BOOLD CELLS SORTING USING COTTON THREADS

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A dissertation submitted in partial fulfillment of the

requirements for the award of the degree of

Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering

Universiti Teknologi Malaysia

SEPTEMBER 2014

To my adorable parents for all their best things and for their priceless support.

To my lovely sisters and brother for their effort when I needed their help and suggestions.

To all my lecturers who educated me especially to Dear Dr. Fahrul Zaman bin Huyop and Dear Dr. Dedy H.B Wicaksono.

To my beloved Mahdi for being there any moment for me and for his kind encouragement during my study.

ACKNOWLEDGEMENT

In the Name of ALLAH, the Most Gracious, the Most Merciful. First of all I thank the almighty God for giving me, support, guidance, patience and perseverance during my study.

I would like to express my sincere appreciation to my supervisor, Assoc. Prof. Dr. Fahrul Zaman bin Huyop, for his calm encouragement, guidance and tremendous patience in finishing my thesis and guiding me throughout the project.

I would not be finishing this project without the guidance and support of my Co-supervisor, Dr. Dedy H.B Wicaksono Thank you for being a patient teacher and helping me throughout the course of my research.

I would like to thank Sahba Sadir, Mahdi Hozhabri Namin, Radha who not only have given me constructive feedback and advice on my research as well as thesis document, but have also been great friends and stuck by me through both good times and bad.

I would also like to acknowledge the cooperation and assistance of UTM health center staffs especially Mrs. Natrah who helped me to conduct the tests and prepare all requirements of experiments.

ABSTRACT

Microfluidics systems have been developed for pretreatment of whole blood in last decades. Blood pretreatment or blood processing includes blood cell and plasma separation, white blood cell lysis and DNA purification, to name a few. In this project our focus is on blood cells sorting. Various methods have been demonstrated in literature for blood cell sorting and separation as one essential step of blood sample pretreatment in both the macro and micro scale.

In this study we proposed cotton threads as a matrix for fabrication of cell sorting systems. this kind of thread used in this study is inexpensive and fabricated microfluidic device is low volume and easy to use particularly appropriate for the developing world or remote areas, because of their relatively low fabrication costs .threads provide wicking channel for liquid via capillary forces without any external forces as a pump. The use of threads for cell sorting is based on liquid wicking along the gapes inside the threads which can be manipulated by twisting thread. This means more twisting in the same direction of real twist of thread make the gaps smaller which bigger cells cannot pass them and trapped in the channel results their separation from smaller cells. Fabricated device in this study has 3 different zones through the inlet to the outlet which each zone has a different TPI (Twists per Inch) means different sizes of gapes. Blood used to test the ability of fabricated device to sort different size of cells and the result showed efficient separation of red blood cells, white blood cells and plasma. Based on these results threads cab be consider as a proper material in microfluidic devices to sort different cells by their size.

ABSTRAK

Perkembangan dalam sistem mikro jumlah analisis yang menyasarkan pengesanan sampel darah membawa ke arah permintaan penggunaan peranti bendaliran mikro untuk pra-rawatan sampel darah termasuk pengasingan sel darah dan plasma, lisis sel darah putih dan penulenan DNA, antara beberapa aplikasinya. Dalam projek ini, fokus kami adalah pengaturan sel-sel darah. Pelbagai kaedah telah pun didemonstrasikan dalam kesusasteraan untuk pengaturan dan pengasingan sel darah sebagai langkah penting untuk pra-rawatan sampel darah dalam skala makro dan mikro.

Dalam kajian ini, kami mencadangkan penggunaan benang kapas sebagai satu landasan untuk menfabrikasi sistem pengasingan sel. Jenis benang yang digunakan dalam kajian ini ialah tidak mahal dan membolehkan fabrikasi peranti bendaliran mikro yang memerlukan volum sampel yang rendah dan mudah digunakan, sesuai untuk penggunaan dunia membangun atau kawasan terpencil, disebabkan oleh kos fabrikasi yang rendah secara relatifnya. Benang menyediakan saluran untuk aliran cecair melalui tekanan kapilari tanpa memerlukan tekanan luaran seperti pam. Penggunaan benang untuk pengasingan sel adalah berdasarkan aliran cecair melalui ruang dalam benang yang boleh dimanipulasi dengan memintal benang. Ini bermaksud lebih pintalan dalam haluan yang sama dengan pintalan sebenar benang, membuat ruang yang sedia ada semakin kecil. Oleh itu, sel yang bersaiz besar tidak boleh melaluinya dan terperangkap di dalam saluran menyebabkan pengasingan daripada sel-sel yang lebih kecil. Peranti yang difabrikasi dalam kajian ini mempunyai tiga zon yang berlainan melalui saluran masuk ke saluran keluar, di mana setiap zon mempunyai TPI yang berbeza yang bermaksud saiz ruang yang berlainan. Darah digunakan untuk menguji keupayaan peranti yang difabrikasi untuk mengatur sel yang berlainan saiz dan keputusan menunjukkan pengasingan sel darah merah, sel darah putih dan plasma yang efisien. Berdasarkan keputusan yang diperoleh, benang boleh dipertimbangkan sebagai bahan yang sesuai untuk menfabrikasi peranti bendaliran mikro untuk mengatur sel yang berlainan berdasarkan saiznya.

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LIST OF SYMBOLS

RBC	-	Red Blood Cell
WBC	-	White Blood Cell
TPI	-	Turns Per Inch
L	-	Wetted length
γ	-	Surface tension
θ	-	Contact angle between liquid and yarn surface
D	-	Capillary diameter
t	-	Time
μ	-	Liquid viscosity
M		Molar Concentration

CHAPTER 1

1 INTRODUCTION

1.1 Introduction

This chapter illustrates the background, problem statement, objectives, scopes and research methodology of the project. The thesis outline also included in this chapter as well.

1.2 Project Background

Cell sorting is a pre requirement in many analytical assays in basic research as well as for diagnostic applications (Thiel, Scheffold *et al*. 1998). As an example, isolation of small population of cells from background populations is a necessary step in clinical diagnosis and cell biology research. In the context of cell biology experiments, sorting can be a way to select a desired population of cells or can be a tool to analyze the results of an experiment (Ibrahim and Van Den 2003).

1.2.1 Blood:

Blood is a specialized fluid that delivers necessary substances such as nutrients and oxygen to the cells and tissues and transports metabolic products away from those

same cells and tissues. It contains a huge amount of information because it is draining every single part of the body.

Blood is a bodily fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells.

Main component of human blood is plasma 55 % and Red blood cells, white blood cells and platelets which make 45% of blood all together which are suspended in the plasma. Plasma is a yellowish liquid consist of 95% water, proteins, clotting factors, hormones, electrolytes and carbon dioxide. RBCs function is carrying oxygen to tissues. WBCs have major role in the immunity system and platelets play main role in the blood clotting. Blood adapts to the body's requirements through circulatory system. For example in the case of infection blood provides more immune cells for the infection sites to suppress harmful invaders. Analyzing of blood elements such as RBCs and WBCs numbers has been used to indicate various diseases. For example decrease in the number of Red blood cells indicates to anemia and increase in the number of White blood cells has been seen in the case of infection and tumors. In addition, the number of platelets indicates whether bleeding or clotting is likely to occur (Chen, 2010).

As mentioned before 45% of blood is cells which from this population 99% are RBCs (erythrocytes). They are most common type of cells in blood, 4-6 million in each cubic millimeter of blood which gives red color to blood. Mature RBCs are between 6-8 μm in diameter and without nucleus can easily pass smallest vessels. Lack of nucleus in red blood cells provides more capacity of oxygen storage by hemoglobin.

Granulocytes, lymphocytes and monocytes are three groups WBCs (leukocytes) which are in different shapes and sizes. Three kinds of granulocytes consist of neutrophils, Eosinophils and basophile mostly first group kill invaders by digesting them (Daniels and Bromilow 2007).

Eosinophils and basophiles are involved in allergic reactions. Eosinophils fight with parasites. T cells and B cells which are lymphocytes play main part of immune system. T cells are divided to two groups; killer T cells and helper T cells, and direct the activity of the immune system. The principal functions of B cells are to make antibodies against antigens. Recently suppressor activity of B cells is discovered. Monocytes are the largest groups of leukocytes ın the diameter between 12-20 μm which converts to macrophages in the tissues and digest foreign bacteria and damaged and dead cells of body. All leukocytes have a role in the immune response. When body is damaged, immune system circulates leukocytes in the blood in response. Signals include interleukin 1plays a central role in the regulation of immune and inflammatory responses to infections which is expressed by macrophages, monocytes, B lymphocytes and natural killer cells and form an important part of the inflammatory response of the body against infection. Other example is histamine which is released by basophiles and mast cells in the tissue involved in allergic reactions. Thrombocytes or platelets help blood to clot to cover a wound. They are smallest type of blood cells at only 2 or 3 microns (Daniels and Bromilow 2007).

1.2.2 Blood processing:

Blood samples analysis are important steps in either medical or science applications and propose a central role in the diagnosis of many physiologic and pathologic conditions due to this fact it is containing a massive amount of information about the function of all tissues and organs. Blood sample need to undergo pretreatment before analysis for clinical and scientific applications since its complexity. In Whole blood similar to many other biologically relevant samples such as saliva separation is often an essential part of any analytical process, necessary in order to avoid problems of cross-sensitivity, because the targets for detection can be present in extremely low concentrations (Chen 2010). We can consider separation in two methods which are preparative methods and analytical methods. In first method aim is collecting separated particles whereas in latter one analyses are done on samples without collecting each of separated particles. Prior to separate unusual particles in a mixture should be able

analyze and identify different components in a complex mixture. Based on this fact separation methods are consider in parallel with diagnosis since by using these methods we can measure a special feature of a components as we are separating them by that feature. This is true for microfluidics which are being used for blood separation and also for our device proposed in this thesis which can be used for analytical purposes for diagnosis as they separate different cells (Chen 2010).

1.2.3 Microfluidic:

We can define fluid as a substance that constantly transform under the effect of shear stress. Microfluidics means the science and engineering of small scale systems in which fluid behave different from conventional flow theory.

Recently application of microfluidics increased in cell biology and biological assays since they made possible the controlling of environment properties at the cell scale. Based on this fact researchers agree that microfluidics will have critical contribution in biological researches and point of care diagnosis including cell sorting. In last decade new concepts were proposed for cell sorting using microfluidics which has progressive improvement and drastically expanding.

The main advantage of microfluidics is the ability to design the structure space adequate to cell size which is being processing. They also provide user-friendly automation, reduction of sample treatment time on-chip, reagents consumption and chemical waste which is definition of lab on chip concept (Autebert, Coudert *et al.* 2012). Therefore these principles introduce microfluidics as a great choice for mammalian cells sorting.

The main purpose of developing of the lab-on-chip concept is to present devices diagnosis ability without using extensive laboratory testing in short time and wherever the patient happens to be, since they provide portable systems for in the field detection.

Another advantage of using these macro scale devices is increase the speed of analysis which is important in point of care issues.

In the last two decades, microfluidics has been used as ideal tools to handle small volumes of proteins or DNA solution or cell suspensions which our focus is on the last one in this thesis.

Microfluidics has been spotlighted for some reasons that it has the potential to retransform the way we approach cell biology research. Microfluidics enabled interfacing and analyzing single or small populations of cell. Also, it has a large variety of microfluidic devices that is available for cell analysis (Kim, Lee *et al*. 2008). Thus, microfluidic systems have started to play an increasingly important role in discoveries for cancer diagnosis, cell biology, neurobiology, cell transplantation, and tissue engineering.

The major advantages of micro fabricated systems for cell study are the ability to design cellular microenvironments, precisely control fluid flows, and to reduce the time and cost of cell culture experimentations (Autebert, Coudert *et al.* 2012). Microfluidic methods are an effective means to investigate the constituents of biological fluids for diagnostic purposes, just as they are useful for precise measurements and assays for other analytical processes, such as drug screening, nucleic acid amplification, and enzymatic reactions (Dong, Skelley *et al*. 2013).

1.3 Problem Statement

Currently, conventional cell separation methods using fluorescence-activated cell sorter (FACS) had many limitations. They are prolong time for analysis that can up to several hours, limited capacity by the speed of analysis and sorting that is about 5000 cells per second and very high cost for instrumentation (Thiel, Scheffold *et al.* 1998).Current cell sorting equipment are very expensive (Baret, Beck *et al.* 2010),

(Orfao and Ruiz-arguelles 1996) and (Meital Reches, Dickey *et al*. 2012). So, in this project, we have come with a new idea to develop a cell sorting device using thread that both raw material and fabrication process are low cost and simple.

1.4 Research Objectives

Several objectives had to be taken into account in this project. The objective of this project consists of:

i. To find out better design for a cell separation device based on Cotton threads.
ii. To demonstrate the structural parameters of threads and their effects in cell sorting or separation property.
iii. To apply designed device for blood to separate plasma and cells.

1.5 Scope of Research

Several scopes had been outlined in order to accomplish the objective of this project.

1) In this study cotton threads, glass slides, glass cover and double sides sticker were used to fabricate the microfluidic device
2) Healthy human blood will be used for tests in this study. Subjects are one 30 years old male and one 25 years old female. Blood will be collected in EDTA tubes.
3) For threads treatment anhydrous sodium carbonate (Na2CO3) and Millipore water were used.
4) To dilute the blood citrate anticoagulant were used.

5) For data collection Scanning Electron Microscopy (SEM) and Fluorescent Microscopy were used.

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