various target analytes, motivate the creation of an entirely brand-new category that hybrids miniaturization technology and medical diagnostics, towards reimagining the future of rapid health care via instant monitoring. However, as the idea of miniaturization and technology expansion continues to widen, highly advanced and sophisticated devices could be realized, granting to cheaper, flexible and denser diagnostic tools.

POC diagnostics grants testing on patients anywhere, be in hospitals, home, and field or at the physician's desk. By having fast test results, immediate treatment to the patient is applicable. Engaging physicians to make choices at the "POC" could significantly influence the health care conveyance and focus on the difficulties of well-being inconsistencies. The achievement of a potential transfer from therapeutic drug, to prescient, customized, and pre-emptive pharmaceutical could depend on the advancement of versatile handheld monitoring devices for POC diagnostics (National Institutes of Health, 2010).

In the beginning days of medicine, POC means the basic health care provided by the visit of physician to the patient's place. Then, throughout the years, from a few medical discoveries, POC technique was transferred to hospitals with devices used directly on patient by external probe attachments such as heart rate monitoring, electrocardiogram measurements and brain signals. Later in 1950s, systematic centralized laboratories for blood sample analysis were established as cost saving development

(Huckle, 2008). Within this system, special methods and chemicals preparation were required before the analysis could be carried out. Hence, these attempt in developing POC uses patient samples which is not in real time, and in some cases it was an insignificant step before a successful breakthrough. It was reported that one of the first commercial immunoassay introduced in 1968, exhibits radioactive signals back in a decade ago (Yalow and Berson, 1959). Pregnancy test kits were also one of the earliest focus on POC, which have been developed for prenatal care (Chard, 1992).

Today, as necessity for health care becomes large priority, POC focused more on early detection of disease or therapy to provide precautions and a way of managing serious health conditions. Therefore, POC now specifically defined as non-involvement of the laboratory equipment and staffs to provide the test results (Gubala et al., 2012). Recent health care delivery approaches to be more patient-centered, and aims to give immediate results without using laboratory settings. Tremendous support on latest technologies has been overwhelming, as low-cost imaging, micro-device based sensor systems were developed, incorporating wide variety of science discipline.

Hence, the background of this research represents the motivation for the design and development of multiplexed detection and quantification of multiple analytes, utilizing polysilicon based nanogap structure on a single lab-on-chip platform, assisted by single drop fluid delivery through microchannels for prenatal care application.



## 1.2. Problem Statement

Improvement in maternal healthcare has been insisted as one of the prime goals by the United Nation's Millennium Development Goals (MDGs) to save lives of more than 16 million women and children by 2015. Recent MDG statistical report has shown that only half of women in developing regions received the recommended health care and

almost 300,000 women died globally in 2013 by pregnancy related issues and during childbirth (Garenne et al., 2013; Ki-moon, 2010; Lincetto et al., 2013; Say et al., 2014; United nation, 2005). It indicates that there is still millions of women does not receive adequate prenatal care and as recommended by the World Health Organization, a minimum of four prenatal care visits are necessary to ensure the well-being of mothers and newborns, at the same time diagnostics to predict/detect early stage abnormalities is mandatory (Lincetto et al., 2013; United nation, 2005). To make it practical, the usage of point-of-care (POC) diagnostic devices is recommended for early prenatal care, which could be an influencing factor to reduce the maternal mortality rate (Balakrishnan et al., 2015; Gubala et al., 2012). Besides that, it is also an early precaution strategy to avoid exposing a developing fetus to potentially harmful diagnostic or therapeutic interventions and discontinuing fetotoxic prescribed and non-prescribed drugs (Cole and Ladner, 2009; Greene et al., 2013).



There are many reasons behind why early pregnancy detection is considered to be mandatory. It is to make sure enough attention is paid to the regulation of diet and general well-being of pregnant women. They can be precaution with an early pre-natal care from the initial stages. Human chorionic gonadotropin (hCG), a glycoprotein hormone secreted from the placenta, is a key molecule that indicates pregnancy. Studies on hCG levels are important to keep the health of the baby and the mother under monitoring all the time. Miscarriage possibilities during pregnancy could also be determined from the rise and decline of hCG levels. Methods for the detection of hCG at the early stage of pregnancy are of utmost importance and are currently an important area of research interest lately. However, the anomalies of most medical diagnostics kits are still carried in clinical laboratories and requires the use of large equipment.

Despite tremendous effort made for promising experimental results, fundamental mechanism of electrical sensing of biomolecule and the sensor design consideration are remain poorly understood and the current detections often rely on fluorescent (expensive read out in optics), time consuming, detecting single protein. In this context, nano-lab-on-chip; direct label-free electronic sensing using silicon biosensors for biomolecular analysis become significant. In other words, biosensors for the detection of multi-parameter of complex molecule during the course of diseases, produced efficiently with low cost, quantitative, sensitive, real time and quick detection scalable in arrays for nano-lab-on-chip with multi-parameter measurements. An effortless approach of sample employment on biosensor for detection purpose, could be made possible by utilizing microfluidic channels as fluid delivery tool. Most fluidics sensing experiments are primarily conducted in ceroscopy chambers, either closed or open. Nevertheless, integration of microfluidics module on a chip offers several advantages, including reagents consumption and small solvents, portability, short reaction times and ease of integration with other devices miniaturized. The common solutions for pumping the fluid in the micro channels rely on external peristaltic, syringe and pressure pumps.

The healthcare and medical markets are worth of hundreds of billions (Euros) every year. Diagnosis and pharmaceuticals equipment account for the wide majority with global sales amounting $400.6 billion US in the year 2010, expects to increase the investment in the biotechnology and healthcare industry over the next few years, innovation will be continued to fuel the sector and provide new opportunities for the market (“Point of Care Diagnostics,” 2012). With these, nearer is patient testing, is one of the fastest-growing segments. At present, pregnancy sensing kits in market has lower sensitivity and apparently most of them shows promising results upon the pregnancy period of two months and above. Pregnancy could be detected as early as one week, as

hCG is excreted from the trophoblastic cell which present in urine eventually (Cole, 2011). Further, sensing kits in the market is fundamentally based on qualitative detection and from enabling quantitative detection, the hCG detection could be much more derived based on the readable data acquired. This readability of hCG levels may open a wide accessible information that is necessary to be evaluated for the application in prenatal care diagnostics.

## 1.3. Thesis Objectives

### 1.3.1. Research Objective

The main objective of this research was to develop a novel, point-of-care nano-diagnostic device based on lab-on-chip biosensing using nanostructured polysilicon nanogap electrode, assisted by single drop fluid delivery via tripartite microchannels for multi-analyte diagnosis called 'Prenatal-Care-on-Chip'. The specific research objectives are as following:

i. A lab-on-chip (LOC) is developed to achieve a multiplexed status as a single analyte determination from a single drop of sample.
ii. Selective immobilization process is designed to study, analyze and explore the correlation between dielectric mechanism and vibrational characteristics, for the selectivity and sensitivity effect on aminosilane and epoxysilane modified polysilicon nanogap (PSNG) LOC.
iii. Size expansion technique is employed to examine the effect of different gap sizes on electrical characterization, and the effect of prepared hCG, pregnant and non-pregnant clinical urine samples on aminosilane modified PSNG electrodes were analyzed based on quantitative detection method.

iv. Size reduction technique is employed to explore and examine quantitatively using clinical saliva samples for glucose detection, before and after meals on chemically modified aminosilane PSNG LOC, and its effect on various gap sizes.

### 1.3.2. Research Scopes

The polysilicon nanogap based lab-on-chip biosensor requires certain procedures and steps to achieve the objective. Therefore, the scopes of this research is divided into four parts.

SCOPE 1. To develop a design for the multi electrode point-of-care lab-on-chip polysilicon nanogap biosensor to function as a single device. Therefore, utilization of microfluidic channels, sensing electrodes and contact pads for electrical read-out is a must to design the device. Subsequently, chrome masks for photolithography is designed and developed to fabricate the parts.

SCOPE 2. To fabricate the designed lab-on-chip using conventional photolithography for polysilicon nanogap electrodes and soft-lithography for microfluidic channels. Both structures are coupled ultimately. To perform early and fundamental studies prior to biomolecule detection, fabrication is done based on customization of polysilicon electrode by annealing process and gap sizes via size reduction and expansion technique.

SCOPE 3. To chemically modify polysilicon nanogap surface with biomaterials and nanomaterials for specific bio-capturing of target analytes. Ultimately, multiple targets are aimed to be diagnosed on a single platform using similar and specific surface modification.

SCOPE 4. To perform morphological, structural, electrical and analytical performance analysis on the biomolecule detection. Carry out morphological analyses using AFM, SEM, FESEM and UV-Vis. Structural investigation via XRD, EDS and FTIR. Electrical analysis using IV measurement, dielectric analyzer and impedance spectroscopy. Finally, device validation performed based on sensitivity, selectivity, specificity, repeatability and reproducibility analysis.



## 1.4. Organization of Thesis

This thesis is divided into four individual chapters. The first chapter presents an overview of the research background, problem statement, objectives and scope of the overall work executed. Briefly, it highlights the significance of study, problems associated to the existing methods, approaches to solve current limitations, significance of proposed method over existing.

In chapter 2, literature review based on the present work is discussed and the recent developments published in this area were analyzed. The electrochemical sensing strategy, involving amperometric characterization, application of nanotechnology in constructing nanostructures, the advantages of polysilicon as sensing electrode and the idea of multiplexing detection mechanism on single platform for application in prenatal care was reviewed.

In the third chapter, all published works that reporting the proposed study were compiled together. The chapter briefly starts with an introduction and a synopsis of overall publications. Then, it is followed by three articles that is published in leading journals.

Finally, in the last chapter, a brief summary of the major findings of this work and perspective on future directions of this research is included here.