
**UNIVERSITI PUTRA MALAYSIA**

***PHOTOLITHOGRAPHIC MODIFICATION OF POLYETHYLENE GLYCOL SILANE MONOLAYER FOR DEVELOPMENT OF LABEL-FREE DENGUE BIOSENSOR***

**NOR ZIDA BINTI ROSLY**

**FS 2015 63**

# PHOTOLITHOGRAPHIC MODIFICATION OF POLYETHYLENE GLYCOL SILANE MONOLAYER FOR DEVELOPMENT OF LABEL-FREE DENGUE BIOSENSOR

**By**

**NOR ZIDA BINTI ROSLY**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science**

**December 2015**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**Chairman : Shahrul Ainliah Alang Ahmad, PhD**
**Faculty : Science**

Dengue disease becomes critical global health issue after millions were infected worldwide every year, which lead to fatality due to late detection. Hence, a new technique has been explored to improve the performance of the detection in term of time, price, sensitivity and selectivity. In order to develop the diagnostic devices, electron beam (e-beam) or ultra-violet (UV) lithography were used to fabricate small-sized patterns to provide surfaces with high sensitivity and selectivity to biomolecules. For this particular project, polyethylene glycol (PEG)-silane monolayer, which was the best-known antifouling polymer was used as a lithographic template due to its non-polar, non-toxic and non-immunogenic properties of the surface. The monolayer formed was thoroughly characterized with contact angle, atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS). The AFM and contact angle data showed a uniform surface and hydrophilic properties of PEG-silane monolayer obtained which confirmed by the XPS. The e-beam and UV irradiations were subsequently performed, which generated the aldehyde functional groups. These offers conjugation sites for the immobilization of DNA dengue. Further studies were done to verify the presence of aldehyde functionality by testing with reducing agent and 2-amino-1,1,1-trifluoroethane (TFEA). The TFEA test showed an increased value of contact angle from 42° to approximately 74°, suggesting the hydrophobicity of surface that corresponds to the bonding of carbon atom to three fluorine atoms was formed. The reduction test studied the reduction aldehyde to alcohols which resulted in the decrease of contact angle values. The properties of gold nanoparticles (AuNPs) on particles sizes, shape, morphology and elemental composition were investigated which was utilized as a colorimetric probe for the determination dengue-DNA. For the DNA hybridization, target DNA was attached directly to probe DNA that had been immobilized on irradiated modified surfaces in which an amine-terminus (or N-terminus) from the DNA bound with aldehyde on modified surfaces. Gold enhancement process was introduced for naked eye detection after inducing electrostatic interaction between positively charged AuNPs and negatively charged target DNA to probe DNA. Control experiments were performed with mismatch DNA sequences to confirm the selectivity of the sensor. The effect of target DNA concentration was studied in the hybridization of DNA. The results obtained indicate an efficient and selective device have been successfully developed for label-free dengue detection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**PENGUBAHSUAIAN FOTOLITOGRAFI POLIETILINA GLIKOL SILANA EKALAPIS UNTUK PEMBANGUNAN LABEL BEBAS DENGGI BIOSENSOR**

Oleh

**NOR ZIDA BINTI ROSLY**

**Disember 2015**

**Pengerusi : Shahrul Ainliah Alang Ahmad, PhD**
**Fakulti : Sains**

Penyakit denggi menjadi isu kesihatan global yang kritikal selepas berjuta-juta manusia dijangkiti di seluruh dunia setiap tahun, yang membawa kepada kematian disebabkan pengesanan yang lewat. Oleh itu, satu teknik baru telah diterokai untuk meningkatkan prestasi pengesanan dari segi masa, harga, kepekaan dan pemilihan. Dalam usaha untuk membangunkan diagnostik yang sebegini, alur elektron (e-beam) dan litografi sinar ultra-lembayung (UV) telah digunakan untuk fabrikasi corak bersaiz kecil yang mampu menghasilkan permukaan dengan sensitiviti yang tinggi dan pengecaman biomolekul tertentu. Dalam kajian ini khususnya, polietilena glikol (PEG)-silana ekalapis, yang merupakan polimer antifouling yang paling terkenal digunakan sebagai templat lithografi kerana sifatnya yang tidak berkutub, tidak beracun dan tidak imunogenik. Ekalapis yang terbentuk dengan sempurna dicirikan dengan ukuran sudut sentuh, mikroskopi daya atom (AFM), dan spektroskopi fotoelektron sinar-X (XPS). Data daripada AFM dan sudut sentuh menunjukkan permukaan ekalapis yang seragam dan hidrofilik ekalapisan PEG-silana diperolehi dan ini disahkan oleh XPS. E-beam dan penyinaran UV kemudiannya dilaksanakan, telah menjana kumpulan berfungsi aldehid. Kumpulan ini menyediakan laman konjugasi untuk imobilisasi denggi DNA. Selanjutnya ujian telah dilakukan untuk mengesahkan kehadiran kumpulan berfungsi aldehid dengan 2-amino-1,1,1-trifluoroethane (TFEA) dan ujian penurunan. Ujian TFEA menunjukkan terdapat peningkatan sudut sentuh daripada 42 ° kepada kira-kira 74 °, mencadangkan kehidrofobikan permukaan yang sepadan dengan ikatan atom karbon yang mempunyai tiga atom fluorin. Ujian penurunan dikaji menunjukkan aldehid menurun kepada alcohol yang menghasilkan penurunan bacaan sudut sentuh. Sifat-sifat nanopartikel emas (AuNPs) ke atas saiz zarah, bentuk, morfologi dan komposisi unsur telah dikaji yang digunakan sebagai kajian kolorimetrik bagi menentukan denggi DNA. Untuk penghibridan DNA, sasaran DNA telah diikat langsung dengan prob DNA yang telah diimobilisasi pada permukaan radiasi diubahsuai di mana amina-terminal (atau N-terminal) daripada DNA terikat dengan aldehid pada permukaan yang diubahsuai. Proses peningkatan emas telah diperkenalkan untuk pemerhatian secara mata kasar selepas menjalankan interaksi elektrostatik antara AuNPs bercas positif dan DNA bercas negatif terhadap DNA prob. Eksperimen kawalan telah dijalankan dengan urutan DNA tidak sepadan untuk mengesahkan pemilihan sensor. Kesan kepekatan sasaran DNA telah dikaji dalam penghibridan DNA. Keputusan yang diperoleh menunjukkan kejayaan peranti yang cekap dan selektif dibangunkan pada label bebas denggi diagnostik.

**ACKNOWLEDGEMENTS**

Firstly, I would like to thank to my supervisor and co-supervisor Dr. Shahrul Ainliah Alang Ahmad, Prof Nor Azah Yusof and Dr. Jaafar Abdullah for their encouragement and support. Without their guidance, support, encouragement and supervision, this study would not be materialised.

Special dedication to my beloved parents, mak and abah, for supporting me to continue my study. Without them, it is not easy to achieve my dream. To my siblings thank you for financial support and advice.

My gratitude is also extended to my friends and colleagues, especially Ms Nur Izzah Roslan, Ms 'Iffah Syafiqah Abdullah, Ms Siti Rosnah Mustapa, Ms. Nor Hafizah Rappek, Ms Hernita Katni and Ms Nor Idayu Sidek, for sharing the laughter and tears during this period as a master student. Last but not least, a special dedication to my labmates, Mrs Siti Nurzawani Mohd Zain, Mrs Hayati Mukhair and Ms Izsdihar Ilzam for their advises and support to finish my journey in this study.

I certify that a Thesis Examination Committee has met on 14 December 2015 to conduct the final examination of Nor Zida binti Rosly on her thesis entitled "Photolithographic Modification of Polyethylene Glycol Silane Monolayer for Development of Label-Free Dengue Biosensor" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| µCP | Microcontact printing |
| AFM | Atomic force microscopy |
| Au | Gold |
| AuNP | Gold nanoparticles |
| DF | Dengue fever |
| DHF | Dengue hemorrhagic fever |
| DNA | Deoxyribonucleic acid |
| DPN | Dip-pen nanolithography |
| DSS | Dengue shock syndrome |
| e-beam | Electron beam |
| FE-SEM | Field emission scanning electron microscope |
| FTIR | Fourier transform infrared spectroscopy |
| OEG | Oligo ethylene glycol |
| PDMS | Polydimethylsiloxane |
| PEG | Poly(ethylene glycol) |
| PEG-silane | 2-[Methoxy(polyethyleneoxy)propyl] trimethoxysilane |
| RNA | Ribonucleic acid |
| RT-PCR | Reverse-transcription-polymerase chain reaction |
| SAMs | Self-assembled monolayers |
| $SiO_2$ | Silicon dioxide |
| TEM | Transmission electron microscopy |
| UV | Ultraviolet |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Biosensor technologies have been well developed due to demands of devices with high sensitivity, fast detection, small and easy to use. Sensing of biomolecule (antibodies, enzymes, nucleic acids) and biological systems (receptors, cells) can be performed through transducer (e.g optical, electrochemical, mass sensitive devices) (Wang, 2000). The chemical and physical transducers translate the event into important electrical signal such as light, current and frequency. There are two types of biosensor; classified by the nature of recognition event. The first type is bioaffinity devices which use a surface confined ligand partner (e. g antibody, oligonucleotide) for the attachment of target analyte. Otherwise, biocatalaytic devices based on enzyme for recognizing the target substrate (Junhui *et al*., 1997).

Biosensors apply self-assembled monolayers (SAMs) as interfaces between solid surfaces and biomolecules. Self-assembled monolayers (SAMs) are formed when amphiphilic surfactant molecules are adsorbed spontaneously onto solid surface of interest by specific interaction of surface chemistry. The adsorption of molecules on substrate can be performed in variety of solvents (e.g polar or non polar) which allow a greater flexibility in molecular design, therefore, providing surfaces properties that can be modified and controlled. Monolayers have been witnessed as successful model surfaces in biosensor's research since homogenous on thin films can be formed.

Over the past few decades, a lot of scientific studies have witnessed dramatic changes for detection of deoxyribonucleic acid (DNA) sequence, genomic analyses and early diagnosis for critical diseases. At that time, DNA hybridization biosensor was introduced by recognition of nucleic acid on transducer surfaces towards the goal for a rapid, simple and inexpensive devices (Junhui *et al*., 1997). DNA biosensor can be fabricated using various strategies including electrical, optical and mechanical.

Optical strategy especially fluorescent method shows a great potential due to high sensitivity and easy accessibility to commercialized equipment (Csa *et al*., 2000). However, this method requires expensive equipment and complex steps for label analytes (fluorophores) which are not suitable for shorter detection time. Therefore, label free naked eye detection is developed as an alternative to fluorescence technique. The detection is significantly simplified by simply using by naked eye or with a flat-scanner (so called scanometric detection). The technique used metal enhancement as a signal amplification process to detect conjugation between AuNPs and DNAs. The method shows a great potential to be developed since the analysis used instrument (e.g flat scanner) which is cost-effective and enhancement process takes a few minutes to operate.

## 1.2 Dengue Virus

Dengue diagnostic devices has been rapidly developed in order to meet the requirement of worldwide demand due to the increase of death in tropical and sub-tropical regions, mostly in urban and semi-urban countries, leading to approximately 50-100 million infections per year. The infection occurs when the four serotypes (DEN-1, DEN-2, DEN-3, DEN-4) transmitted in human body by *Aedes aegypti* mosquito, members of the Flavivirus family. In recent years, number of cases for Dengue fever (DF), Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS) are getting increased among children in some Asian and Latin American country (Weaver & Reisen, 2010).

So far, infection with dengue virus has no apparent symptoms or clinical signs since it can be confused with other vector borne viral and parasitic diseases such as influenza, chikungunya, malaria and zika viruses (Zhang *et al*., 2010). There is no available vaccine and therapy for the treatment of dengue infection, to date, the early prevention is the only way to control the disease caused by dengue virus. Hence, laboratory diagnoses are required to identify the disease rapidly and reliably, thus treat the disease at early stage of infection.

Currently, the techniques used for diagnosis of dengue infections are through virological detection and serological test. Virological test allows the identification of specific viral molecules. Meanwhile, serological test is to identify specific dengue antibodies such as IgG and IgM. However, the sufficient amounts of antibodies are not always presence in the body at early stage of infection and there are available at least 5 days after onset of illness. Additionally, the test should be confirmed with two or more serum sample since it has similarity with other Flaviviruses.

Recently, a new technique, reverse transcription polymerase chain reaction (RT-PCR) was created with high sensitivity, low risk of contamination and rapid real time assays as compared to serological test. The technique used agarose gel electrophoresis to detect dengue viral RNA. However, the process is time-consuming and hazardous. The summary of the available techniques are shown in Table 1.

**Table 1: Advantages and limitations of current dengue diagnostics (Peeling *et al.*, 2010)**

| Diagnostic tests | Advantages | Limitations |
|---|---|---|
| Viral isolation | - confirmed infection<br>- specific<br>- serotypes detection | - needs acute sample (0-5 days post onset)<br>- needs expertise and appropriate equipments<br>- takes more than 1 week<br>- does not differentiate between primary and secondary infection<br>- expensive |
| RNA detection | - confirmed infection<br>- sensitive and specific<br>- serotypes and genotypes detection<br>- results in 24-48 hours | - potential false-positives owing to contamination<br>- needs acute sample (0-5 days post onset)<br>- needs expertise and positive laboratory equipment<br>- does not differentiate between primary and secondary infection |
| Antigen detection | | |
| Clinical specimens (for example, using blood in an NS1 assay) | - confirmed infection<br>- easy to perform<br>- less expensive than virus isolation and RNA detection | - not sensitive as virus isolation or RNA detection |
| Tissues from fatal cases ( for example in immunohistochemistry) | - confirmed infection | - not sensitive as virus isolation or RNA detection |
| Serological tests | | |
| IgM or IgG seroconversion | - confirmed infection<br>- least expensive<br>- easy to perform | - IgM levels can be low in secondary infections<br>- confirmation needs two or more serum samples |
| IgM detection (single sample) | - probable Dengue cases detection<br>- useful for surveillance, tracking outbreaks and monitoring effectiveness of interventions | - IgM levels can be low in second infections |

## 1.3 Surface lithography

Lithography has been widely used as it offers potential applications in a wide variety of fields, including biosensor, biomedical, bioelectronics and fundamental studies of cell biology. It ables to create patterns down to smaller (micrometer to nanometer) scale precision are which providing surfaces with high sensitivity and specific biomolecular recognition. Patterning is important for modification of surfaces and it is useful to build functional nanostructures completely or partially on the exposed surfaces (Smith *et al*., 2004).

Lithography is performed to functionalize surfaces either by removing particular adsorbates (Mulder *et al*., 2001), placement of adsorbates (Buoninsegni *et al*., 1998), or by the particular reaction of adsorbates (Nan Li & Ho, 2008). Once the adsorbates placed on sub-monolayer regions, the remaining surface with the exposed area can interact with a new adsorbate. It creates adsorbates with various groups on the same adsorbent.

Currently, there are many techniques exist to create patterns onto chip substrate such as contact lithography (dip pen lithography (DPN), atomic force microscope (AFM), microcontact printing (μCP), scanning–near field photolithography, soft lithography, electron beam (e-beam) lithography *(*Agarwal *et al*., 2003; Demers *et al*., 2002; Mendes *et al*., 2004; Whitesides *et al*., 2001; Zhang *et al*., 2004). Most of the techniques used both physical adsorption and covelent reaction.

### Microcontact printing (μCP)

Microcontact printing (μCP) has proven a valuable technique for patterning DNA (Lange *et al*., 2004), proteins and cells (Turner & Shain, 1998). The technique were pioneered by Whitesides and the group where adsorbates were transferred to a surface by using flexible, polymeric stamps with pattern reliefs (usually made from polydimethylsiloxane, PDMS) (Whitesides *et al*., 2001). The stamps are dipped in ink (alkanethiol) and dried before brought to contact with a surface like gold. However, one major limitation of such μCP based approaches is the uncontrollable contamination of the stamp due to changes in environment condition (humidity, pH) during the biomolecules transferring process (Tan *et al*., 2004).

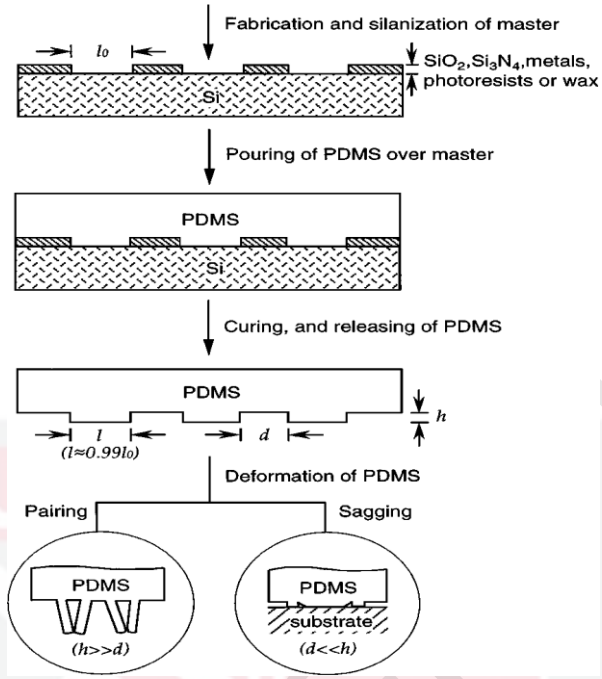


**Figure 1: Microcontact printing by PDMS stamp on silicon surfaces (Whitesides *et al*., 2001)**

**Dip-pen nanolithography (DPN)**

Dip-pen nanolithography (DPN) is a technique that performed under ambient condition without using any large electron magnetic field and shear effect. The technique was introduced by Piner and co-workers as new scanning probe lithography for fabricating thiol molecules on Au surfaces (Piner *et al*., 1999). The technique used AFM tip where was first coated with chemical reagents by immersing the cantilever in a solution or by evaporation. The coated tip was then transporting chemical reagents to nanoscopic regions on substrate of interest in one step, which is an advantage over serial techniques. Although the technique is simple but it is much more expensive due to powerful instrument for transporting chemical reagents from AFM tip to substrate.

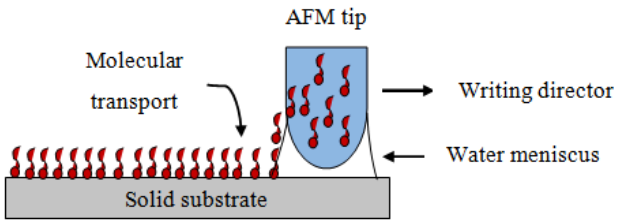


**Figure 2: Schematic illustration of dip-pen nanolithography (DPN) process**

**Electron beam (e-beam) lithography**

Electron beam (e-beam) lithography has been exploited as a maskless technique for patterning biomolecules with featured sizes ranging from several micrometers to nanometer, in addition to the possibility of designing the arbitrary shapes on the surfaces. In general, the patterning is performed by first coating the substrate with a polymeric thin film or resist and then exposing it to the e-beam (Glezos *et al*., 2002).

The high energy electrons present in the e-beam causes the changes in the chemical bonding by cross-linking or degrading the elements available at the surface. The exposed substrate is then rinsed with a developer solution to remove the unattached soluble portion of the resist. The technique is likely preferred for fabricating surfaces since it gives high resolution small scale patterns (Kolodziej & Maynard, 2012). Recently, the used of scanning electron microscope (SEM) equipped with the technique enable to scan electron beam spot within desired area, thus generate nanoscale pattern.

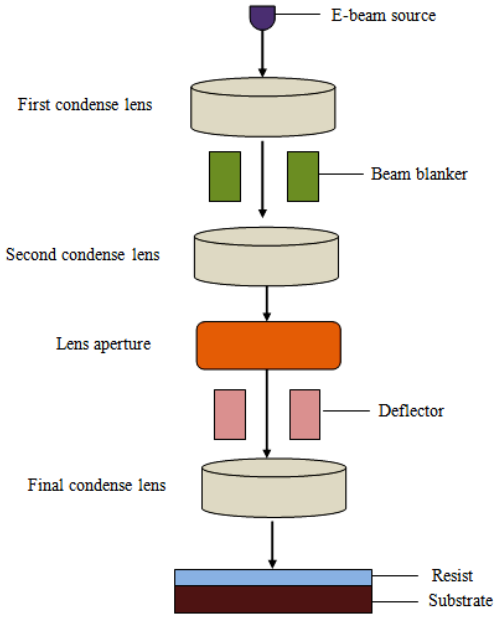


**Figure 3: Schematic illustration of electron beam (e-beam) lithography process**

**Photolithography**

Photolithography is another option for fabrication of monolayer surfaces. It has been employed to create a three-dimensional topography of resist layer and can provide chemical attachment on modified surfaces. The conventional technique utilizes an exposure of photo-resist layer to ultraviolet (UV) through photomask with opaque features (e.g. Cu grid). Commonly, UV light with wavelength 193-436 nm is performed in order to affect the terminal end group at the designated regions of substrate. The technique becomes attractive due to high resolution (sub-micrometer line width) and excellent control of feature dimensions. It also has a big potential to be used in industrial exposure tools due to the high quality and high resolution pattern such as deep UV ( $\lambda$ < ~250 nm) (Dulcey *et al.*, 1991).

The principle of this method is by irradiate the top of monolayer by UV light through the photomask which ables to create pattern on surfaces. It has been demonstrated on various materials including alkylthiolates (Montague *et al.*, 2007) and phosphonic acids as well as films of Au (Tizazu *et al.*, 2009), polymers (Hurley *et al.*, 2010) and metallic nanoparticles (Sun *et al.*, 2006). Light plays an important tool for surface immobilization due to big success in attachment of biomolecules (Blawas & Reichert, 1998; Yang *et al.*, 2006). The main purpose for irradiating monolayer under UV light is to generate functional groups such as aldehyde and carboxylic acid that available for immobilization of biomolecules.

The UV light have ability to shorten the alkyl chain and dissociate the oxygen molecules and create active oxygen species such as oxygen free radical thus oxidize the alkyl chain ( Xue & Yang, 2010). Therefore, UV lithography method is well established technique and can be used repeatedly on the same substrate with different protein to create multiple protein patterns (Lee *et al*., 2003).

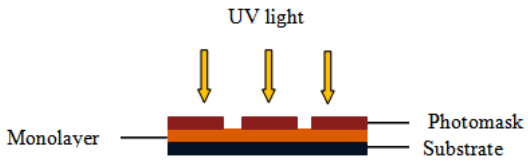


**Figure 4: Schematic illustration of photolithography process**

## 1.4 Problem statements

Dengue is a serious tropical disease that causes death to human if it is not treated promptly. Therefore, many strategies have been developed to detect dengue for the requirement of rapid detection, high sensitivity and selectivity, inexpensive and portable devices. However, the challenge dealing with dengue viruses is the unstable structure of virus and the differences among the four serotypes (DEN-1, DEN-2, DEN-3, DEN-4), causing the dengue viruses can not be controlled. Moreover, the existing of conventional techniques for dengue detection is focusing on specific anti-Dengue antibodies which are developed in response to Dengue viruses. Unfortunately, these antibodies produce at least 5 days after the onset of illness, whose the process is time-consuming for early detection (Vijayakumar *et al*., 2005). The advancement today's technology provides new opportunities for improving the performance of biosensors in term of time, price, sensitivity and selectivity which enable it to be used as portable device.

Optical biosensors are powerful technological developments that have a great potential for the direct, real-time and label-free detection of biomolecules especially nucleic acid. Optical biosensors are high specification, sensitivity, inexpensive, rapid and easy for users. The development of such sensors should be given high attention to improve the bio-recognition where the researchers require developing a new technique that can give a well respond to the selective biomolecules element such as nucleic acid without any interference from non-specific proteins element.

## 1.5 Objective of the study

The main objective of this study is to develop naked eye detection of DNA-dengue biosensor on modified 2-[Methoxy(polyethyleneoxy)propyl] trimethoxysilane (PEG-silane) monolayer. The detection facility incorporates the use of AuNPs to form Au/DNA

composites. The following specific objectives are designed to achieve the main objective:

i. To prepare and characterize PEG-silane monolayer on silicon oxide or glass surfaces.
ii. To identify the reactive functional groups formed and hence the surface chemistry by exposing e-beam or UV onto SAMs surfaces
iii. To immobilize and hybridize dengue DNA on photo-modified surfaces.
iv. To make the targeted spot visible by Au enhancement process.
v. To identify the response of various target base length and various target DNA concentrations in hybridization process by naked eye detection.

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