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DEVELOPMENT OF MICROWEIR STRUCTURE BY LITHOGRAPHIC FABRICATION PROCESS FOR MALARIA CELL SEPARATION

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By

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

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

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March 2016

Chairperson : Fakhrul Zaman Rokhani, PhD
Faculty : Engineering

Malaria, a mosquito-borne deadly disease invades millions of lives yearly and consumes the socio and economic resources for drug invention and treatment. Besides early disease detection is hampered by limited resources and unavailability of portable detection system in rural areas. Hence the present work concentrates on improving the available impedance based malaria detection system through red cell separation. To maximise the detection efficiency narrow microchannels are designed to suit the dimension of a single red blood cell (RBC) which demands white blood cell (WBC) separation.

RBC separation is achieved in the present work using the passive and crossflow filtration by microweir components. It works based on the principle of laminar flow under different pressure gradients, creating the necessary suction force to drag the red cell to the appropriate location. It offers the benefits of easy fabrication, reproducibility and good cell separation with unaltered cell property. Therefore the development of microweir structure for malaria cell separation is been studied in this work to understand its efficiency and limitations. The key parameters influencing weir separation efficiency are including flow rate, channel length and channel width are derived through the numerical study. They are then optimized through simulation made on three dimensional models built in Comsol Multiphysics software. The simulation results are analysed using Anova test to find the significance among the different values being simulated for each parameter. Later the microweir separation chip is fabricated through the traditional ultraviolet (UV) lithographic process on the positive photoresist AZ1518. The experiment on separation is made on beads of sizes 2, 3 and 5 μm at three different flow rate values of 0.03ml/hr, 0.05 ml/hr and 0.07 ml/hr.

The simulation results showed the buffer flow rate reduced to a tenth of particle flow rate, side channel length of 100 μm and side channel width of 40 μm produced higher bead count value at the secondary channel while reducing the value at primary channel. The simulation of different weir height could not be effectively analysed due to the limitations of the chosen study physics and simulation software used. The experiment and simulation results are then analysed using imageJ image processing software to

calculate the bead count and velocity magnitude. The experiment result shows an efficient separation of 5 µm beads from the 2 µm and 3 µm beads qualitatively. The analysis of bead count at the secondary channel shows 45.6%, 34.2% and 16.7% separation efficiency and 113 µm/s, 168 µm/s and 350 µm/s as the average bead velocity at 0.03 ml/hr, 0.05ml/hr and 0.07 ml/hr respectively. Later the simulation bead count and velocity is compared with experimental result by running T-test on SPSS statistical software. The results show a $P < 0.05$, implying no significant differences between the experiment and simulation results.

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

PEMBANGUNAN STRUKTUR BENDUNG MIKRO MENGGUNAKAN PROSES FABRIKASI LITOGRAFI BAGI PENGASINGAN SEL MALARIA

Oleh

LALITHA SIVARAJ

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Malaria, penyakit bawaan nyamuk berbahaya menyerang berjuta-juta nyawa setiap tahun dan menggunakan banyak sumber sosio-ekonomi bagi mencipta ubatan dan rawatan. Selain itu, pengesanan peringkat awal penyakit dihalang oleh sumber yang terhad dan ketiadaan sistem pengesanan mudah alih di kawasan luar bandar. Oleh itu, kajian ini menumpukan kepada peningkatan sistem pengesanan malaria berdasarkan galangan melalui pemisahan sel darah merah. Untuk memaksimumkan kecekapan pengesanan, saluran mikro direka untuk memenuhi dimensi sel tunggal darah merah manusia (RBCs) yang memerlukan pemisahan sel darah putih(WBCs).

Pemisahan bendalir mikro sel darah merah dicapai dalam kajian ini dengan menggunakan komponen pasif bendung mikro. Ia berfungsi berdasarkan prinsip aliran laminar di bawah kecerunan tekanan yang berbeza , mewujudkan daya sedutan untuk mengheret sel merah ke lokasi yang sesuai .Ia menawarkan faedah fabrikasi mudah, keboleh-ulangan dan kemahiran pemisahan sel dengan sifat sel tidak diubah. Oleh yang demikian, pembangunan struktur bendung mikro untuk pemisahan sel malaria dikaji untuk memahami kecekapan dan batasannya. Parameter utama yang mempengaruhi kecekapan pemisahan adalah kadar aliran , panjang saluran, lebar saluran dan ketinggian bendung mikro . Parameter ini dioptimumkan melalui simulasi model tiga dimensi yang terbina dalam perisian Multifizik Comsol. Keputusan simulasi dianalisis menggunakan ujian Anova bagi mencari signifikasi di antara nilai-nilai yang berbeza yang telah disimulasi untuk setiap parameter. Kemudian, cip pemisahan bendung mikro direka melalui proses tradisional litografi ultraviolet (UV) pada rintangan foto positif AZ1518. Ujian pengasingan dibuat pada manik bersaiz 2 , 3 dan 5 mikron di tiga nilai aliran yang berbeza iaitu 0.03ml / jam , 0.05 ml / jam dan 0.07 ml / jam.

Hasil kajian menunjukkan pengurangan kadar aliran penampan kepada darah kesepuluh bagi aliran zarah, panjang saluran sisi 100 mikron dan saluran sisi lebar 40 mikron menghasilkan nilai kiraan manik lebih tinggi pada saluran sekunder di samping mengurangkan nilai pada saluran utama . Simulasi ketinggian dasar bendung yang berbeza tidak dapat dianalisis dengan berkesan kerana kekangan kajian fizik dan perisian simulasi yang dipilih. Keputusan eksperimen dan simulasi kemudiannya

dianalisis dengan menggunakan perisian pemprosesan imej ImageJ untuk mengira kiraan manik dan halaju magnitud. Keputusan eksperimen menunjukkan pemisahan yang cekap bagi 5 mikron manik berbanding 2 mikron dan 3 mikron manik secara kualitatif. Analisis kiraan manik di saluran sekunder menunjukkan 45.6 %, 34.2 % dan 16.7% kecekapan pemisahan dan 113 mikron /s, 168 mikron /s dan 350 mikron /s sebagai halaju manik pada purata 0.03 ml / jam, 0.05ml / jam dan 0.07 ml / jam masing-masing. Kemudian simulasi pengiraan manik dan halaju dibandingkan dengan keputusan eksperimen dengan menjalankan ujian-t pada perisian statistik SPSS. Keputusan menunjukkan $P < 0.05$, yang menunjukkan tiada perbezaan yang signifikan antara eksperimen dan keputusan simulasis.

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I certify that a Thesis Examination Committee has met on 23 March 2016 to conduct the final examination of Lalitha.S on her thesis entitled "Development of Microweir Structure by Lithographic Fabrication Process for Malaria Cell Separation" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

RBC	Red Blood Cell
iRBC	Infected Red Blood Cell
WBCs	White Blood Cell
WHO	World Health Organisation
LoC	Lab on Chip
FACS	Fluorescent Activated Cell Sorting
MACS	Magnetic Activated Cell Sorting
HGMF	High Gradient Magnetic Field
PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Test
DEP	Dielectrophoresis
FFF	Field Flow Fractionation
MEMS	Microelectromechanical System
DRIE	Deep Reactive Ion Etching
PDMS	Polydimethylsiloxane
DLD	Deterministic Lateral Displacement
PMC	Paramagnetic Capture
FFA	Field Flow Acoustophoresis
EDTA	Ethylenediaminetetraacetic Acid.
TDS	Transport of Diluted Species
FPT	Particle Tracing for Fluid Flow
EIS	Electrical Impedance Spectroscopy

LIST OF NOMENCLATURES

μ	fluid dynamic viscosity
ρ	fluid density
τ	shear stress
γ	shear rate
Re	Reynold number
v	mean velocity
D_r	hydraulic diameter
R_h	hydraulic resistance
ΔP	pressure difference and
w	channel width
d	channel depth
l	channel length
r	particle diameter
D	diffusion coefficient
F	frictional coefficient
K	Boltzmann constant
T	absolute temperature

CHAPTER 1

INTRODUCTION

Malaria is a fatal, blood borne parasitic disease encountering millions of lives yearly[1]. Vector control and early disease detection are proven effective to control mortality rate [1]. Yet the goal is not reached due the unavailibility of powerful detection system in rural areas[2]. The present work focuses on developing a passive microweir separation chip to enhance impedance based malaria detection system. Since the malaria parasite affects the red cell population, single red blood cell passage through the constricted detection channel is needed for active detection [33]. Hence the bigger white blood cells must be filtered from the whole through agile separation method to refrain clogging. Therefore this work concentrates on investigating the design parameters involded in microweir separation through simulation and validating it through real experiment. This chapter provides an overview about human blood and its composition, cell separation techniques and the importance of cell separtion. Subsequently some light is shed on malaria parasite and its lifespan. The major objectives of the work, hypothesis and scope of this research work are also being discussed here.

Blood : Functions and Composition

Blood is the essential bodily fluid critical to make the internal transportation of oxygen, hormones, metabolites and nutrients and for the excretion of carbon di-oxide and metabolic wastes. It constitutes 7 – 8 % of an adult's body weight and has an average pH of 7 at 38^0 C mean temperatures [3]. It carries a myriad of information for body functioning and most importantly it plays a critical role in the immune system through the transport of white blood cells and antibody. Indeed, pathologically, it is an important vector of parasite, virus and bacterial infection. Blood-borne infections like Malaria, AIDS, Hepatitis B and C, and Sepsis pose severe treat to mankind and needs immediate treatment.

Human blood is composed of four major components: erythrocytes or red blood cells (RBCs), leukocytes or white blood cells (WBCs), platelet and plasma [3]. By volume, blood cells constitute about 45% and plasma constitutes about 55% of the blood volume [4]. From the rheological point of view, the most important constituents are the blood plasma and the red blood cells as they help to determine the blood flow behaviour. Typically plasma consists of 90 % (w/w) water, 7% (w/w) is proteins [5]. Plasma has a specific gravity of 1.026 and viscosity of 1.2×10^{-3} Pa.s which is similar to water and hence it behaves like a Newtonian fluid. The majority of the blood cell volume is occupied by RBCs ranging from $4.7 - 6.1 \times 10^6$ cells per µl of blood accounting for the non-Newtonian characteristic of blood [6, 7]. The erythrocyte or RBC volume fraction is expressed as hematocrit and the average hematocrit ranges from 40 – 52 % for men and 36 – 48% for women. RBCs are biconcave shaped cells, composed of cytoplasm and cell membrane [8]. The membrane is made of protein and lipids while the cytoplasm is rich in haemoglobin, a paramagnetic oxygen binding molecule. The RBCs lack nucleus and are marked with extreme deformability. This

deformability makes it advantage while separating RBCs from WBC and their size difference also but their sizes overlap at the lowest limit of WBCs. White blood cells counts very low as 4000 – 11,000 cells per µL of blood and they are involved in defending the body against foreign organisms [7]. Platelets meant for blood clotting mechanism counts for 2 - 5 x 10^5 cells per microliter. The key blood constituents of whole blood are listed in the following table 1.1.

Table 1.1 Constituents of human blood [6]

Constituent	Size	Composition	Concentration
Red blood cell	6-8 µm diameter; 2 µm thick		$4.7 – 6.1 \times 10^6$ μL^{-1}
White blood cell	7-20 µm		$4 -11 \times 10^3$ μL^{-1}
Platelet	2-3 µm		$2 – 5 \times 10^5$ μL^{-1}
Plasma		Water, Salt, glucose nutrients and Proteins:	
		• Albumins	$4.5\text{-}5.7 \times 10^{-5}$ g/µL[3]
		• Globulins	$1.3\text{-}2.5 \times 10^{-5}$ g/µL[3]
		• Fibrinogen	$1.3\text{-}2.5 \times 10^{-5}$ g/µL[3]

1.2 Cell separation and its need

Cell separation is the ability to sort cells according to their intrinsic or physical properties. Physical sorting utilizes parameters like size, shape (morphology), behaviour and deformability while active sorting is based on the antigen status or inherent paramagnetic or electrical behaviour in the presence of external magnetic or electric field [9]. The traditional cell separation has been enabled through centrifugation, membrane filtration, fluorescent activated cell sorting (FACS) and Magnetic activated cell sorting (MACS). The current researchers focus more on microfluidic cell sorting due to their numerous advantages like cost efficiency, low reagent or sample consumption, quick sorting and portable nature [10]. Besides there are several issues like clogging after long run, troubleshoots in fabrication, separation efficiency and high throughput issues needs to be resolved to build a novel sorting device.

The separation of blood cells into individual components becomes an inevitable component of many biological assays and Lab on Chip (LoC) devices. Blood contains a myriad of information about the basic functioning or diseased metabolism of human body. Hence, complete blood analysis has been the primary diagnostic test in the health care system. Research involving cell analysis frequently requires isolation of certain cell types either as a final objective or as a preparative tool for further assays [11, 12]. With the advancement in microfluidic technology, more efficient micro-separators are being researched to meet the on-chip demand. Besides providing the necessary

component for analysis, these micro cell separating devices connected to LoC devices, also eliminates the irreversible cell storage threats like fragmentation and oxidation.

A diverse number of applications rely on the individual blood cell properties. For example platelets with their coagulation properties are needed during surgeries, organ transplantation and trauma treatment [11]. Similarly, WBCs are more appreciated for haematological analysis and in diagnostic tests for monitoring disease progression [12]. Treating diseased conditions like leukaemia also needs removal of WBCs [13]. Fractionation of RBCs from other blood components plays a major role in different field including disease diagnosis for infections like malaria, dengue, hepatitis, HIV and for scientific research [14].

1.3 Malaria Disease

The malaria parasite is carried by 30 different species of female Anopheles mosquito. The human infecting parasites which belongs to the Plasmodium genus are P.falciparum, P. vivax, P.ovale, P.malariae and P.knowlesi [15]. Among these, infection due to P.falciparum and P.vivax is more predominant with P.falciparum regarded as the deadliest parasite invading the majority of lives. P.knowlesi parasite which mainly infects the long tailed macaque is found to affect human since 1931 [16]. The infection due to P. knowlesi is found mostly in the South-East Asia region only. The intra-erythrocyte cycle of these parasites varies with the shortest cycle noted in P.knowlesi infection as 24 hours. P.falciparum and P.vivax infection spikes after 48 hours and P.malariae infection spikes after 72 hours. P.vivax and P.ovale parasites can form hypnozoites causing a relapse period of weeks to months after infecting human which is not found among the other infecting parasites. Also P. vivax can sustain much lower temperature of 10^0 C and higher altitudes [16]

Malaria is a bidirectional transmitted disease between human and mosquito. The parasites lifecycle starts, when an infected female mosquito bites human. The sporozoites present in the mosquito's saliva are transmitted to the human blood stream. These sporozoites invade the liver cells where they undergo asexual reproduction to form merozoites. The merozoite invades healthy human red blood cell and travels through a series of stages: ring stage, trophozoite and schizont which finally yield 8 - 24 daughter merozoites and hence continue infecting other red blood cells [15]. Upon reproduction, some merozoites develop into gametocytes which are transmitted to mosquito during its blood meal, thus the parasite initiates its life cycle inside another mosquito. Inside the mosquito, the male and female gametocytes fuse to form ookinete, which then develops into sporozoite and migrate to the salivary gland [17]. Hence the mosquito now turns to be the vector to transmit the disease further.

1.4 Research Questions

The growing mortality rate due to malaria disease demands a portable, inexpensive and disposable device to be available on all drug stores in rural area [17]. Microfluidic impedance-based detection chip has been designed for easy malaria detection [33]. Yet, it lacks a sample pre-processing system attached to it to separate the RBCs and hence it

is not completely available for malaria detection at rural setup. Although a crossflow filtration using microweir structure enable label-free RBC separation [67], lack of study in utilizing it for malaria cell separation system motivates the current work to test its feasibility to enable separation. Hence this work addresses the following research question.

- How effective could a microweir based separation chip be fabricated to sort the beads of sizes similar to the blood cells thereby making the current system as a good alternative for the conventional separators?

1.5 Objectives of the Research

The aim of this research is to assist the current impedance based malaria detection system through fabricating an adaptable cell sorter based on weir separation mechanism to be attached to it. Hence the following objectives needs to be achieved

i. To investigate the key parameters influencing weir separation by simulating them through a desired range of values for each parameter.
ii. To optimize the parameters using Anova test to achieve higher particle count or particle separation efficiency and the target particle velocity.
iii. To validate the simulation through statistically analyzing its significance with the experiment using Independent sample T-test.

1.6 Scope and Limitations of the Research

This microweir sorting device would act as a rapid, user friendly and low cost cell separation system suitable for easy malaria cell separation and diagnosis. This prototype could also extends its scope to any LOC devices requring cell sorting.

The device fabrication is made for a larger geometry and compared with simulation results obtained for the same geometry. Scaling down the geometry is also been studied through simulation while not in experiment. Also the present system is experimented only with microbeads and hence it should be further investigated on real blood sample to avoid possible mishaps. The number of particles is only calculated using image processing steps and the result could be further improved by using cell counting equipment.

1.7 Thesis Outline

The thesis is divided into five chapters and their sub sections. Chapter 1 is the Introduction which presents general information about the research topic, research questions, objectives and scope of the work. Chapter 2 is the Literature Review, where the reviews about several separation technique and malaria detection methods are presented. Since the separation of red blood cell is the primary objective of the current study, the review focuses mainly on them. Chapter 3 talks about the Methodology. The procedure for microchannel and micro weir fabrication and their bonding procedure to make a complete microfluidic system are discussed. The experimental setups for

measuring the separation efficiency using microbeads are discussed here. Multiphysics simulation using COMSOL software to optimise the key process parameters is also been discussed. Chapter 4 presents the Results obtained from the simulation and experiment made. In addition, this chapter compares the experimental and simulated results and also discusses and related the findings with supporting literature works. The concluding chapter, Chapter 5 summarises the present work being done, and makes recommendations for further studies.

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