MAGNESIUM ZINC OXIDE DUAL GATE THIN FILM TRANSISTOR-BASED BIOSENSOR FOR MONITORING THE BIOFILM FORMATION

By

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ABSTRACT OF THE DISSERTATION

MAGNESIUM ZINC OXIDE DUAL GATE THIN FILM TRANSISTOR-BASED BIOSENSOR FOR MONITORING THE BIOFILM FORMATION

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Recently, increasing attention has been paid to transistor-based biosensors due to their preferred advantages, especially the high signal gain induced by the active three-terminal device. Thin film transistor (TFT) is a special kind of field-effect transistor (FET) made by depositing thin films over a supporting substrate. Due to its low fabrication temperature, the substrate of TFT can be selected from non-conducting materials such as glass and plastics, which fuels the realization of low-cost flexible and/or wearable electronics for biomedical applications. In the traditional bottom-gate staggered TFT biosensor, the top channel surface acts as the biological receptor. However, such configuration lacks the flexibility on sensing surface modification; furthermore, it is difficult to sense the analyte in aqueous environment. In this dissertation, we demonstrate a magnesium zinc oxide dual gate TFT (MZO DGTFT) with extended nanostructured MZO (MZO$_{\text{nano}}$) modified sensing pad for the dynamic monitoring of biofilm formation. MZO$_{\text{nano}}$ is used to enhance the sensitivity and biocompatibility of the biosensor. The MZO$_{\text{nano}}$ sensing pad is electrically connected to the top gate of the DGTFT. Such extended sensing pad design allows the separation of the DGTFT device from the harsh biochemical environment and different
sensing pads according to the detection tasks can be connected to the same transducer sequentially.

The MZO DGTFT biosensor is firstly implemented for the early stage detection of *Staphylococcus epidermidis* (*S. epidermidis*) biofilm formation. Biofilm formation is a serious issue in the clinical treatment of bacterial infections, because once matured, biofilms show 500 – 5000 times more tolerant to antibiotics in contrast to the free-floating bacteria of the same kind. Therefore, the earlier the detection, the more effective the treatment will be. *S. epidermidis* bacteria were cultured *in vitro* on the MZO$_{\text{nano}}$ modified sensing pad. Charge transfer occurs between the microbial cells and the MZO$_{\text{nano}}$ during the initial bacterial adhesion stage. Such electrical signals, which represent the onset of biofilm formation, were dynamically detected by the DGTFT where its bottom gate was used for biasing the device into the optimum characteristic region for high sensitivity and stable operation. The testing results show that a current change of ~80% is reached after ~200 minutes of bacterial culturing. The crystal violet staining-based assay shows that tiny bacterial microcolonies just start to form at 200 minutes, and that it would take approximately 24 hours to form matured biofilms. This technology enables medical professionals to act promptly on bacterial infection before biofilms get fully established.

Despite the early stage detection, the full-scale dynamic monitoring is also important because the long-term growth kinetic profile of biofilm development can serve as a feedback signal for future medical treatment studies. Therefore, we have developed the MZO$_{\text{nano}}$ modified multifunctional biosensing system for the full-scale dynamic monitoring of *Pseudomonas aeruginosa* (*P. aeruginosa*) biofilm formation. In this system, the DGTFT serves as an electrical sensor for early stage detection while the quartz crystal
microbalance (QCM) as an acoustic sensor for long-term monitoring. The sensing surfaces of both devices were modified with the same MZO\textsubscript{nano} to enhance the sensitivity and biocompatibility. \textit{P. aeruginosa} bacteria were cultured \textit{in vitro} on both sensing surfaces. The early stage detection is realized by sensing the charge transfer from cell membrane to the MZO\textsubscript{nano} during bacterial adhesion using the DGTFT biosensor while the monitoring of the long-term evolution is achieved through sensing of mass loading and viscoelastic transition during biofilm development using the MZO\textsubscript{nano} modified QCM. The drain current of DGTFT starts to change at the beginning of the test and levels off after ~6.5 hours of bacterial inoculation, whereas the signals of MZO\textsubscript{nano} modified QCM become detectable after ~5 hours and then lasts for 24 hours. The full-scale process of biofilm development covering from bacterial adhesion to maturation is thus dynamically monitored using this MZO\textsubscript{nano} modified multifunctional sensing technology.

In addition, MZO DGTFT biosensor is used for the determination of modified folic acid, hexadecyl alkynated folic acid (HAFA). 11-azidoundecanoic acid (AA) was bonded onto the MZO\textsubscript{nano} sensing surface as the linker layer, resulting in negative charges to MZO\textsubscript{nano} due to carboxylic acid binding chemistry. HAFA was then immobilized on AA/MZO\textsubscript{nano} via click reaction with AA. HAFA is a polar molecule with the positive end adjacent to AA/MZO\textsubscript{nano}, and therefore, the electrostatic condition of MZO\textsubscript{nano} is impacted again. Such changes were detected by DGTFT that displayed significant drain current variations. Fourier-transform infrared spectroscopy (FTIR) imaging confirms such successful chemical processes. This MZO DGTFT biosensor with HAFA is promising for potential applications in the detection of folate receptor (FR) overexpressed cancer cells.
Such MZO DGTFT biosensor with extended sensing gate design demonstrates the feasibility of sensing analytes in aqueous environment with modified sensing surface, and it can be potentially applied to various sensing applications where electrochemical reactions occur, such as charge transfer or electrical dipole.
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Table of Contents

ABSTRACT OF THE DISSERTATION ........................................................................... ii

Acknowledgements ........................................................................................................ vi

Table of Contents ........................................................................................................... viii

List of Tables ............................................................................................................... xii

List of Illustrations .................................................................................................... xiii

Chapter 1. Introduction ................................................................................................. 1

1.1 Motivation .................................................................................................................... 1

1.2 Objectives .................................................................................................................... 4

1.3 Dissertation Organization ............................................................................................ 6

Chapter 2. Technical Background .................................................................................. 8

2.1 Thin Film Transistor-based Biosensor Technology .................................................. 8

2.1.1 TFT Technology ....................................................................................................... 8

2.1.2 TFT-based Biosensor Technology .......................................................................... 11

2.1.3 TFT Biosensors with Extended Sensing Gate ....................................................... 14

2.1.4 Dual Gate Thin Film Transistor (DGTFT) ............................................................ 17

2.2 Multifunctional Semiconductors: ZnO and MZO .................................................... 22

2.2.1 As TFT Channel Material ..................................................................................... 22

2.2.2 As Sensing Material ............................................................................................. 23

2.3 Bacterial Biofilm and its Development Stages ......................................................... 24
Chapter 2. Existing Technologies of Biofilm Detection

2.4  Existing Technologies of Biofilm Detection ............................................. 28

2.4.1  Conventional Detection Methods ......................................................... 28

2.4.2  Mass-based Sensors .......................................................... 29

2.4.3  Impedance-based Sensors .......................................................... 32

2.5  Biosensing Platform Functionalized with Folic Acid ................................. 34

2.6  Stepwise Functionalization of ZnO-based Nanostructures .......................... 35

Chapter 3.  Early Stage Detection of Biofilm Formation .................................... 38

3.1  Background ......................................................................................... 38

3.2  Design of the MZO DGTFT Biosensor .................................................. 38

3.2.1  Fabrication of the MZO DGTFT ......................................................... 41

3.2.2  Fabrication of the ZnO_{nano} and MZO_{nano} Sensing Pads ................. 41

3.3  Biological Sample Preparation and Protocols ........................................... 42

3.3.1  Bacterial Biofilm Incubation .......................................................... 42

3.3.2  Crystal Violet Staining Assay .......................................................... 43

3.4  Bio-measurement and Parameter Extraction ............................................. 43

3.5  Result Analysis .................................................................................. 44

3.5.1  Electrical Characterization of the MZO DGTFT .................................. 44

3.5.2  Real-time Monitoring of the Biofilm Early Stage Formation .................. 48

3.5.3  Microscope Characterization and Bacterial Quantification .................... 55

3.5.4  Effects of Nanostructures on Sensing Performance .............................. 58

ix
Chapter 4. Full Scale Monitoring of Biofilm Development.......................... 63

4.1 Background........................................................................................................ 63

4.2 Design of the Hybrid Technology (MZO DGTFT + MZO\textsubscript{nano} modified QCM) 65

4.2.1 Fabrication of the MZO\textsubscript{nano} modified QCM ........................................ 67

4.2.2 Modification of the QCM Sensing Surface with MZO\textsubscript{nano}.................. 67

4.3 Biological Sample Preparation ........................................................................... 67

4.3.1 Bacterial Biofilm Incubation .......................................................................... 67

4.3.2 Crystal Violet Staining Assay.......................................................................... 68

4.4 Electrical Measurement and Signal Processing ................................................. 68

4.4.1 Measurement and Parameters Extraction for the MZO DGTFT ............... 68

4.4.2 MZO\textsubscript{nano} Modified QCM Measurement and Data Analysis ............ 69

4.5 Result and Discussion........................................................................................ 70

4.5.1 Device Characterization for Biosensing Operations .................................... 70

4.5.2 Full-scale Dynamic Monitoring of Biofilm Development .......................... 76

4.5.3 Microscopy Characterization of Crystal Violet Stained Biofilms............. 80

4.6 Summary............................................................................................................ 82

Chapter 5. Sensitive Determination of Modified Folic Acid.............................. 83

5.1 Background......................................................................................................... 83
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>Chemical Synthesis</td>
<td>84</td>
</tr>
<tr>
<td>5.3</td>
<td>Electrical Dipole Simulations</td>
<td>88</td>
</tr>
<tr>
<td>5.4</td>
<td>Results and Discussions</td>
<td>88</td>
</tr>
<tr>
<td>5.4.1</td>
<td>Monitoring of the HAFA Immobilization on MZO\textsubscript{nano}</td>
<td>88</td>
</tr>
<tr>
<td>5.4.2</td>
<td>Time Effect of Click Reaction</td>
<td>99</td>
</tr>
<tr>
<td>3.2</td>
<td>Summary</td>
<td>101</td>
</tr>
<tr>
<td>6.1</td>
<td>Conclusion</td>
<td>102</td>
</tr>
<tr>
<td>6.2</td>
<td>Suggestions for Future Work</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>Publications</td>
<td>118</td>
</tr>
</tbody>
</table>
List of Tables
Table 3-1 For $V_{BG} = 0, 2, 10$ and $15$ V, the slope of the I-V curve when $t = 0$, the maximum relative current change, and the average standard deviation were shown in the table. A trade-off between sensitivity and dispersion is obvious with different bottom gate biases. [65] ........................................................................................................................................ 52
Table 5-1 The values of threshold voltage and on-current for the three different processing steps. [113] ........................................................................................................................................ 90
List of Illustrations

Figure 2-1 Schematic diagrams of TFT structures: (a) coplanar top gate structure; (b) staggered top gate structure; (c) inverted coplanar bottom gate structure; (d) inverted staggered bottom gate structure. ................................................................. 10

Figure 2-2 The schematic presentation of the operating mechanism of a bioTFT immunosensor: (a) drain current vs gate bias for fixed drain bias of 10 V. Step 1: bare device, step 2: EGFR-antibody immobilization, and step 3: EGFR protein detection; (b)–(d) schematic of the carrier modulation mechanism for steps 1 to 3, respectively. [15] .. 13

Figure 2-3 Schematic of the IGZO-TFT biosensor with extended sensing gate. The device is divided into two separate parts. The IGZO-TFT is for electrical signal readout, while the sensing pool is for applying biological analyte. [25] ................................................................. 16

Figure 2-4 (a) Comparison of transfer characteristics of two dual-gate a-IGZO TFTs under single- and double-gate driving [40]. (b) The drain current of a dual gate transistor is presented on a semi logarithmic scale as a function of the bottom gate bias. The top gate bias is varied from left to right in steps of 5 V starting at + 5 V to - 10 V. The inset is a schematic of the dual gate transistor. [37] ................................................................. 19

Figure 2-5 A simplified cross-sectional view of the DGTFT. ........................................ 22

Figure 2-6 The development process of biofilm formation on a solid surface is shown in three steps: 1) bacterial adhesion, 2) bacterial expansion, and 3) biofilm maturation. EPS stands for extracellular polymeric substances. [66] ........................................................................ 26

Figure 2-7 (a) Schematic of the integrated microsystem of the SAW sensor and electrodes for induction [76]. (b) Schematic of the QCM system for monitoring the biofilm formation. [77] ........................................................................ 31
Figure 2-8 (a) An image of the biosensor and a mask detail of the interdigitated microelectrodes. (b) A cross-section view of the equivalent circuit model for the impedance measurement. [79]

Figure 3-1 (a) A schematic drawing of the MZO DGTFT biosensor system. The system consists of two parts: an MZO DGTFT as the transducer and a ZnO or MZO nanostructure modified sensing pad as the receptor. (b) The cross-sectional view of the DGTFT. m-MZO layer means the combination of the MgO diffusion barrier and the MZO active channel layer. (c) The optical microscope top view of the DGTFT. Four terminals (bottom gate, top gate, source and drain) are labeled accordingly. SEM images of the MOCVD-grown (d) ZnO and (e) MZO nanostructured films. [66]

Figure 3-2 The electrical transfer characteristics of an MZO DGTFT during the *S. epidermidis* growth in TSB media recorded at different times. Left axis presents the drain current in logarithm scale whereas the right axis presents same data in linear scale. [66]

Figure 3-3 (a) The relative variation of drain current reduction during the *S. epidermidis* biofilm growth were extracted from the DGTFT transfer characteristics. Three different bottom gate biases (*V*<sub>BG</sub> = 2 V, 10 V, and 15 V) are presented as a function of incubation time. No significant current variation was observed without bacterial inoculum. (b) The fitting curve of relative current reduction when *V*<sub>BG</sub> = 2 V was plotted with its first-order derivative. The current change saturates at about *t* = 200 min, which indicates the end point of *S. epidermidis* biofilms early stage detection. [66]

Figure 3-4 (a) Optical images of crystal violet-stained *S. epidermidis* biofilm on MZO film, respectively recorded at *t* = 0, 100 min, 200 min, 8 h, 16h, and 24 h. (b) Quantification of
the biofilm formation process at different times of culture by measuring the absorbance at 590 nm. [66]............................................................................................................. 57

Figure 3-5 (a) The optical absorption measurements were performed for both ZnO and MZO films on glass substrates, respectively. The absorption coefficient squares $\alpha^2$ are plotted against photon energy $h\nu$ for ZnO and MZO films. The bandgaps of ZnO and MZO films are determined to be 3.26 eV and 3.36 eV, respectively, which indicates the $y$ value in $\text{Mg}_y\text{Zn}_{1-y}\text{O}$ to be $\sim 0.04$ (4%). (b) $S. \text{epidermidis}$ biofilms were respectively incubated on bare sensing pads, ZnO$_{\text{nano}}$ modified pads, and MZO$_{\text{nano}}$ modified pads for 60 min. The same MZO DGTFT was used to conduct the experiments. Bottom gate voltage was set at 2 V. The relative variations of drain current reduction were obtained comparing with the same type of pad without bacteria incubation. [66]............................................................................................................. 60

Figure 4-1 The schematic of the hybrid and multifunctional biosensing system consisting of an MZO DGTFT with an extended MZO$_{\text{nano}}$ gate for the bacterial adhesion (early stage) detection and an MZO$_{\text{nano}}$ modified QCM for monitoring the subsequent stages. [107]. 66

Figure 4-2 (a) The electrical transfer characteristics of MZO DGTFT were tested with the top gate electrode connected to various top gate biases. The inset shows the detailed characteristics with $V_{BG}$ ranging from $-3$ V to 2 V. The I-V curves keep right shifting with the increasing values of negative top gate bias owing to the electrostatic field-effect. (b) The electrical signal response of the MZO DGTFT biosensor ($2.5 \text{ V} < V_{BG} < 7.5\text{V}$) with its top gate electrically connected to the sensing pad where bacterial incubation occurred. Drain current keeps decreasing as the incubation time increasing until $t = 390$ min. [107]........................................................................................................................................ 73
Figure 4-3 The signal variations during the full-scale development of *P. aeruginosa* biofilm formation, represented by the percentage change of drain current of DGTFT, motional resistance and frequency shift of MZO$_{\text{nano}}$ modified QCM. [107] ................................. 79

Figure 4-4 Optical microscopic images of crystal violet stained *P. aeruginosa* biofilm formation on MZO$_{\text{nano}}$ modified sensing pad at different times. [107] ................................. 81

Figure 5-1 Scheme of the functionalization of the MZO$_{\text{nano}}$ films. The structures in red mark the functional groups engaged in the click reaction. The SEM image shows the surface morphology of the MZO$_{\text{nano}}$ films. The inset figure shows the 3D structure of HAFA/AA bound on the MZO$_{\text{nano}}$ surface. The calculations show the combined HAFA/AA molecule has a dipole moment pointing from the positively charged folate pteroate moiety to the negatively charged glutamate moiety. [112] ................................. 87

Figure 5-2 The transfer characteristics of the MZO DGTFT biosensor with different processing steps. Step 0: bare MZO$_{\text{nano}}$ sensing pad, Step 1: AA linker binding on MZO$_{\text{nano}}$, and Step 2: HAFA immobilization on AA/MZO$_{\text{nano}}$. [112] ................................. 90

Figure 5-3 (a-c) The schematic of the carrier modulation mechanism from Steps 0 to 2, respectively. $E_t$ and $E_b$ denote the electrical fields in the top and bottom dielectric layers, respectively. [112] ................................. 93

Figure 5-4 The decibel changes of drain current between Step 1 and 2 as a function of bottom biasing voltage. [112] ................................. 96

Figure 5-5 (a) FTIR-ATR spectrum of neat AA (solid, black line), neat HAFA (short dot, red line) and representative single pixel FTIR spectrum of AA/MZO$_{\text{nano}}$ (dash dot, orange line) and HAFA/AA/MZO$_{\text{nano}}$ (dash dot, blue line). (b) FTIR images of the integrated band
area of the azido region 2212 - 2064 cm\(^{-1}\) (±STD), and the N-H stretching region 3460 - 2988 cm\(^{-1}\) (±STD) of MZO\(_{\text{nano}}\) film under each step. [112]

Figure 5-6 (a) The decibel changes of drain current in terms of different times of HAFA click reaction under \(V_{BG} = -1.7\) V. (b) FTIR images of the integrated band area of the azido region 2212 - 2064 cm\(^{-1}\) (±STD) of the MZO\(_{\text{nano}}\) film with each step. Three different reaction times of Step 2 were examined, respectively. [112]
Chapter 1. Introduction

1.1 Motivation

In past decades, disease diagnosis and detection have been one of the most critical research topics in the biomedical engineering field. Several detection methods have been developed and implemented into clinical practice, such as blood test [1], tumor markers [2], surface plasmon resonance [3], and spectrophotometric assay [4]. Recently, much attention has also been paid to transistor-based biosensors due to their favorable characteristics, including sensitivity, speed, miniaturization, and low cost. This interest is evident in numerous studies that have monitored biological events such as antigen-antibody binding, protein-protein interactions, and nucleic acid hybridizations [5].

A thin-film transistor (TFT) is a special kind of field-effect transistor made by depositing thin films of an active semiconductor layer as well as the dielectric layer(s) and metallic contacts over a non-conducting substrate. Unlike Si-based MOSFET, the fabrication process for TFT technology does not require high temperature steps; even room temperature is possible. Therefore, TFTs can be built on various substrates, including glass and plastics which are light and cost-effective. Multifunctional TFT based biosensors built on glass or flexible substrates have the potential to be integrated into a single chip and form a biosensor array.

The dual gate TFT (DGTFT) is comprised of a secondary gate electrode and its dielectric layer in additional to the single gate structure of regular TFTs. The operation conditions of DGTFT can be preferably set by the primary gate biasing voltage, while at the same time the secondary gate electrostatically modifies the carrier distribution in the channel accumulated by the primary gate and hence influence the electrical characteristics
of the device. This unique feature of DGTFT is exceptionally suitable for biosensing applications to achieve high sensitivity.

By adopting DGTFT as the actuator, the biological receptor can be separated from the transducer by electrically connecting an extended sensing pad to the top gate of DGTFT. Therefore, the transducer (i.e., DGTFT) can be separated from the harsh biochemical environment and different sensing pad can be connected to the transducer sequentially, which fuels the realization of a plug-in-card type of biosensor. Moreover, the separated sensing pad can be freely modified based on the specific biomolecules to achieve high sensitivity.

In this study, we explore of using magnesium zinc oxide (Mg$_x$Zn$_{1-x}$O, i.e., MZO) DGTFT with extended Mg$_y$Zn$_{1-y}$O nanostructure (MZO$_{nano}$) modified sensing pad to achieve the early stage and full-scale dynamic monitoring of biofilm formation.

Although aseptic and sterilization precautions are used, indwelling devices present suitable conditions for free floating bacteria to attach to their surfaces [6]. As the division of bacterial cells and the production of Extracellular Polymeric Substances (EPSs) occur, bacterial microcolonies form at the sites of infection and then eventually develop into matured biofilms [7]. EPS houses the microbes and offers a unique biochemical environment for them. Once the biofilms are fully established, the conventional antibiotics therapies are largely ineffective since biofilms are characterized to show from 500 to 5,000 times more tolerant to antibiotic agents than free-floating bacteria of the same kind [8]. This is mainly because the EPS acts as a protecting layer against the diffusion of antibiotic agents. The strong antimicrobial resistance of biofilms results in long-term antibiotic
treatment and possible repeated surgical procedures to eradicate. This would place a heavy burden on patients due to the undesirable stress and considerable extra medical expenses.

*Staphylococcus epidermidis* (*S. epidermidis*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) are gram-positive and gram-negative opportunistic pathogens, respectively. *S. epidermidis* is a common cause of infections associated with prosthetic devices and indwelling catheters. It is also a frequent infectious agent in many non-biomaterial-related infections including peritonitis, neonatal sepsis, and native valve endocarditis [9]. *P. aeruginosa* biofilms would form readily on the surface of implants and indwelling devices, such as urinary catheters and ventilator tubes, resulting in serious risks to patients [10]. It is even worse that such biofilm induced infections are often difficult to treat effectively and thus frequently life threatening.

To minimize or even prevent the bacterial infections on medical indwelling devices, a need has emerged for high-throughput analysis and techniques that allow for the detection and monitoring of the biofilm development in real time. In addition, bacterial cells are not encapsulated in EPS at the early stage of biofilms formation; therefore, antimicrobial agents are still effective to kill the bacteria. As a result, the sooner an infection is detected, the more effective treatment will be. Thus, the development of an early stage biofilm detection method with high sensitivity is critically important for timely clinical treatment of bacterial infections.

Despite the high accuracy and sensitivity for detecting the early stage of biofilm formation, the long-term monitoring capability for detecting the complete process should not be overlooked. Methods that can acquire data in the long run provide insightful information about the dynamic kinetic properties of biofilm development and hence would
allow medical and biological professionals to study the antimicrobial resistance effect of biofilms at different stages.

In addition to the biofilm detection, we have also demonstrated the sensitive determination of modified folic acid (FA) using the MZO DGTFT biosensor.

Cancer is a leading cause of death around the globe. The rising burden of cancer places enormous strains on the health care systems especially to the developing countries. In order to get improved access to affordable and effective cancer treatment, screening by detecting the early stage of cancer development is important. Improved outcomes and fewer medical expenses are expected when treatment is initiated at the early stage. As a result, the rise in cancer burden can be alleviated.

The use of biosensors for detecting cancer-related biomarkers is a common way of early diagnosis. Such sensors can provide a point-of-care testing that is rapid and low-cost with high sensitivity and selectively. Folate receptor (FR), also known as folic binding protein, is a typical tumor-associated antigen which is almost absent in normal tissues but overexpressed in numerous human tumor cells to meet the folate demand of rapidly dividing cells [11]. Therefore, FR has been widely used as a strategic target in the detection of cancer. In order to detect the presence of FR, a biosensing platform functionalized with folic acid (FA)-containing molecules is essential because FA specifically binds FR with high affinity (association constant in the order of $10^9$ - $10^{10}$ M$^{-1}$) at 1:1 stoichiometry [12]. The realization of a DGTFT biosensor functionalized with modified FA has potential applications in cancer diagnosis and treatment.

1.2 Objectives
The objectives of this research are to design, fabricate and characterize the novel MZO DGTFT biosensor, then to enable the prototype device for dynamic monitoring of biofilm formation. To realize these objectives, the following topics are included in this dissertation.

- Design and fabricate the novel MZO DGTFT with an $\text{MZO}_{\text{nano}}$ modified extended sensing pad.
- Analyze the electrical characteristics of MZO DGTFT and find the optimized operation point with high sensitivity and stable operation.
- Demonstrate the early stage detection of $S. \text{epidermidis}$ biofilm formation using the MZO DGTFT biosensor.
- Investigate the effects of nanostructures ($\text{ZnO}_{\text{nano}}$ vs $\text{MZO}_{\text{nano}}$) on sensing performance.
- Demonstrate the full-scale monitoring of $P. \text{aeruginosa}$ biofilm formation with DGTFT as an electrical sensor and QCM as the acoustic sensor of which the sensing surfaces are both modified with the same $\text{MZO}_{\text{nano}}$.
- Demonstrate the sensitive determination of HAFA using such MZO DGTFT biosensor.

Such MZO DGTFT biosensor allows the separation of the electrical transducer with the biological receptors and enables the modification of the sensing surface for higher sensitivity. The biological event can also be monitored in aqueous environment because the active electrical device is isolated from it. This MZO DGTFT biosensor can be adapted to a wide variety of potential scenarios where electrochemical interactions occur, and in-vitro detections are required.
1.3 Dissertation Organization

The research motivation and objectives of this dissertation is given in Chapter 1. Chapter 2 presents the technical background for this dissertation, which includes the introduction of thin film transistor-based biosensor technology, multifunctional semiconductor MZO and its nanostructures, introduction to bacterial biofilm and its development, existing technologies of biofilm detection, biomarker detection technologies functionalized with FA, and the stepwise functionalization of MZO\textsubscript{nano}. Chapter 3 and 4 cover the experimental work and the main findings; early stage detection of \textit{S. epidermidis} biofilm formation in Chapter 3 and full-scale monitoring of \textit{P. aeruginosa} biofilm formation in Chapter 4. In Chapter 3, we present the design of the MZO DGTFT biosensor, biological preparation of \textit{S. epidermidis} and related experimental settings of the bio-measurement. We carried out electrical characterizations of the MZO DGTFT, followed by the real-time early stage monitoring of biofilm formation. Crystal violet staining assay was used to verify the existence of biofilms and showed its process of long-term evolution. The effects of the nanostructures (ZnO\textsubscript{nano} vs MZO\textsubscript{nano}) on sensing performance were studied next to validate the advantages of using MZO\textsubscript{nano} as the biological receptor. In Chapter 5, we describe the design of a hybrid and multifunctional technology to full-scale monitor the development process of \textit{P. aeruginosa} biofilm formation; MZO DGTFT biosensor for the early stage and MZO\textsubscript{nano} modified QCM for the long-term monitoring purpose. The biological sample preparations of \textit{P. aeruginosa} and related measurement setups are presented, followed by the discussion of experimental results. Various small top gate biases were used to characterize the dual gate function of the MZO DGTFT and then it was used to detect the early stage formation of \textit{P. aeruginosa} biofilms. The subsequent
stages of the biofilm formation were dynamically monitored by the frequency shift and motional resistance change of the MZO\textsubscript{nano} modified QCM. The full-scale process of \textit{P. aeruginosa} biofilm development was thus recorded. In Chapter 5, we demonstrate the sensitive determination of hexadecyl alkynated folic acid (HAFA) using the MZO DGTFT biosensor. The chemical synthesis procedures are presented followed by the DGTFT measurement results. The biosensor exhibited its maximum drain current variation of 68.8 dB caused by HAFA. Fourier-transform infrared spectroscopy (FTIR) imaging also confirmed the successful chemical processes. The progress of the click reaction was also investigated using the DGTFT biosensor. Finally, conclusion and suggestions for future work are given in Chapter 6.
Chapter 2. Technical Background

In this chapter, we firstly introduce the TFT-based biosensing technologies. The use of extended sensing gate design in regular TFT-based biosensors is then reviewed, followed by the introduction of DGTFT. The dual gate configuration of DGTFT provides the flexibility in operation point tuning using one gate and biological signal sensing using the other gate. Next, the advantages of the multifunctional MZO and its nanostructures are discussed in terms of using them as the active channel layer for TFTs and sensing materials for biosensing applications, respectively. Then, the introduction of bacterial biofilm and its development are discussed, followed by a literature review of the existing detection technologies of biofilms. Next, a review of biomarker detection technologies which functionalized with folic acid is given. Finally, we review the stepwise functionalization of MZO nanostructures.

2.1 Thin Film Transistor-based Biosensor Technology

2.1.1 TFT Technology

As stated in Chapter 1, a TFT is a special kind of field-effect transistor made by depositing thin films of an active semiconductor layer as well as the dielectric layer(s) and metallic contacts over a non-conducting substrate. TFTs are mainly used in the high-volume large-area electronics with low-cost substrates, such as active-matrix liquid crystal displays (AMLCDs) and active-matrix organic light emitting diodes (AMOLEDs). Other applications include photovoltaics [13], wearable electronics [14], and sensing devices [15]–[17].

TFTs have a similar structure as MOSFETs. Besides the substrate, it has gate(s), dielectric layer(s), active channel layer and source/drain, but without body contact in
comparison with MOSFET. The fabrication process of MOSFET usually involves high temperature steps, including oxidation, implantable, and diffusion. The highest temperature often exceeds 1000°C. Single-crystalline Si is chosen as the most suitable material for MOSFET. Its main applications are in the field of logic circuits for high performance processing units. However, the fabrication process of TFT does not involve high temperature steps. Thin film deposition can even be achieved at room temperature. Therefore, TFTs can be built on various substrates, including glass and plastics. TFTs are mainly used in the high-volume large-area electronics with low-cost substrates. Owing to the low temperature and large-area advantages of TFT, multifunctional TFT based biosensors have the potential of being integrated on glass or flexible substrates in a single chip and form a biosensor array. Researchers have paid much attention to this field.

There are four basic structures of TFT (Figure 2-1) distinguished by the electrode placement; top gate coplanar type, top gate staggered type, bottom gate coplanar type, and bottom gate staggered type. Process integration-related issues can motivate the use of different structures. For biosensing applications, the bottom gate inverted staggered type of TFT is usually constructed and bio-functionalize its top channel surface to achieve a gating effect. The decorated top channel surface serves as the biological receptor whereas the transistor acts as an electrical transducer. Without the use of bulky instruments or special labelling, minute potential alternation at the semiconducting layers can be converted into detectable electrical signal instantly resulting from the applied charged biomolecules on the top channel surface.
Figure 2-1 Schematic diagrams of TFT structures: (a) coplanar top gate structure; (b) staggered top gate structure; (c) inverted coplanar bottom gate structure; (d) inverted staggered bottom gate structure.
The operation principles of TFT are similar to that of MOSFET. A transistor acts as an electrical switch of which the resistance depends on the voltage applied to the gate electrode. A positive voltage applied at the drain causes an electrical field with the direction from drain to source and makes the current flow. At the same time, the applied gate bias determines whether the charged carriers are accumulated in the semiconductor (ON state) or if the charges are depleted (OFF state). The threshold voltage is the gate bias at which significant carriers are accumulated in the dielectric-channel interface. The drain current versus gate voltage (transfer characteristic) at a fixed source and drain voltage exhibit logarithm linear behavior in the regime just above the threshold voltage. When used in sensing applications, the sensitivity of the device mainly depends on the subthreshold swing ($S_S$). $S_S$ is defined as the reciprocal value of the subthreshold slope. Smaller $S_S$ represents more current variations for the same gate voltage alternations, leading to higher sensitivity.

2.1.2 TFT-based Biosensor Technology

For biosensing applications, researchers usually construct bottom gate inverted staggered type of TFT and bio-functionalized the top channel surface to achieve a gating effect. The decorated top channel surface serves as the recognition surface whereas the transistor acts as an electrical transducer. Thus, minute potential alternation at the semiconducting layers can be converted into detectable electrical/voltage signal instantly resulting from the applied charged biomolecules, without the use of bulky instruments, as well as no need of special labeling.

In 2011, Reyes et al. demonstrated the first type of BioTFT for the use of EGFR antigen-antibody interaction detection [15]. The top channel surface of the BioTFT was
functionalized with amine-terminated EGFR mAbs as linkers which selectively bond with EGFR proteins. EGFR proteins with the concentration of 10 fM were detected by the device in both pure state and selectively in a concentration serum solution containing various other protein species. This BioTFT enabled bias-controlled operation through its bottom gate configuration. The high sensitivity of the device was attributed to its high on-off ratio, and the output trend was explained by the pseudo-double gating effect. The conductance of the transistor was controlled by variations in the charge density or electrical field at the channel region, as the electrical field which resulted from the binding of a charged molecule to the oxide surface is analogous to applying a voltage via a gate electrode. The schematic presentation of the operating mechanism of it is shown in Figure 2-2.
Figure 2-2 The schematic presentation of the operating mechanism of a bioTFT immunosensor: (a) drain current vs gate bias for fixed drain bias of 10 V. Step 1: bare device, step 2: EGFR-antibody immobilization, and step 3: EGFR protein detection; (b)–(d) schematic of the carrier modulation mechanism for steps 1 to 3, respectively. [15]
Similar mechanism has also been utilized by other sensing applications, such as [18]–[21].

However, there are several drawbacks of using the pseudo double-gating effect, i.e., implementing the top channel surface of TFT as the sensing surface. For a biosensor, it is preferred that the sensing of biomolecules occurs in an aqueous solution. However, a semiconducting channel layer, such as ZnO, in the TFT generally would degrade when expose to moisture. In addition, the modification of the top channel surface is difficult to further increase the sensitivity, as it may significantly degrade the device performance. Also, the transistor is not reusable for another biomolecule detection as the channel layer has already been specifically functionalized. Fortunately, when implemented with real dual gate structures and extended sensing gate design, TFTs should have an additional dimension of control [22], [23], and hence provide more flexibilities in sensing applications. We will introduce the extended gate design and the dual gate TFT (DGTFT) in the following sections.

2.1.3 TFT Biosensors with Extended Sensing Gate

As mentioned, the use of the top channel surface as the sensing area has several intrinsic drawbacks, mainly resulting from the lack of flexibility in device design. To overcome these shortcomings, several extended sensing gate designs were proposed [16], [17], [24]–[27] and an example of its schematic diagram is shown in Figure 2-3 [24]. Extended gate TFT separates electrical components (signal transducer) from the sensing area (biological receptor), preventing the electrical degradation from ions and liquids. Different sensing pads according to the detection tasks can be connected to the transducer sequentially, which fuels the realization of a plug-in-card type of biosensor. In our current
biosensor, the extended sensing pad is separated from the signal transducer, i.e., DGTFT. This design enables the MZO DGTFT device reusable and the sensing pad disposable. However, for the practical applications requiring low-cost mass production and high throughput analysis, it is preferred to integrate the multiple TFT devices with the MZO$_{\text{nano}}$ sensing pad on the same substrate, making the entire biosensor disposable and cost-effective. The unique features of our devices make it feasible: (i) both DGTFT and MZO$_{\text{nano}}$ pad are built on glass substrate; (ii) they use the same material (MgZnO). (iii) the low-cost in materials and fabrication processing. Moreover, the separated sensing pad can be freely modified based on specific biomolecules to achieve high sensitivity and selectivity.

In this dissertation, we utilize this design to separate the DGTFT transducer from the harsh biochemical environment that is for the bacterial biofilm culturing. Also, the sensing surface of the extended gate is modified with MZO$_{\text{nano}}$ to enhance its sensitivity. The extended gate design separates the sensing pad from the device and prevents electrical performance degradation of DGTFT resulted from the subsequent nanostructure deposition.
Figure 2-3 Schematic of the IGZO-TFT biosensor with extended sensing gate. The device is divided into two separate parts. The IGZO-TFT is for electrical signal readout, while the sensing pool is for applying biological analyte. [25]
2.1.4 Dual Gate Thin Film Transistor (DGTFT)

The design of DGTFT was initially put forward to solve the threshold voltage problem of TFT. Unlike CMOS transistors, the threshold voltage of TFT cannot be set by the amount of doping applied by ion implantation. To get around this constraint of TFT and to externally set the threshold voltage, a secondary gate was used to modulate the carrier distributions within the channel layer and hence provides one more dimension to control the device [28].

The first dual gate TFT was based on cadmium selenide (CdSe) and demonstrated by Luo et al. in 1981 for the use of flat panel displays [29]. In 1982, a-Si:H was used in DGTFT by Tuan et al. [30] and reproduced in 1992 by Kaneko et al. [31]. Entering the 21st century, organic and metal oxides semiconductor materials attract lots of attention. In 2005, the first organic DGTFT based on pentacene was reported by Cui and Liang [32], and in 2009, ZnO-based DGTFTs were demonstrated by various groups from Korea [33]–[35]. The unique features of DGTFT have been implemented into various applications, such as logic circuits [22], [36], pixel driving circuits [37], [38], as well as sensors [39].

In addition to the regular structure of TFTs, DGTFTs have an extra top gate electrode (TG) and its top dielectric. There are two ways of operating the DGTFT, namely the double-gate driving mode and the single-gate driving mode. For the double-gate driving mode [40], [41], a DGTFT operates in a way that the top and bottom gates are electrically tied together, two channels are formed thereafter, and the charge carriers tend to accumulate/deplete in the center of the channel with the vertically aligned electrical field. Therefore, the channel carriers are less influenced by scattering at the channel-dielectric interfaces, and as shown in Figure 2-4(a), the electrical characteristics of device, including
the on-current, subthreshold swing, can be significantly improved. The turn-on voltage also approaches zero by adopting this measurement setting. With dual gate operation, the driving capability, switching speed, and the electrical stability can be greatly enhanced. For the single-gate driving mode [42]–[44], a constant bias applies to the secondary gate while a sweep voltage applies to the primary gate. The secondary gate electrostatically modifies the charge carrier distribution in the channel accumulated by the primary gate. Hence the secondary gate can accurately set the threshold voltage, but at the cost of an extra electrical contact in the circuit and of additional steps in the fabrication process.

An example of the single gate driving mode is presented here using [37]. A schematic layout of a dual gate transistor and its transfer characteristics of various constant top gate biases ($V_{TG}$) are shown in Figure 2-4(b). The top gate bias is varied from left to right in steps of 5 V starting at +5 V to -10 V. The current in the DGTFT at a given source-drain bias is determined by the interplay between the biases on the two gate electrodes. When a positive top gate bias ($+V_{TG}$) is applied to a n-type channel, an extra constant drain current is added to the transfer curve, because additional charge carriers are introduced at the top channel-dielectric interface brought by the top gate. The top channel is only depleted by the bottom gate at a bias that beyond the original threshold voltage. Thus, the entire transfer curve is shifted to the right. On the other hand, a negative top gate bias ($-V_{TG}$) depletes the accumulated charges in bottom channel. Effectively, the transfer curve is shifted to the left. We will use this mode for the biosensing applications in this dissertation.
Figure 2-4 (a) Comparison of transfer characteristics of two dual-gate a-IGZO TFTs under single- and double-gate driving [40]. (b) The drain current of a dual gate transistor is presented on a semi logarithmic scale as a function of the bottom gate bias. The top gate bias is varied from left to right in steps of 5 V starting at +5 V to -10 V. The inset is a schematic of the dual gate transistor. [37]
To quantify the relation between $V_{th}$ and $V_{TG}$, an analysis using a simplified device structure (Figure 2-5) was proposed by [38].

**Step no. 1:** the difference of electrical potential is expressed as the integral of the electrical field:

$$\int_{y_0}^{y} E(y) dy = V(y_0) - V(y) \quad (2.1)$$

Integration from $y_0 = -t_{BI}$ to $y = 0$ gives

$$t_{BI} E_1 = (V_{BG} - \Delta \varphi_B) - V_{ch} \quad (2.2)$$

where $V_{ch}$ is the voltage at the channel surface ($y = 0$).

Since $C_{BI} = \varepsilon_{BI}/t_{BI}$, the above equation can be expressed as

$$\frac{\varepsilon_{BI} E_1}{C_{BI}} = (V_{BG} - \Delta \varphi_B) - V_{ch} \quad (2.3)$$

In addition, the integration from $y_0 = 0$ to $y = t_{IGZO} + t_{TI}$ gives

$$\frac{\varepsilon_{TI} E_4}{C_{TI}} + \frac{\varepsilon_{semi} E_3}{C_{semi}} = V_{ch} - (V_{TG} - \Delta \varphi_T) \quad (2.4)$$

Here, the $\Delta \varphi_B$ and $\Delta \varphi_T$ represent the work function difference of the semiconductor film at top gate and bottom gate electrodes, respectively.

**Step no. 2:** applying Gauss’ law to the interface $y = 0$ and $y = t_{semi}$ yields

$$\varepsilon_{semi} E_2 - \varepsilon_{BI} E_1 = Q_{TB} + Q_{ch} \quad (2.5)$$

$$\varepsilon_{TI} E_3 - \varepsilon_{semi} E_2 = Q_{tT} \quad (2.6)$$
where \( Q_{B} \) and \( Q_{T} \) are trap densities at bottom and top channel surface, respectively. \( Q_{ch} \) is the mobile charge density of the channel layer. \( E_1, E_2, \) and \( E_3 \) are the electrical fields at the drawn points in Figure 2-5.

**Step no. 3:** The mobile charge density \( Q_{ch} \) can be expressed as follows if substitute Eq. (2.5) – (2.6) into Eq. (2.3) – (2.4).

\[
Q_{ch} = -C_{BI}V_{BG} - \left( \frac{C_{TI}C_{semi}}{C_{TI} + C_{semi}} \right) V_{TG} - \frac{C_{semi}}{(C_{TI} + C_{semi})} Q_{TT} + C_{BI} \left( 1 - \frac{C_{TI}C_{semi}}{C_{BI}(C_{TI} + C_{semi})} \right) V_{ch} + C_{BI} \Delta Q_{B} + \frac{C_{TI}C_{semi}}{(C_{TI} + C_{semi})} \Delta Q_{T}
\]

When the device is just about to turn on, we can let \( Q_{ch} = 0 \) and \( V_{BG} = V_{th} \).

Defining \( \beta = \frac{C_{TI}C_{semi}}{C_{BI}(C_{TI} + C_{semi})} \)

We can obtain

\[
V_{th} = -\beta V_{TG} - \frac{Q_{TB}}{C_{BI}} - \frac{\beta}{C_{TI}} Q_{TT} + (1 - \beta) V_{ch} + \Delta Q_{B} + \beta \Delta Q_{T}
\]

We define \( V_{th}(0) \) as the TFT’s threshold voltage for top gate voltage \( V_{TG} = 0 \)

\[
V_{th}(0) = -\frac{Q_{TB}}{C_{BI}} - \frac{\beta}{C_{TI}} Q_{TT} + (1 - \beta) V_{ch} + \Delta Q_{B} + \beta \Delta Q_{T}
\]

Therefore,

\[
V_{th} = V_{th}(0) - \beta V_{TG}
\]
\( \beta \) is the function of capacitances, which is related to the dielectric constants and thicknesses of the channel and the gate dielectrics.

![Diagram](image)

Figure 2-5 A simplified cross-sectional view of the DGTFT.

It has been shown that TFTs have an additional dimension of control when implemented with dual gate structures, and hence provide more flexibilities in sensing applications [22], [23]. In this dissertation, we use the single gate operation feature of DGTFT for the early stage detection of biofilm formation. The bottom gate of the DGTFT is used as the biasing gate which serves to optimize the operation point, whereas the top gate is used as the sensing gate which is connected to the extended sensing pad. While the bacterial cells adhere themselves onto the sensing pad surface, the electrochemical interactions make a certain portion of cell surface charge transfer downwards to the supporting substratum. Then the transferred charge induces a micro bias to the top sensing gate and impact the channel current. Thus, the onset of biofilms formation can be monitored.

2.2 Multifunctional Semiconductors: ZnO and MZO

ZnO and its related alloy MZO are wide bandgap semiconductors which can be made as multifunctional materials through proper doping.

2.2.1 As TFT Channel Material
ZnO has been widely employed in thin-film transistors (TFTs) as the active channel layer, due to its high electron mobility, transparent to visible light, and low-cost properties. Moreover, ZnO is a partial ionic semiconductor material in which the carrier transportation is non-directional, and therefore the carrier mobility of amorphous and polycrystalline ZnO is much higher than that of the same type of Si [45]. This is especially advantageous for biosensing applications because ZnO-based devices can simultaneously achieve high electrical performance and low-cost properties. In 2011, Reyes et al. reported a ZnO bioTFT immunosensor for the detection of EGFR antigen-antibody interaction [15]. The ZnO TFT device possessed excellent and repeatable characteristics with a ~10^8 on-off ratio which provided high sensitivity of the device to the charge modulation within the ZnO channel. However, due to the nature of oxide semiconductors, pure ZnO suffers from thermal instability and negative bias stress instability which could result in unstable operations for sensing applications. A small amount of Mg is doped into ZnO to form ternary Mg_{1-x}Zn_{x}O (MZO) as the TFT channel material, which suppresses the oxygen vacancy in the active channel benefit from the stronger Mg-O bonding [46], [47]. MZO TFTs are used in a variety of applications, including converters/inverters in BIPV [13], [48], frequency modulators in surface acoustic wave (SAW) device [49], and negative capacitance TFTs for low power consumption electronics [50].

2.2.2 As Sensing Material

Despite the unique advantages of using ZnO-based semiconductors as the active channel layer of TFT, nanostructured ZnO can also be used as a biosensing material.

ZnO-based nanostructures are known to be bio-compatible functional nanostructures used in biosensor technologies [51]. Besides biomolecules, ZnO based sensors have also
demonstrated high sensitivity to various species, including gases, chemicals, pH, and UV light [52]. ZnO nanostructures (ZnO\textsubscript{nano}) can be grown with various surface morphologies [53] on different substrates, including glass and metal electrode surface. The controls of wettability of ZnO\textsubscript{nano} are achieved to improve the sensitivity of biosensors [54]. A small percentage of Mg composition is introduced into ZnO to form the ternary compound Mg\textsubscript{y}Zn\textsubscript{1-y}O (MZO). MZO keeps the main advantages of the pure ZnO but is able to sustain a larger pH range, which is particularly suitable for the bio-testing process comparing with pure ZnO [55]. In addition, ZnO\textsubscript{nano} would release Zn\textsuperscript{2+} ions into aqueous solutions such as cell culture medium, and the Zn\textsuperscript{2+} ions are toxic to bacteria. In contrast, MZO\textsubscript{nano} could effectively reduce the toxicity by suppressing the Zn\textsuperscript{2+} ions [56], [57]. Owing to the above advantages, ZnO and MZO have been demonstrated for high sensitivity and compatibility with various biochemicals such as DNA, oligonucleotides, antibodies, proteins, as well as live biological species such as mammalian cells and bacterial strains [15], [56], [58]–[60].

2.3 Bacterial Biofilm and its Development Stages

Biofilms are densely packed communities of microbial cells that grow on a surface and around themselves with extracellular polymeric substances (EPSs). EPS acts as a protecting layer against the diffusion of antibiotic agents. Therefore, once the biofilms are fully established, they are characterized to show from 500 to 5000 times more tolerant to antibiotics than the free-floating bacteria of the same kind. First, the early stage detection of biofilm formation is critical, because the sooner the onset of biofilm formation is detected, the more effective the prevention of infection will be. Once passes the early stage, its growth kinetic profile should also be monitored during the long-term evolution of biofilm formation because such information can serve as a feedback signal.
The formation process of bacterial biofilms can be schematically presented by three stages as shown in Figure 2-6: bacterial adhesion stage (early stage), bacterial expansion stage (growth stage), and biofilm maturation stage (final stage). In the bacterial adhesion stage, bacterial cells start with reversible attachment on the solid surface with the forces being van der Waals force, electrostatic forces and hydrophobic interactions [61]–[63]. During this initial contact, bacteria can still be removed by fluid shear forces such as rinsing [64]. Then, the cells change from reversible attachment to irreversible adhesion with the help of various short-range forces, including covalent and hydrogen bonding as well as hydrophobic interactions [65]. The cells then begin to excrete EPS as they grow and divide [66]. EPS acts as a protection layer against the diffusion of antibiotic agents, which causes the biofilms to have antimicrobial resistance. Finally, the bacterial cells create biofilms that have a set size and shape for a given set of environment conditions. Biofilms at this stage are referred to as being mature.
Figure 2-6 The development process of biofilm formation on a solid surface is shown in three steps: 1) bacterial adhesion, 2) bacterial expansion, and 3) biofilm maturation. EPS stands for extracellular polymeric substances. [67]
As indicated in [68], [69], charge transfer between bacterial cell surface and the (semi)conducting substratum surface plays an important role in the initial bacterial adhesion stage and hence influences the subsequent biofilm development process. Bacterial cell surface consists of a variety of different macromolecules including proteins which contain electrochemical active groups; particularly carboxylate functions that facilitate charge transfer. During adhesion, substratum free electrons are present which give rise to short-range electrochemical interactions with the adhering microorganisms. The charge transfer phenomenon happening during the initial bacterial adhesion stage provides an important perspective on detecting the biofilm formation at the very early stage, as the bacterial adhesion indicates the onset of biofilm formation. At the early stage of biofilms formation when bacterial cells are not encapsulated in EPS yet, antimicrobial agents are still effective to kill the bacteria. Therefore, the sooner an infection is detected, the more effective the treatment will be. Thus, the development of an early stage biofilm detection method with high sensitivity is very critical for timely clinical treatment of biofilms, so that one does not have to wait until the biofilm ultimately forms. Instead, the biofilm development at the early stage can be decisively predicted by detecting the charge transfer effect using electrochemical sensors with high sensitivity such as the MZO DGTFT biosensor presented in this dissertation.

While entering the bacterial expansion stage towards to the biofilm maturation stage, daughter cells from cell division spread outward from the sites of attachment to form clusters. Stronger bonding between cells is formed partly due to the excretion of EPS. Therefore, the mass of biofilms is gradually increasing with viscoelastic transitions, which can be easily detected by QCM.
2.4 Existing Technologies of Biofilm Detection

2.4.1 Conventional Detection Methods

There are three major types of conventional biofilm detection methods, namely microbiological and molecular methods, physical and chemical methods, and microscopy methods [70].

The determination of colony forming units (CFU) on agar plates is the most widely used microbiological technique to estimate the biofilm cell viability. This method is based on the universal dilution series approach to quantify cells, so it is easy to perform and available in all microbiology labs. However, it is time-consuming and thus low efficiency. Besides, quantitative polymerase chain reaction (q-PCR) and PMA-qPCR are typical ways of molecular methods, which significantly shorten the duration of experiments, but the measurement apparatus is costly.

Physical methods such as biomass weight measurement and ultrasonic time-domain reflectometry, on the other hand, are cost-effectively and very easy to perform. In addition, chemical methods such as biofilms disruption methods combined with staining assays, say crystal violet staining assay, have also been employed to visualize and detect biofilms and bacterial cells [71]. For both the physical and chemical methods, the low sensitivity and low accuracy disadvantages prevent their applications from strictly required measurement tasks.

Microscopy technique is also an important way for studying biological samples, including bacterial biofilms. According to different experimental settings and the level of information desired to acquire from the sample, researchers need to select the right
technique of microscopy imaging among various options, such as phase-contrast microscopy, differential interference contrast microscopy, and confocal scanning laser microscopy (CLSM). Among these, CLSM has been used by microbiologists to visualize biofilms and its EPS structure because it can capture multiple two-dimensional images at different depths in a sample. Quantitative structural parameters such as the biofilm bio-volume, thickness and roughness can also be extracted and presented with a 3D model of biofilms [70], [72]–[75]. These unique advantages of CLSM make it stand out from other microscopy methods. However, the high intensity of laser beam can seriously damage the cells. The complexity and cost of confocal systems have also limited their use.

These conventional methods are either slow, highly labor intensive, or very costly and complex. To minimize or even prevent bacterial infections on medical implants for clinical applications, a need has emerged for high-throughput analysis and techniques that allow for the rapid detection of biofilm development in real time. So far, two major types of dynamic sensors have emerged alleviating the limitations of the conventional methods. These are namely the mass-based sensors [76]–[78] and the electrical impedance sensors [79]–[84].

2.4.2 Mass-based Sensors

The mass-based sensors are capable of precisely measuring the mass accumulation on its surface using acoustic wave devices, including the surface acoustic wave (SAW) sensors and the quartz crystal microbalances (QCMs). Nanogram ranges of mass change can be easily detected by these devices in online and real time modes. Recently, Y. W. Kim et al. demonstrated aluminum oxide (Al$_2$O$_3$) passivated ZnO thin film SAW sensor to detect the biofilm formation [76] and the biofilm removal through bioelectric effect [77] as shown in
Figure 2-7(a). On the other hand, the QCM technique was employed to identify the sequence of events occurring in the biofilm formation process and probed the time response under different environmental conditions [78] as shown in Figure 2-7(b). Both SAW and QCM sensors have been mainly used for monitoring the long-term behavior of biofilm formation because they are intrinsically limited by the sensitivity, i.e., the smallest mass these sensors can detect.
Figure 2-7 (a) Schematic of the integrated microsystem of the SAW sensor and electrodes for induction [77]. (b) Schematic of the QCM system for monitoring the biofilm formation. [78]
2.4.3 Impedance-based Sensors

Electrical impedance sensors involve the changes in the resistance and reactance of the device due to the modulation of the charges on the sensing area introduced by the bacterial cell attachment and biofilm formation. Several works have been reported utilizing the detection of impedance variations caused by different phases of biofilm formation [79]–[86]. An example of the biosensor as well as its equivalent circuit model is shown in Figure 2-8. As the biofilms develop on the device, the capacitance and conduction of the sensor device would vary due to the bacterial adhesion and their metabolism activities, leading to a long-term monitoring capability. The low frequency range was found to be the best setting for analyzing the culture changes and meeting the power consumption requirements for implantable applications. In addition, some efforts have been made to employ the impedance measurement in different experimental setups mimicking real field environment of bacterial biofilm cultures [84]. The EIS is primarily used for the long-term monitoring of biofilm development on the devices, however there have been some works reporting the detection of early stage biofilm formation by [79], [81], [85], [86]. These reports all involved EIS as the mechanism for detection combined with various electrode configurations and materials. However, the passive nature of two-terminal electrical impedance devices still limits the sensitivity because of the lack of signal gain. To further enhance the detection sensitivity, which is especially needed for the early stage detection of biofilm formation, a transistor type of three-terminal active device with high signal gain is desired to offer intrinsic amplification to the sensor signals.
Figure 2-8 (a) An image of the biosensor and a mask detail of the interdigitated microelectrodes. (b) A cross-section view of the equivalent circuit model for the impedance measurement. [80]
As introduced in Section 2.3, the charge transfer phenomenon happening during the initial bacterial adhesion stage provides an important perspective on detecting the biofilm formation at the very early stage, as the bacterial adhesion indicates the onset of biofilm formation. As the charge transfer is tiny and hard to characterize directly, an electrical transducer with high gain is needed to convert and amplify such small biological signals to discernible electrical signals.

Some effort has also been made to realize both early stage detection and long-term monitoring in a single device. For example, cyclic voltammetry was used to differentiate bacterial attachment and biofilm formation through their different signal responses [87]. In the case of bacterial attachment, cell attachment to the electrodes results in an increase of current in the cyclic voltammograms due to bacterial metabolism activities, whereas in the case of biofilm formation, a decrease of current occurs due to the reduction of electrode surface area. However, the measurements of bacterial adhesion and biofilm formation were separately conducted by controlling the nutrient supply, thereby not representing a true dynamic and real-time monitoring of the full-scale development of biofilm formation. For clinical applications, a full-scale continuous monitoring technique is needed to guide treatment of biofilm infections promptly and effectively.

2.5 Biosensing Platform Functionalized with Folic Acid

Folate receptor (FR) is a tumor-associated antigen which over-expresses in many human tumors. As FR is absent in normal tissues, the detection of FR can serve as an early stage cancer diagnostic step. Folic acid (FA), also called folate, is an important component of nucleic acids and necessarily needed for the methylation of DNA, proteins, and lipids. FA specially binds FR with high affinity (association constant of $10^9 - 10^{10}$ M$^{-1}$) at 1:1
stoichiometry. Therefore, a biosensing platform functionalized with FA-containing molecules is essential for the detection of FR. Several works have demonstrated the use of FA for building such biosensing platform.

Two main techniques have been employed to realize such detection, namely fluorescence imaging and electrochemical sensing. Fluorescence imaging is an important optical analytical tool for biomolecule detection, because of its high sensitivity and spatiotemporal resolution. As FA possess low fluorescence quantum yield, much efforts have been made on the immobilization of FA on fluorescent nanomaterials, such as carbon [88], [89], gold [90], and ZnS-based quantum dots [91], [92]. Moreover, fluorescence imaging is easily affected by the surrounding environment. Electrochemical sensing, such as cyclic voltammetry and impedance spectroscopy, has been widely used for the detection of disease biomarkers due to simplicity, convenience, and low cost. Au nanoparticles and graphene functionalized with FA are commonly used to decorate the electrodes to enhance the sensitivity and selectivity of the device [93]–[96]. However, their sensitivity can be further enhanced by utilizing three-terminal active devices, such as TFT, due to the electrical signal gain induced by gate.

2.6 Stepwise Functionalization of ZnO-based Nanostructures

The immobilization of FA on the active sensing surface is critically important to achieve highly selective and reliable devices for the detection of FR. Many of the above-mentioned references used non-covalent binding processes, such as physisorption, which are simple, fast, and do not require synthetic modifications [88]–[90], [92], [95]. However, weakly-bound FA molecules may desorb from the platform. Covalent bonding can provide
a stable and uniform interface that can undergo further reactions in comparison with non-covalent bonding.

ZnO$_\text{nano}$ was firstly functionalized using bifunctional linkers by Taratula et al. in 2006 [55]. The study indicated that the (carboxylic acid group) COOH is a suitable anchoring group for binding to the ZnO$_\text{nano}$. However, the linkers with acidic functional groups “dissolve” the ZnO$_\text{nano}$ films, because the films can be etched at low pH (pH<4). It also found that by alloying ZnO with MgO (Mg$_y$Z$_{1-y}$O$_\text{nano}$, $y = 5 – 10 \%$) its resistance to acids can be enhanced while staying identical to the ZnO$_\text{nano}$ in other aspects. The research opens the possibility to develop the ZnO-based nanostructures with a variety of dyes, redox-active molecules or biomolecules, and leading to novel ZnO-based sensing technologies. In 2009, as an extension to the work in 2006, Taratula et al. demonstrated a three-step procedure to functionalize the ZnO$_\text{nano}$ with DNA, followed by the hybridization with complementary and fluorescein-tagged ssDNA [97]. The new approach allowed the sequential reactions on the ZnO$_\text{nano}$ surface which can be extended to other molecules and biomolecules. Surface engineering was also used to explore the morphology effect of ZnO$_\text{nano}$ for hybridizing DNA on the films [53]. In 2013, Cao et al. demonstrated the stepwise functionalization of ZnO$_\text{nano}$ through click reaction [98]. A long chain saturated carboxylic acid capped with an azide end group was bound to the ZnO$_\text{nano}$ surface through the COOH moiety, leaving the N$_3$ group available for click reaction with an alkyne. In 2017, MZO$_\text{nano}$ was used to demonstrate such functionalization process and FTIR microscopic imaging was firstly used for characterization [99].
In this dissertation, the hexadecyl alkynated folic acid (HAFA) was covalently anchored onto the MZO_{nano} surface through linkage chemistry and click reaction. Detailed experimental process and measurement results are presented in Chapter 5.
Chapter 3. Early Stage Detection of Biofilm Formation

3.1 Background

Early stage detection of biofilm formation is an important aspect of microbial research because once formed, biofilms show serious tolerance to antibiotics in contrast to the free-floating bacteria, which significantly increases the difficulty for clinical treatment of bacterial infections. The early stage detection technology is desired to improve the efficiency of medical treatments. In this chapter, we demonstrate a biosensor consisting of an MZO DGTFT with an extended MZO\textsubscript{nano} modified sensing gate for the early stage detection of \textit{S. epidermidis} biofilms. \textit{S. epidermidis} bacteria were cultured \textit{in vitro} on the nanostructure modified sensing pad. Charge transfer occurs between microbial cells and the MZO\textsubscript{nano} during the initial bacterial adhesion stage. Such electrical signals, which represent the onset of biofilm formation, were dynamically detected by the DGTFT where the top gate electrode was connected to the extended MZO\textsubscript{nano} sensing pad and the bottom gate was used for biasing the device into the optimum characteristic region for high sensitivity and stable operation. This technology enables medical professionals to act promptly on bacterial infection before biofilms get fully established.

3.2 Design of the MZO DGTFT Biosensor

The schematic diagram of the MZO DGTFT biosensor is shown in Figure 3-1(a). It consists of two parts: (1) an MZO DGTFT as the signal transducer, and (2) an MZO\textsubscript{nano} modified sensing pad as the biological receptor. Figure 3-1(a) and (b) present the cross-sectional and top view of an MZO DGTFT, respectively.
As mentioned in Chapter 2, we utilize the double gate design of TFT, extended-type of sensing gate, and ZnO-based materials as both the active channel layer of DGTFT and the sensing materials, to realize a robust biosensor for the early stage detection of biofilm formation. In DGTFT, the bottom gate is used as the biasing gate which serves to optimize the operation point, whereas the top gate is used as the sensing gate which is connected to the extended sensing pad. While the bacterial cells adhere themselves onto the sensing pad surface, the electrochemical interactions make a certain portion of cell surface charge transfer downwards to the supporting substratum. Then the transferred charges induce a micro-bias to the top gate and impact the channel current through the field-effect. Thus, the onset of biofilm formation can be monitored. The dual gate design allows the TFT device to perform at the optimum biasing region to achieve the best sensitivity and to ensure the stable operation.

Bacterial growth including biofilm formation can be affected by electric fields [77] especially if the bacterial samples are placed directly on the active sensing device. However, our MZO DGTFT has the sensing pad not directly on the active layer of the transistor and hence the only effect the sensor detects is the field produced by the biofilm and the transistor does not interact electrically with the biofilm. The extended sensing pad design allows the separation of TFT device from the harsh biochemical environment and different sensing pads according to the detection tasks can be connected to the transducer sequentially, which fuels the realization of a plug-in-card type of biosensor. Moreover, the separated sensing pad can be freely modified based on specific biomolecules to achieve high sensitivity and selectivity.
Figure 3-1 (a) A schematic drawing of the MZO DGTFT biosensor system. The system consists of two parts: an MZO DGTFT as the transducer and a ZnO or MZO nanostructure modified sensing pad as the receptor. (b) The cross-sectional view of the DGTFT. m-MZO layer means the combination of the MgO diffusion barrier and the MZO active channel layer. (c) The optical microscope top view of the DGTFT. Four terminals (bottom gate, top gate, source and drain) are labeled accordingly. SEM images of the MOCVD-grown (d) ZnO and (e) MZO nanostructured films. [67]
3.2.1 Fabrication of the MZO DGTFT

A 50-nm-thick Cr layer was first deposited using electron beam evaporation and then patterned using the standard photolithographic method to form the bottom gate electrode on a glass substrate. Then a SiO$_2$ film (100 nm) was deposited on top of the Cr bottom gate as the gate dielectric layer using plasma enhanced chemical vapor deposition (PECVD). A 5 nm MgO interfacial layer followed by a 40 nm Mg$_{0.03}$Zn$_{0.97}$O channel layer was deposited using metal organic chemical vapor deposition (MOCVD) at ~400°C, to form a MZO /SiO$_2$/MgO structure. The ultra-thin MgO layer acts as a barrier to minimize the Zn$^{2+}$ ions diffusion into the SiO$_2$ dielectric layer, in order to enhance the TFT characteristics and stability [13], [49]. In MOCVD, DeZn (Diethylzinc), MCp$_2$Mg (bis-(methyl-cyclopentadienyl) magnesium), and ultra-high purity (99.999%) oxygen gas were used as the Zn metalorganic source, Mg metalorganic source, and oxidizer, respectively. The active mesa area ($W/L = 160 \, \mu m/15 \, \mu m$) was formed through wet etching. The source and drain contacts were formed through metallization process (5 nm Ti/85 nm Ti/35 nm Au) followed by a normal lift-off process. Then the top SiO$_2$ dielectric layer (70 nm) was deposited by PECVD again. Next, a 150-nm-thick Al layer was deposited by electron beam evaporator as the top gate electrode and then patterned. Finally, VIA openings were made using buffered oxide etch solution.

3.2.2 Fabrication of the ZnO$_{\text{nano}}$ and MZO$_{\text{nano}}$ Sensing Pads

In this biosensor, the ZnO$_{\text{nano}}$ or MZO$_{\text{nano}}$ pad is used as the extended sensing gate, which is connected to the top gate of DGTFT device. The nanostructured surface is designed due to its giant effective sensing area which enables high sensitivity. The
fabrication started from depositing a 50 nm Au/5 nm Cr metal layer using electron beam evaporation process on glass substrate, which serves as the conducting electrode. The metal layer was then patterned and wet etched to form squares of 120 mm². The interface between biological materials and the micro-electronics is a key aspect to achieve high sensitivity. For comparisons, 400-nm-thick ZnO and Mg₃Zn₁₋₃O nanostructured films (ZnO_{nano} and MZO_{nano}) were grown on the Au/Cr coated glass pad using MOCVD at ~500°C [100]. Same precursors and oxidizer as the channel layer were used, however, the y value in Mg₃Zn₁₋₃O nanostructures was kept at ~0.04 to enhance the etching resistance, reduce the diffusion of Zn²⁺ ions into the incubation solution and hence reduce the toxicity to the bacterial cells. The scanning electron microscope (SEM) images of the ZnO_{nano} and MZO_{nano} films are shown in Figure 3-1(d) and (e), respectively. The roughness of the surface ensures the biosensor with high sensitivity [56]. The nanostructured layer was then patterned and wet etched, while leaving a small area of the metal pad open for electrical contact. Then the MZO_{nano} sensing pads underwent UV illumination to get the super-hydrophilic characteristics in order to achieve high sensitivity and minimize the bio-sample consumption [101]. The resulting MZO_{nano} and ZnO_{nano} sensing pads possess optimized morphology and wettability, which leads to higher sensitivity of the device.

ZnO and MZO films were then respectively grown on glass substrates to experimentally characterize the y value in Mg₃Zn₁₋₃O using the optical absorption measurement. The absorption spectra of ZnO and MZO films were acquired at room temperature by use of a Beckman DU 530 UV-visible spectrometer.

### 3.3 Biological Sample Preparation and Protocols

#### 3.3.1 Bacterial Biofilm Incubation
S. epidermidis ATCC 35984 was inoculated into Tryptic Soy Broth (TSB; Becton Dickinson) and then grown at 37°C for 16 –18 h with shaking at 200 rpm. The pH of the culture medium is 7.4 which is close to neutral when prepared according to the manufacturer’s prescription. The stationary phase cultures were diluted 100-fold into fresh TSB medium pre-loaded in 10 cm quad-plate petri dish, with each sector containing 5 mL cultures. The MZO\textsubscript{nano} and ZnO\textsubscript{nano} sensing pads were sterilized and placed into the bacterial cultures, with the sensing area being up to allow bacterial adhesion. With the increase of culture time, the bacteria would adhere to the sensing surface first, followed by bacterial expansion and biofilm maturation.

3.3.2 Crystal Violet Staining Assay

The same procedure was followed as describe in Section 3.3.1 but adding one more sensing pad incubated in TSB medium without bacteria as a control. The dishes were incubated statically at 37°C for biofilm formation. After incubation for 100 min, 200 min, 8 h, 16 h and 24 h, the sensing pads from the cultures were washed three times with 5 mL 0.9% NaCl to remove planktonic cells. Biofilms attached on the sensing surface were then stained using 0.2% crystal violet for 10 min, followed by washing three times with 0.9% NaCl. 100 μL of 30% ethanol was added to each sensing pad to release bound crystal violet when the crystal violet staining solution was removed. The absorbance value was measured at 590 nm using absorption plate reader.

3.4 Bio-measurement and Parameter Extraction

The MZO DGTFT was placed inside a light-tight measurement station. Its three terminals, i.e., source, drain, and bottom gate, were electrically connected to the
semiconductor parameter analyzer HP-4156C. A bottom gate biasing voltage $V_{BG}$ was swept between -5 V to 15 V to optimize the operation point of the MZO DGTFT. The drain was set at 0.1 V whereas the source was grounded. The drain-source voltage $V_{DS}$ acts as a pump to drive current across the channel. The top sensing gate of the DGTFT was connected to the MZO$_{nano}$ or ZnO$_{nano}$ sensing pad outside of the measurement station. With the increasing of culture time, bacteria adhere themselves onto the MZO$_{nano}$ or ZnO$_{nano}$ sensing pad. This interaction process generates the charge transfer downwards, resulting in a micro-bias applied on the top gate of the DGTFT.

The threshold voltage $V_{th}$ of the device was extracted using the linear fitting method for 10% - 90% of the maximum drain current. The subthreshold slope $S.S.$ value is extracted from a 3-decade range in the subthreshold region ($I_D = 10^{-12} - 10^{-9}$ A) of the transfer characteristics in logarithm scale.

3.5 Result Analysis

3.5.1 Electrical Characterization of the MZO DGTFT

Before we utilize the MZO DGTFT biosensor to dynamically monitor the formation of *S. epidermidis* biofilms, we need to determine three aspects, i.e., the electrical characteristics of the MZO DGTFT without bioreaction, the change of the electrical characteristics during the growth of *S. epidermidis* on the extended sensing gate, and the proper biasing voltage on the bottom gate of DGTFT to attain the best combination of high sensitivity and stable operation. The MZO DGTFT biosensor was initially tested by fixing the drain voltage at 0.1 V and sweeping the bottom gate voltage from -5 V to 15 V while the top gate was connected to the extended MZO$_{nano}$ sensing pad that was immersed in TSB medium and let the signal stabilize to baseline value. *S. epidermidis* bacterial culture
solution of predetermined volume was introduced into the sensing pad with TSB medium at time $t = 0$ and let the device incubate for biofilm formation. The transistor transconductance plot (i.e., I-V curves of drain current vs bottom gate bias voltage) were recorded at times $t = 0, 50, 125, 200, \text{ and } 375$ min of incubation time. Figure 3-2 shows the electrical transfer characteristics of an MZO DGTFT during *S. epidermidis* growth in TSB media. The curve at $t = 0$ shows the threshold voltage of 8.4 V, subthreshold swing of 510 mV/dec, and on-off ratio of $\sim 10^8$. The steep slope (small value of subthreshold swing), and high on-off ratio indicate high signal gain, therefore high sensitivity of the biosensor.

From the I-V curves in Figure 3-2, the drain current dramatically decreases as incubation time increases from $t = 0$ to 200 min; however, afterwards, i.e., $200 \text{ min} < t < 375$ min, there is no significant change. It should point out that the DGTFT offers the flexibility to tune the sensitivity through the biasing. This initial setting serves as the calibration of the device. In the next section, we show that the bottom gate biasing conditions are optimized to achieve the best operation point where the early stage detection of biofilm formation is enabled. The observed modulation of the drain current of the MZO MGTFT biosensor as a function of biofilm formation time is attributed to the electron charge transfer, resulted from the bacterial adhesion to the MZO$_{nano}$ surface.

The bottom gate biasing voltage is used to optimize the sensor’s operation point. The I-V curves in the right side of Figure 3-2 shows the electrical characteristics as a function of incubation time plotted in linear scale, where it seems the drain current change as a function of time at any bottom gate biasing voltage between 10 V to 15 V could be used with good sensitivity. However, if the same curves are plotted in the logarithmic scale as shown in the left side of Figure 3-2, we can see that there is a narrower range of bottom
gate voltages that would be most suitable for sensor operation with high sensitivity. The slope of I-V transconductance plot at the triode region is much larger, and hence the current changes are more discernible. Beyond this range, the TFT enters the saturation region, where the current does not change significantly as incubation time increases, in comparison with the triode region.
Figure 3-2 The electrical transfer characteristics of an MZO DGTFT during the *S. epidermidis* growth in TSB media recorded at different times. Left axis presents the drain current in logarithm scale whereas the right axis presents same data in linear scale. [67]
3.5.2 Real-time Monitoring of the Biofilm Early Stage Formation

We demonstrate the real time monitoring of the early stage formation of *S. epidermidis* biofilms using the MZO DGTFT sensor. Three MZO<sub>nano</sub> sensing pads were prepared and the detection of *S. epidermidis* biofilms was repeated three times using the same MZO DGTFT device under the same microbial culture conditions. The *S. epidermidis* bacteria were injected at *t* = 0. For a field-effect transistor (FET) type of biosensor, the sensitivity of the device is characterized as the relative variation (Rel. Var.) of drain current at a certain operation condition [5]. Eq. (3.1) was used to calculate it as a function of time:

\[
\text{Rel. Var.} = \frac{I_D(t) - I_D(0)}{I_D(0)} \times 100\% \tag{3.1}
\]

where *I*<sub>D</sub>(*t*) is the drain current measurement at time *t*, and *I*<sub>D</sub>(0) is the drain current measurement at the start time. The mean value and standard deviations of these relative variations were then taken from the three experiments.

As mentioned in the previous section, the bottom gate biasing voltage is used to optimize the operation point of the measurement. In the triode region of the I-V characteristic curves, the plots possess a steep slope which enhances the sensitivity; however, in the saturation region of the I-V curves, the sensitivity dramatically decreases as the slope is much smaller. To investigate the effect of bottom gate biasing voltage on the sensitivity for detection of biofilm formation, the sensor was operated at *V*<sub>BG</sub> of 0, 2 V, 10 V, and 15 V, respectively. For each sample, the measurements were made sequentially every 25 min for a total of 375 min of the incubation time. The mean values of drain current variation as a function of incubation time and their corresponding error bars for standard deviation are presented in Figure 3-3(a). The current variation at *V*<sub>BG</sub> = 0 is not shown in
Figure 3-3(a) due to large error bars (average standard deviation: 24.6%). For all four bottom gate biasing voltages, the percentage change in drain current shows significant decreasing values starting from the introduction of the bacterial culture on the MZO\textsubscript{nano} sensing pad ($t = 0$) until saturation at about $t = 200$ min. However, it can be seen that there is a trade-off between the sensitivity and stable operation for different bottom gate biases. At $V_{BG} = 15$ V, the sensor exhibits smallest standard deviations indicating higher accuracy compared to the other biasing conditions. This is due to the fact that under large bottom gate bias, the TFT enters into the saturation region, where the conduction channel is fully occupied by electrons. On the other hand, the biosensor’s sensitivity, which represents by the total percentage change of drain current, is higher when operated at a lower bottom gate biasing as compared to the one at $V_{BG} = 15$ V, which could be attributed to the steep slope in the triode region of the characteristic curve. Table 3-1 lists the experimental results on the slope of the transfer characteristic, maximum current change, and the average standard deviation at these four different bottom gate voltages. From the table, although the highest drain current change was found when bottom gate was grounded (i.e., $V_{BG} = 0$), the standard deviation (24.6%) is too large to make the stable and reliable operation. Therefore, $V_{BG}$ of 2 V is selected as the optimized operation point. At this condition, the device provides high sensitivity in terms of the total percentage reduction in drain current (82.9%) and the stable operation represented by a small standard dispersion (10.1%). Figure 3-3(b) represents the fitting curve of drain current variation as a function of incubation time for bottom gate bias $V_{BG}$ of 2 V. The current change saturates at about 200 min which signifies the detection point of the onset of biofilm formation. The maximum current reduction of 82.9% is reached at incubation time $t = 375$ min.
In Figure 3-3(b) we can see that the sensor’s drain current decreases rapidly as incubation time goes from $t = 0$ to 200 min, after which the current levels off. The decrease in drain current signifies that electron transfer has occurred between the bacterial cells and the MZO nanostructures. According to Patil et al. [102], electrons are produced by the bacterial cells during metabolism. In the case of cell adhesion, these bacterial electrons are transferred to the electrodes or semiconducting layer such as the MZO nanostructures through a pathway called direct electron transfer (DET). The DET mechanism creates a pathway for the bacterial electron to the sensor’s electrode via the bacteria’s outer membrane cytochromes and conductive cell appendages. These cytochromes and conductive appendages are a product of the cell membrane due to the bacterial cell adhesion to the surface of the electrode.
Figure 3-3 (a) The relative variation of drain current reduction during the *S. epidermidis* biofilm growth were extracted from the DGTFT transfer characteristics. Three different bottom gate biases ($V_{BG} = 2 \text{ V}, 10 \text{ V}, \text{ and } 15 \text{ V}$) are presented as a function of incubation time. No significant current variation was observed without bacterial inoculum. (b) The fitting curve of relative current reduction when $V_{BG} = 2 \text{ V}$ was plotted with its first-order derivative. The current change saturates at about $t = 200 \text{ min}$, which indicates the end point of *S. epidermidis* biofilms early stage detection. [67]
Table 3-1 For $V_{BG} = 0, 2, 10$ and 15 V, the slope of the I-V curve when $t = 0$, the maximum relative current change, and the average standard deviation were shown in the table. A trade-off between sensitivity and dispersion is obvious with different bottom gate biases.

<table>
<thead>
<tr>
<th>Bottom gate voltage $V_{BG}$ (V)</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope of the I-V curve when $t=0$ (V/dec)</td>
<td>0.44</td>
<td>0.93</td>
<td>6.35</td>
<td>12.62</td>
</tr>
<tr>
<td>Maximum current change (%)</td>
<td>-88.0</td>
<td>-82.9</td>
<td>-54.9</td>
<td>-26.4</td>
</tr>
<tr>
<td>Average standard deviation (%)</td>
<td>24.6</td>
<td>10.1</td>
<td>7.6</td>
<td>5.4</td>
</tr>
</tbody>
</table>
While the *S. epidermidis* bacterial cells are adhering to the sensing pad and proliferate gradually, more and more negative charges are transferred from bacterial cell surface to the semiconducting $\text{MZO}_{\text{nano}}$ sensing pad. The transferred charges introduce negative micro-bias, acting onto the MZO DGTFT’s top gate. It decreases the current in the MZO channel through the electric field effect. The equivalent top gate micro-bias due to the adhered *S. epidermidis* cells can be determined from the threshold voltage shift $\Delta V_{TH}$ using Eq (3.2) [38].

$$V_{TG} = -\frac{C_{BI}(C_{TI} + C_{MZO})}{C_{TI}C_{MZO}} \Delta V_{th} \tag{3.2}$$

where $V_{TG}$ is the micro-bias at the top gate due to the charge transfer, $\Delta V_{th}$ is the threshold voltage shift, $C_{BI}$, $C_{TI}$, and $C_{MZO}$ represent the bottom gate, top gate, and the channel layer capacitance per unit area respectively. The induced charge $Q$ at the top gate electrode was calculated using Eq. (3.3):

$$Q = A C_{TI} V_{TG} \tag{3.3}$$

where $A$ is the area of the top gate capacitor. From Figure 3-2, the threshold voltage is found to shift in the positive voltage direction by $\sim 1.6$ V from $t = 0$ to 375 min; therefore, approximately $10^{-12}$ C amount of equivalent charge is induced to the top gate-dielectric interface through the micro-bias caused by the attached bacterial cells on the $\text{MZO}_{\text{nano}}$ sensing pad.

Both the threshold voltage change and the drain current variation could be used as the signals to represent the biofilm development process. However, the drain current varies much more significantly ($\sim 80\%$ current variation when $V_{BG} = 2$ V) in comparison with the threshold voltage change ($\sim 19\%$ threshold voltage variation). This contrast is especially
obvious in the triode region of the transfer characteristics, which is selected as the operation region for sensing. Therefore, the drain current variation is chosen as the signal to represent the biofilm development process with high sensitivity.

The first-order derivative curve of the drain current reduction versus time is shown in Figure 3-3(b). The DGTFT drain current dramatically decreases at the beginning of the test (large first-order derivative), resulting from the adhesion of \textit{S. epidermidis} bacteria. Although the biofilms were still developing and far from maturation, the charge transfer effect between bacterial cell surface and substratum MZO\textsubscript{nano} surface was being inhibited gradually (decreasing first-order derivative), and diminished at about 200 min. The charge transfer between the bacterial cells and the substratum is an interfacial phenomenon, and once the sensing area is covered by the interfacial adhesion cells, there will be no more charge transfer. In addition, the bacterial adhesion could induce a change in the electrical potential of substratum, and the potential change induced per adhering bacterium would decrease during the adhering process and eventually becomes zero, i.e., further charge transfer is hampered by the initial amount of charge that has been transferred. The similar phenomena has been observed and reported by Poortinga et al [68], [69]. There is no lag phase exhibited by the bacterial culture and can be attributed to having the bacterial culture introduced are already in a metabolically active state and does not require time to enter cell division [76].
3.5.3 Microscope Characterization and Bacterial Quantification

Crystal violet staining assay was used to verify that the biofilms indeed were formed on the \( MZO_{nano} \) sensing pads and showed the time dependent long-term process of biofilm development. \( MZO_{nano} \) films were grown on the same glass substrates as the sensing pads, then they were introduced in the same bacterial biofilm incubation process. Optical images of the \( MZO_{nano} \) coated glass substrates were respectively taken at incubation time \( t = 0 \), 100 min, 200 min, 8 h, 16 h, and 24 h (as shown in Figure 3-4(a)). For each times of culture, its absorbance values were also measured at the wavelength of 590 nm after the bound crystal violet was released (as shown in Figure 3-4(b)). The absorbance measurement was repeated three times with an average standard deviation of 0.015. The value of crystal violet absorbance increases from 0.12 at \( t = 100 \) min to 0.28 at \( t = 200 \) min. Concomitantly, the current of the DGTFT biosensor decreases from \( 6.60 \times 10^{-9} \) A at \( t = 100 \) min to \( 3.00 \times 10^{-9} \) A at \( t = 200 \) min. Therefore, the statistical correlation between these two results obtained by different techniques is achieved. From the biosensor standpoint, \( t = 200 \) min is the moment when the signal starts to saturate, indicating that cellular adhesion has taken place. It is defined as the onset of biofilm formation. However, at 200 min, the crystal violet staining assay results show that tiny bacterial microcolonies just start to form at the sites of adhesion as shown in Figure 3-4(a). Afterwards, with the EPS production and bacterial cell proliferation, it took approximately 24 h to develop into the matured biofilms.

Initial bacterial adhesion is the onset of biofilm formation, and it is detected no longer than 200 min by using the novel MZO DGTFT biosensor. Crystal violet staining assay shows bacterial microcolonies just form up at 200 min and the biofilms get matured at approximately 24 h. Thus, the trend of biofilm formation has been predicated at its early
stage, allowing medical professionals to act ahead of time to inhibit the subsequent biofilm formation.
Figure 3-4 (a) Optical images of crystal violet-stained *S. epidermidis* biofilm on MZO film, respectively recorded at $t = 0$, 100 min, 200 min, 8 h, 16h, and 24 h. (b) Quantification of the biofilm formation process at different times of culture by measuring the absorbance at 590 nm. [67]
3.5.4 Effects of Nanostructures on Sensing Performance

The effects of DGTFT using the different nanostructures as the extended sensing gate are studied. The extended sensing gates are made up of MZO \(_\text{nano}\) and ZnO \(_\text{nano}\), respectively. It is well known that ZnO can alloy with MgO to form the ternary compound Mg\(_y\)Zn\(_{1-y}\)O to extend the energy bandgap. The direct energy bandgap of wurtzite-structured Mg\(_y\)Zn\(_{1-y}\)O can be tuned up to \(\sim 4.0\) eV \((y = 0.34)\). The energy bandgap of Mg\(_y\)Zn\(_{1-y}\)O follows Vegard's law \[103\]:

\[
E_g(Mg_xZn_{1-x}O) = xE_g(MgO) + (1 - x)E_g(ZnO)
\]

where \(E_g(Mg_xZn_{1-x}O)\), \(E_g(MgO)\) and \(E_g(ZnO)\) are the energy bandgaps of MZO, MgO and ZnO, respectively. For small \(y\) value, the \(E_g(Mg_yZn_{1-y}O)\) can be estimated by the linear approximation:

\[
E_g(Mg_xZn_{1-x}O) = E_g(ZnO) + bx
\]

where \(b\) is a constant number from fitting.

In this work, the energy bandgaps of MZO and ZnO are experimentally determined by optical absorption measurements. Near the absorption edge, the relationship between the absorption coefficient \(\alpha\) and the photon energy \(h\nu\) is given by Eq. 3.6:

\[
\alpha \propto (h\nu - E_g)^{1/2}
\]

where \(\alpha\) is the absorption coefficient, \(h\nu\) is the photon energy, and \(E_g\) is the energy bandgap. By measuring \(\alpha^2\) versus \(h\nu\) respectively for Mg\(_x\)Zn\(_{1-x}\)O (MZO) and ZnO, and then curve fitting, the energy bandgaps \(E_g\) can be determined \[104\]. The absorption spectra of ZnO and MZO were acquired at room temperature using the Beckman DU 530 UV-visible
spectrometer. Figure 3-5(a) shows the measured curves of $a^2$ versus $h\nu$, where the bandgaps of ZnO and Mg$_x$Zn$_{1-x}$O (MZO) are determined to be 3.26 eV and 3.36 eV, respectively. The linear relationship in Eq. 3.5 is then used to decide the y value in Mg$_y$Zn$_{1-y}$O to be ~0.04 (4%), where $b \approx 2.5$.

*S. epidermidis* biofilms were cultured on the bare sensing pads (Au/Cr coated glass, i.e., metal/glass), pure ZnO nanostructures (ZnO$_{nano}$ on metal/glass), and MZO nanostructures (MZO$_{nano}$ on metal/glass) modified sensing pads for 60 min under the same conditions, respectively. These three different sensing pads were then separately connected to the top gate of the same MZO DGTFT. The electrical measurements were performed for each of them. The bottom gate voltage was set at $V_{BG} = 2$ V, and the relative drain current reductions were obtained in comparison with the same type of pad without biofilm incubation. The same experiment was repeated three times for each type of the sensing materials. Shown in Figure 3-5(b) are the mean values of the drain current reduction and the standard deviation error bars of the DGTFT with each sensing pad, respectively. The bare sensing pad without nanostructure coating essentially failed bacterial measurements due to low sensitivity while both devices with the ZnO$_{nano}$ or MZO$_{nano}$ sensing pad are able to detect the drain current reduction; 19% and 66%, respectively.
Figure 3-5 (a) The optical absorption measurements were performed for both ZnO and MZO films on glass substrates, respectively. The absorption coefficient squares $\alpha^2$ are plotted against photon energy $h\nu$ for ZnO and MZO films. The bandgaps of ZnO and MZO films are determined to be 3.26 eV and 3.36 eV, respectively, which indicates the $y$ value in $\text{Mg}_y\text{Zn}_{1-y}\text{O}$ to be $\sim 0.04$ (4%). (b) $S.\ epidermidis$ biofilms were respectively incubated on bare sensing pads, ZnO$_{\text{nano}}$ modified pads, and MZO$_{\text{nano}}$ modified pads for 60 min. The same MZO DGTFT was used to conduct the experiments. Bottom gate voltage was set at 2 V. The relative variations of drain current reduction were obtained comparing with the same type of pad without bacteria incubation. [67]
From the comparison, it is clear that the modification of sensing surface with the ZnO and MZO nanostructures can achieve higher sensitivity; therefore, enabling the early stage detection of biofilm formation. The surface roughness impact on the biofilm formation can be explained by two factors. First, the bacteria prefer to start adhesion at somewhere sheltered from shear forces so that they have time to change from reversible to irreversible attachment [105]. Second, the effective area for adhesion is significantly increased due to the roughening of surface. In addition, the ZnO\textsubscript{nano} and MZO\textsubscript{nano} underwent UV light illumination and thus exhibited super-hydrophilicity [106]. The super-hydrophilicity enables less liquid sample consumption and higher sensitivity.

The above comparative studies also demonstrate that the sensing pad with the MZO\textsubscript{nano} modification offers much high sensitivity over the pure ZnO\textsubscript{nano} counterpart. ZnO would release Zn\textsuperscript{2+} ions in acidic environment, and that the Zn\textsuperscript{2+} ions are toxic to bacterial cells [57]. The Zn\textsuperscript{2+} ion formation proceeds as follows:

\[ \text{ZnO} + 2\text{H}^+ \rightarrow \text{Zn}^{2+} + \text{OH}^- + \text{H}^+ \rightarrow \text{Zn}^{2+} + \text{H}_2\text{O} \]  \hspace{1cm} (3.7)

where, the Zn-O bond in ZnO\textsubscript{nano} can be easily attacked by hydronium ions in acidic solutions. In our case, as the biofilms secreted by \textit{S. epidermidis} is acidic with a pH value between 4 - 5 [107], the chemical reaction holds. The use of pure ZnO\textsubscript{nano} sensing pad could decrease the sensitivity of the biosensor as the Zn\textsuperscript{2+} ions migrating into the growth medium and thus the cells are killed to some extent. By adding small composition of MgO (~4%) into pure ZnO to form the MZO, Zn\textsuperscript{2+} ions are significantly reduced due to the stronger bonding between Mg and the metal oxide surface compared to Zn. We observed similar phenomenon in [56]. In this work, MZO\textsubscript{nano} is chosen as the optimized sensing material.
over pure ZnO$_{nano}$ to reduce the toxicity to *S. epidermidis*; therefore, improve the device sensitivity.

3.6 Summary

A biosensor consisting of an MZO DGTFT and an extended MZO nanostructure modified sensing gate was developed for the early stage detection of *S. epidermidis* bacterial biofilm formation. The Mg$_{y}$Zn$_{1-y}$O ($y = 0.04$) nanostructures (MZO$_{nano}$) with properly controlled surface morphology and wettability provide the giant effective surface area and preferred conditions for bacteria adhesion, concomitantly reduce the biotoxicity to *S. epidermidis* bacteria by suppressing Zn$^{2+}$ ions in the culture medium. The extended sensing gate design isolated the bacterial incubation from the DGTFT device, and thus allowed the continuous and dynamic detection in aqueous environment. The dual gate design of the MZO DGTFT possess the biasing flexibility and stable operation, enabling the device to operate at the proper characteristic region. The bottom gate biasing voltage $V_{BG} = 2$ V was selected to achieve high sensitivity and stable operation. A drain current change of $\sim 80\%$ (with an average standard deviation of $\sim 10\%$) is achieved after $\sim 200$ minutes of *S. epidermidis* bacteria culturing, which signified the detection point for the onset of biofilm formation. In comparison, the crystal violet staining assay shows the matured biofilms would take approximately 24 h to form. This MZO DGTFT biosensor is promising in clinical applications as it could alert medical professionals to treat bacterial infections promptly and more effectively.
Chapter 4. Full Scale Monitoring of Biofilm Development

4.1 Background

*Pseudomonas aeruginosa* (*P. aeruginosa*) is a gram-negative opportunistic pathogen, which causes severe chronic nosocomial infections particularly to the immunocompromised patients. *P. aeruginosa* biofilms would form readily on the surface of implants and indwelling devices, such as urinary catheters and ventilator tubes, resulting in serious risks to patients [10]. It is even worse that such biofilm induced infections are often difficult to treat effectively and thus frequently life threatening. The case fatality rate for patients infected with *P. aeruginosa* is close to 50% [10], mainly due to two reasons: (ⅰ) the onset of its biofilm formation is difficult to detect with routine clinical methodologies, and (ⅱ) once the biofilms are established, the strong antimicrobial resistance (AMR) effect could lead to long-term high dose antibiotic treatments or even repeated surgical procedures to eradicate the biofilms.

As mentioned, the formation of biofilms generally takes places in three phases. Knowledge of the dynamic behavior and characteristics of the biofilms of a particular bacterial strain in all three phases is highly desirable for studying and the treatments of biofilm infections, as well as for developing the coating materials that prevent biofilm formation. In order to effectively battle the *P. aeruginosa* bacterial biofilms, a full-scale dynamic biosensing technology is required. First, the early stage (i.e., bacterial adhesion stage) detection of biofilm formation is critical, because the sooner the onset of biofilm formation is detected, the more effective the prevention of infection will be. Once passes the early stage, its growth kinetic profile should also be monitored during the long-term
evolution of biofilm formation because the development of biofilms can serve as a feedback signal for the subsequent medical treatments.

In this chapter, we demonstrate the dynamic monitoring of the full-scale process of *P. aeruginosa* biofilm formation using multifunctional sensing technology. The technology consists of an MZO DGTFT electrical sensor and a QCM acoustic sensor. Both devices use the same MZO\textsubscript{nano} as the sensing layer to enhance the sensitivity. The MZO DGTFT with an extended MZO\textsubscript{nano} modified gate detects the onset of biofilm development utilizing the electrical charge transfer mechanism, whereas the MZO\textsubscript{nano} modified QCM is a bulk acoustic wave device which monitors the kinetic growth profile of the subsequent developing stages through mass accumulation and viscosity transitions. In Chapter 3, it has been demonstrated that the DGTFT biosensor enables to detect the early stage formation of *Staphylococcus epidermidis* (*S. epidermidis*) biofilms. During the initial bacterial adhesion stage, the charge transfer phenomenon between bacterial cells and the MZO\textsubscript{nano} coated gate electrode signals the onset of biofilm formation. However, DGTFT is not capable of detecting the subsequent change of biofilm development beyond the early stage because the electrical charge transfer is just an interfacial phenomenon. To remedy this shortcoming, the MZO\textsubscript{nano} modified QCM biosensor is used in conjunction with the DGTFT biosensor. QCM is a bulk acoustic wave (BAW) device with high sensitivity to the mass loading and viscosity change. In 2010, Chen et al. monitored the long-term behaviors of the *Pseudomonas fluorescens* (*P. fluorescens*) biofilm development using QCM [78]. However, during the early stage of biofilm formation, the mass loading is too small to generate detectable output signal response. In contrast, the electrical charge transfer during the early stage (i.e., bacteria adhesion) is much more significant than that
of the mass loading. The unique features of DGTFT and QCM complements each other. The full-scale development of biofilm formation can thus be realized by combining these two technologies: DGTFT for the early-stage charge detection while QCM for the mass loading and viscosity changes for long-term monitoring. Furthermore, both devices use the same critical MZO\textsubscript{nano}. The MZO\textsubscript{nano} was grown on the sensing area of both DGTFT and QCM, serving as the biomolecules interface via surface modification to enhance sensitivity. Biofilms were incubated simultaneously on each the MZO\textsubscript{nano} of both sensing device with the same culture conditions. Through the signal processing, the measurement results are presented continuously as the function of time.

This multifunctional biosensing technology dynamically detects the details of the biofilm formation process, ranging from bacterial adhesion at the early stage to biofilm maturation at the final stage. In clinical applications, such hybrid technology would benefit the \textit{in-vitro} study of biofilm formation on implantable devices and help medical professions to promptly treat patients with effective therapies.

4.2 Design of the Hybrid Technology (MZO DGTFT + MZO\textsubscript{nano} modified QCM)

A schematic description of this hybrid biosensing system is presented in Figure 4-1. It consists of two biosensors: (i) the DGTFT for the early stage detection of biofilm formation, and (ii) the QCM for the long-term dynamic monitoring for the subsequent stages of biofilm development. The sensing surfaces of both devices were modified with the same MZO\textsubscript{nano} layer. Then, they were immersed in the bacterial incubator. The full-scale process of \textit{P. aeruginosa} biofilm development is thus be monitored continuously using this complementary biosensing technology.
Figure 4-1 The schematic of the hybrid and multifunctional biosensing system consisting of an MZO DGTFT with an extended MZO$_{nano}$ gate for the bacterial adhesion (early stage) detection and an MZO$_{nano}$ modified QCM for monitoring the subsequent stages. [108]
The MZO DGTFT biosensor has an MZO DGTFT as the signal transducer and an MZO\textsubscript{nano} modified sensing pad as the biological receptor. The fabrication process of the DGTFT biosensor has been described in Section 3.2.1.

4.2.1 Fabrication of the MZO\textsubscript{nano} modified QCM

The MZO\textsubscript{nano} modified QCM device consists of an MZO\textsubscript{nano} modification layer that is integrated with a commercial AT-cut QCM (International Crystal Manufacturing, Inc.) by growing them directly on the surface of the sensing electrode of the QCM using MOCVD. A schematic drawing of the top and cross-sectional multilayer view of the MZO\textsubscript{nano} modified QCM is shown in right side of Figure 4-1. The standard QCM’s quartz layer is sandwiched by two 100 nm gold electrodes. The sensing area of the QCM is 20.47 mm\textsuperscript{2}. The operation frequency of the standard QCM is 10 MHz, while the MZO\textsubscript{nano} modified QCM has an operating frequency of 9.912 MHz.

4.2.2 Modification of the QCM Sensing Surface with MZO\textsubscript{nano}

The MZO\textsubscript{nano} layers were deposited on the top electrodes of QCM and the extended pad of DGTFT to serve as the sensing surfaces to enhance their sensitivity. The MOCVD growth process of the nanostructures has been described in Section 3.2.2.

4.3 Biological Sample Preparation

4.3.1 Bacterial Biofilm Incubation

*P. aeruginosa* KD 4053 w PAO1 as inoculated into Mueller Hinton Broth (MHB; Fisher Scientific) and grown at 37°C for 16 - 18 h in test tubes placed in a shaking incubator operating at 200 rpm. The stationary phase cultures were diluted 100-fold into the fresh MHB medium and then pre-loaded in the Teflon cell culture well. The extended MZO\textsubscript{nano}
sensing pads of DGTFT and the MZO\textsubscript{nano} modified QCMs were sterilized and placed into the wells.

4.3.2 Crystal Violet Staining Assay

We used the crystal violet staining to verify biofilm formation on the MZO\textsubscript{nano} surfaces. MZO\textsubscript{nano} was deposited on glass substrates and then divided into two sets: one for control and one for testing. Both sets were prepared through the same procedure as outlined in Section 4.3.1, however, for the control set the MZO\textsubscript{nano} glass substrates were incubated with only MHB growth medium but without the bacteria. The separate petri dishes containing the control and testing sets containing multiple samples were placed in a static incubator at 37°C to induce biofilm formation. At various time points during incubation, a sample from the control and testing sets were retrieved for crystal violet staining. The time points of retrieval were 0, 1.7 h (100 min), 3.3 h (200 min), 5 h (300 min), 8 h, 15 h and 24 h, which represent the biofilm development at different times. After removal from the culture, the samples were washed three times with 5 mL of 0.9% NaCl solution to remove planktonic cells to assure that only the biofilms were attached to the sample surface. Biofilms attached on the sensing surface were then stained with 0.2% crystal violet for 10 min, followed by washing three times with 0.9% NaCl. The microscopic images were then taken for each time point.

4.4 Electrical Measurement and Signal Processing

4.4.1 Measurement and Parameters Extraction for the MZO DGTFT

In the first step of measurement, the basic electrical characteristics of the MZO DGTFT were examined. The transistor’s transconductance plots (i.e., drain current $I_{DS}$ vs
bottom gate biasing voltage $V_{BG}$) were recorded under various top gate biasing voltages $V_{TG}$. Next, the transfer characteristic variations as a result of the early stage detection of $P. aeruginosa$ biofilm formation are given. As the bacterial membrane electrons transferring to the MZO$_{nano}$, the equivalent micro biasing voltage is applied to the top gate of the DGTFT. Such top gate bias changes the conducting current $I_{DS}$ flowing through the channel layer through the electric field effect. By extracting the current variations under proper bottom gate biasing $V_{BG}$, the optimized signal output can be realized with the best combination of high sensitivity and stability. At last, the time-dependent signal response with standard deviation error bars is presented in combination with the QCM’s results to form the full-scale monitoring results.

The electrical testing setup of the DGTFT has been given in Section 3.4.

4.4.2 MZO$_{nano}$ Modified QCM Measurement and Data Analysis

The MZO$_{nano}$ modified QCM was used to monitor the long-term (24 h) development process of $P. aeruginosa$ biofilms. The MZO$_{nano}$ modified QCM was sterilized and deployed inside a Teflon cell-growth well and seeded with growth media with $P. aeruginosa$ cells. The cell was then placed in a standard bacterial incubator with controlled ambient environment. The characterization of MZO$_{nano}$ modified QCM was conducted using an HP-8573D network analyzer, which was connected via IEEE-488 general purpose interface bus (GPIB) to the universal serial bus (USB) of a microprocessor running of a LabView data acquisition program. The impedance transmission spectrum $Z_{21}(\omega)$ of the device was automatically measured at fixed time intervals and digitally stored while the $P. aeruginosa$ biofilms were developing on the MZO$_{nano}$ modified QCM inside the incubator. The sensor’s output signals were in the form of time-frequency set. These signals were then
analyzed by extracting the peak frequency shifts $\Delta f$ and variations of the motional resistance amplitude $R_{\text{Load}}$ relative to their starting frequencies and amplitudes resulted from the mass change and viscoelastic transitions on the sensing surface.

4.5 Result and Discussion

We start with the characterizations of the electrical performance of DGTFT, followed by the extraction of signals which represent the status of early stage biofilm formation. Next, the frequency shift and motional resistance of QCM is discussed. The full-scale dynamic signal response resulted from $P. \text{aeruginosa}$ biofilm formation is then demonstrated, where the early stage is detected by DGTFT biosensor and the long-term process is monitored by MZO nano modified QCM. For comparison, crystal violet staining is used to show the microscopic images of biofilm development at different incubation times.

4.5.1 Device Characterization for Biosensing Operations

**Electrical performance of the MZO DGTFT biosensor**

The transfer characteristics of the MZO DGTFT were firstly tested with its top gate electrode electrically connected to a DC power supply. We chose $V_{\text{TG}}$ from 0 to -1 V with a step of -0.2 V as the setting to demonstrate the highly sensitive signal response of the device as a result of the voltage alternation on its top gate electrode. Such $V_{\text{TG}}$ was chosen because the bacteria tend to donate only a small fraction of their membrane electrons to the supporting substratum [68], [69], and thus the equivalent top gate bias induced by bacterial adhesion should be negative and small.
The measured results are shown in Figure 4-2(a). The curve at $V_{\text{TG}} = 0$ V shows the threshold voltage of 7.47 V, and subthreshold slope of 637 mV/dec. The low threshold voltage ensures the device with low power consumption. The steep subthreshold slope enables high sensitivity owing to the high electrical signal gain of the device. To have a closer look at the variations of the I-V curves due to different top gate biases, the inset figure in Figure 4-2 (a) shows the detailed characteristics in the range of $-3 \text{ V} < V_{\text{BG}} < 2 \text{ V}$ where the device just about to turn on. It can be clearly seen that these transfer curves exhibit parallel right shifts with respective to the increasing values of $V_{\text{TG}}$. The threshold voltage $V_{\text{TH}}$ positively shifts ~10% from 7.47 V at $V_{\text{TG}} = 0$ V to 8.25 V at $V_{\text{TG}} = -1$ V. Such right shifting can be explained as follows:

MZO is a kind of n-type semiconductor material. The bottom gate bias introduces a vertical electrical field which accumulates electrons at the bottom channel/dielectric interface. However, the negative top gate voltage would partially deplete the accumulation channel. To compensate the depletion and turn on the device, the threshold voltage must be adjusted by an equivalent positive shift.

The right shifting of threshold voltage also lowers the drain current under a certain bottom gate biasing condition, especially in the triode region of the transfer characteristics. For example, the drain current at $V_{\text{BG}} = 0$ V decreases nearly one decade from $1.37 \times 10^{-9}$ A at $V_{\text{TG}} = 0$ V to $1.41 \times 10^{-10}$ A at $V_{\text{TG}} = -1$ V (shown in the inset of Figure 4-2(a)). The steep slope in the triode region of the transfer characteristics results in large current variations within a small range of bottom gate biases, and hence provides high current amplification when the top gate bias varies. Comparing to the threshold voltage change, the drain current change of DGTFT is particularly favorable for using as the signal to
represent the early stage development of biofilm formation and is defined as the sensitivity of the biosensor.

The high electrical sensitivity of the device enables the DGTFT to detect the early stage of biofilm formation. Next, the MZO DGTFT biosensor was used to detect the onset of \( P. \ aeruginosa \) biofilm formation with its top gate electrode electrically connected to the sensing pad where bacterial incubation occurs. Three MZO_{nano} modified sensing pads were prepared. The detection of \( P. \ aeruginosa \) biofilms was performed on each of these pads using the same DGTFT device under the same microbial culture and measurement conditions. The sensing pad was immersed in MHB medium and let the signal stabilize to baseline before the \( P. \ aeruginosa \) culture solution was introduced at time \( t = 0 \). The measurements of transfer characteristics were made sequentially for a total of 900 min of incubation time.

In obtaining the signal that represents the status of biofilm development, we’ve talked about the balancing between sensitivity and stable operations when choosing the best point of operation in Section 3.5.3.5.1. Using similar method, \( V_{BG} = 5 \text{ V} \) is determined as the optimum operation point in this study. For better visualization, part of the transfer characteristics \((2.5 \text{ V} < V_{BG} < 7.5 \text{ V})\) of a single set of measurements are shown in Figure 4-2(b). It can be clearly seen that the drain current keeps dropping until \( t = 390 \text{ min} \) where it shows no significant variation comparing with the one at \( t = 900 \text{ min} \). DGTFT is a highly sensitive electrical device. Drain current drops as a result of the negative bias applied on its top gate electrode. In the biosensing experiment, the current change is attributed to the electron charge transfer resulted from the bacterial adhesion to the MZO_{nano} surface during the early stage development of the biofilm formation [68], [69].
Figure 4-2 (a) The electrical transfer characteristics of MZO DGTFT were tested with the top gate electrode connected to various top gate biases. The inset shows the detailed characteristics with $V_{BG}$ ranging from -3 V to 2 V. The I-V curves keep right shifting with the increasing values of negative top gate bias owing to the electrostatic field-effect. (b) The electrical signal response of the MZO DGTFT biosensor ($2.5 \, V < V_{BG} < 7.5 \, V$) with its top gate electrically connected to the sensing pad where bacterial incubation occurred. Drain current keeps decreasing as the incubation time increasing until $t = 390 \, \text{min}$. [108]
However, the testing results demonstrate no significant signal variation after $t = 390$ min, and hence limiting the long-term monitoring capability of the device. The biofilm development process beyond the early stage is essentially difficult to detect using this MZO DGTFT biosensor. We next employ the MZO$_{\text{nano}}$ modified QCM for monitoring the long-term biological evolutions of biofilm formation and complement the results of the biofilm monitoring.

**Significance of frequency shift and motional resistance of QCM**

MZO$_{\text{nano}}$ modified QCM was used to monitor the long-term process of *P. aeruginosa* biofilm development. The impedance transmission spectrum $Z_{21}(\omega)$ of the device was automatically measured at various time points for a fixed time interval. The impedance spectrum of the MZO$_{\text{nano}}$ modified QCM can report the biophysical properties of the bacterial culture as a function of time. The spectrum features two important parameters, namely the peak frequency shift ($\Delta f$) and the motional resistance ($R_{\text{load}}$). When mass accumulates on the sensing surface of the MZO$_{\text{nano}}$ modified QCM, the resonant frequency of the device shifts proportionately to the amount of mass change. The frequency shift parameter $\Delta f$ of the MZO$_{\text{nano}}$ modified QCM is determined by measuring the absolute value of the difference in resonant frequency between the case without and with mass loading. It can be calculated with the following relation [109]:

$$\Delta f = \frac{2f_0^2}{v_q \rho_q A} \Delta m$$  \hspace{1cm} (4.1)

where $\Delta f = f_0 - f(t)$ is the frequency shift and $f_0$ is the intrinsic operating frequency of the device, $v_q$ and $\rho_q$ are the acoustic velocity and mass density of the AT-cut quartz layer, $A$
is the sensing area of the top electrode, and \( \Delta m \) is the mass change, i.e., the accumulated mass on the sensing electrode.

The second parameter extracted from the impedance transmission spectrum is the motional resistance \( (R_{\text{load}}) \). This parameter is obtained from the real part of the complex amplitude in the impedance transmission spectrum, i.e., \( R_{\text{load}} = \text{Re}\{Z_{21}(\omega)\} \). With the development of biofilm on the MZO\(_{\text{nano}}\) modified QCM sensing surface, the biofilm undergoes viscoelastic transitions due to the attachment and metabolism activities of bacterial cells and the formation of the EPS. These viscoelastic transitions manifest themselves as modulations in the amplitude of the impedance transmission spectrum, specifically on \( R_{\text{load}} \). According to [110] and discussed in biological context by [59], there is a direct relationship between the \( R_{\text{load}} \) (which is an electrical quantity) and the load impedance (a mechanical quantity) describing the viscoelastic property of the accumulated mass on the sensing surface:

\[
R_{\text{Load}} = \frac{\pi}{4K^2\omega_0C_0Z_{BAW}}\text{Re}\{Z_{\text{mechL}}\} \tag{4.2}
\]

where \( K^2 \) is the coupling coefficient of the piezoelectric layer, \( \omega_0 \) is the resonant angular frequency of the QCM with no mass loading, \( C_0 \) is the capacitance of the device, \( Z_{BAW} \) is the impedance of the device without the mass accumulation, and \( Z_{\text{mechL}} \) is the mechanical load impedance due to the biofilm. The value of \( R_{\text{load}} \) therefore reflects the mechanical property of the biofilm formation on the sensing surface, more specifically the piezoelectric energy dissipation due to the viscoelastic transitions of the biofilm. Moreover, to remove the effect of the growth medium in the value of \( R_{\text{load}} \), we separately setup the sensor with growth medium only (as a control) and incubated it in the same conditions as test setup and monitored the signal for 24 h. The signal due to the MHB growth medium served as the
background signal and was subtracted from the test measurements so that the measured values correspond directly to the biofilm.

4.5.2 Full-scale Dynamic Monitoring of Biofilm Development

In this section, we present the full-scale dynamic monitoring results of the biofilm development and formation. The early stage detection capability of the MZO DGTFT biosensor has been discussed in Chapter 3. Owing to the high electrical signal gain provided by the active device, its drain current variation as a result of bacterial charge transfer was utilized as the output signal to realize the early stage detection of biofilm formation. However, our results show the signal saturates after a certain period. On the other hand, MZO$_{nano}$ modified QCM is capable of precisely measuring mass accumulation and viscoelastic transitions on its surface, and thus provides high sensitivity of monitoring the subsequent stages of biofilm evolution.

To obtain the full-scale dynamic profile of biofilm evolution, both MZO DGTFT and MZO$_{nano}$ modified QCM biosensors were used to monitor the progress of biofilm development under the same microbial conditions.

The time-evolving signal response of the MZO DGTFT and the MZO$_{nano}$ modified QCM are plotted in Figure 4-3. Percentage change of drain current is used as the signal of the DGTFT biosensor to represent the early stage development of biofilms. Using similar method as described in Section 3.5.1, $V_{BG} = 5$ V is determined as the optimum operation point here. No lag phase is exhibited by the bacterial culture, which can be attributed to having the bacterial culture introduced already in a metabolically active state and does not require time to enter cell division [76]. The percentage change of drain current keeps
increasing as incubation time goes from $t = 0$, after which the currents level off at about $t = 6.5$ h (390 min) which signifies the detection point for the onset of biofilm formation. Although the biofilms were still growing and far from maturation when $t = 6.5$ h, as will be shown in the microscopic images, the charge transfer effect between bacterial cell surface and the substratum surface was being gradually prohibited and diminished. The charge transfer only takes place at the interface between bacterial cells and the substrate because the biofilms of *P. aeruginosa* are detected to be poorly conductive [111], [112]. After $t = 6.5$ h, the slope of the current change is approaching zero and the signal eventually becomes independent of time. The signal of the DGTFT here demonstrates a maximum current change of 57% with an average standard deviation of 5.1%. Such early stage detection could alert medical professionals to treat the biofilms in a timely manner.

The MZO$_{nano}$ modified QCM’s resonance frequency shift $\Delta f$ and the motional resistance $R_{load}$ are also plotted in Figure 4-3 with the same time axis as the DGTFT’s results. The average standard deviations of $\Delta f$ and $R_{load}$ are 2.47 KHz and 11.68 $\Omega$, respectively (they are too small to be shown in the figure scale). As shown in the figure, the QCM barely detects the early stage, especially before $t = 5$ h. The small mass and viscoelastic transition the sensor can detect limit its sensitivity for the early stage biofilm monitoring. The MZO DGTFT biosensor, however, already sees 52% drain current reduction at $t = 5$ h when $V_{BG} = 5$ V. Large increment in the QCM signal values starts to be observed after $t = 5$ h. The $R_{load}$ plot shows a rapid growth phase from $t = 5$ h to 15 h, but the signal stops increasing and saturates at about 15 h with a maximum change of $\Delta R_{load} = 560$ $\Omega$. The motional resistance saturation indicates the viscosity of the biofilms reaches its peak value even the biofilms were still increasing in mass as shown in the frequency
shift data. The frequency shift $\Delta f$ exhibits a steady increase all the way towards the end of the experiment ($t = 24$ h) and reaches 160 KHz, meaning the biofilms were still evolving and keep gaining weight during the course of development.

The MZO DGTFT biosensor shows a significant signal variation (52%) in the first 5-hour of biofilm development, but the signal diminishes after the certain period. The $\text{MZO}_{\text{nano}}$ modified QCM has difficulties detecting the initial formation of biofilms but shows the ability of monitoring the long-term process of biofilm development which represented by two important characteristics of $\text{MZO}_{\text{nano}}$ modified QCM; frequency shift and motional resistance. The change of both parameters demonstrates the development of biofilms, whereas the saturation of motional resistance corresponds the final biofilm maturation stage as will be shown in the next section.
Figure 4-3 The signal variations during the full-scale development of \( P.\ aeruginosa \) biofilm formation, represented by the percentage change of drain current of DGTFT, motional resistance and frequency shift of \( \text{MZ}_\text{nano} \) modified QCM. [108]
4.5.3 Microscopy Characterization of Crystal Violet Stained Biofilms

Crystal violet staining assay was used to verify the formation of *P. aeruginosa* biofilms and show the process at different times. The MZO$_{\text{nano}}$ modified glass substrates were used as the support substrates for biofilm incubation. The same growth conditions were applied as used for sensing. The optical microscopic images of the biofilm formation process on the MZO$_{\text{nano}}$ coated glass substrate were taken at times of $t = 0, 1.7$ h (100 min), 3.3 h (200 min), 5 h, 8 h, 15 h, and 24 h as shown in Figure 4-4.

From the microscopic images in Figure 4-4, we can hardly distinguish if there is bacterial adhesion on the sensing pad when $t = 1.7$ h. However, the result of the MZO DGTFT biosensor does show the obvious current reduction of 21% at $t = 1.7$ h, indicating bacterial adhesion. Afterwards, it can be clearly seen that more bacterial microcolonies were formed at the sites of adhesion from $t = 3.3$ to 5 h and the biofilms began to take shape at $t = 8$ h. However, $t = 8$ h is the time point where the DGTFT already shows a signal saturation value of 57%. With the increasing of incubation time, the microcolonies shown at $t = 8$ h kept developing and showed darker color when time reached $t = 15$ h. During this time period, DGTFT biosensor does not show signal variation whereas the MZO$_{\text{nano}}$ modified QCM does show obvious change in both the frequency shift and motional resistance. At $t = 15$ h, a set of size and shape of biofilms can be seen now, and biofilms at this stage are referred to as being “mature” [66]. This is also the point where the motional resistance signal of MZO$_{\text{nano}}$ modified QCM reaches plateau. Finally, the biofilms covered the majority of the surface (~ 64%) when $t = 24$ h.
Figure 4-4 Optical microscopic images of crystal violet stained *P. aeruginosa* biofilm formation on MZO$_{nano}$ modified sensing pad at different times. [108]
4.6 Summary

We have demonstrated a multifunctional sensor technology for the full-scale detection of *P. aeruginosa* biofilm formation, ranging from bacterial adhesion to biofilm maturation. The detection system consists of an MZO DGTFT electrical sensor and an MZO\textsubscript{nano} modified QCM acoustic wave sensor. Both devices use the same MZO nanostructures as the sensing surface to achieve high sensitivity and biocompatibility. The two devices function in the complementary mode. The MZO DGTFT with the extended MZO\textsubscript{nano} gate pad detects the early phase biofilm formation through the charge transfer mechanism. Drain current starts varying at the beginning of the test and its percentage change reaches 52% at $t = 5$ h. On the other hand, the MZO\textsubscript{nano} modified QCM enables to monitor the long-term (for 24 h) development of biofilm beyond the early stage to the mature stage. It gives the mass accumulation induced frequency shift up to 160 KHz and the viscoelastic transitions induced motional resistance change of 560 $\Omega$. Combining both the DGTFT and QCM sensing, the full-scale and dynamic monitoring of the biofilm formation process is demonstrated; early stage (0 – 5 h) by current change of DGTFT, through growth stage (5 – 15 h) by motional resistance and frequency shift of QCM, to the biofilm maturation (15 – 24 h) by frequency shift of QCM.
Chapter 5. Sensitive Determination of Modified Folic Acid

5.1 Background

In this chapter, we use the MZO DGTFT biosensor for the sensitive determination of modified folic acid (FA). Hexadecyl alkynated folic acid (HAFA) can be directly bound with FR in the same way as FA as HAFA contains COOH groups, but the conversion of FA into HAFA has several advantages: 1) improves solubility in organic solvents by introducing the alkyl chain, 2) enables the click reaction by introducing the alkyne group, and 3) prevents competitive displacement of the bound linker layer by protecting the carboxylic group as an amide. Stepwise functionalization technology was used to attach the HAFA on the MZO$_{nano}$ surface through the introduction of a linker layer. The MZO$_{nano}$-modified sensing pad was connected to the top gate of the DGTFT, and the entire immobilization process was monitored by the biosensor. 11-Azidoundecanoic acid (AA) was firstly bound onto the MZO$_{nano}$ films through the carboxylic acid as a reactive linker layer, leaving the surface decorated with click-ready azido groups available for further reactivity. Net negative charges were donated to the MZO$_{nano}$ due to carboxylate binding, and such impact suppressed the electron carriers within the MZO channel of DGTFT. In the second step, HAFA was immobilized on the AA via copper Cu(I) catalyzed azide-alkyne click reaction (CuAAC). The click reaction, occurring on the top sensing gate, compensated a portion of the negative charges and again electrostatically modified the charge carrier distribution in the MZO channel accumulated by the bottom biasing gate. The entire immobilization process was thus monitored by measuring the signal variations of the DGTFT device. This stepwise functionalization process was also monitored by
Fourier-transform infrared spectroscopy (FTIR) microscopic imaging, which is proven to be an efficient method to study surface chemistry with high spatial resolution [99].

5.2 Chemical Synthesis

Synthesis of 11-azidoundecanoic acid (AA): Sodium azide was added to a 11-bromoundecanoic acid in DMF solution. The reaction mixture was kept at 80°C for 3 h, then diluted with CH₂Cl₂, and washed by diluted aqueous HCl solution. Finally, the organic layer was dried over Na₂SO₄ and evaporated in vacuum.

Synthesis of alkynated folic acid (AFA): Folic acid was dissolved in DMF. N-hydrosuccinimide and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were then added, and the mixture was stirred while cold for 30 min. Propargylamine was dissolved in DMF and the solution was added to the folic acid solution. The mixture was then warmed to room temperature. Next, the reaction mixture was poured into water. The crude product mixture was then filtered, washed, and finally dried in vacuum to yield AFA.

Synthesis of hexadecyl alkynated folic acid (HAFA): AFA was dissolved in DMF. N-hydrosuccinimide and EDC were then added, and the mixture was stirred while cold for 30 min. 1-Hexadecylamine was dissolved in DMF and the solution was added to the AFA solution. The mixture was warmed to room temperature and stirred for 24 h. The reaction mixture was poured into water and stirred for 30 min. The crude product mixture was then filtered, washed, and dried.

The binding of AA and immobilization of HAFA on MZO_{nano} was processed as follows. Scheme of the functionalization process is depicted in Figure 5-1.
Binding of AA onto the $\text{MZO}_{\text{nano}}$ sensing pads. A small volume of a 10 mM solution of AA in 3-methoxypropionitrile (3-MPN) was dropped onto the sensing pads and let it react for 22 h. $\text{MZO}_{\text{nano}}$ on r-sapphire substrates were also used for FTIR imaging purpose. Then, the samples were thoroughly rinsed with neat solvent to remove weakly bound molecules and were dried under nitrogen flow.

Immobilization of HAFA onto the $\text{MZO}_{\text{nano}}$ sensing pads with AA. $\text{CuSO}_4$-tris[(1-benzyl-1H-1,2,3-triazol-4-yl) methyl] amine (TBTA) and ris(carboxyethyl)phosphine were added to a 10 mM HAFA solution in DMSO. The azido-functionalized samples were immersed suspended face-down in this solution, which was continuously shaken for different reaction time periods (2, 4, and 6 h). The samples were then thoroughly rinsed by neat solvent and dried under nitrogen flow. The structures in red (Figure 5-1) mark the functional groups that engaged in the click reaction process. The 3D structure of HAFA/AA bound on the $\text{MZO}_{\text{nano}}$ surface is shown in the inset figure of Figure 5-1, with a dipole moment pointing from the positively charged folate pteroate moiety to the negatively charged glutamate moiety.

The synthesis process was optimized to minimize potential contaminations, such as to have the sample face-down during the immobilization process. After the two-step reactions, energy-dispersive X-ray spectroscopy (EDS) analysis was carried out for investigating possible residues absorbed on the $\text{MZO}_{\text{nano}}$ surface. The results showed a small amount of Cu residues on the $\text{MZO}_{\text{nano}}$ surface with an atomic percentage of 0.9%. Figure 5-1 shows a SEM image of the nanostructured surface of $\text{MZO}_{\text{nano}}$. The existence of the Cu residues may be due to the over-reducing of Cu(II) to copper metal that was trapped between the MZO nanostructures. However, such copper metal does not impact the electrical sensing
results because they neither initiate charge transfer nor carry electrical dipole that could cause the shift of DGTFT's electrical characteristics.
Figure 5-1 Scheme of the functionalization of the MZO$_{nano}$ films. The structures in red mark the functional groups engaged in the click reaction. The SEM image shows the surface morphology of the MZO$_{nano}$ films. The inset figure shows the 3D structure of HAFAA/AA bound on the MZO$_{nano}$ surface. The calculations show the combined HAFAA/AA molecule has a dipole moment pointing from the positively charged folate pteroate moiety to the negatively charged glutamate moiety. [113]
5.3 Electrical Dipole Simulations

Density functional theory calculations were performed to investigate the dipole properties of HAFA and HAFA/AA using the GAMESS(US) software package [114] using Becke3-Lee- Yang-Par (B3LYP) three parameter DFT theory [115], [116]. Geometries of local minima on the potential energy surface were calculated with a 6-31G basis set [117].

5.4 Results and Discussions

5.4.1 Monitoring of the HAFA Immobilization on MZO_{nano}

We demonstrate the detection of the stepwise functionalization of HAFA on MZO_{nano} surface using the MZO DGTFT biosensor. The electrical characteristics of the DGTFT with unfunctionalized MZO_{nano} sensing pad was firstly examined. As shown in the Step 0 of Figure 5-2, the transfer curve shows that the bare DGTFT biosensor is a normally off enhancement mode device. Its basic electrical parameters include threshold voltage $V_{th}$ of 4.89 V, subthreshold swing S.S. of 439 mV/dec, and on-off current ratio of $\sim 10^9$.

In Step 1, the MZO_{nano} sensing surface was modified with the bifunctional linker layer, i.e., AA. The carboxylic acid group of AA enables it to be anchored to the MZO_{nano} surface, and the azido group at the other end enables the upcoming click reaction with alkyne moiety of HAFA in the next step. As shown in the Step 1 of Figure 5-2, the transfer characteristics show a threshold voltage of 1.87 V and an on-current of $2.8\times10^{-6}$ A. The curve shifts in the positive direction with a lowered drain current comparing to Step 0.

As Step 2, the azido functionalized MZO_{nano} was then reacted with the HAFA via CuAAC for 6 h. The electrical characteristics now exhibit a threshold voltage of 3.15 V with an on-current of $1.3\times10^{-5}$ A. Comparing to Step 1, the threshold voltage negatively
shifts 1.28 V, and the drain current is significantly increased. For comparison, we list the values of threshold voltage and on-current of each processing step in Table 5-1.
Figure 5-2 The transfer characteristics of the MZO DGTFT biosensor with different processing steps. Step 0: bare MZO_{nano} sensing pad, Step 1: AA linker binding on MZO_{nano}, and Step 2: HAFA immobilization on AA/MZO_{nano}. [113]

Table 5-1 The values of threshold voltage and on-current for the three different processing steps. [113]

<table>
<thead>
<tr>
<th>Processing Step</th>
<th>Threshold Voltage</th>
<th>On-current</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: MZO_{nano}</td>
<td>4.89 V</td>
<td>7.6×10^{-5} A</td>
</tr>
<tr>
<td>1: AA/MZO_{nano}</td>
<td>1.87 V</td>
<td>2.8×10^{-6} A</td>
</tr>
<tr>
<td>2: HAFA/AA/MZO_{nano}</td>
<td>3.15 V</td>
<td>1.3×10^{-5} A</td>
</tr>
</tbody>
</table>
The mechanism of the threshold voltage and drain current variations under different processing steps is presented in Figure 5-3. At Step 0, the positive bottom biasing gate voltage induced an electrical field which causes the electrons of the n-type MZO channel to accumulate near the channel/bottom dielectric interface. Such accumulation layer facilitated a conduction path for the electrons flowing from source to drain. However, when AA was immobilized on the MZO\textsubscript{nano} sensing surface in Step 1, electrons were transferred from the molecules to the MZO\textsubscript{nano} surface due to the carboxylic binding chemistry. It has been found there are two pathways of charge transfer in the carboxylic binding chemistry; one is the O atoms of COOH gain electrons from the sensing surface by Zn-O bonding, and the other is the H atoms of COOH donate electrons to the sensing surface to generate O-H groups. The net effect of these two pathways is that the oxide surface gains electrons from the carboxyl group [118], [119]. Such transferred electrons cause a negative micro-bias on the top sensing gate and a relatively small electrical field with the same direction is formed within the top dielectric layer. Therefore, a certain portion of the electrons in the MZO channel layer are depleted. The device’s drain current is reduced to a certain extent, and a more positive bottom gate bias is required to turn on the device. To elucidate the role of the HAFA molecule in Step 2, density functional theory calculations have been employed on both the isolated HAFA molecule and combined AA/HAFA product formed via the click reaction. As shown in the inset of Figure 5-1, the calculations demonstrate that both HAFA (magnitude of dipole moment: 7.9 D) and AA/HAFA are polar molecules with dipole moment directed from the positively charged folate pteroate moiety to the negatively charged glutamate moiety. Since the positive end of the HAFA is directed towards the AA/MZO surface, the negative micro-bias induced by AA is partially
compensated by HAFA, and more carriers are accumulated in the channel layer. The drain current was boosted almost an order, and less bottom gate voltage is needed to turn on the device.
Figure 5-3 (a-c) The schematic of the carrier modulation mechanism from Steps 0 to 2, respectively. $E_t$ and $E_b$ denote the electrical fields in the top and bottom dielectric layers, respectively. [113]
Both the threshold voltage change and the drain current variation could be used as the signal to represent the immobilization process. However, it can be seen from Table 5-1 that the on current varies much more significantly in comparison with the threshold voltage change. The on current indicates the drain current of DGTFT in the saturation region of the transfer characteristics, but this contrast is even more obvious in the triode region which is selected as the operation region for sensing.

The current variation behaves differently with respect to different bottom biasing voltages. To find out the point with highest sensitivity, we repeated the experiments of Step 1 and 2 three times. The transconductance curve of AA/MZO_{nano} (Step 1) is used as the baseline to characterize the signal variations caused by the introduction of HAFA (Step 2). The device’s sensitivity is represented by the decibel change of drain current \( \Delta I_D \) (dB), as described by Eq. (5.1).

\[
\Delta I_D (dB) = 20 \log \left[ \frac{I_D (Step \, 2)}{I_D (Step \, 1)} \right]
\]  

where \( I_D (Step1) \) and \( I_D (Step2) \) indicate the drain currents under certain bottom biasing voltage of Step 1 and 2, respectively. Decibel change is used because the current varies in such a wide range that calculating the commonly used percentage change is not suitable. It is plotted as a function of bottom biasing voltage as shown in Figure 5-4. It can be seen that the maximum current variation is achieved at \( V_{BG} = -1.7 \) V with a value of 68.8 dB. The standard deviation at this point is 6.7 dB. \( V_{BG} = -1.7 \) V is also the point where the transconductance curve of Step 1 about to turning on (Figure 5-2), which facilitates the maximum current change. Then, the decibel changes of drain current gradually decrease as leaving the triode region. The DGTFT exhibits steep slope in the triode region of its transfer
characteristics, and such slope provides high sensitivity for sensing applications. $V_{BG} = -1.7 \, \text{V}$ is chosen as the optimized bottom biasing voltage with highest sensitivity.
Figure 5-4 The decibel changes of drain current between Step 1 and 2 as a function of bottom biasing voltage. [113]
Next, FTIR microscopic imaging was used to characterize the binding process on the MZOnano surface, as shown in Figure 5-5(a). Free acid AA has a broad OH stretching band observed in the 3300 – 2500 cm⁻¹ region and a carbonyl stretching band at 1706 cm⁻¹. However, after binding onto the MZOnano surface, both features disappeared, and new bands of carboxylate moiety emerged near 1540 cm⁻¹ and 1480 cm⁻¹. Also, the azido stretching band of AA shifts from 2096 cm⁻¹ to 2130 cm⁻¹ after binding onto the MZOnano surface. Those observations confirm the successful binding of AA on the MZOnano. During the immobilization of HAFA through azido-alkyne click reaction for 2 h (Step 2), new N-H stretching band appeared at 3292 cm⁻¹, which indicates the chemical reaction with HAFA. The FTIR images displayed in Figure 5-5(b) show the spatial distribution of the integrated areas of the azido band and N-H stretching band during the stepwise functionalization. The FTIR image of azido band in Step 1 demonstrates a fully covered MZOnano film with AA. In Step 2, azido reacted with alkyne group of HAFA and its signal decreased, but instead the N-H stretching band raised indicating the successful bonding of HAFA on the AA/MZOnano surface.
Figure 5-5 (a) FTIR-ATR spectrum of neat AA (solid, black line), neat Hafa (short dot, red line) and representative single pixel FTIR spectrum of AA/MZO\textsubscript{nano} (dash dot, orange line) and Hafa/AA/MZO\textsubscript{nano} (dash dot, blue line). (b) FTIR images of the integrated band area of the azido region 2212 - 2064 cm\textsuperscript{-1} (±STD), and the N-H stretching region 3460 - 2988 cm\textsuperscript{-1} (±STD) of MZO\textsubscript{nano} film under each step. [113]
5.4.2 Time Effect of Click Reaction

To investigate the progress of the click reaction, we immersed the azido functionalized MZO\textsubscript{nano} pads faced down in the click solution and let shaking for 2, 4, and 6 h, respectively, then rinsed and dried. After the reaction, we sequentially connected these sensing pads to the top gate of the same MZO DGTFT while using the pad with AA functionalization only as the control. The bottom biasing voltage was set at -1.7 V because it shows the highest sensitivity as we discussed above.

The decibel changes of drain current were plotted against the duration of HAFA click reaction, as shown in Figure 5-6(a). The current change at $t = 2$ h shows a dramatic increase comparing to the one at $t = 0$. Then, the current change reaches saturation at $t = 4$ h and keeps no significant change at $t = 6$ h.

The reaction progress was also monitored by FTIR microscopic imaging. Figure 5-6 illustrates the reacting of AA/MZO\textsubscript{nano} film with HAFA imaged at different reaction times. The FTIR image shows that no azido band was detected after 4 h, and therefore, the click reaction was completed between 2 h and 4 h. Such observations are consistent with the sensor’s results.
Figure 5-6 (a) The decibel changes of drain current in terms of different times of HABA click reaction under $V_{BG} = -1.7$ V. (b) FTIR images of the integrated band area of the azido region $2212 - 2064 \text{ cm}^{-1}$ ($\pm \text{STD}$) of the MZO$_{\text{nano}}$ film with each step. Three different reaction times of Step 2 were examined, respectively. [113]
3.2 Summary

We have demonstrated a DGTFT-based biosensing platform for the sensitive determination of HAFA on the sensor’s extended MZO$_{\text{nano}}$ sensing surface. HAFA was covalently bond on the MZO$_{\text{nano}}$ surface through linkage chemistry. The mechanism of electrical field alteration caused by the stepwise functionalization process was discussed. The biosensor exhibited its maximum drain current variation of 68.8 dB caused by HAFA at $V_{BD} = -1.7$ V. The successful binding of linker and HAFA was confirmed by FTIR spectra and FTIR imaging. The progress of the click reaction was also monitored by DGTFT and FTIR. The realization of the MZO DGTFT immobilized with HAFA has potential applications in cancer diagnosis and treatment.
Chapter 6. Conclusion and Suggestions for Future Work

6.1 Conclusion

Every year, substantial number of device-associated infections happens due to the bacterial adhesion and the subsequent biofilm formation. Matured biofilms show 500 to 5000 times more tolerance to antibiotics than the free-floating bacteria of the same kind. However, the sooner an infection is detected, the more effective treatment will be. In addition, methods that acquire data in the long run also provide insightful information about the dynamic kinetic properties of the biofilm formation process and hence allow medical professionals to study the antimicrobial resistance effect of biofilms at different stages.

In this dissertation, we have demonstrated the design of the novel MZO dual gate thin film transistor (DGTFT) with MZO\textsubscript{nano} modified extended sensing gate and its biosensing applications of early stage detection and full-scale monitoring of bacterial biofilm development. The MZO DGTFT has also been implemented for sensitive determination of modified folic acid.

The MZO DGTFT possesses two gates: the bottom gate is used to adjust the operation point of the device whereas the top gate is electrically connected to the extended sensing pad. Biological receptor sends electrical signals through the top gate while the best combination of sensitivity and stable operation is achieved by adjusting the bottom gate bias. The extended sensing pad design isolates the TFT device from the harsh biochemical environment and different sensing pads can be connected to the device sequentially according to the detection tasks. Moreover, the separated sensing pad is disposable, and therefore, increasing the testing accuracy and reduces the cost of testing.
The MZO DGTFT enables the early stage detection of *S. epidermidis* biofilm formation. A drain current change of ~80% was achieved after ~200 minutes of bacteria culturing, which signified the detection point for the onset of biofilm formation. In comparison, the crystal violet staining assay showed the matured biofilms would take approximately 24 h to form. We also compared ZnO\textsubscript{nano} vs MZO\textsubscript{nano} as the sensing material. It shows that the Mg doping in MZO\textsubscript{nano} can enhance the pH tolerance of the material and hence suppresses the release of Zn\textsuperscript{2+} into the culturing medium which reduces the extent of toxicity to the bacterial cells, and eventually the sensitivity of the device increases significantly.

We demonstrated the full-scale dynamic monitoring of the *P. aeruginosa* biofilm formation ranging from bacterial adhesion to biofilm maturation: the MZO DGTFT as an electrical sensor for the early stage detection and the QCM sensor as an acoustic sensor for the long-term monitoring. The sensing surfaces of both devices uses the same MZO\textsubscript{nano} to achieve high sensitivity and biocompatibility. The drain current of DGTFT started to change at the beginning of the test and leveled off at time $t = 6.5$ h of bacterial incubation, whereas the signals of the MZO\textsubscript{nano} modified QCM became detectable after ~5 h and then lasted for 24 h. It gave the mass accumulation-induced frequency shift up to 160 KHz and the viscoelastic transition-induced motional resistance change of 560 $\Omega$. The full-scale process of biofilm development covering from bacterial adhesion to maturation is thus monitored using this MZO\textsubscript{nano} modified multifunctional sensing technology.

The MZO DCTFT has also been explored for the sensitive determination of modified FA, HAFA, using the MZO DGTFT biosensor. HAFA was covalently bond on the MZO\textsubscript{nano} sensing surface through stepwise functionalization technique. The electrical field
alternations on the DGTFT are caused by charge transfer during the linker binding step and electrical dipole induced by HAFA after immobilization. The biosensor exhibited a drain current variation of 68.8 dB caused by HAFA. FTIR were used to confirm the successful binding of linker and immobilization of HAFA. The realization of the MZO DGTFT functionalized with HAFA has potential applications in cancer diagnosis and treatment.

Comparing to the convention design of the single-gate TFT sensors, the MZO DGTFT with extended sensing pad design enables the modification of the sensing surface, optimization of the operation point, and the capability of sensing in aqueous environment. Overall, this novel MZO DGTFT technology may lead to a broad impact on a wide variety of potential applications where electrochemical interaction occur and in vitro detection are required.

6.2 Suggestions for Future Work

The following works are suggested for future study to develop the DGTFT biosensor with higher sensitivity, more robust system integration, and further sensing applications.

Optimization of layer thickness of DGTFT for sensing applications

The DGTFT device structure can be further optimized to enhance the sensitivity of the biosensor. As described in Eq. (3.2), the threshold voltage tunnability of the device depends on the capacitances of the bottom dielectric layer, the channel layer, and the top dielectric layer. With material and channel W/L unchanged, it is more preferrable to have thicker bottom SiO$_2$ layer, thinner MZO channel layer, and thinner top SiO$_2$ layer. Detailed investigation with simulations should be conducted for exploring the optimized thickness of each layer. Talking about the bottom SiO$_2$, though thicker thickness leads to greater
threshold voltage tunnability, the degradation of S.S. would also lead to the decrease of sensitivity in our case. We need to find the balanced point. In terms of the MZO channel layer, the interdiffusion during the MOCVD and PECVD processes consumes a certain amount of channel layer and an over thin channel also leads to a significant reduction of drain current. Therefore, for further improving the device’s sensitivity, an optimized channel thickness also needs to be found. As for the top SiO₂ layer, special attention needs to be paid to the side coverage during the PECVD deposition because short circuit may happen through the source - top gate - drain path when side coverage is poor, and thickness is thin. The deposition process needs to be done as conformal as possible.

Optimization of the interconnect between DGTFT and the extended sensing pad

The biosensor used in this study consists of two parts: 1) the MZO DGTFT as the signal transducer, and 2) the extended MZO nano modified sensing pad as the biological receptor. The extended sensing pad was electrically connected to the top gate (TG) of the DGTFT through a coaxial cable with two micro-positioners (one needle probing TG, and the other needle probing sensing pad). Such electrical connection approach could introduce the parasitic impedance. To improve the optimized and stable operation, current interconnection and electrical contacts should be changed. First, we plan to design and make a “plug-in-card” type of connection, which shortens the distance to cm level. In this approach, the DGTFT transducer is reusable, and the sensing pad is disposable. Second, wire bonding plus the printed circuit board technology will be used to improve the contacts so that the sensing pad and the TFT can be on the same substrate. It is estimated that the resistance from both the contact and the bonding wire could be in the single digit mΩ range. Our final goal is to further miniaturize the device through on-chip integration. In such a
case, the interconnect issue can be completely resolved. The MZO DGTFT sensor technology can be used as the real testing tool for commercial applications.

Integration of DGTFT and extended sensing pad

For experimental demonstration, we interconnected two probes and let one of them contact with the top gate of DGTFT and the other probe contact with the extended sensing pad. However, we must replace such setup with more robust integration for real product. Plug-in-card is one of the ideal designs. The extended sensing pad is disposable after each use, and the DGTFT transducer is reusable. Such setup can maximize the repeatability of test and minimize the cost of device. On the one hand, multiple DGTFTs can be built as transducer array on a glass substrate with their top gates exposed for connection with the sensing pad. On the other hand, multiple metal pads can be patterned with MZO\textsubscript{nano} on top on another glass substrate as the biological receptor. A small area of metal is exposed for contact with the transducers’ pad. With this plug-in-card type of design, multiple tests can be done simultaneously with low-cost.

Detection of DNA

The development of DNA sensors has recently gained wide interests in the research aeras of gene analysis, gene disorder detection, and tissue matching applications. Various techniques were developed to detect DNA, but most conventional methods utilize fluorescence detection of labeled molecules which is time-consuming and not easy to use. Jung et al. have demonstrated a label-free DNA sensor with quantitative analysis based on InZnO (IZO) TFT in 2014 [21]. Order level current variations were observed after the immobilization of DNA, and such signal changes were attributed to DNA oxidation
associated with the reactive oxygen species on the IZO surface that supplies excess electrons to the device. However, as described in Chapter 2, the use of the top channel surface as the sensing layer hinders its improvement of sensitivity and further applications. As one of the future works, the detection of the immobilization and hybridization of DNA on MZO$_{nano}$ surface using the MZO DGTFT biosensor could be the next topic. In 2009, Taratula et al. and Reyes et al. demonstrated the stepwise functionalization of ZnO$_{nano}$ with DNA and applied the technology on QCM for biosensing purpose [58], [97]. This previous work paves the way of immobilizing the hybridizing DNA on the MZO$_{nano}$ modified sensing surface of the DGTFT biosensor. The sensor’s drain current is expected to change due to charge transfer between the DNA and the MZO$_{nano}$ surface. With this plug-in-card type of DGTFT biosensor, the detection of DNA could be more convenient and cost-effective.
References


W.-C. Hong et al., “MgZnO high voltage thin film transistors on glass for inverters in building integrated photovoltaics,” *Sci. Rep.*, vol. 6, 2016, doi: 10.1038/srep34169.


[61] B. Carpentier and O. Cerf, “Biofilms and their consequences, with particular


S. E. Astorga, L. X. Hu, E. Marsili, and Y. Huang, “Electrochemical Signature of Escherichia coli on Nickel Micropillar Array Electrode for Early Biofilm


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