

**LOW-COST AND RAPID PROTOTYPING OF  
ELECTROCHEMICAL MICROFLUIDIC BIOSENSORS**

**BY**

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**A thesis submitted in fulfilment of the requirement for the  
degree of Master of Science (Electronics Engineering)**

**Kulliyyah of Engineering  
International Islamic University Malaysia**

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## ABSTRACT

Electrochemical microfluidic biosensor is a widely used category of bioanalytical microdevices, with applications ranging from home-use glucometers to advanced blood analysis devices. They enable powerful microscale analyses in biology, physics and chemistry. Conventionally, the methods to fabricate these devices are either screen-printing, inkjet printing, or cleanroom-based photolithography. All these methods have slow iteration times, and cleanroom facilities are especially expensive and are limited in access to researchers in low-and-middle-income (LMIC) countries. In this thesis, a low-cost, accessible and rapid fabrication process of electrochemical microfluidic biosensors has been developed. This work leverages the accessibility of consumer-grade electronic craft cutters as the primary tool for patterning of sensor electrodes and microfluidic circuits, while commodity materials such as gold leaf, conductive silver ink, double-sided tape, vinyl sticker, plastic transparency films, and fabric adhesives are used as its base structural materials. The process enables fabrication of gold electrodes with dimensions as small as 450  $\mu\text{m}$  and gaps of 110  $\mu\text{m}$ , silver electrodes with dimensions as small as 600  $\mu\text{m}$ , and fluid microchannels as small as 300  $\mu\text{m}$ . Micro-volume hydrogen peroxide concentration measurements were performed as validation of biosensor performance, which achieved a limit of detection of 0.713 mM and sensitivity of 82.002  $\mu\text{A mM}^{-1} \text{cm}^{-2}$  from 2  $\mu\text{L}$  samples. The rapid process allows an iterative design-build-test cycle in less than 2 hours. This method is applicable in typical university laboratories and costs less than RM2100 to set up, enabling lower access barriers into the biosensor field for academic and industry researchers in low-resource settings.

## خلاصة البحث

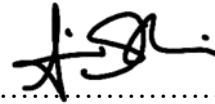
إنّ المستشعرات الحيوية الكهروكيميائية المائعة الدقيقة هي إحدى فئات أجهزة التحليل الحيوي الدقيقة المستخدمة على نطاق واسع، وتطبيقاتها تتراوح بين أجهزة قياس السكر في الدم للاستخدام المنزلي وأجهزة تحليل الدم المتقدمة. هذه المستشعرات تمكّن من إجراء تحليلات فعالة على المستوى المجهرى في مجالات الأحياء والفيزياء والكيمياء. ومن الطرق المألوفة لتصنيع هذه الأجهزة استخدام طباعة الشاشة الحريرية، أو الطباعة النافثة للحبر، أو الطباعة الليثوغرافية الضوئية في غرف الأبحاث النظيفة. كل هذه الطرق لها أوقات تكرار بطيئة، كما أنّ مرافق غرف الأبحاث باهظة الثمن بشكل استثنائي، وتتم بمحدودية الوصول لها من قبل الباحثين في البلدان ذات الدخل المنخفض والمتوسط. في هذه الأطروحة، تم تطوير عملية تصنيع منخفضة التكلفة، وسهلة الوصول، وسريعة، للمستشعرات الحيوية الكهروكيميائية المائعة الدقيقة. يستفيد هذا العمل من إمكانية الوصول إلى أجهزة القطع الحرفية الإلكترونية المصممة للمستهلكين كأداة أساسية لتصميم وتشكيل أقطاب المستشعر ودوائر الموائع الدقيقة، بينما المواد الأولية مثل الأوراق الذهبية، والحبر الفضي الموصل، والشريط اللاصق ذو الوجهين، ولاصق الفينيل، والأفلام الشفافة البلاستيكية، واللواصق القماشية، فإنها تستخدم كمواد هيكلية أساسية. وتتيح هذه العملية تصنيع أقطاب كهربائية ذهبية بأبعاد صغيرة تصل إلى 450 ميكرومتر، وفجوات مقدارها 110 ميكرومتر، وأقطاب فضية بأبعاد صغيرة تصل إلى 600 ميكرومتر، وقنوات مائعة صغيرة تصل إلى 300 ميكرومتر. وقد أجريت قياسات لتركيز بيروكسيد الهيدروجين ذات الحجم الصغير للتحقق من صحة أداء المستشعر البيولوجي، وحققت القياسات دقة في الكشف بلغت 0.713 ملليمولار، وحساسية بمقدار 82.002 ميكروأمبير/مليمولار/سم<sup>2</sup>، وذلك باستخدام عينات بحجم 2 ميكرو لتر. وتسمح هذه العملية السريعة بدورة (تصميم وبناء واختبار) تكرارية في أقل من ساعتين. هذه الطريقة قابلة للتطبيق في مختبرات الجامعة النموذجية وتكلفة تجهيزها أقل من 2100 رنحت ماليزي، مما يمكن من تذليل العقبات وتسهيل الوصول إلى مجال استخدام المستشعر الحيوي للباحثين الأكاديميين والصناعيين في الأماكن منخفضة الموارد.

## ABSTRAK

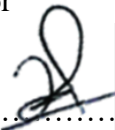
Penderia elektrokimia mikrobendalir ialah salah satu kategori peranti bioanalisa mikro yang diguna secara meluas, dengan penggunaan yang merangkumi glukometer persendirian sehingga peranti analisa darah termaju. Alatan ini membolehkan analisa berskala mikro berkuasa tinggi untuk biologi, fizik dan kimia. Kaedah konvensional dalam pembuatan peranti-peranti ini bergantung kepada teknik percetakan skrin, teknik percetakan pancut dakwat, atau fotolitografi dalam bilik bersih (*cleanroom*). Semua teknik-teknik tersebut mempunyai kitaran ulang yang perlahan. Fasilitas bilik bersih terutamanya berkos tinggi dan mempunyai ketercapaian terhad bagi para penyelidik dari negara-negara berpendapatan rendah dan sederhana. Dalam tesis ini, proses pemprototaipan penderia elektrokimia mikrobendalir yang pantas, mudah capai, dan berkos rendah telah dihasilkan. Kajian ini mengeksplotasi kemudahperolehan penyurih-potong elektronik gred pengguna sebagai alat utama untuk pembentukan pola elektrod penderia dan litar mikrobendalir. Bahan-bahan komoditi seperti kerajang emas, dakwat perak konduktif, perekat dwimuka, pelekat vinil, kepingan plastik slaid lutsinar, dan perekat fabrik digunakan sebagai bahan struktur asas. Proses ini membolehkan fabrikasi elektrod emas dengan dimensi sekecil 450  $\mu\text{m}$  dan sela selebar 110  $\mu\text{m}$ , elektrod perak dengan lebar sekecil 600  $\mu\text{m}$ , dan salur mikrobendalir sekecil 300  $\mu\text{m}$ . Pengesanan prestasi biopenderia dijalankan melalui pengukuran kepekatan hydrogen peroksida berisipadu mikro berjaya mencapai had terendah pengesanan 0.713 mM dan kepekaan 82.002  $\mu\text{A mM}^{-1} \text{cm}^{-2}$  dari sampel 2  $\mu\text{L}$ . Proses pantas ini membolehkan kitaran rekabina-uji beriterasi dalam masa kurang dua jam. Kaedah ini terpakai di makmal universiti biasa dan berkos kurang RM2100 untuk dipasangsedia, sekaligus merendahkan sekatan penglibatan ke dalam bidang biopenderia bagi para penyelidik akademik dan industri dari sekitaran bersumber rendah.

## APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Electronics Engineering).



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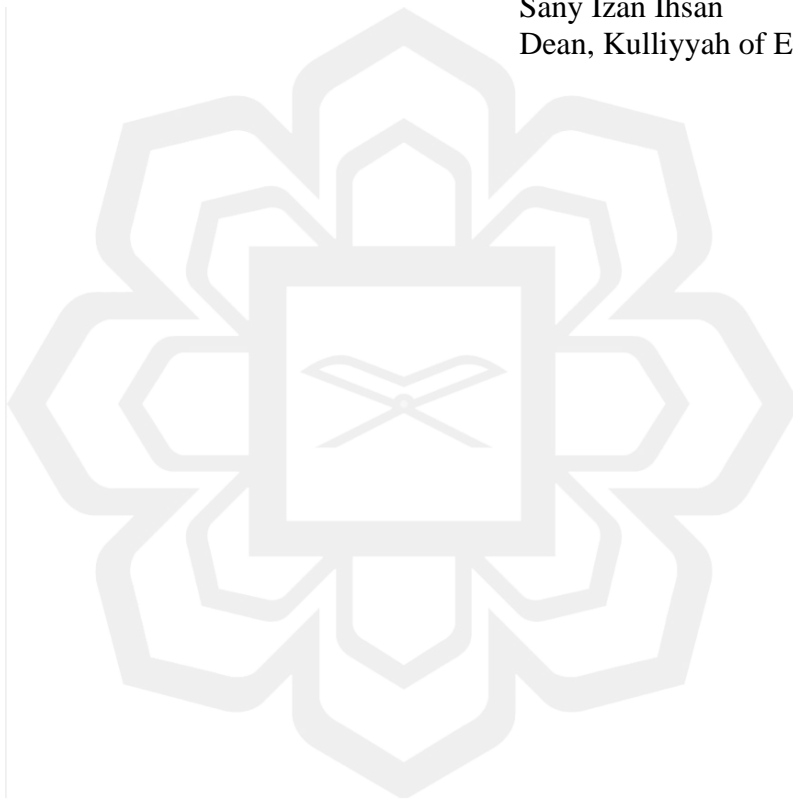
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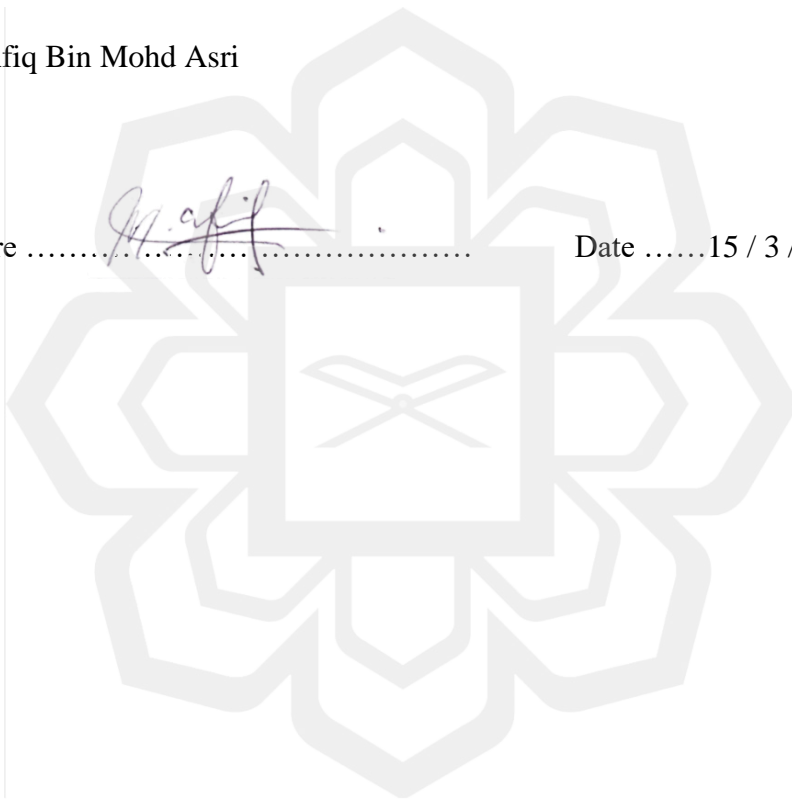


## DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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## LIST OF ABBREVIATIONS

$\mu$ TAS	Micro-total analysis system
$[\text{FeCN}_6]^{3-/4-}$	Ferri/ferrocyanide redox couple ion
$\mu$ PAD	Micro-paper analytical devices
24K	24 karats
2DPN	Two dimensional paperfluidic networks
3D	Three dimensional
AgCl	Silver chloride
AgNP	Silver nanoparticles
ASSURED	Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end users
CAD	Computer Aided Design
CE	Counter Electrode
CMOS	Complementary metal-oxide semiconductor
CNC	Computer numerical control
COC	Cyclic olefin copolymer
CV	Cyclic voltammetry
DI	Deionised water
DIW	Direct ink writing
ELISA	Enzyme-linked immunosorbent assay
ESCARGOT	Embedded SCAffold RemovinG Open Technology
FDM	Fused deposition modelling
GOx	Glucose oxidase
$\text{H}_2\text{O}_2$	Hydrogen peroxide
HNB	Heat 'N' Bond iron-on adhesive
IDT	Interdigitated electrodes
IJP	Inkjet printing

IPA	Isopropyl alcohol
LDI	Laser desorption ionisation
LFA	Lateral flow assay
LMIC	Low- and middle-income countries
LOC	Lab on a chip
LOD	Limit of detection
MEMS	Microelectromechanical systems
PAAm	Polyacrylamide
PBS	Phosphate buffered saline
PCB	Printed circuit board
PCL	Print-cut-laminate
PCL	Polycaprolactone
PDMS	Polydimethylsiloxane
PET	Polyethylene terephthalate
PLA	Polylactic acid
PMMA	Polymethyl methacrylate
POC	Point of care
POCT	Point of care testing
PSA	Pressure sensitive adhesive
PVA	Polyvinyl acetate
PVD	Physical vapor deposition
R&D	Research and development
RACER	Research Acculturation of Early Career Researchers
RE	Reference electrode
RIGS	Research Incentive Grant Scheme
SAM	Self-assembling monolayer
VIA	Vertical interconnect access
WE	Working electrode

## LIST OF SYMBOLS

$\mu$	Dynamic viscosity, $\text{N m}^{-2} \text{s}$
$\mu_{\text{blank}}$	Mean value of blank measurement, A
A	Cross-sectional area, $\text{m}^2$
C	Concentration, $\text{M / mol cm}^{-3}$
D	Diffusion coefficient, $\text{cm}^2 \text{s}^{-1}$
$E_{1/2}$	Half-cell potential, mV
$E_{\text{p-p}}$	Peak-to-peak separation, mV
$i_p$	Peak current, A
$I_p^*$	Specific polar moment of inertia
J	Peak current density, $\text{A mm}^{-1}$
L	Characteristic length, m
l	Length, m
n	Number of replicates
Q	Flow rate, $\text{m}^3 \text{s}^{-1}$
R	Electrical resistance, $\Omega$
$r^2$	Coefficient of determination
Re	Reynolds number
u	Fluid front velocity, $\text{m s}^{-1}$
$\nu$	Kinematic viscosity, $\text{m}^2 \text{s}^{-1}$
$\nu$	Scan rate, $\text{V s}^{-1}$
V	Applied potential, V
w	Width, m
$\Delta E_p$	Peak separation, mV
$\Delta P$	Pressure drop, $\text{N m}^{-2}$
$\theta$	Angle, $^\circ$
$\sigma_{\text{blank}}$	Standard deviation of blank measurement, A

# CHAPTER 1

## INTRODUCTION

### 1.1. INTRODUCTION

Micro-total analysis systems ( $\mu$ TAS), more commonly known as “lab-on-a-chip” (LOC), is a powerful emerging technology used for analytical applications in biology, chemistry and physics. These devices exploit the special physical properties in nature at the microscale, such as laminar flow and diffusion-dominated kinetics to engineer features such as low resource consumption, rapidness, and high precision when doing analytical techniques. The use of LOC enables multiple applications across the research laboratory, including biological/chemical analysis, chemical synthesis, high-throughput screening, precise liquid manipulation and creation of new tools to pursue novel scientific questions. Additionally, they expand the laboratory capabilities into the clinic and in the field outside of the laboratory (Kovarik et al., 2013).

#### 1.1.1. Electrochemical Microfluidic Biosensors

One such category of LOC devices are electrochemical microfluidic biosensors, which is of high interest in the fields of point-of-care diagnostics, clinical chemistry, environmental monitoring, and precision cellular and molecular analysis. Electrochemistry-based biosensors holds several advantages over their optical-based and electromechanical-based counterparts, but most particularly for its relatively inexpensive instrumentation (compared to optical-based biosensing) and reduced unit cost at scale (compared to electromechanical-based biosensing) (Rackus et al., 2015).

The most commonly used and most commercially successful example of electrochemical microfluidic biosensor is the glucometer (Turner, 2013).

### **1.1.2. Conventional and New Approaches to Microfabrication**

The majority of LOC are made using cleanroom-associated technologies, which was first developed for fabrication of semiconductor devices such as diodes and transistors, and later adopted by the micro-electromechanical systems (MEMS) in the 1980s that produces accelerometers, miniature pressure and temperature sensors and GPS integrated devices (Reyes et al., 2002). Among cleanroom-associated equipment are photolithographic mask aligners and thin film deposition machines, such as metal sputtering chambers and plasma-enhanced chemical vapor deposition. Cleanrooms are not necessarily easy to access, especially to researchers in low-and-middle-income countries (LMIC), and the facilities and equipment involved are expensive (Pan & Wang, 2011; Walsh et al., 2017). Additionally, with biosensors, often there are region-specific modifications on the device involving bio-recognition capture molecules, such as immobilised proteins, antibodies, and nucleic acid hybridisation probes. These molecules are often functionalised onto the device or sensors using microarray spotter, a costly robotic instrumentation meant for customising nucleic acid microarrays (Park et al., 2008). Each of these instruments may cost anywhere between RM 250,000 to RM 3 million, and even membership access to the few available cleanroom facilities may cost from RM 5000 to RM 10,000 per annum in Malaysia, non-inclusive of equipment per use basis fees. These associated costs and access barriers hinder prototyping through iterative design process, which inadvertently delays product delivery and discourages LOC development and applications in LMICs.

To overcome these financial and access barriers, several independent works have been developed to build electrochemical sensors and microfluidic devices, including gold leaf lamination (Thompson, Birch, Nelson, et al., 2016), inkjet printing (Kawahara et al., 2014), heat-sensitive adhesive lamination (Birch et al., 2017), and xurography (Bartholomeusz et al., 2005; Martínez-López et al., 2016; Yuen & Goral, 2010) – all to a certain degree of limitations in geometric resolution, material versatility, and complexity.

### **1.1.3 The Frugal Approach to Science Tools**

The concept of frugal science and innovation has recently gained momentum. The approach of ‘constraint-based science’, often starting by asking questions about costs, accessibility and inclusivity, leads to an innovator’s ability to reframe problems and solutions (Ahuja, 2014; Reardon, 2013). Some of the prominent frugal innovations in science tools include the 50 cents paper microscope i.e. the Foldscope (Cybulski et al., 2014), a 125,000 rpm paper centrifuge i.e. Paperfuge (Bhamla et al., 2017), a gas lighter-based electroporator i.e. ElectroPen (Byagathvalli et al., 2020), a nebuliser powered by bicycle pump (Dzwonczyk et al., 2015), and a solar-powered medical oxygen concentrator (Hawkes et al., 2018). These tools have already revolutionised chemical and life sciences, as well as being used in real world applications.

Within the field of microdevices and biosensors, a common approach is to develop “tools to create tools”, usually kits containing modular parts for non-specialists to build custom microfluidic circuits and diagnostic kits. A few well known example of this is Ampli, which is based on laser-cut lateral flow assay modules (Phillips et al., 2018), and micromachined Lego bricks (Owens & Hart, 2018). For microfabrication in

general, several ‘cleanroom-to-makerspaces’ approaches have been introduced, as reviewed by Walsh et al (Walsh et al., 2017).

This research seeks to explore a combination of these various techniques to compensate each techniques’ limitation, to develop a novel, composite process to fabricate electrochemical microfluidic devices.

## **1.2. PROBLEM STATEMENT**

Lab-on-a-chip (LOC) are emerging technologies that has been enabling powerful microscale analyses in biology, physics and chemistry. This technology has given rise to point-of-care (POC) diagnostics, single cell-associated physiological studies, and rapid, low-consumption chemical/bio-reactor systems. Most LOC components such as sensors and microfluidic circuits rely on traditional microfabrication methods associated with cleanrooms, most notably photolithography and sputtering techniques; and/or robotic handling methods such as microarray spotting. Cleanrooms are not necessarily easily accessible, especially to researchers in low-and-middle-income (LMIC) countries, and the facilities and equipment involved are expensive for early stage prototyping. In Malaysia, the rental costs for cleanrooms may go up to RM7500 per annum, and fees for use of equipment may range between RM50 to RM300 per hour or process (see Appendix A).

Alternatives to cleanroom-based prototyping include industrial and research-grade material inkjet printers such as the Dimatix DMP-2800 series, and screen-printing. The Dimatix printer, while overall cheaper than a cleanroom, may cost up to RM300,000 (conservative estimate), which is still cost limiting for majority of researchers. Furthermore, the Dimatix involve a complex process optimisation for each given type of ink and substrate (A. A. Zainuddin et al., 2017). Meanwhile, screen-

printing enables a low-cost mass manufacturing option for fabrication of sensors. However, during early prototyping phase where iterative design is often required, fabrication of masks for screen-printing is a time-consuming process which may take several hours (if fabricated in-house) to over a week (if made to order). Additionally, both methods require functional inks which are either custom formulated in-house or purchased from specialty manufacturers which are often expensive. Specific to electrochemical sensors, another alternative is present in commercial screen-printed electrodes, such as ones commercialised by DropSens. A limitation presented by these commercial sensors is that they are sold according to manufacturer specified designs, which are non-customisable and not necessarily integrable into microfluidic systems.

These associated costs and access barriers extend the turnaround time of each iteration during the device prototyping phase, which delays product completion and delivery. Given that the typical seed funding for academic research in countries such as Malaysia has a small quantum – the Research Incentive Grant Scheme (RIGS), for example, funds at RM20,000 over two years, with only RM5000 allocated for materials and supplies – exacerbating the need for frugal approaches to microfabrication for those intending to pursue such endeavor.

### **1.3. HYPOTHESIS**

We hypothesise that a systematic combination of various frugal approaches will enable a reasonable alternative to the cleanroom-based techniques in miniaturised chemical and biological systems at a significantly lower cost, with more accessible set of instrumentation.