INTEGRATED NANONEEDLE-MICROFLUIDIC SYSTEM FOR SINGLE CELL INTRACELLULAR TEMPERATURE MEASUREMENT

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Engineering (Electrical)

Faculty of Electrical Engineering
Universiti Teknologi Malaysia

JULY 2015

"I dedicate this to my beloved parents; Abdullah Mohammed Binsilm and Nasra Salim Daer, my husband Iyad Hamed Alsaqqaf, my brother and his family for their support and encouragement throughout my education. Thank you for always being there for me"

ACKNOWLEDGEMENT

In the name of Almighty Allah, the most Gracious, the most Merciful, for giving me the determination and will to complete my master research.

My deepest gratitude goes to my supervisor Dr. Mohd Ridzuan bin Ahmad for giving me the opportunity to carry out the research under his supervision. I consider myself lucky to be able to have the proper guidance, great support, encouragement, motivation, inspirations and kindness throughout the research

My heartiest and utmost gratitude goes to my parents and my family for their patience, sacrifices, understanding, constant concern, moral support and prayers during my journey.

I also want to thank my friends and colleagues who have been very helpful throughout my research journey especially Amelia Ahmad Khalili, Abdul Hafiz Mat Sulaiman, Md Habibur Rahman, Ida Laila, Siti Nadia, Arbab Alamgir and Muhammad Asyraf Mandor. Thank you very much for the support that had been given either directly or indirectly until the very end.

ABSTRACT

Single cell analysis has become an important field of research in which cell properties are studied for an improved understanding of cellular processes. Cell intracellular temperature has proven to be a vital element in most cellular activates, chemical reactions and cell survival. An integrated nanothermal sensor-microfluidic system has been proposed to characterize the internal temperature of single cells. A finite element analysis study based on resistance temperature detectors has been studied. The first stage was to optimize the sensor design and dimensions where tungsten was chosen as a sensing material. Results show that a rectangular shape with a gap of 10.8 μm gave an equally distributed current density within the sensor, and 90 nm^2 cross sectional area caused minimal damage to the cell. Further mechanical characterization has been done and the results show that the nanoneedle could resist ramp force applied before failure, up to 22.5 μN. The second stage was to test the nanoneedle ability to measure the temperature of a cell. Electrical measurement on yeast cell was done and the results show that the nanoneedle conductivity was independent of cell conductivity. The nanoneedle proved to be able to measure the temperature with a current difference of 50 nA and the resolution was 0.015 °C in the range of 24-60 °C. The nanoneedle detected temperature change of 0.02 °C in 10 ms. The third stage was to integrate the nanoneedle with the microfluidic system and to study water flow behaviour in the microfluidic channel. Results show that 63 $μm^2$ microchannel cross sectional area was optimum and flow rate of 24.6 pl/min allowed successful cell penetration with minimal cell damage. The developed system can be a good candidate to be used in early disease diagnoses. Also, the system has the potential to measure electrical properties of cells and to be used for single cell drug delivery.

ABSTRAK

Analisis sel tunggal telah menjadi satu bidang yang penting dalam penyelidikan di mana pencirian sel dikaji bagi memahami proses selular dengan lebih baik. Suhu intraselular sel telah terbukti merupakan elemen penting dalam kebanyakan aktiviti selular, tindak balas kimia, dan kelangsungan hidup sel. Suatu sistem bersepadu pengesan nanoterma-*microfluidic* telah dicadangkan untuk mencirikan suhu dalaman sel tunggal. Suatu kajian analisis unsur terhingga berdasarkan rintangan pengesan suhu telah dijalankan. Peringkat pertama adalah pngoptimuman reka bentuk sensor dan dimensi di mana tungsten telah dipilih sebagai bahan penderiaan. Keputusan menunjukkan bahawa bentuk segi empat tepat dengan jurang sebanyak 10.8 μm memberi ketumpatan arus sekata diedarkan dalam sensor, dan 90 nm^2 kawasan keratan rentas menyebabkan kerosakan minimum kepada sel. Pencirian mekanikal lanjut telah dilakukan dan keputusan menunjukkan bahawa, *nanoneedle* yang dapat menahan daya tanjakan digunakan sebelum kegagalan, sehingga 22.5 μN. Peringkat kedua adalah untuk menguji keupayaan *nanoneedle* untuk mengukur suhu sel. Pengukuran elektrik pada sel yis yang telah dilakukan dan keputusan menunjukkan bahawa kekonduksian *nanoneedle* itu tidak dipengaruhi oeh kekonduksian sel. *Nanoneedle* terbukti dapat mengukur suhu dengan perbezaan semasa 50 nA dengan resolusi 0.015 °C dalam lingkungan 24-60 °C. *Nanoneedle* berupaya mengesan perubahan suhu sebanyak 0.02 °C dalam 10 ms. Peringkat ketiga adalah untuk mengintegrasikan *nanoneedle* dengan sistem *microfluidic* dan mengkaji sifat aliran air dalam saluran *microfluidic*. Hasil kajian menunjukkan bahawa luas keratan rentas 63 $μm^2$ mikro adalah optimum dan kadar aliran sebanyak 24.6 pl/min berjaya membenarkan penembusan sel dengan kerosakan sel yang minimum. Sistem yang dibangunkan boleh menjadi calon yang baik untuk digunakan dalam diagnosis penyakit awal. Selain itu, sistem ini mempunyai potensi untuk mengukur sifat elektrik sel dan digunakan untuk penyampaian ubat kepada sel.

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LIST OF SYMBOLS

"V"	-	Voltage
"I"	-	Current
"R"	-	Resistance
"T"	-	Temperature
"P"	-	Pressure
"E"	-	Young Modulus
"d"	-	displacement
"i"	-	Moment of inertia
"Q"	-	Heat Flux
"k"	-	Thermal conductivity
"v"	-	Velocity
"q"	-	Flow rate

LIST OF ABBREVIATIONS

Nps	-	Nano Particles
PEG	-	Polyethyleneglycol
QDs	-	Quantum dots
Pdots	-	Polymer dots
RhB	-	Rhodamine B
PMMA	-	Polymethylmethacrylate
PAH	-	Polyallylaminehydrochloride
SPE	-	Single Photon Excitation
TPE	-	Two Photon Excitation
QGY	-	Human Hepatocellular Carcinoma
KB	-	Human Nasopharynx Carcinoma
OS	-	OrganoSilica
PEG	-	Polyethylene glycol
Cr	-	Color difference of red
Cb	-	Color difference of blue
Eu-TTA	-	Europium (III) thenoyltrifluoro-acetonate
CHO	-	Chinese hamster ovary
ACh	-	Acetylcholine
FPT	-	Fluorescent Polymeric Thermometer
MBs	-	Molecular Beacons
GFP	-	Green Fluorescent Proteins
FPA	-	Fluorescence Polarization Anisotropy
NV	-	Nitrogen Vacancy
MWCNTS	-	Multi Walled Carbon Nanotubes
PU	-	Polyurethane
BFCs	-	Brown fat cells
NE	-	Norepinephrine
NMR	-	Nuclear Magnetic Resonance
CNT	-	Carbon nanotube
NTC	-	Negative Temperature Coefficient

TCR	-	Temperature Coefficient of Resistance
LOC	-	Lab on a chip
MEMS	-	Microelectromechanical Systems

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CHAPTER 1

INTRODUCTION

1.1 Research background

Traditional microbiological studies have been done at a population level in which information on how cells interact with each other, react to external stimuli and undergo complex processes such as gene expression was always obtained from population study data [1]. However, such information does not consider the identity and the importance of individual cells, which may lead to low level of precision and accuracy in the resulted data. The recent emerging techniques that have higher sensitivity and the need for cellular heterogeneity has driven research towards focusing on developing techniques that support the study of individual cells. Single cell analysis has become an important field of research that allows the differentiation between cells in living organisms and the ability to relate them to different biological functions and disease progression. Different cell properties such as electrical [2-4], mechanical [5-7] and thermal [8-10] are currently being studied for individual cell profiling that can help in the identification of rare cell types and identify the health condition of specific cells that has the potential for early disease diagnosis applications.

1.2 Temperate effect on cells and natural systems

Temperature is an important physical property of a matter that can determine the internal energy contained within a system; it can be defined as the measurement of the average kinetic energy of molecules in an object or system. It plays an important role governing many physical and chemical processes humans and plants undergo throughout their lifecycles [11-14]. For instance, in plants when increasing the temperature, a noticeable growth is observed in specific areas as compared to the rest of the plant such as, elongation in stem, growth in the leaf area, and the plant

biomass [15]. In humans, the environmental temperature shows a direct relationship with the rate of food consumption by humans. During the summer the rate of food intake is considered lower compared to days when the weather is cold. This is due to the difficulties the body undergoes when trying to lose heat to the surroundings. On the other hand, in winter the body needs extra heat to protect itself against hypothermia [16].

A single cell level, temperature shows the ability to differentiate cells with an abnormality i.e. cancerous cells and cells that are healthy. The human body is made up of billions of cells that grow and divide to produce more cells to keep the body healthy. However, sometimes cells become sick when the genetic material (DNA) is damaged or changed and cause mutations that affect normal cell growth and division. This process of mutation is accompanied by extraordinary heat production, which makes the internal cell temperature higher compared to the healthy cells. These mutated cells tend to become dangerous after several divisions, usually at that late stages when patients seek medical attention, but in most cases it is too late to be cured. Being able to measure the temperature of a cell at early stages of division can help in the early disease detection and probably save many lives [17].

1.3 Problem statement

Many attempts have been carried out in order to determine the internal temperature of single cells. Some of these techniques rely on materials that use the fluorescence properties as the determining factor with the change of the temperature. These sensors tend to show a high level of sensitivity. However, several factors still discourage their use, for instance, photo-bleaching in which the fluorescence is lost through irreversible alteration of the sensor's molecular structure by photo-damage, enzymatic degradation, and chemical damage [18], the insertion of material into the cell and the movement of material inside cell which can damage the cell [19], and the possible of toxicity due to the material degradation [20, 21].

There are few other attempts to measure the internal temperature that are not based on the luminescence properties [22]. However, such methods depend on the use of bulky operating systems that need highly trained operators and a constrained experimental environment. These systems are with no doubt important because of their novelty, but their significance can only be evident in the early stages of conducting a

research. These systems require upgrading and further improvements otherwise can be very time consuming, impractical and costly. Also the need for highly skilled operators makes them inefficient and not user-friendly. Besides, some of these systems are restricted to specific cell types which limit their implementation diversity.

The need to develop a system that has the ability to improve single cell studies is critical in the upcoming years of research by being portable, easy to use, inexpensive, and produces quantitative results in a prompt manner that does not require user interpretation.

1.4 Significance of the project

This project focuses on developing a nano-thermal sensor microfluidic system that can measure the internal temperature of a variety of single cells efficiently and in a more manageable procedure. The use of microfluidic devices facilitate sequential sample pre-treatment and increase sample throughput through parallel analysis. They also have added the advantages of improved portability due to miniaturization, reduced sample and reagent consumption, and accelerated speed of reaction and analysis [23, 24].

By knowing the temperature of a single cell, several important advancements in the fundamentals of cell biology and its cellular activities can be investigated. Heat generation inside cells has been used as one of the measures to understand the function of all constituent parts of living organisms and, ultimately, understand the chemistry of life.

Integrating a nano-thermal sensor into a microfluidic chip can facilitate the research studies and allow the researchers to carry out experiments in a more convenient way without the need of using bulky equipment. In addition, this system has the potential to be used for many other applications, for instance, early disease diagnoses, drug delivery, and many others. It will help produce a device that has multiple implementations and high reproducibility in a short time.

1.5 Objectives of the project

The main objective of this project is to develop an integrated nanoneedle-microfluidic system for measuring single cell temperature. The sub objectives are as follows:

- To design and optimize a thermal nano-sensor for single cell's internal temperature measurement.
- To integrate the nano-sensor with a microfluidic system.
- To measure the internal temperature of a single cell using the integrated thermal-microfluidic system.

1.6 Scope of the project

The project is simulation based study that is mainly about designing and optimizing a thermal nano-sensor that measures the internal temperature of single cells. It is divided into three main parts: firstly, the sensor design and optimization which covers the shape, dimensions and structure of the sensor. It also includes the electrical and mechanical characterizations of the sensor. The second part is about the electrical characterization of a cell and the nanoneedle ability to measurement the temperature. The third part covers the integrating of the sensor into a microfluidic channel which study the water flow direction, water flow rate in the proposed channel design. The research study was done using a finite element analysis and designing softwares, mainly ABAQUS and Solidworks, and the cell model used was based on *Saccharomy cerevisiae* (2.5.2).

1.7 Organization of the thesis

The thesis is divided into 6 chapters. First chapter is an introduction of the project, explaining the problems and motivations that encouraged to conduct this research, objectives and the scope of the research. The second chapter is a thorough discussion of the current techniques used in the single cell intracellular temperature measurements with a table that summarize the advantages and disadvantages of the conventional techniques, and theoritical studies explaining different sensing

mechanisms. The third chapter is a discussion on the methodology undertaken during the research with a detailed explanation on the system concept and the reasons for selecting of each part. The forth chapter is the nanoneedle design and characterization which covers topics in the thermal/electrical and mechanical characterization of the nanoneedle and cell temperature measurement. The fifth chapter is the microfluidic channel integration discussing the parameters needed in the integration of the nanoneedle microfluidic system. The last chapter is the conclusion and future recommendations for this research.

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