Ministry of Higher Education and Scientific Research
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Control and Systems Engineering Departments
Medical Control System Engineering Department

"DESIGN AND FABRICATION OF BLOOD FILTRATION, PLASMA AND TUMOR CELLS SEPARATION DEVICE"

Graduation project submitted to the control and systems Engineering Department in partial fulfillment of B.Sc.Degree in Medical systems Engineering

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ABSTRACT

The current project focuses on the development and fabricated of micro-device using three different designed methods of filtration and separating for blood and cancer cells separation and filtration or for any other chemical substances particles separation.

The response surface methodology (RSM), the full factorial design (FFD) and the expert system 11.0 software program were selected to design the experimental work improved and verified the experimental results.

The experimental results showed that the blood and cancer cells filtration and separating quantities are increasing with increasing the air pressure level and the microfluidic pore size. The best results for the highest quantity of blood separated and free of tumor and cancer cells were obtained at 150 mbar air pressure and the use of horizontal separation system, which is higher by 282% compared with the use of the vertical separation system.

The highest quantity of blood white cell (BWC) separated were obtained at 150 mbar air pressure and the use of horizontal separation system, reached 17.14 mL., which is higher by 32% than when use of the microfluidic blood white cell (BWC) separation system and by 343 % compared with the use of the vertical separation system.

The largest quantity of blood red cell (BRC) separated were obtained at 150 mbar air pressure and the use of horizontal separation system, reached 5.71 mL., which is higher by 90 % compared with the use of the vertical separation system.The highest quantity of blood cell filtration from the virus cells separated were obtained at 100 mbar air pressure and the use of vertical separation system,

reached 2.50 mL., which is higher by 9 times compared with the use of the horizontal separation system.

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Cell separation devices are used in various biomedical applications to identify individual cell types within a mixed population, enrich a single cell type into subpopulations of similar types for downstream diagnostic analysis or therapeutic applications. Blood analyses, in particular, requires the separation of different components such as red blood cells, (RBCs), white blood cells, (WBCs), platelets and cell-free plasma for analysis or DNA purification from WBCs [1].

Enrichment and separation of blood is often the first step in blood analysis, as each component of blood provides critical information for both diagnostics and therapeutics. For example, diseases like anemia, hemolysis, thalassemia, and spherocytosis are diagnosed by determining physiological and quantitative changes in RBCs (red blood cells) levels.

WBCs (white blood cells) play a significant role in the immune system, and abnormal levels or any form of deformation of WBCs indicate immune disorder, infection, or blood cancer. It is not surprising that the most common tests in clinical diagnostics involve sorting blood components, such as CBC (complete blood count), clotting test, blood-chemistry test, and blood enzyme test. In addition to clinical diagnostics, sorting of blood cells is also important in cell-research and therapeutics [2].

Blood accounts for 7% of the human body weight [3-4], with an average density around 1060 kg/m3, very close to pure water's density of 1000 kg/m3. The average adult has a blood volume of roughly 5 liters [5], which is composed of plasma and several kinds of cells. These blood cells (which are also called corpuscles or "formed elements") consist of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%.

Blood performs many important functions within the body, including: Supply of oxygen to tissues , Supply of nutrients , Removal of waste, Immunological functions , Coagulation , Messenger functions , Regulation of core body temperature and Hydraulic functions. Blood can be vital transfusions for people with medical conditions or who are having surgery. But blood transfusions can also improve the quality of life for people whose illness has no cure to give patients a better quality of life and the energy and ability.

There are several ways to measure or count cells in the blood, including: Microscopic Method, Automatic Optical Method and Electrical Conductivity Method.

1.2 BLOOD COMPONENTS AND TRANSPORT

Blood is a body fluid that circulates in the heart, arteries, capillaries, and veins from all parts of the body in humans and other animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells [5].

Human blood contains a number of cells that are present in a fluid called plasma (blood without pellets) and form. These cells account for 45% of blood volume while plasma makes up 55%.

Blood is made up of a number of components, including red blood cells, platelets and plasma. Each of these can be used to treat many different conditions. Blood is usually separated into its individual components or parts, so a patient can be given the particular component they need. This makes the most of every blood donation, as the components in one unit of blood (or one donation) can be used to treat different patients.

Donated blood or components are given to a patient in a blood transfusion. Blood transfusions are given via an intravenous line into a blood vessel. In 2014, according to hospital usage [6]:

- 67 was used to treat medical conditions including anemia, cancer and blood disorders
- %27 was used in surgery, including cardiac surgery and emergency surgery
- %6 was used to treat blood loss after childbirth.

The demand for blood from hospitals has fallen due to increased efficiency, but new donors are always needed to make sure there is enough blood to treat those who need it. In general, the blood is composed of:

1.2.1 Red Blood Cells (RBC)

Red cells containing hemoglobin whose primary function is to store and transport oxygen to the tissues. It is also referred to as erythrocytes, are the most common type of blood cell and the vertebrate organism's principal means of

delivering oxygen (O_2) to the body tissues via the blood flow through the circulatory system. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries. These cells' cytoplasm is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the blood's red color.

In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles. New 2.4 million erythrocytes are produced per second. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells [7].

1.2.2 White Blood Cells (WBC)

White blood cells whose principal role is to identify, destroy and remove any foreign material that has entered the body. White blood cells are part of the immune response. WBCs come in many different shapes and sizes. Despite their differences in appearance, all of the various types of WBCs have a role in the immune response. They circulate in the blood until they receive a signal that a part of the body is damaged. In response to these signals, the WBCs leave the blood vessel by squeezing through holes in the blood vessel wall. They migrate to the source of the signal and help begin the healing process.

1.2.3 Plasma Blood Cells (PBC)

Platelets which play a major role in the blood clotting mechanism.

1.3 THE CANCER HEALTH PROBLEM

Cancer is one of the most pervasive diseases and a major health concern all over the world. Each year, a lot of people die from cancer due to its high fatality ratio [8]. According to American Cancer Society, one in four deaths in this country is as a result of cancer [9] and this rapid growth will rise by 50% in the next few years due to population aging.

There is growing interest in developing efficient CTC separation techniques. There are technical challenges facing CTC separation devices such as extremely low percentage of CTCs in patient blood sample, different behavior of cancer cells and etc. Thus, more detailed studies of the cell process in such devices are required before designing and fabricating useful devices.

Sometimes cancer cells break away from the original tumor and travel to other areas of the body, where they keep growing and can go on to form new tumors. This is how cancer spreads. The spread of a tumor to a new place in the body is called metastasis.

The spreading of tumor cells is one of the primary causes of recrudescence at distant sites and of death from cancer. Thus, the detection of circulating metastatic cells is important to predict recurrence and improve survival [10]. Cancer cells usually congregate or come together to form tumors. The tumor becomes a growing mass of cancer cells that can destroy normal cells around the tumor and damage healthy tissues in the body. This can make someone very sick.

The most challenging goal in the Circulating Tumor Cells (CTC) field is their unbiased and reliable detection when they are extremely rare, namely at the beginning of the invasion process. At clinical level, this goal implies the possibility

to detect invasive cancers when they are still curable, raising the hope of tremendously reducing cancer mortality. At biological level, the initial spread of CTC may provide an outstanding source of material to understand the biology of early tumor invasion.

However, the equipment required for CTC detection system is very bulky, expensive, and difficult to operate at the point-of care. Therefore, the development of alternative sensitive, speedy, specific and low cost methods for CTC detection is important for cancer prognosis.

1.4 METHOD OF CELLS COUNTING (MCC)

Separation of cells is a critical process for studying cell properties, disease diagnostics, and therapeutics. There are several methods for cell counting. Some are primitive and do not require special equipment, so can be done in any biological laboratory, whereas others rely on sophisticated electronic appliances. Over the years researchers have come up with several ways to measure or count blood cells

1-Microscopic Method

2-Automatic Optical Method

3-Electrical Conductivity Method

The microfluidic device offer an alternative blood-separation approach to conventional plasma extraction techniques such as centrifugation, blood filtration or CD-like platforms. It depends in operation on several biomechanical separation

principles that are combined to produce a separation between plasma and whole blood within micro-channels [11],

Briefly, the three principles of separation are outlines as follows:

(a) Laminar flow: When blood flows at relatively low Reynolds number (0.01 to 1) in micro-channels of dimension comparable to the cells dimension, red blood cells (RBCs) exhibit a number of flow behaviors that causes the cells to concentrate at the center of a microfluidic channel, creating a plasma-rich layer adjacent to the channel walls.

(b) Flow focusing: The movement of RBCs in the blood flow is dependent on the magnitude of shear forces exerted on the cells. This relation has led to the assumption that the existence of a constriction in the blood flow will create a zone of high shear stress, which pushes the cells to the middle of the flow creating a flow-focusing effect as shown in Figure (1-1).

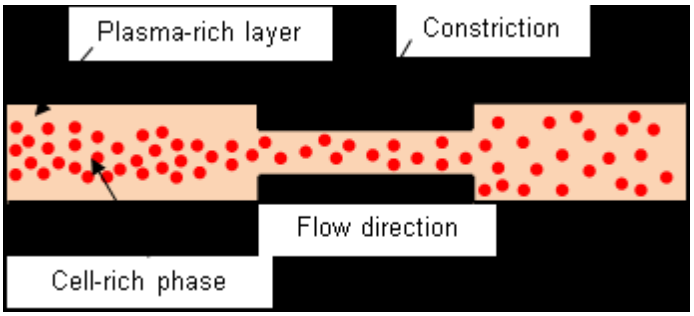


Figure (1-1): The flow-focusing effect pushes the blood cells to the middle of the flow [11]

(c) Bifurcations: RBCs exhibit a specific behavior in bifurcations. As shown in figure (2-1). At bifurcations, RBCs have the tendency to travel to the high-flow-rate channels, with the largest cross section, whilst plasma tends to move in low-

flow rate channels. One condition for this effect is that the flow rate ratio is at least 2.5:1

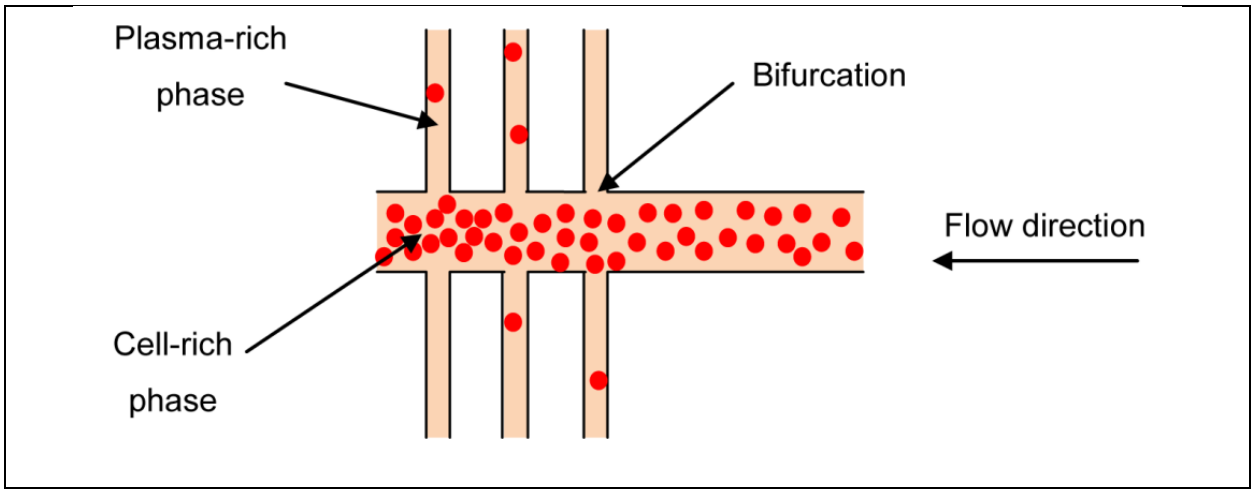


Figure (2-1): The illustration of the plasma-separation effect caused by bifurcations

1.5 THE FILTRATION PROCESSES

Filtration is any of various mechanical, physical or biological operations that separate solids from fluids (liquids or gases) by adding a medium through which only the fluid can pass. The fluid that passes through is called the filtrate [12]. In physical filters oversize solids in the fluid are retained and in biological filters particulates are trapped and ingested and metabolites are retained and removed. However, solids will be contaminated with some fluid and filtrate will contain fine particles (depending on the pore size, filter thickness and biological activity). Filtration occurs both in nature and in engineered systems; there are biological, geological, and industrial forms. For example, in animals (including humans), renal filtration removes wastes from the blood, and in water treatment and sewage treatment, undesirable constituents are removed by the filter medium, as in sand filtration.

Filtration, as a physical operation is very important in chemistry for the separation of materials of different chemical composition and widely used as one of the unit operations of chemical engineering. It is also important in filtration by sieving, where separation occurs at a single perforated layer (a sieve). Filtration can be done by adsorption by the effects of surface charge. Filtration for removal of magnetic contaminants from fluids with using of magnets (typically lubrication oil, coolants and fuel oils).

I.6 APPLICATION THE SEPARATION OF BLOOD

Microfluidic devices have several applications in different fields, such as chemistry, medicine and biotechnology. Many research activities are currently investigating the manufacturing of integrated microfluidic devices on a mass-production scale with relatively low costs. This is especially important for applications where disposable devices are used for medical analysis.

1.7 THE PROJECT OBJECTIVES

The main significant project objectives were focused on designing and manufacturing a microfluidic device for blood filtration and cells separation, which is including the following:

1- Study the works of the former researchers on developments that have been achieved in different aspects of microfluidic devices

1- Designing and fabricating of a low cost, easily operable, multifunctional device for the purification and separation of blood and other industrial components to fulfill the growing demands.

2- Conducting experiments to determine the efficiency of separation rates of separation.
3- The experimental results by were analyzing and improving by the statistical Expert 10.0 software programs.

CHAPTER TWO

MODERN MICROFLUIDIC BLOOD AND CANCER CELLS SEPARATION METHODS

2.1. INTRODUCTION

Separation of cells is a critical process for studying cell properties, disease diagnostics, and therapeutics. Many techniques are available to separate cells based on physical properties. Cell sorting offers a means to separate cells on the basis of their size and physical properties in a label-free, contactless, and biocompatible manner. The main blood separation methods are as in the following:

1- Separation of blood by filters.
2- Separation of blood by Microfluidic.
3- Cell separation using acoustic waves.
4- Separation of blood by Micro-magnetic.
5- Separation of blood by the electric field.

The need for efficient cell separation, an essential preparatory step in many biological and medical assays, has led to the recent development of numerous micro-scale separation techniques. This review describes the current state-of-the-art in microfluidics-based cell separation techniques. Some of these modern methods of separation will be explaining in the following items.

2.2 DETECTION OF CIRCULATION TUMOR CELLS (CTC)

Detection of circulating tumor cells (CTC) is a promising method for both diagnosis and clinical management of cancer patients through monitoring treatment efficacy before metastasis occur [13]. However, CTCs are extraordinarily rare

(estimated at one CTC per billion normal blood cells) which make it difficult to develop a suitable assay [14]. CTC detection techniques can be classified as biochemical and biophysical methods [8]. Biochemical methods are often based on biological markers or antibodies for recognition and separation of cysts. One of the major challenges in these methods is the heterogeneity and genetic instability of cancer cells that make it difficult or in some cases unlikely to find unique markers of cells [9]. Entirely on other biochemical approaches using physical markers such as cell size, shape, density and others to distinguish cysts from blood cells.

Hence, side filter detection method was development to improve the low purity of size-based filtration. The filters of device are installed on the side of main channel to wash out the captured cells that were not deformed into the trap and also to avoid direct strong flow. This approach could significantly enhance the detection of CTCs using filtration for cancer diagnosis and prognosis.

Recently, there have been increasing interests in the use of microfluidic techniques to develop free label devices. Using this technology, high capture efficiency and purity of isolation can be reached because it enables us to accurately control the device parameter in the cellular scale. Additionally, the microfluidics has a great advantage over working in the laminar flow system which provides more precise control over the process.

2.3 SEPARATION OF BLOOD CELLS BY MICROFILTRATION DEVICES

Filtration is any of various mechanical, physical or biological operations that separate solids from fluids (liquids or gases) by adding a medium through which only the fluid can pass. The fluid that passes through is called the filtrate [15], as shown in figure (2-1), where the oversize particles in the feed cannot pass through

the lattice structure of the filter, while fluid and small particles pass through, becoming filtrate.

Fluids flow through a filter due to a difference in pressure fluid flows from the high-pressure side to the low-pressure side of the filter, leaving some material behind. Pressure in the form of compressed air on the feed side (or vacuum on the filtrate side) may be applied to make the filtration process faster, though this may lead to clogging or the passage of fine particles.

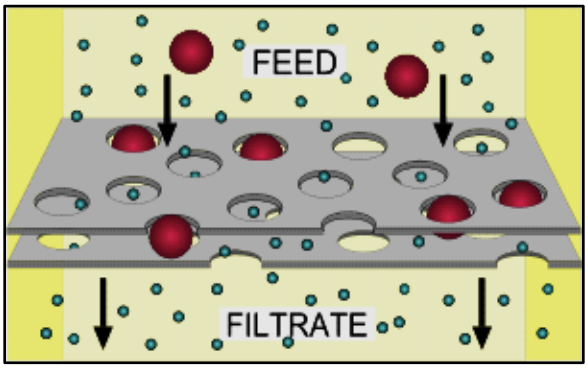


Figure (3-1): The Diagram of simple filtration

The microfiltration device is fabricated with standard soft lithography techniques from polydimethylsiloxane (PDMS) micro-channels bonded to semipermeable polycarbonate membranes coated with a solution of aminopropyltriethoxysilane (APTES) [16]. This approach was previously used and developed to separate plasma from whole blood cells using a single membrane [17].

In order to create the multi-compartment device, different pore sized membranes were aligned across the microfluidic structures in order to create the multi-compartment structure. A prototype device containing 3 membrane pore sizes of 5 μm, 2 μm and 400 nm is schematically shown in Figure (2-2). The device is designed so that the lowest stringency filtration will occur at the left inlet

reservoir with a progressively smaller pore sized membrane used in each compartment from the left to right through the device to increase the filtration stringency. This device can enrich different blood components such as platelets or white blood cells for clinical diagnostics**.**

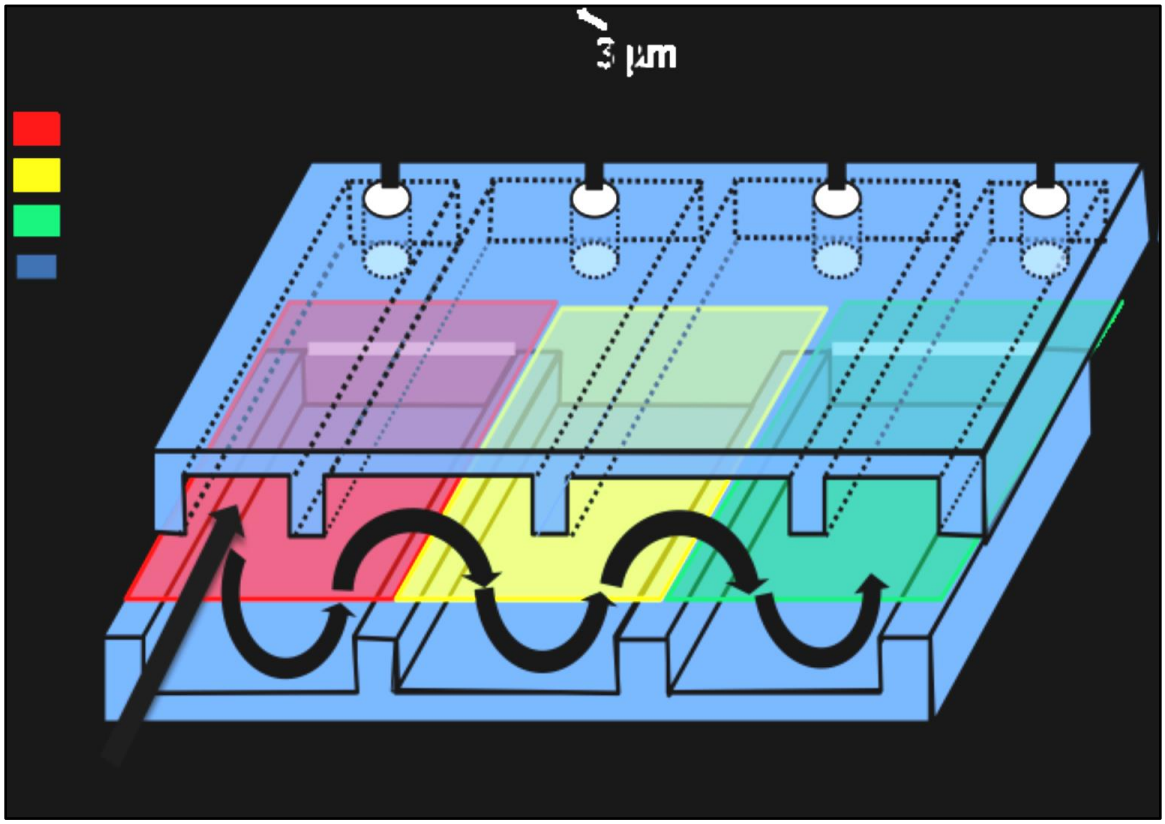


Figure (2-2): Schematic of the device showing the individual compartments and flow path to separate different compartments.

2.4 MICROFLUIDIC DEVICES FOR BLOOD FRACTIONATION

Microfluidics-based sorting offers numerous advantages, including reducing sample volumes, faster sample processing, high sensitivity and spatial resolution, low device cost, and increased portability. The techniques presented are broadly classified as being active or passive depending on the operating principles [18].

Microfluidic methods realized to successfully fractionate one or more blood components. The most common way of separating plasma from blood cells using microfluidic devices is based on the principle of particle retention. These devices frequently involve the use of micro-machined filters or meshes to retard or block blood cell movement, thereby allowing the collection of the plasma portion of blood.

In particular, comb and weir-type filter structures with well-defined geometries have been designed and demonstrated [19-21]. Designing weirs with a gap size of 0.5 μm a cross-flow based microfluidic device for continuous plasma separation [20]. The 0.5 μm gap permits the flow of plasma while impeding the RBCs, leukocytes and platelets. The device is capable of operating continuously for an hour extracting ~8% plasma at 0.65 μL/min throughput and <0.1% hemolysis.

The microfluidic device design consists of arrays of micro-pillar structures placed within the main flow channel leading to the formation of multiple cell streams based on size. Cells larger than the critical diameter $dc = 20\% \times 2w$ (w is the separation gap between two adjacent pillars) follow a deterministic path while smaller cells remain unperturbed moving in an average downward flow direction.

Several microfluidics devices have been developed to isolate RBC using intrinsic markers such as size and deformability, and hemoglobin content. The RBC sizes are around 9–12 μm and similar in size with leukocytes (10–15 μm), but larger than bulk of the RBC (~6–8 μm in diameter). A simple micro-device consists of an array of micro-pillars of different gap sizes (2.5 to 15 μm) to separate the fetal RBC from blood based on size and deformability [22].

Figure (2-3) shows the cells separation from blood using microfluidics. the schematic (A) of the cross flow filtration principle for RBCs separation. RBCs are continuously filtered out through the inclined filters with a 4 μm gap while the bigger RBCs cannot deform through the filters and are made to roll along the filter upstream and collected separately [23]. Schematic design (B) of a microfluidic device for CTCs isolation and enumeration. Placing crescent-shaped cell traps in the isolation regions traps the larger tumor cells while allowing smaller blood components to sieve through [24]. The layout (C) of the CTC chip designed consisting of functionalized micro-post array for affinity capture of CTCs. Inset showing a scanning electron micrograph of a lung cancer cell captured between two micro-posts [25]. The (D) schematic illustrating the separation principle using a micromagnetic-microfluidic

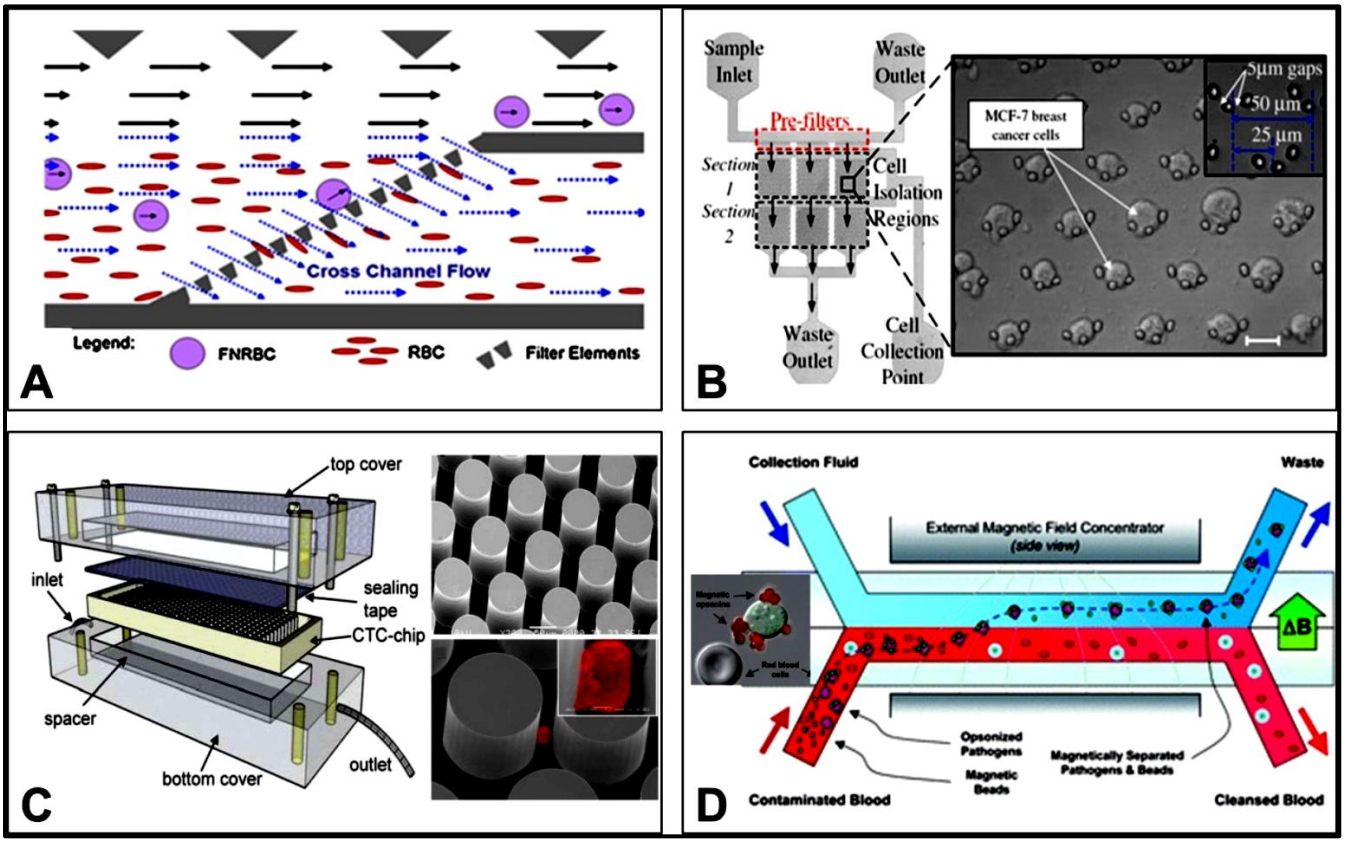


Figure (2-3): The microfluidics cells separation from blood methods

based blood cleansing device to selectively remove pathogens from contaminated blood by create magnetic opsonins which are removed continuously in the presence of magnetic field gradients [26].

2.5 SEPARATION OF BLOOD CELLS USING ACOUSTIC WAVES

Cell sorting by acoustic waves offers a means to separate cells on the basis of their size and physical properties in a label-free, contactless, and biocompatible manner [27]. The acoustic separation has not been widely used in practical cell-separation applications due to their relatively low separation sensitivity and efficiency however, thereby restricting their widespread application in research and health diagnostics. Surface acoustic waves (SAW), which are oriented at an optimally designed inclination to the flow direction in the microfluidic channel. Acoustic separation is often achieved by establishing a standing acoustic field within a flow channel for successfully separated 2- and 10-μm diameter polystyrene beads with a separation efficiency of ∼99%, and separated 7.3- and 9.9-μm-polystyrene beads with an efficiency of ∼97%. The effectiveness of the present technique for biological–biomedical applications by sorting MCF-7 human breast cancer cells from nonmalignant leukocytes, while preserving the integrity of the separated cells.

This method offers a route for separating circulating tumor cells, and for label-free cell separation with potential applications in biological research, disease diagnostics, and clinical practice.

The microfluidic channel consists of three inlets and two outlets. The pair of IDTs were deposited in a parallel arrangement with respect to each other, and

aligned at a specific angle with respect to the channel and flow direction. A radio frequency (RF) signal was imposed at each IDT to generate two identical surface acoustic waves (SAWs). These two SAWs propagate toward each other and interfere to form an SSAW in between the IDTs located within the micro-channel as shown in figure (2-4), where figure (A) shows the SAW-based cell-separation device, while (B) and (C) show the separation process for 10- and 2-μm-diameter polystyrene beads in the SAW working region and the outlet region, respectively [28].

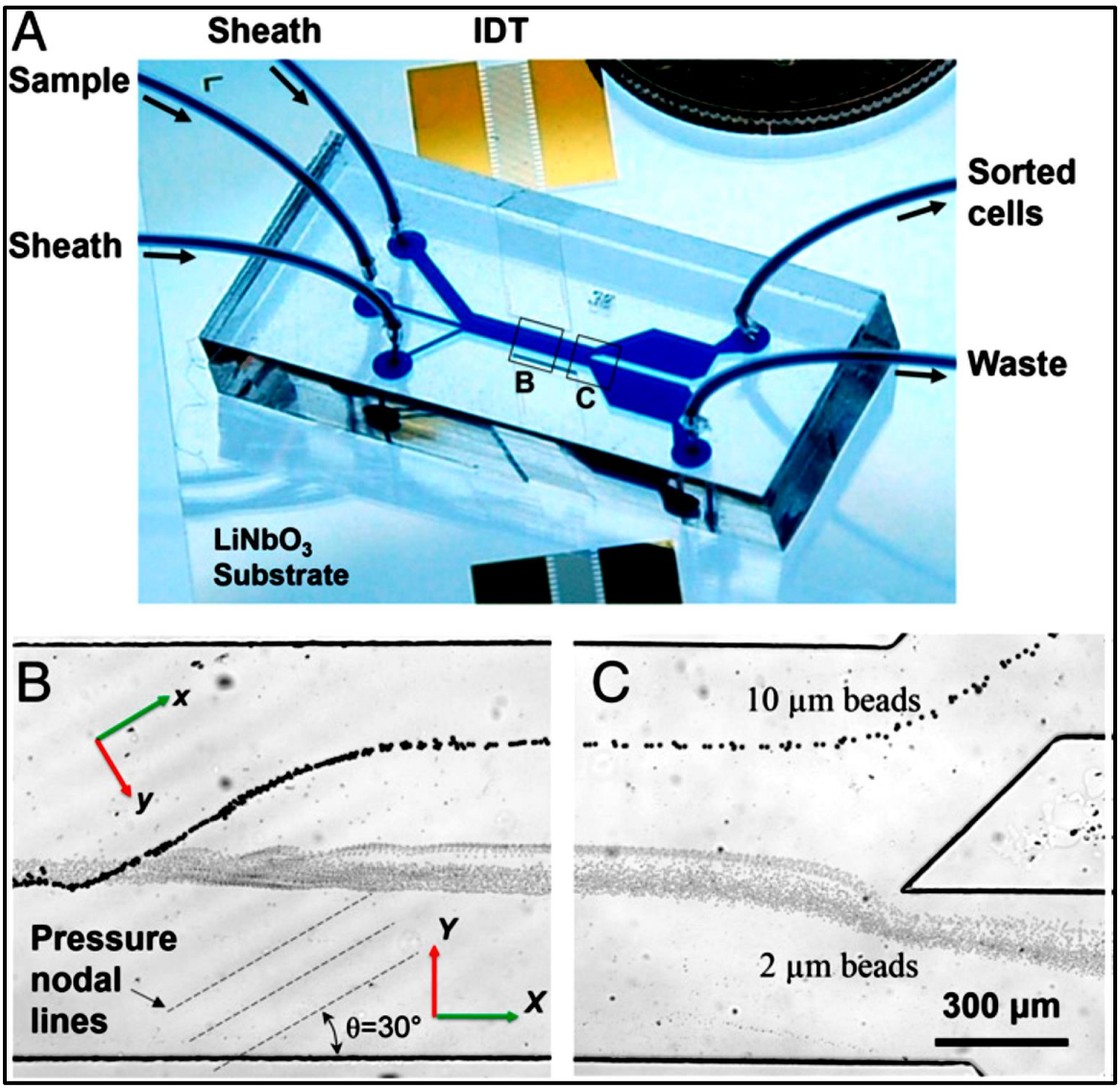


Figure (2-4): The surface acoustic waves (SAWs) schematic working principle and device structure.

2.6 MAGNETIC ACTIVATED MICRO-CELL SORTERS

Magnetics based flow detection can enable several improvements in cytometry-based analyses. Magnetic detectors are extremely small (tens of micrometers) and rugged, representing an intriguing opportunity to reduce the size and complexity of cytometers for field-deployment. The small footprint also points to the potential to create highly multiplexed systems with hundreds of parallel channels and dramatically increase sample throughput.

Table (2-1): The microfluidic activated micro-cell sorters for isolating low cancer cells

Cell capture structures	Targeted cells	Carrier medium and control cells	Target cell recovery rate	Capture purity
A nickel micropillar array + magnetic beads functionalized with wheat germ agglutinin	Human lung cancer cells of A549	Human RBCs + culture medium	62% - 74%	93% (initial ratio A549:RBCs 1/4 1:10)
A paramagnetic array of 80% Ni and 20% Fe^{+} magnetic beads complementary to anti-CD10 antibodies in chamber 1 and anti-PSMA antibodies in chamber 2	Human prostate cancer cells of LNCaP incubated with PSMA antibodies	LNCaP incubated with CD10 antibodies + PBS	50% - 70% of LNCaP incubated with PSMA antibodies in chamber 2	10% of LNCaP incubated with CD10 antibodies in chamber 2 (initial mixture ratio of 1:1)
External permanent magnet + magnetic beads coated with Anti-EpCAM (Ber-EP4)	Human ovarian cancer cells of BG-1 and lung cancer cells of AS2	Blood samples from healthy donors (10^6 cells per ml)	95.1% for BG-1 cells and 92.7% for AS2 cells	NA
An array of magnetic dots + self-assembled magnetic beads coated with anti-CD19 antibodies	Human lymphoma cells of Raji CCL-86	Human lymphoma cells of Jurkat TIB152 + PBS (2×10^6 cells per ml)	97% ± 2% of Raji cells	<2% (capture of Jurkat TIB152 cells)
An array of magnetic dots + self-assembled magnetic beads coated with anti-CD19 antibodies	B-cell hematological malignant tumors (leukemia and lymphoma)	Clinical samples (blood, pleural effusion, and fine needle aspirates) from chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma and two healthy volunteers	Consistent immunophenotype and morphology results with those obtained by flow cytometry	
External permanent magnet + self-assembled magnetic bead patterns coated with 5D10 antibodies	Human breast cancer cells of MCF-7	Human lymphoma cells of Jurkat TIB152 + PBS (10^6cells per ml for both MCF-7 and Jurkat cells)	85% ± 10% of MCF-7	<5% (capture of Jurkat TIB152 cells)

Magnetic activated cell sorting relies on the interaction between cell surface antigens and antibodies conjugated to suspended magnetic particles

Compared to cell-affinity micro-chromatography, where the retrieval of captured cancer cells can be difficult, magnetic bead-based techniques readily permit the manipulation of captured cancer cells using local magnetic fields. Table (2-1) demonstrated the first microfluidic device for isolating low abundance cancer cells from a red blood cell (RBC) suspension using magnetic cell separation.

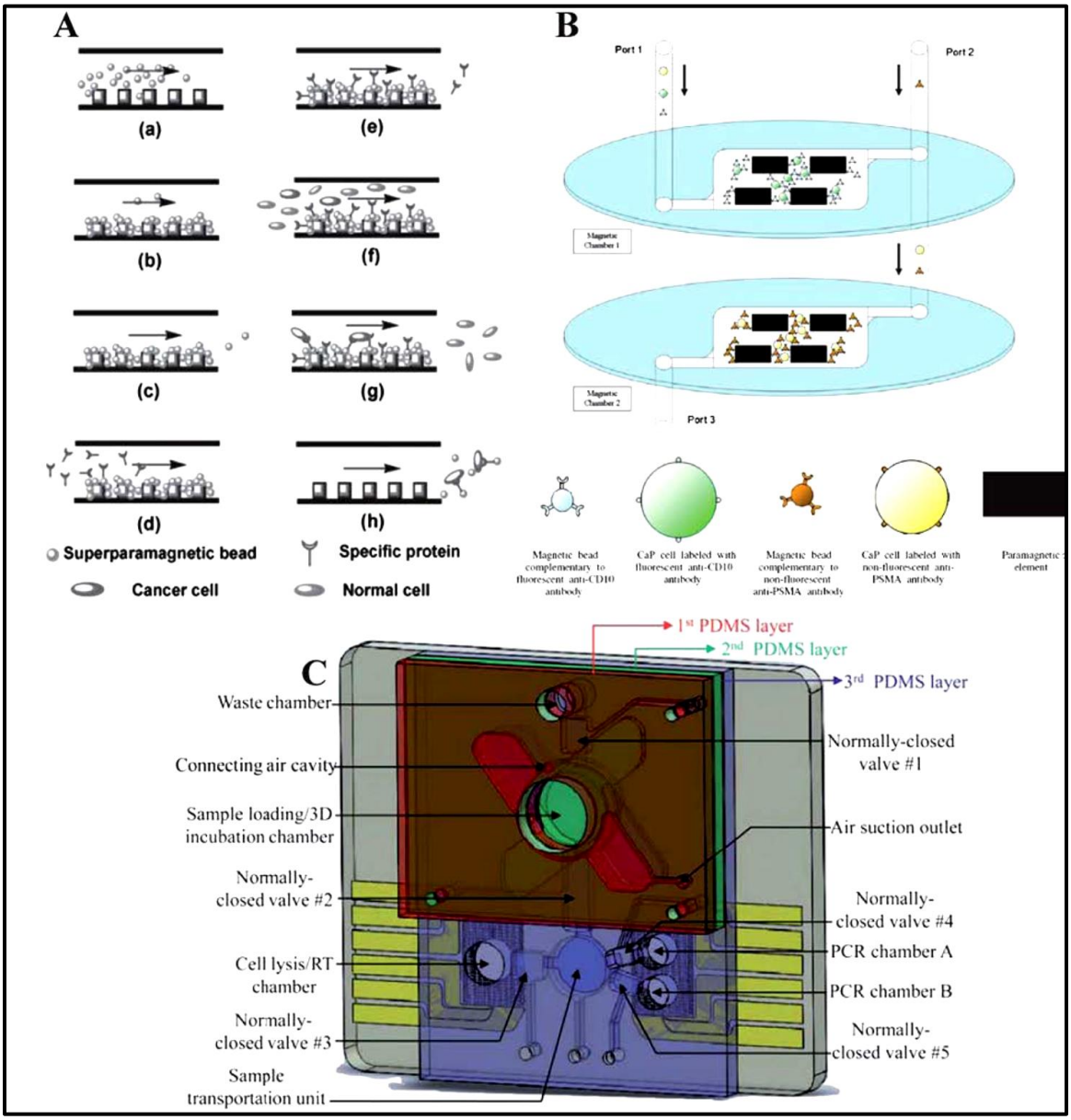


Figure (2-5): The magnetic activated micro-cell sorters.

Figure (2-5) shows a magnetic activated micro-cell sorters. Figure (A) shows a step by step illustration of the first magnetic activated micro-cell sorter for cancer cell capture. In this system, a hexagonal array of nickel micro-pillars was integrated onto the bottom of a micro- fluidic channel and used to generate magnetic field gradients to efficiently trap super paramagnetic beads.

Figure (B) demonstrated the schematic of a microfluidic device for serial selection of cellular subpopulations by the use of antibody-coated magnetic beads. Figure (C) shows the integrated magnetic-based cancer cell capture platform, consisting of an incubator for the magnetic beads to capture cancer cells, a control module for sample transportation, and a nucleic acid amplification module for cell lysis and genetic identification.

2.7 DIELECTROPHORESIS (DEP) BASED CANCER CELL SEPARATION

Dielectrophoresis (DEP) uses the polarization of cells in non-uniform electrical fields to exert forces on cells. DEP forces depend on factors such as cell membrane and cytoplasm electrical properties as well as cell size. Table (2-2) illustrated devices have been developed for separating cancer cells, based on differences in cells' response to electric fields [29].

A simplified microfluidic device for particle separation is designed by using two consecutive steps:

- Induced charge electro-osmotic prefocusing and
- Dielectrophoretic separation:

Continuous dielectrophoretic separation is recognized as a powerful technique for a large number of applications including early stage cancer

Table (2-2): the DEP based cancer cells separation microfluidic devices

Cell capture structures	Targeted cells	Carrier medium and control cells	Target cell recovery rate	Capture purity
An electrode affinity column with interdigitated micro-electrodes	Human leukemic cells of HL-60	Blood cells + sucrose solution	NA	~80% (initial mixture of 2×10^7 HL-60 and 3×10^7 blood cells)
A dielectric affinity column with interdigitated micro-electrodes	Human breast cancer cells of MDA-231	Blood samples + sucrose solution	NA	~95% (initial mixture of 1×10^7 MDA-231 and 3×10^7 blood cells)
A dielectric affinity column with an interdigitated micro-electrodes	Human breast cancer cells of MDA-231	Blood samples + sucrose solution	>95%	NA
A dielectric affinity column with reconfigurable electrodes	Human cervical cancer cells of HeLa	Human peripheral blood cells + sucrose solution	NA	NA
A dielectric affinity column with a micro-electrode array	Human monocytic cells of U937, lymphoma cells of Jurkat, HTLV-1, tax-transformed human T cells of Ind-2, glioma cells of HTB, and neuroblastoma cells of SH-SY5Y	Peripheral blood mononuclear cells + sucrose solution	47% - 79%	>95%
DEP field flow fraction with interdigitated electrodes	Human leukemic cells of HL-60	WBCs from blood samples + sucrose solution	NA	NA
DEP field flow fraction with interdigitated electrodes	Human breast cancer cells of MDA-435	Hematopoietic CD34+ stem	NA	>99% (initial MDA-435: stem cells = 2:3)
DEP field flow fraction with interdigitated electrodes	Human breast cancer cells of MDA-435	Hematopoietic CD34+ stem cells + sucrose solution	NA	>99% (initial MDA-435: stem cells = 2:3)
DEP field flow fraction with interdigitated electrodes	Human breast cancer cells of MDA-435	Blood samples + sucrose solution	NA	>98% of MDA-435 (initialMDA-435: blood cells = 2:3)
DEP field flow fraction with interdigitated electrodes	Human breast cancer cells of MDA-435, MDA-468 and MDA-231	Peripheral blood mononuclear cells + sucrose solution	>90%	NA
Microscope slides coated with electrode arrays with changing frequencies	Human breast cancer cells of MDA-435 and leukemic cells of HL-60	Blood samples + sucrose	NA	NA
Microscope slides coated with electrode arrays with changing frequencies	Cancer cells from biopsy	Biopsied cells + sucrose solution	NA	NA
3D-asymmetric micro-electrodes with a continuously varied electric field	Mouse P19 embryonic carcinoma cells	Mouse RBCs + PBS	NA	81.5% ± 7.6% of P19 EC and 94.1% ± 4.3% RBCs (initial ratio 1:1)
3D-asymmetric micro-electrodes with a continuously varied electric field	Human breast cancer cells of MCF-7 and MCF-10A	PBS	86.67% of MCF-7 and 98.73% of MCF-10A	NA
DC-dielectrophoresis	Fixed WBCs and human breast cancer cells of MCF-7	Trehalose solution	NA	NA
Guided DEP with a pair of planar electrodes	Human leukemic cells of Jurkat and cervical cancer cells of HeLa	Sucrose solution	NA	NA
Planar interdigitated Microelectrodes	Clones of mouse melanoma B16F10 cells	Sucrose solution	NA	NA
A planar electrode pair with	Human colorectal cancer cells			

diagnosis, water quality analysis, and stem-cell-based therapy. Generally, the prefocusing of a particle mixture into a stream is an essential process to ensure all particles are subjected to the same electric field geometry in the separation region. However, accomplishing this focusing process either requires hydrodynamic squeezing, which requires an encumbering peripheral system and a complicated operation to drive and control the fluid motion, or depends on dielectrophoretic forces, which are highly sensitive to the dielectric characterization of particles.

An alternative focusing technique, induced charge electro-osmosis (ICEO), has been demonstrated to be effective in focusing an incoming mixture into a particle stream as well as nonselective regarding the particles of interest. Encouraged by these aspects, a propose a hybrid method for micro-particle separation based on a delicate combination of ICEO focusing and dielectrophoretic deflection. This method involves two steps: focusing the mixture into a thin particle stream via ICEO vortex flow and separating the particles of differing dielectric properties through dielectrophoresis as shown in figure (2-6).

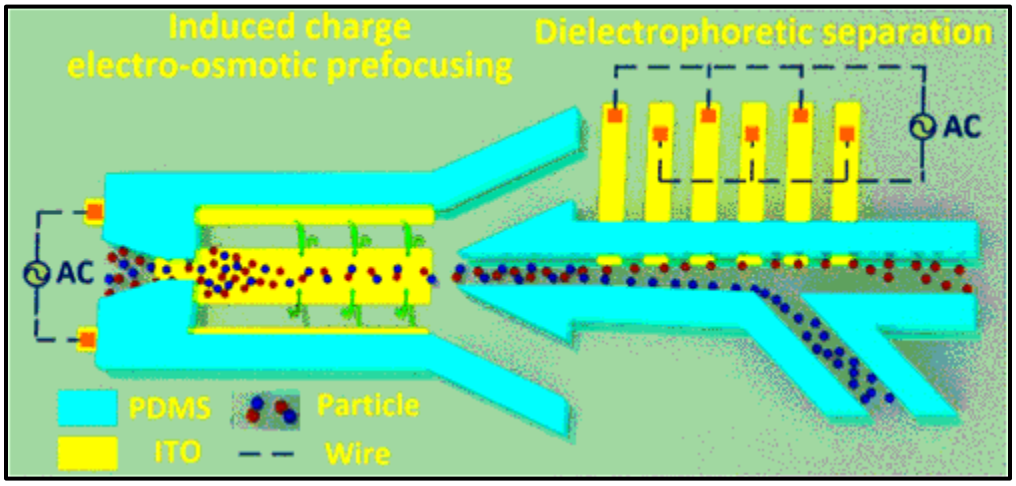


Figure (2-6): The hybrid method for micro-particle separation based on a delicate combination of ICEO focusing and dielectrophoretic deflection

Figure (2-7) shows the microfluidic DEP devices for cancer cell separation in which human leukemia cells suspended within normal blood cells were retained on microelectrode arrays while normal blood cells were eluted [29]. The cancer cells were subsequently released for collection by the removal of the DEP field. Figure (A) shows a dielectric affinity column for cancer cell separation where large cancer cells are trapped on electrode tips while small blood cells are eluted. Figure (B) demonstrated the DEP-FFF combines DEP, sedimentation and hydrodynamic forces to influence cell positions in the hydrodynamic flow profile. Figure (C) shows a 3D-asymmetric microelectrode system for DEP cell separation and figure (D) shows a continuous separator integrates multiorifice flow fractionation and DEP.

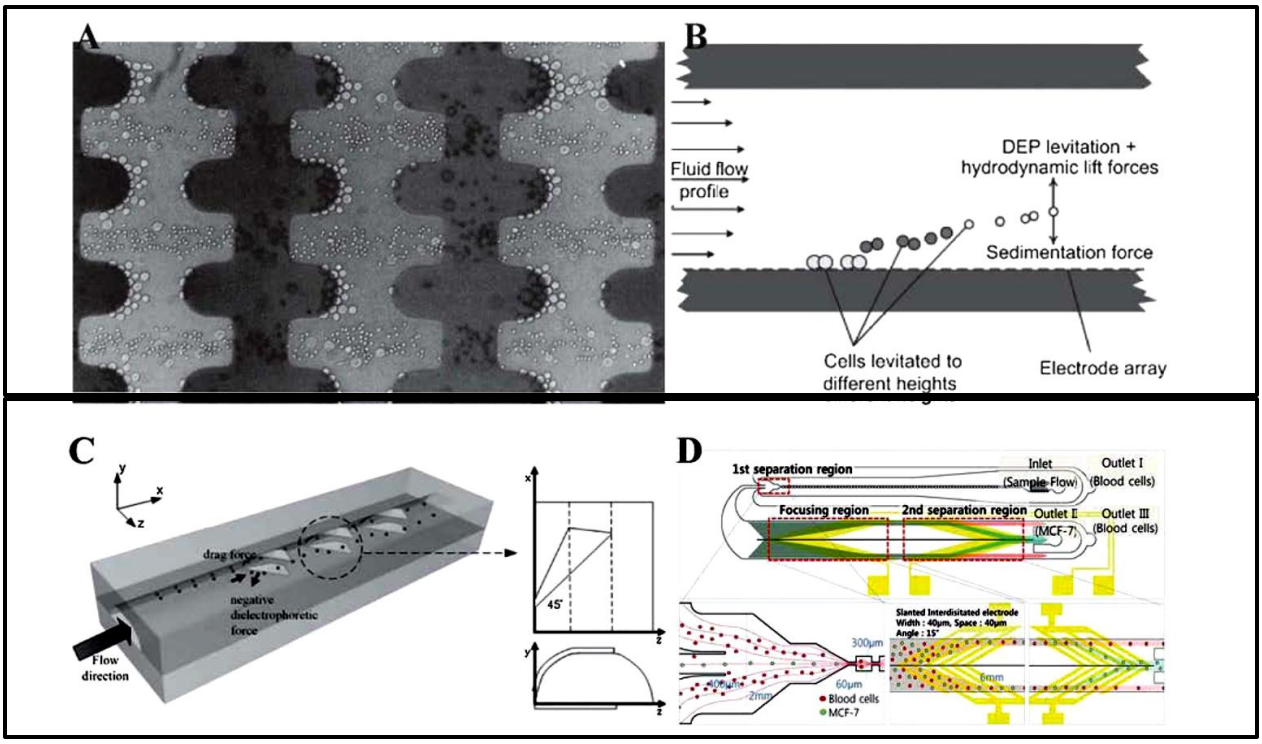


Figure (2-7): The microfluidic DEP devices for cancer cell separation.

CHAPTER THREE

DESIGN AND FABRICATION OF MICROFLUIDIC BLOOD CELL FILTRATION AND SEPARATION DEVICE

3.1 INTRODUCTION

Microfluidic cell separation and sorting techniques have been studied with relevance to cell biology researches or various diagnostic and therapeutic applications. This work describes the development of a label free, low cost, high throughput device which uses cross flow filtration in order to fractionate particles based on size.

The device is a multi-compartment device with porous membranes of varying pore sizes incorporated between the compartments within the microfluidic device to perform staged blood cells filtration and separation, where nonhomogeneous cell mixtures are fractionated into different compartments and collected for further analysis. By varying the pore size between compartments, cells and particles larger than the pores cannot pass through the membrane to the adjacent compartment and are selectivity enriched. This device was tested by infusing a mixture of polymer particles of varying nano and micro sizes samples. The sizes of the powder molecules are simulate the sizes and the types of blood and cancer cells and pathogenic viruses to examine size selectivity and particle recovery efficiency where cancer, white, red and platelets blood cells and were selectivity separated and enriched.

Various lab on a chip devices have been developed recently to detect and separate circulating tumor cells (CTCs) detach from a primary tumor structure and circulate in the blood, spreading to other parts of the body, which is the major cause of metastasis [2, 3]. Detection and enumeration of these cells in the early stages of cancer can be used for screening, diagnosis, and prognosis, and isolated cells can also be used to test the efficacy of personalized cancer treatment. Although current clinical techniques for isolating CTCs from whole blood are introduced by flow cytometry, density gradient centrifugation, and immune-affinity capture using magnetic beads, [11]. These techniques require pretreatment processing of samples, which can cause cell loss and is expensive and labor intensive.

Microfluidics is a well-suited technique for cell separation because of their favorable properties such as low cost, simple procedure, and small amount of space needed and also allows better control of the microenvironment during the cell separation. Immunoaffinity-based capturing approach has been reported for isolation of CTCs with microchips containing micro posts and herringbone structures coated with a human epithelial cellular adhesion molecule (EpCAM) antibody [13]. Although the device shows effective capturing of CTCs, it must be operated slowly to maintain capture efficiency and the retrieval of viable CTCs is difficult due to the strong binding of cells to the micro post surface. Because the number of CTCs in the blood is very small compared to the number of other blood cells, as small as one CTC per billion blood cells.

CTC isolation technique requires high throughput to process blood samples on the milliliter scale with high recovery [15]. CTC capturing methods using

immunomagnetic nanoparticles demonstrated successful capturing at high throughput. However, such methods require dilution of the blood sample and washing steps, which essentially increase the total volume of the sample that needs to be processed, and possibly cause rare cell loss during the washing steps. Also, to effectively capture rare cells that exist among billions of other blood cells, the amount of magnetic particles required can significantly increase, depending on the amount of blood sample that needs to be treated. Common biological characteristic of CTCs is their diameter (15−30 μm), which is generally larger compared to that of other blood cells (2−12 μm) [18], which gives the possibility of size-based separation. Micro filtering approaches with a cutoff pore size of approximately 8, 5, 2 and 0.4 μm have been demonstrated with a high flow rate and high enrichment concentration. To overcome the shortcomings of immunoaffinity-based capturing or filtration method to isolate and recover CTCs, a label-free and continuous high throughput separation technique is needed in the microfluidic separator.

Recent advances in inertial microfluidics using inertial effects such as inertial lift force and Dean flow have allowed the continuous high throughput size-based cell sorting without using external forces. Some inertial separators for isolating cancer cell lines as models of CTCs have been reported in particular geometries such as straight, spiral, and multi orifice structures, using migration differences according to the cell size and cell deformability. In the straight channel, cells being influenced by shear-induced inertial lift force and wall-induced inertial lift force result in distinct equilibrium positions, depending on their size and deformability. Using this mechanism, various cancer cell lines were isolated from diluted blood cells, resulting in a cancer cell recovery rate.

3.2 Governing Equations for Blood Flow

Blood was treated as a multi-component system, comprised of red blood cells (RBC) and plasma. White cells (WBC) are considered too dilute to affect the flow system. The plasma was treated as a Newtonian fluid.

For unclogged flow of blood sample through the device, the total inlet pressure P_{total} applied on the device should overcome two main pressure drop components: pressure drop due to flow of the medium and pressure drop due to surface tension of the cells when they are in contact with the wall of microfilter Or to overcome two types of resistances: 1) viscous resistance and 2) resistance caused by surface tension, which acts to preserve the integrity of the cell surface [19-20]:

$$\Delta P_{total} = \Delta P_{flow} + \Delta P_{surface\ tension} \quad (1)$$

3.2.1 Pressure drop due to flow (or viscosity) of the medium (ΔP_{flow} or P_{vis})

The pressure drop due to flow of medium mainly consists of the pressure drop due to viscosity (ΔP_{hyd}) and the pressure drop due to contraction and expansion at microfilter ($\Delta P_{contraction-expansion}$):

ΔP viscous dissipation of mechanical energy of the fluid due to internal friction results in a pressure drop in the flow direction. This pressure loss can be calculated using Hagen-Poiseuille law [40], which relates the viscous pressure drop and flow rate of the flow by introducing hydraulic resistance factor. The Hagen-Poiseuille law is as follow:

$$\Delta P_{flow} = \Delta P_{hyd} + \Delta P_{contraction-expansion} \quad (2)$$

where, ΔP_{hyd} is the viscous pressure drop of the conical channel, R_{hyd} is the hydraulic resistance, and Q_v is the volume flow rate. Akbari et al [41] developed a

general model for predicting low Reynolds number flow pressure drop in non-uniform microchannels. Based on their model, the hydraulic resistance is calculated from the following relationship:

$$\Delta P_{hyd}=R_{hyd}\times Q_v \tag{3}$$

$$R_{hyd} = 16\pi^2\mu \int_{x1}^{x2} \frac{I_P^*}{A(x)^2} dx, \tag{4}$$

where μ is the viscosity of the fluid, $A(x)$ is the cross sectional area, and $I_P*=I_pA^2$ with $I_p= \int (y^2+z^2)\, dA$ is called the specific polar moment of cross-sectional inertia. Based on the geometry of conical channel, $I_P*=2/\pi$ and A(x) can be calculated as follows:

Using equations $(4-5)$, the hydraulic resistance over the length of the channel can be determined by the following formula:

$$A(x)=\pi r(x)^2= \pi(Ltan(\theta)+Ra-xtan(\theta)) \tag{5}$$

$$R_{hyd} = \frac{8\mu}{3\pi \tan(\theta)} \left(\frac{1}{R_a^3} - \frac{1}{(Ra+Ltan(\theta))^3}\right). \tag{6}$$

In addition, $\Delta P_{contraction-expansion}$ is caused by sudden contraction at the inlet of the filtering channel and sudden expansion at the outlet of filtering channel which can be calculated using the following relation:

$$\Delta P_{contraction-expansion} = K_1 \frac{\rho U_1^2}{2} + K_2 \frac{\rho U_2^2}{2}. \tag{7}$$

where U_1 and U_2 are the flow velocity at the inlet and outlet of the filtering channel, K_1 is the constriction coefficient and K_2 is the expansion coefficient. These coefficients are set to be 0.5 and 1 respectively. It is worth to mention that pressure drop in the entrance and outlet chambers are not considered since they are negligible in comparison to two other types.

3.2.2 Pressure drop due to surface tension (P_{sur})

Calculating the maximum pressure due to surface tension which is also referred to threshold pressure depends on whether cell is going in the forward direction or reverse (backward) direction as shown in figure ((3-1) a and b)), respectively in an alternating pressure field. Figure(3-1) shows the dissection of cell going through the filtering channel at critical moment at two possible directions.

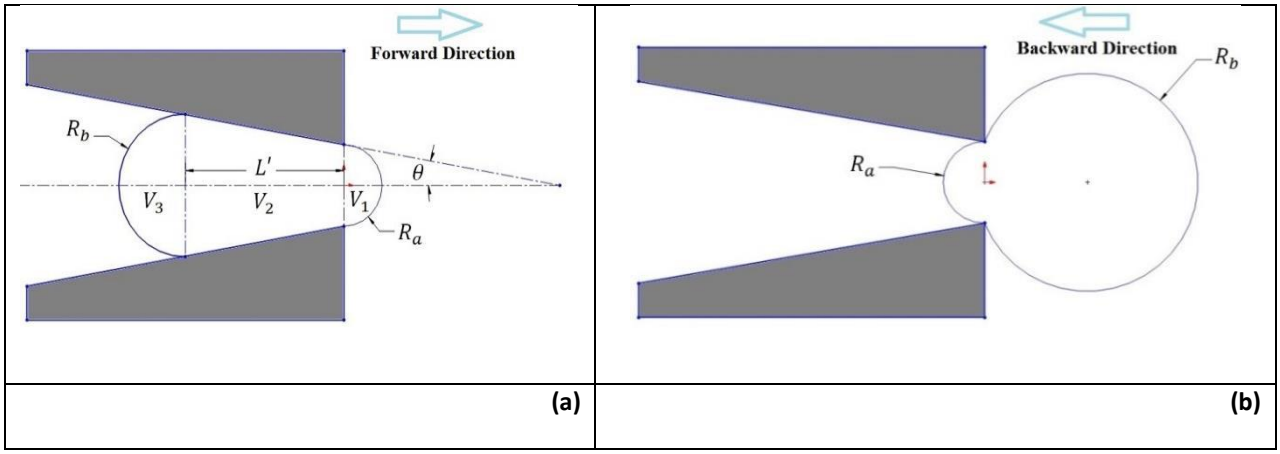


Figure (3-1): The 2D Cross section of microfilter at critical moment (pressure), (a) Forward passing direction (b) Backward passing direction

For both cases, the threshold pressure can be calculated using Laplace law, which relates the pressure difference between inside and outside of the cell to the cortical tension of the cell membrane by radius of curvature of the cell in the critical position.

$$P_{threshold} = 2\sigma\left(\frac{1}{R_a} - \frac{1}{R_b}\right), \quad (8)$$

where σ is the surface tension of the cell membrane and Ra and Rb are the curvature radius of the leading and trailing edge respectively.

In both cases, threshold pressure occurs when the length of extension of the drop (R_a) out of filtering channel (forward direction) or into the filtering channel (backward direction) equals the radius of the filter pore radius ($D/2$). Therefore by knowing the surface tension of the cell, the only unknown parameter in equation (8) is R_b which is different for each case.

CHAPTER FOUR

EXPERIMENTAL WORK

4.1 INTRODUCTION

Cell separation devices are used in various biomedical applications to identify individual cell types within a mixed population, enrich a single cell type into subpopulations of similar types for downstream diagnostic analysis or therapeutic applications. Blood analyses, in particular, requires the separation of different components such as red blood cells, (RBCs), white blood cells, (WBCs), platelets and cell-free plasma for analysis or DNA purification from WBCs.

In this work, we present the development of a label free, high throughput, micro-device using a staged cross flow filtration module which fractionates particles based on size. The separation mechanism of the devices uses different pore size membranes incorporated between each compartment as physical barriers to fractionate particles from the sample specimen so that the size of particles which are to pass through to, or to are blocked from the next compartment is controlled and subpopulations can be collected for further analysis.

The fabricated device is configured in three different methods of filtration and separating of cancer and blood cells or any other liquid chemical substances. And these methods are:

1-Parallel microfluidic methods for blood and cancer cell filtration and separation.

2-Vertical microfluidic methods for blood and cancer cell filtration and separation.

3-Microfluidic method for blood white cell (BWC) separation.

4.2 FABRICATION OF BLOOD AND CANCER CELL SEPARATION DEVICE

The fabricated of the designed microfiltration device consists of a sub-device for collection and mixing of blood or any chemical substance whose molecules are to be separated. The device also consists of the air pressure group to generate the appropriate pressure for the separation process. The device as has been noted in advance were contain of three sub-devices for the separation and purification of blood cells, and each contains of a set of micro filters, connection tubes and control valves for controlling the blood passes during the work of these sub-devices.

The device contains of a power supply and electronic circuits to generate ultrasonic waves, which helps in the separation process in some stages of the operation of the device. The device contains a number of containers to collect blood, ventilation and balancing air pressure tubes. The parts and components of the cell separation device were assembled on a 700x500x100 mm structure. The device structure consists of a total transparent assembled from the polymer panels of Perspex thickness of 5 mm, selected for easy washing and design to suit the sterilization requirements within the protocols (FDA) for food, drag and association and ISO organization. The complete designed and fabricated device is shown in figure (4-1). The main components and specifications of the main parts of the fabricated separation device are:

4.2.1 Collection and Mixing of Blood Sub-Device

This sub-device contains the following parts:

1- Blood mixed pump type 4XD2 for mixing the blood sample with a duration time of 5 minutes to obtaining a homogenous property blood or fluid mixture, as shown in figure (4-2) with the following technical specification:

- Structure: Diaphragm vacuum pump

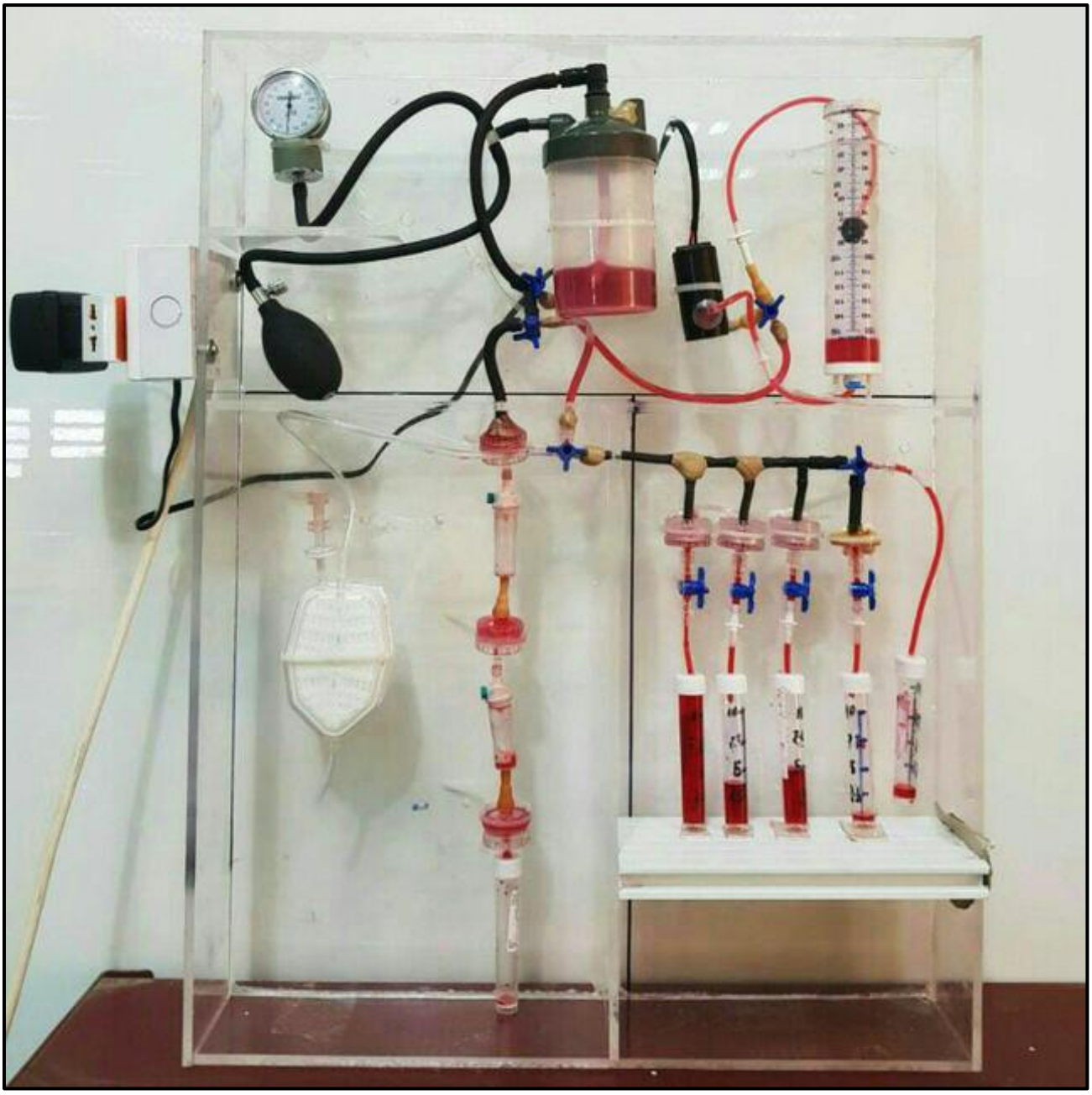


Figure (4-1): The designed and fabricated designed microfiltration device for blood and cancer cell filtration and separation

- Rated voltage: DC 12V
- Rated current: 350 mA
- Maximum vacuum: 420 mm Hg

Figure (4-2): The blood mixed pump type 4XD2

2- Blood container capacity (Infusion Set – Burette) 150 ml for blood mixing as shown in figure (4-3).

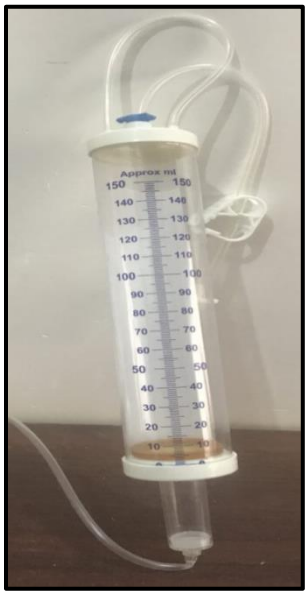


Figure (4-3): The blood container capacity 1500 ml for blood mixing

3- Connection 4mm inner diameter tubes, joints and 3-way control valves for controlling the blood flowing.

4.2.2 Air Pressure Generating Sub-Device

This sub-device contains the following parts:

1- Medical pressure infusion bag, with pulse injection pressure, 500 ml capacity, with gauge 0 - 500 mbar and hand pump ball with spoon as shown in figure (4-4) and the main technical specification are given in table (4-1). This device is used to generate the appropriate pressure to achieve the best quantities and specifications for isolating the cells according to experimental procedures prepared for each experiment.

Figure (4-4): The medical pressure infusion bag, 500 ml capacity

Product Name:	Aneroid Sphygmomanometer with Stethoscope	Bladder:	PVC/Latex bladder with two tube,size 22X12cm
Model Number:	BK2001-3001	Carrying Bag:	069 leather bag
Measure scope:	0-300mmHg	Stethoscope:	Single head stethoscope
Accuracy:	+/-3mmHg	Chest piece:	Aluminum chest piece
Sub-division:	2mmHg	Y-tube:	60cm long Y-tube
Aneroid Gauge:	110g/120g/140g	Color:	black,blue,grey,red,green etc
Cuff:	023 Nylon/cotton cuff with/without D-ring 48X14cm/51X14cm		

Table (4-1): The main technical specification of the medical pressure infusion bag, 500 ml capacity

2- Drainage seal container used to collection the liquid blood after mixing. It consists of three gates as shown in figure (4-4). The first is the blood inlet from

the blood mixing of sub-device, the second for pressurized blood outlet to the separation and filtration systems and the third for the pressurized air inlet. It is fabricated from polyethylene polymer with a collecting capacity of 500 ml. It has been modified to fit the basic design of the separation devices systems.

Figure (4-5): The drainage seal container used to collection the liquid blood after mixing

3- Connection 4mm inner diameter tubes, joints and 3-ways control valves for controlling the blood flowing.

4.2.3 Horizontal microfluidic blood and cancer cell filtration and separation system

This Blood separation system analyses contain of four horizontal in parallel micro-filters stages for separation the different cancer and blood components. The first micro-filter of a pore size 13-16 µm is used to separation white blood cells (WBCs) from the cancer cells. The second micro-filter of a pore size 8-12 µm is

used to separation the red blood cells (RBCs). The third micro-filter of a pore size 3-5 µm is used to separation the blood platelets and the fourth nano-filters of a pore size 430 nm is used to filtration and purifying the blood from any bacteria and pathogenic viruses as shown in figure (4-6).

Figure (4-6): The horizontal microfluidic blood and cancer cell filtration and separation system

The main components of this sub-device system consist of the following parts:

1- Filter pad holder type CHM® HTF PTFE filter holder which is used as a pad to place a filter paper inside it. It contains two gates, one representing the inlet and the other outlet as shown in figure (4-6). This filter holder was fabricated for organic solvents and aggressive chemicals and made completely of PTFE. This holder has a broad chemical compatibility and contains no trace elements which could be released into the liquid being filtered, easy cleaning, and autoclavable by dry heat at 180ºC. It is indicated for particle removal from samples and reagents for analytical methods. The construction of the holder ensures leak proof sealing without a sealing ring, and avoids twisting of the membrane filter

when the top is tightened onto the base. The main technical specification of the fourth Nano-filters used to filtration and purifying the blood are given in table (4-2).

Figure (4-7): The filter pad holder used to place a filter paper inside it

TECHNICAL SPECIFICATIONS	
MEMBRANE FILTER	**13 mm**
Filtration area	0.5 cm^2
Flow rates Typical values per cm^2 for water at 1 bar (100 kPa) differential pressure:	10 ml/min (0.2 µm filter) 18 ml/min (0.45 µm filter)
Max. operating pressure	5 bar (500 kPa)
Materials	PTFE top and bottom part
Chemical compatibility	As for PTFE
Sterilization	By autoclaving (max 134 °C) or by dry heat (max 180 °C)
Connectors	Female Luer Lock inlet, Luer slip outlet

Table (4-2): The main technical specification of the Nano-filters used to filtration and purifying the blood

2- Filter papers; The CHM® filter papers, shown in figure (4-8) are used in this work for their quantitative, gravimetric and filtration under pressure analysis. They are made of refined pulp and linters with virtually 100% of alpha-cellulose content. These filter papers are guaranteed free of possible residual acids used in some production methods. The filter paper contains of a arranged secondary filtration layers. These filters have a high wet strengthened. They are made of high-purity cotton linters and other vegetable fibers. These filter papers

have fast or very fast filtration rates. The retention ranges of the technical specification of the used filter papers are illustrated in table (4-3).

Figure (4-8): The CHM® filter papers

GRADE	Properties	Weight g/m2	Thickness µm	Retention range µm	Ash content %
F1001	Medium	85	180	10-13	<0.06
F1002	Medium-Slow	100	190	7-8	<0.06
F1003	Medium-Slow/Thick	200	320	5-8	<0.06
F1005	Very Slow	85	170	3-5	<0.06

Table (4-3): The main technical specification of the used CHM® filter papers

The F1001 GRADE - medium filtration is the most widely used filter paper in the CHM® range. Medium retention and flow rate. This grade covers a wide range of laboratory applications and is frequently used for clarifying liquids. Traditionally this grade is used in qualitative analytical separations for routine laboratory work as well as rapid filtration of fine precipitates such as lead sulphate, calcium oxalate (hot) and calcium carbonate.

In agriculture, it is used for soil analysis and seed testing procedures. In the food industry, Grade F1001 is used for numerous routine techniques to separate solid foodstuffs from associated liquid or extracting liquid. It is widely used in education for teaching simple qualitative analytical separations.

In air pollution monitoring, using circles or rolls, atmospheric dust is collected from airflow and the stain-intensity measured photo metrically. For gas detection, the paper is impregnated with a chromogenic reagent and color formation quantified by optical reflectance.

The F1003 GRADE – is a medium to low rate of filtration with double the thickness comparing with CHM® Grade F1001. It owns a fine particle retention and excellent loading capacity. The extra thickness gives increased wet strength and allows a higher solute loading. Preferably used for liquids hard to clarify, essences, oils, tinctures, particularly useful for use in Buechner-funnels.

3- The piping, control valves and joints accessories;

The device consists of a set of transparent tubing with different internal diameters of 2-5 mm with three gates control valves to control the blood and compressed air supply routes during separation, washing and sterilization of the sub-device systems.

4.2.4 Vertical microfluidic blood and cancer cell filtration and separation system

This system consists of the same components of the parallel separation system, which is arranged sequentially, and vertically to compare and obtain the best and fastest ways to separate and purify the blood cells. The vertical microfluidic blood and cancer cell filtration and separation system is shown in figure (4-8).

4.2.5 Microfluidic Method for Blood White Cell (BWC) Separation System.

In this vertical blood white cell (BWC) separation method, the B-Leukocyte depletion filter was used. This method of blood units prior to storage is currently a systematic procedure followed by blood banks in the majority of developed countries.

Figure (4-9): The vertical microfluidic blood and cancer cell filtration and separation system

Several clinical studies have indicated that the use of leukocyte reduced cellular blood components may prevent some of the harmful side effects classically associated to blood transfusion.

B-Leukocyte depletion filter can be intended for the leukocyte reduction of red blood cell concentrates and whole blood. It contains a filtration for red cells with air vent options and clinically proven filter media technology. It is made of

non-woven polyester, excellent wettability feature and high biocompatibility as shown in figure (4-10).

Figure (4-10): The vertical microfluidic B-Leukocyte depletion filter for blood white cell separation

4.3 THE USED MIXTURE

The three separation systems of the designed and fabricated device, have been tested by prepared a sample to be suitable for use as an alternative of blood mixture that simulates and quantifies the size of cancer, blood cells and blood-related viruses in order to avoid complications of disease tests and blood coagulation in this stage.

The produced granules were then separated by sizes using the FRITSCH ANALYSETTE 3 PRO SPARTAN machine, shown in figure (4-11). It is a simple laboratory sieving for all tasks with optical adjustment of the amplitude on the running instrument with the possibility of automatic evaluation of the sieve

analysis using the extensive FRITSCH evaluation software AUTOSIEVE. It is suitable for quantitative particle size analysis of solids and suspensions of all kinds through dry or wet sieving with woven test sieves. It is convertible into a micro mill for fine comminution of dry laboratory samples or solids in suspensions and for homogenization of emulsions or pastes. It is a “shaking sieve” system in the classic sense in which an electromagnetic drive causes the sieves to oscillate in a vertical direction. The material to be sieved is periodically propelled upward off the sieve fabric and forced through the mesh of the attached test sieve as it falls back down.

Figure (4-11): The FRITSCH ANALYSETTE 3 PRO SPARTAN laboratory sieving machine

The mixture consists of micro alumina powder (AL_2O_3), distilled water and a red food dye. The alumina and dye powders were milled to the desired grain size by using the roller ball mill machine, shown in figure (4-12), for six hours.

Figure (4-12): The roller ball mill machine

The weight of the mixture samples was counted before and after the milling and separation processes and the weighting of the experimental samples by using the digital balance type KERN with a precision level of 0.0001 gm. as shown in figure (4-13)

Figure (4-13): The digital balance type KERN

The alumina powder (AL2O3) is white or colorless and insoluble in water powder. It is available at reasonably priced and occurs naturally like aluminum oxide and is used in the production of aluminum compounds such as glass, plastic and ceramics. Alumina is used in a wide range of applications, especially in aerospace and medical vehicles. The used Al_2O_3 sample of 99.99 % purity, SSA:1^{-6} m^2/g, ture density of 3.95g/cm^3 with typical Impurities; Ca <20 ppm, Si <20 ppm, Fe <20 ppm, K <50 ppm and Na <10 ppm. The other alumina specifications are:

1-Alumina is a lightweight, high-purity metal with a silver-gray look, depending on the roughness of the surface.

2- Alumina Non-magnetized.

3- Good conductivity for heat and electricity and weight less than copper.

4- Easy to operate, retractable and roads where it can be molded relatively easily.

5- Alumina is characterized by its ability to resist corrosion.

6- Stronger alumina alloys are less resistant to corrosion.

4.4 THE 1PCS NE555 PULSE FREQUENCU GENERATOR

The NE555 pulse frequency generators, shown in figure (4-14) is a duty cycle adjustable module, square / rectangular wave signal generator, manufactured from FR4 + electronic components materials. They are used to study the effect of them on the efficiency of the separation of blood cells process and to accelerate the separation of cells because these waves will work to conduct the first separation of cells before reaching the filters of separation. The main technical specifications of these pulse frequency generators are:

1.Input Voltage: 5V-15VDC and when power supply is 5V , the output current can be 15 mA around and when 12V power supply, the output current can 35 mA around;

2.Input current: > = 100MA;

3.Output amplitude: 4.2V V-PP to 11.4V V-PP. (Different input voltage, the output amplitude will be different)

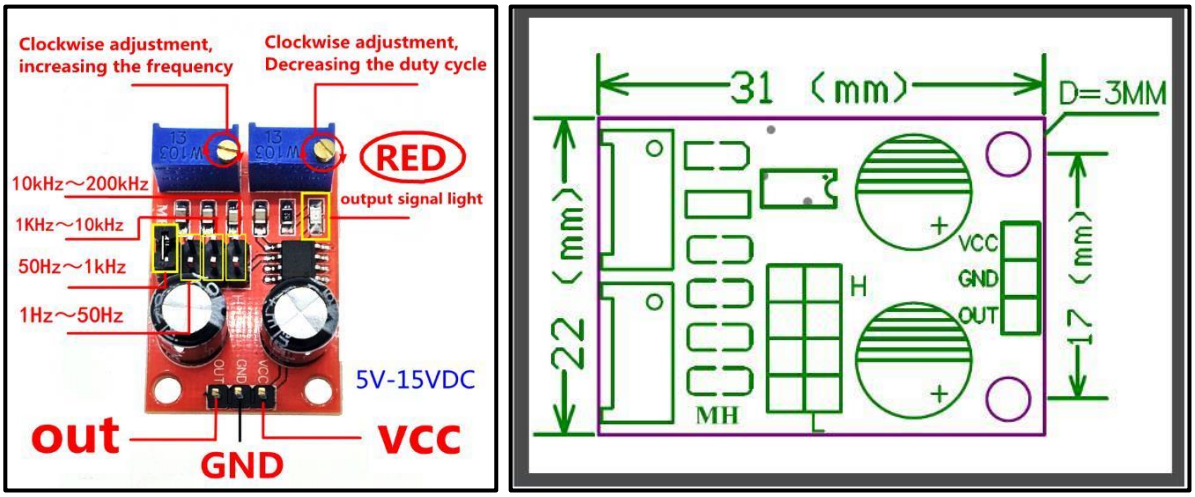


Figure (4-14): The NE555 pulse frequency generators

4.Maximum output current: >=15MA (5V power supply, V-PP greater than 50%), >= 35mA (12V power supply, V-PP greater than 50%)

5.Output with LED indication (low level , LED will on; high level, LED will off; low frequency, the LED flashes);

6.The output requency range is selectable:LF file: 1Hz ~ 50Hz IF file: 50Hz~1kHz High-frequency file: 1KHz ~ 10kHz, M-HF file: 10kHz ~ 200kHz

7.Cycle T=0.7 (RA+2RB) C (RA, RB for 0-10K adjustable);

Low frequency:C=100UF; Intermediate frequency:C=1UF;M-H frequency: C=0.1 UF; High frequency: C=0.001UF. Note that before changing the frequency, must be power off.

The main applications are:

1. Used as a square wave signal generator, offer the square wave signal for the use of the experimental development.
2. Used to produce driving stepper motor drives, offer the square wave signal.
3. Adjustable pulse for the use of MCU.
4. Generate adjustable pulse control circuit.

4.5 THE EXPERIMENTAL DESIGN (DOE)

The response surface methodology (RSM), the full factorial design (FFD) and the expert system 11.0 software program were selected to design the experimental work improved and verified the experimental results. The designed experimental matrix illustrated in table (4-4), using the following designed experimental parameters were selected to test the efficiency of the separation and purification of blood cells for the fabricated device:

1- Separwation method

- Horizontal microfluidic blood and cancer cell filtration and separation system
- Vertical microfluidic blood and cancer cell filtration and separation system
- Microfluidic method for blood white cell (BWC) separation system.

2- Microfluidic Pore Size

The Selected pore sizes diameters are 13 to 16 μm to separate cancer cells, from 8 to 12 μm to separate the white blood cells, from 3 to 5 μm to separate red blood cells and from 2 to 3 μm to separate the blood platelets while nanoparticle filter paper was selected with a Nano pore size 430 nm to purify blood cells from pathogenic viruses and bacteria.

2-Air Pressure Level

In order to determine the highest efficiency of the blood cells and cancer separation and purification processes, four different pressure measurements are used as: 0, 50, 100 and 150 mbar.

3-Using Of Ultrasonic Waves

Four ultrasound wave generators have been added to study the effect of these waves on the efficiency and acceleration of the blood cancer cells separation and blood purification.

Table (4-4): The designed experimental matrix of the presence work

`	Run	Factor 1	Factor 2	Factor 3	Response 1
		Separation method type	Microfluidic Pore Size (µm)	Air Pressure Level (mbar)	Separation quantity (ml)
22	1	Vertical microfluidic separation system	12	150	
26	2	Microfluidic BWC separation system	12	0	
6	3	Horizontal microfluidic separation system	12	0	
34	4	Horizontal microfluidic separation system	12	50	
35	5	Horizontal microfluidic separation system	0.43	50	
15	6	Vertical microfluidic separation system	3	0	
18	7	Vertical microfluidic separation system	8	100	
24	8	Vertical microfluidic separation system	12	50	
17	9	Vertical microfluidic separation system	0.43	150	
1	10	Horizontal microfluidic separation system	0.43	0	
8	11	Horizontal microfluidic separation system	8	150	
12	12	Horizontal microfluidic separation system	12	100	
10	13	Horizontal microfluidic separation system	3	50	

19	**14**	**Vertical microfluidic separation system**	**8**	**50**	
16	**15**	**Vertical microfluidic separation system**	**12**	**0**	
33	**16**	**Vertical microfluidic separation system**	**0.43**	**100**	
36	**17**	**Vertical microfluidic separation system**	**0.43**	**50**	
28	**18**	**Microfluidic BWC separation system**	**12**	**50**	
30	**19**	**Microfluidic BWC separation system**	**12**	**150**	
11	**20**	**Horizontal microfluidic separation system**	**12**	**150**	
29	**21**	**Horizontal microfluidic separation system**	**8**	**50**	
4	**22**	**Horizontal microfluidic separation system**	**0.43**	**100**	
7	**23**	**Horizontal microfluidic separation system**	**0.43**	**150**	
23	**24**	**Vertical microfluidic separation system**	**3**	**100**	
25	**25**	**Vertical microfluidic separation system**	**3**	**150**	
32	**26**	**Vertical microfluidic separation system**	**3**	**50**	
3	**27**	**Horizontal microfluidic separation system**	**3**	**0**	
13	**28**	**Horizontal microfluidic separation system**	**8**	**100**	
2	**29**	**Horizontal microfluidic separation system**	**8**	**0**	
27	**30**	**Microfluidic BWC separation system**	**12**	**100**	
14	**31**	**Vertical microfluidic separation system**	**0.43**	**0**	
31	**32**	**Vertical microfluidic separation system**	**8**	**0**	
9	**33**	**Horizontal microfluidic separation system**	**3**	**150**	
5	**34**	**Horizontal microfluidic separation system**	**3**	**100**	
20	**35**	**Vertical microfluidic separation system**	**8**	**150**	
21	**36**	**Vertical microfluidic separation system**	**12**	**100**	

CHAPTER FIVE

RESULTS AND DISCUSSIONS

The final development and fabricated micro-device using the designed three different methods of filtration and separating for blood and cancer cells separation and filtration or for any other chemical substances particles separation were implemented as shown in figure (5-1).

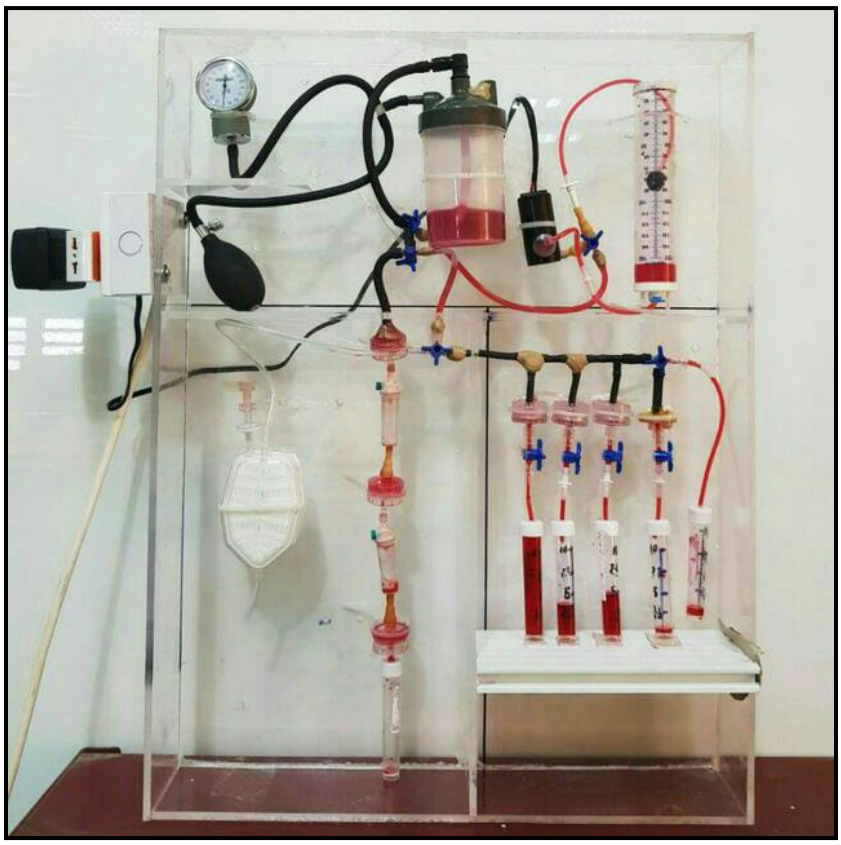


Figure (5-1): The final development and fabricated micro-device for blood, and cancer cells separation and filtration

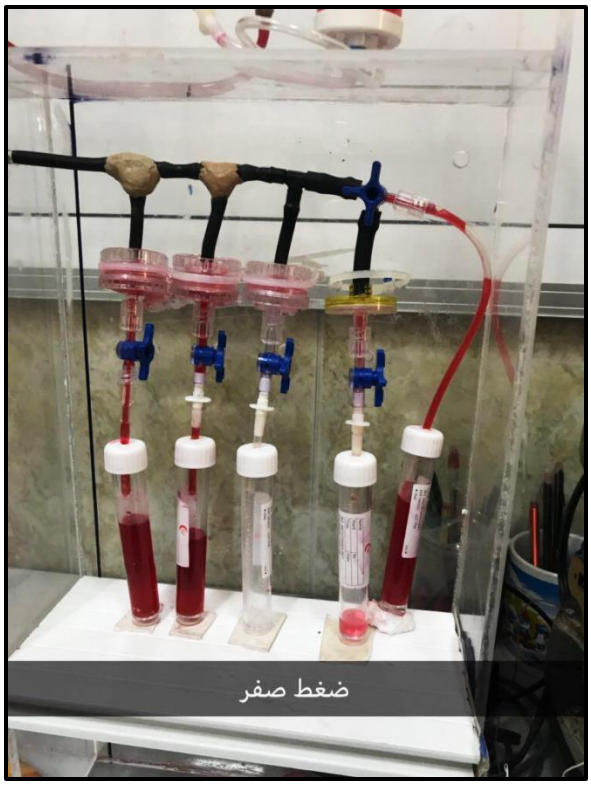
After implemented the designed experiments work, the response surface methodology (RSM), the full factorial design (FFD) and the expert system 11.0 software program, the experimental results are given in table (5-1).

Table (5-1): The experimental results

Std	Run	Factor 1	Factor 2	Factor 3	Response 1
		Separation method type	Microfluidic Pore Size (µm)	Air Pressure Level (mbar)	Separation quantity (ml)
22	1	Vertical microfluidic separation system	12	150	6
26	2	Microfluidic BWC separation system	12	0	5
6	3	Horizontal microfluidic separation system	12	0	4.05
34	4	Horizontal microfluidic separation system	12	50	9.96
35	5	Horizontal microfluidic separation system	0.43	50	0.08
15	6	Vertical microfluidic separation system	3	0	0.81
18	7	Vertical microfluidic separation system	8	100	4.6
24	8	Vertical microfluidic separation system	12	50	3.5
17	9	Vertical microfluidic separation system	0.43	150	2.5
1	10	Horizontal microfluidic separation system	0.43	0	0.05
8	11	Horizontal microfluidic separation system	8	150	17.14
12	12	Horizontal microfluidic separation system	12	100	13
10	13	Horizontal microfluidic separation system	3	50	4.15
19	14	Vertical microfluidic separation system	8	50	3
16	15	Vertical microfluidic separation system	12	0	0.97
33	16	Vertical microfluidic separation system	0.43	100	2.5
36	17	Vertical microfluidic separation system	0.43	50	2
28	18	Microfluidic BWC separation system	12	50	9
30	19	Microfluidic BWC separation system	12	150	13
11	20	Horizontal microfluidic separation system	12	150	16.91

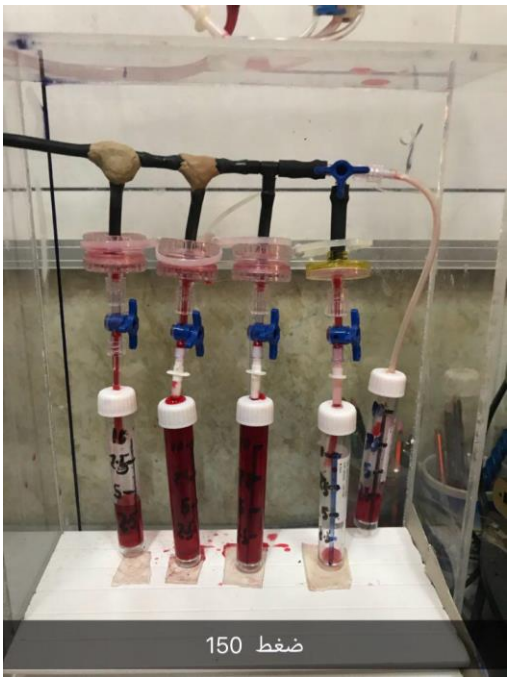
29	**21**	**Horizontal microfluidic separation system**	**8**	**50**	**9.96**
4	**22**	**Horizontal microfluidic separation system**	**0.43**	**100**	**0.1**
7	**23**	**Horizontal microfluidic separation system**	**0.43**	**150**	**0.29**
23	**24**	**Vertical microfluidic separation system**	**3**	**100**	**2.6**
25	**25**	**Vertical microfluidic separation system**	**3**	**150**	**3**
32	**26**	**Vertical microfluidic separation system**	**3**	**50**	**2.5**
3	**27**	**Horizontal microfluidic separation system**	**3**	**0**	**2.45**
13	**28**	**Horizontal microfluidic separation system**	**8**	**100**	**10**
2	**29**	**Horizontal microfluidic separation system**	**8**	**0**	**3.65**
27	**30**	**Microfluidic BWC separation system**	**12**	**100**	**13**
14	**31**	**Vertical microfluidic separation system**	**0.43**	**0**	**0.77**
31	**32**	**Vertical microfluidic separation system**	**8**	**0**	**0.89**
9	**33**	**Horizontal microfluidic separation system**	**3**	**150**	**5.71**
5	**34**	**Horizontal microfluidic separation system**	**3**	**100**	**4.5**
20	**35**	**Vertical microfluidic separation system**	**8**	**150**	**5**
21	**36**	**Vertical microfluidic separation system**	**12**	**100**	**4.7**

Figures (5-2) to (5-4) show the horizontal blood and cancer cells filtration and separating processes at 0, 50 and 150 mbar air pressure levels, respectively, while figures (5-5) to (5-7) show the vertical blood and cancer cells filtration and separating processes at 0, 100 and 150 mbar air pressure levels, respectively. Figure (5-8) shows the microfluidic blood white cell (BWC) separation system.

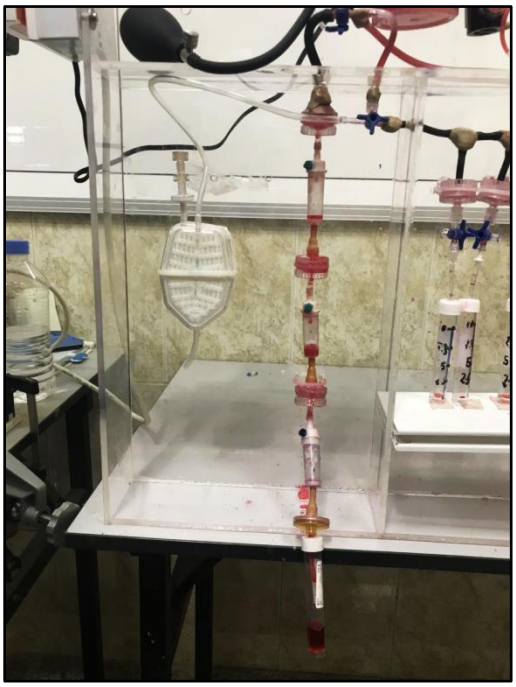


Figures (5-2): The horizontal blood and cancer cells filtration and separating processes at 0 mbar air pressure level

Figures (5-3): The horizontal blood and cancer cells filtration and separating processes at 50 mbar air pressure level



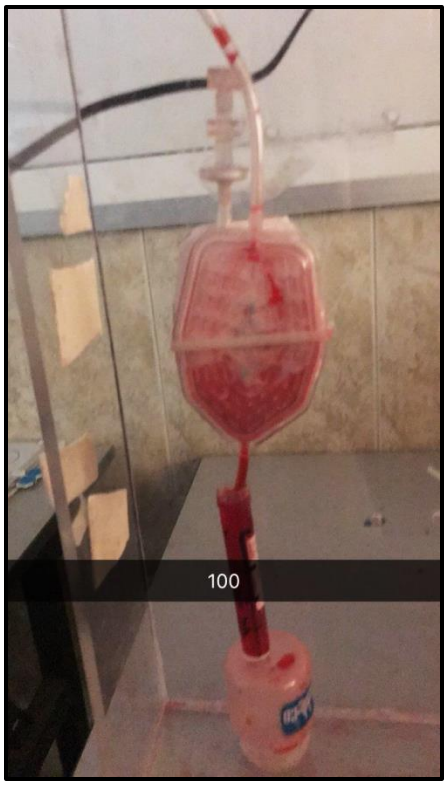
Figures (5-4): The horizontal blood and cancer cells filtration and separating processes at 150 mbar air pressure level



Figures (5-5): The vertical blood and cancer cells filtration and separating processes at 0 mbar air pressure level

Figures (5-6): The vertical blood and cancer cells filtration and separating processes at 100 mbar air pressure level

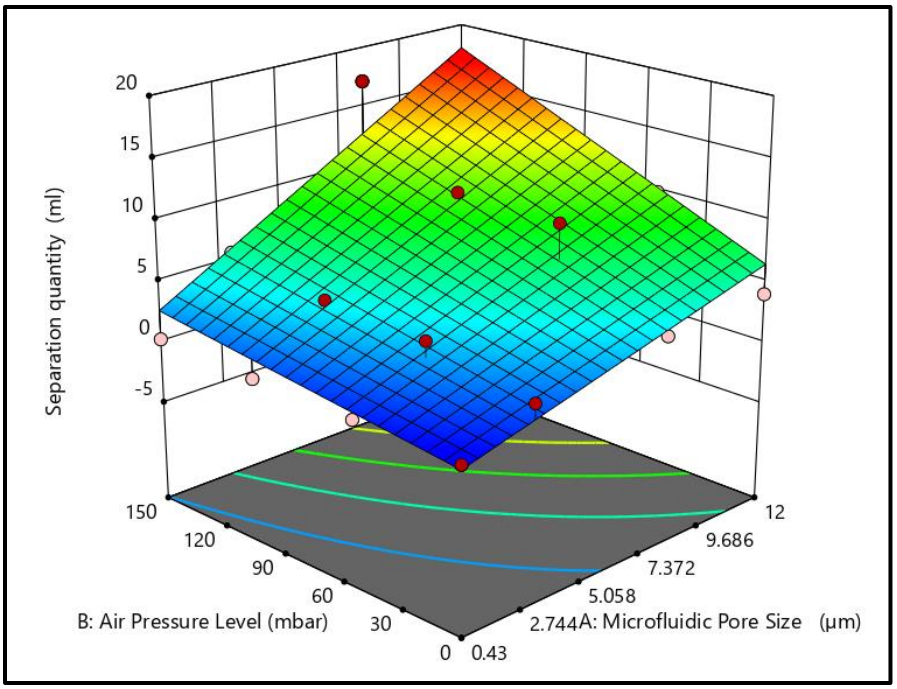
Figures (5-7): The vertical blood and cancer cells filtration and separating processes at 150 mbar air pressure level



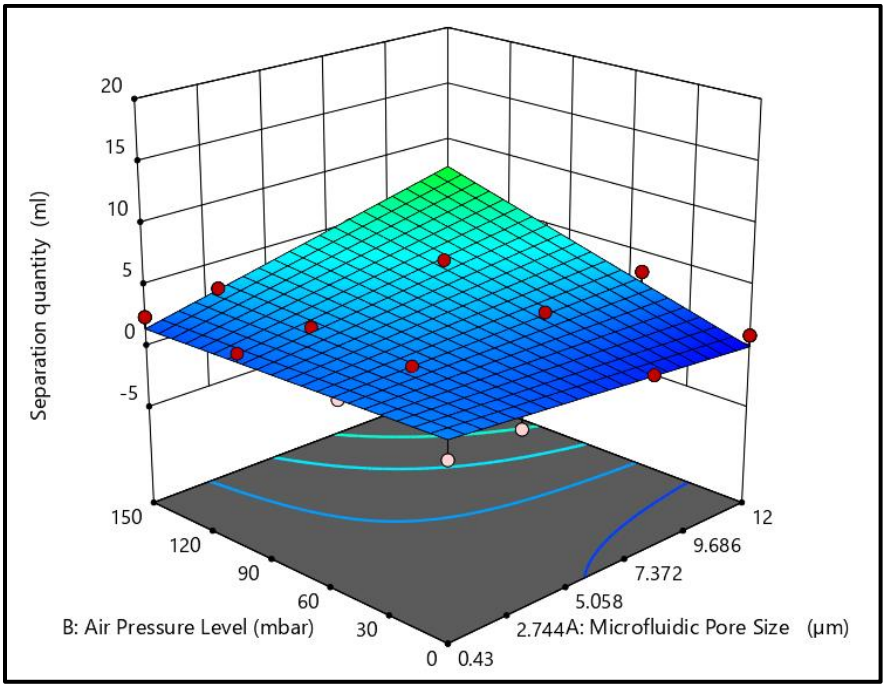
Figures (5-8): The microfluidic blood white cell (BWC) separation system at 100 mbar air pressure level

The 3D graphs for the blood and cancer cells filtration and separating processes are shown in figures (5-9) to (5-11). Figure (5-9) shows that when using the horizontal separation system, the blood and cancer cells filtration and separating quantities are increasing with increasing the air pressure level and the microfluidic pore size and reached its maximum quantity as 16.9l mL.

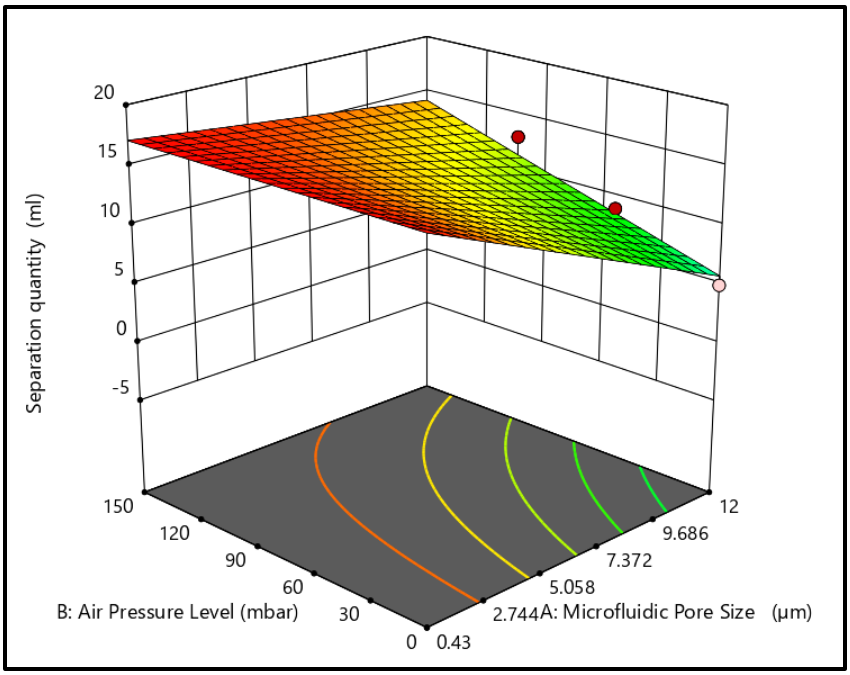
When using the vertical separation system, the blood and cancer cells filtration and separating quantities are increasing with increasing the air pressure level and the microfluidic pore size and reached its maximum quantity as 6.00 mL., as shown in figure (5-10). Figures 5-11shows that when using the microfluidic blood white cell (BWC) separation system, the blood white cell separating quantities are increasing with increasing the air pressure level and reached its maximum quantity as 13.00 mL.,



Figures (5-9): The horizontal blood and cancer cells filtration and separating processes at all air pressure levels and pore sizes



Figures (5-10): The vertical blood and cancer cells filtration and separating processes at all air pressure levels and pore sizes



Figures (5-11): The microfluidic blood white cell (BWC) separation system at all air pressure level

CONCLUSIONS

AND SUGGESTINGS FOR FUTURE WORKS

6.1 Conclusions

The main Conclusions have been achieved through the current work are:

1. The blood and cancer cells filtration and separating quantities are increasing with increasing the air pressure level and the microfluidic pore size.

2. The best results for the highest quantity of blood separated and free of tumor and cancer cells were obtained at 150 mbar air pressure and the use of horizontal separation system, which is higher by 282% compared with the use of the vertical separation system.

3. The highest quantity of blood white cell (BWC) separated were obtained at 150 mbar air pressure and the use of horizontal separation system, reached 17.14 mL., which is higher by 32% than when use of the microfluidic blood white cell (BWC) separation system and by 343 % compared with the use of the vertical separation system.

4.The highest quantity of blood red cell (BRC) separated were obtained at 150 mbar air pressure and the use of horizontal separation system, reached 5.71 mL., which is higher by 90 % compared with the use of the vertical separation system.

5. The highest quantity of blood cell filtration from the virus cells separated were obtained at 100 mbar air pressure and the use of vertical separation system, reached 2.50 mL., which is higher by 9 times compared with the use of the horizontal separation system.

6.2 Recommendations for future works

The main recommendations for future works are:

1. Design and manufacture of blood separation and filtration apparatus in large quantities to serve national and sub-national blood cell banks, and take into account all rules of international organizations such as the FDA and ISO standard and organizations.

2. Study the effect of using of other microfluidic methods of separations like the microfluidic channels.

3. Study the effect of adding other methods of separations to microfluidic systems like the ultrasonic waves, the electrical and magnetic fields.

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Graduation project submitted to the control and systems Engineering Department in partial fulfillment of B.Sc.Degree in Medical systems Engineering

By

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