ÜNAL AKAR DEVELOPMENT OF A LOW-COST MICROFLUIDIC SYSTEM TO DETECT IMMUNOMAGNETICALLY CAPTURED LEUKEMIA CELLS AGU 2020

DEVELOPMENT OF A LOW-COST MICROFLUIDIC SYSTEM TO DETECT IMMUNOMAGNETICALLY CAPTURED LEUKEMIA CELLS

A THESIS

SUBMITTED TO THE DEPARTMENT OF BIOENGINEERING AND THE GRADUATE SCHOOL OF ENGINEERING AND SCIENCE OF ABDULLAH GUL UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

By

Ünal Akar

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M.Sc. thesis titled Development of a Low-Cost Microfluidic System to Detect Immunomagnetically Captured Leukemia Cells has been prepared in accordance with the Thesis Writing Guidelines of the Abdullah Gül University, Graduate School of Engineering & Science.

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ABSTRACT

DEVELOPMENT OF A LOW-COST MICROFLUIDIC SYSTEM TO DETECT IMMUNOMAGNETICALLY CAPTURED LEUKEMIA CELLS

Sensor technologies are used for converting physical properties into measurable signals. With the advances in technology, especially the developments in the semiconductor fabrication caused the emergence of a new technology which is known as microfluidics. Microfluidics is a cutting-edge technology that provides cost and time efficient solutions to conventional methods. They have different areas of applications such as chemistry, biology, information technology, optics, and etc. Acute Lymphoblastic Leukemia (ALL) is the most common malignancy in childhood. If there are few cancer cells still remain in the body after the treatment, doctors cannot detect and observe any physical or other symptoms and these cells can cause relapses which is called Minimal Residual Disease (MRD). Currently, MRD can be detected with flow cytometry and gene-based techniques. Both methods have disadvantages such as being high-cost and requirement of trained personnel. With this project, we aimed to develop a low-cost microfluidic system to capture leukemia cells having specific surface markers (CD10, CD19, and CD45). We used antibody-coated magnetic beads for capturing the target cells. The final step is to immobilize leukemia cells onto gold pads in the microfluidics after the magnetic separation. Then cells were counted to understand the patient response to the treatment process.

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Keywords: immunomagnetic separation, minimal residual disease, microfluidic chip, patient response to leukemia treatment, biosensors

ÖZET

İMMUNOMANYETİK İLE LÖSEMİ HÜCRELERİNİ ALGILAMAK İÇİN DÜŞÜK MALİYETLİ MİKROAKIŞKAN SİSTEM GELİŞTİRİLMESİ

Sensör teknolojileri fiziksel özellikleri ölçülebilir sinyallere dönüştürmek için kullanılır. Özellikle yarı iletken teknolojisindeki gelişmelerle birlikte, mikroakışkan olarak bilinen yeni bir teknoloji ortaya çıktı. Mikroakışkanlar zamandan ve maliyetten tasarruf sağlayan ileri bir teknolojidir. Kimya, biyoloji, bilgi teknolojisi, optik vb. gibi farklı kullanım alanlarına sahiptir. Akut lenfoblastik lösemi kötü huylu bir kan kanseridir, özellikle B öncüllü akut lenfoblastik lösemi çocukluk döneminde çok tehlikelidir. Eğer tedaviden sonra vücut içinde az bir miktar da olsa kanser hücresi kalırsa, doktorlar bu hücreleri fiziksel ya da diğer semptomları inceleyerek tespit edemeyebilirler, bu hücreler kanserin tekrar etmesine sebep olabilir bu duruma Minimal Kalıntı Hastalığı (MRD) denir. MRD, akım sitometrisi ve genetik çalışmalar ile teşhis edilebilir. Bu tür tedavilerin de kendi sınırlamaları vardır; örneğin pahalı olması ve eğitilmiş bir personele ihtiyaç duyulması gibi. Bu proje ile amacımız lösemi hücrelerinin yüzeyindeki belirteçleri antikor ile yakalamak (CD10, CD19 ve CD45) için düşük maliyetli bir mikroakışkan sistem geliştirmektir. Yakalama için antikor kaplı manyetik boncuklar kullanılmıştır. Son adımda; manyetik ayırma işleminden sonra lösemi hücreleri altın kareler üzerinde sabitlenmiştir. Böylece, hastaların tedavi süresince verdikleri dönütleri anlamak için hücreler sayılabilmiştir.

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Temmuz 2020

Anahtar kelimeler: immunomanyetik ayrıştırma, minimal rezidüel hastalık, mikro-akışkan çip, lösemi tedavisine hasta yanıtı, biyosensörler

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To My Family

Chapter 1

Introduction

1.1 Sensors

The sensor is a device that measures or detects a physical, chemical, or biological property and converts it to electrical or optical signals. Sensors convert the physical inputs to a signal which can be measured electrically. The aim of using sensors in a system is to acquire information [1].

Sensing technology has been improved with the help of technological advancements over the years. Evading from being expensive and bulky, the micro-electromechanical systems (MEMS) have been developed to solve various problems for years. Thus, new miniaturized devices such as microfluidic, micromechanical, micro-optical, microchemical, and microthermal systems are applied to solve various problems without performance loss. In biology, reducing the size of the system is an important issue, and it can affect the performance of a system directly [2].

There are many types of sensors and they usually classified based on the application area or transduction type. Some of these systems are biosensors, chemical, electrical, optical, environmental, acoustic, radiation, and pressure sensors.

1.1.1 Biosensors

Sensors have been used to detect various biomolecules in biology and medicine and interdisciplinary efforts are needed to obtain integrated systems including electronic and mechanical components. Biosensors have great potential to address challenges related to health, food, and the environment. They can offer analytical opportunities for industrial and health care services with a low-cost and easy to use fashion [3].

For example, personal (single use) biosensors have played a key role as a result of the optimization of the microfabrication process. A spin-off from the screen-printing technology, new single-use electrode strips for measuring personal blood glucose (Figure 1.1.1.1) are mass-produced by using a thick-film microfabrication process. Each strip involves essential reagents such as glucose oxidizes (GOx) that are usually scattered by ink-jet printing technology and deposited in the dry form. The electrode system collects the electrons which come from the chemical reaction between the enzyme GOx and glucose. A hydrophobic layer allows separating the sample (Blood) from the sensor surface for measuring glucose properly. Although being low cost and rapidly prototyped such sensor strips are based on a high level of optimization [4].

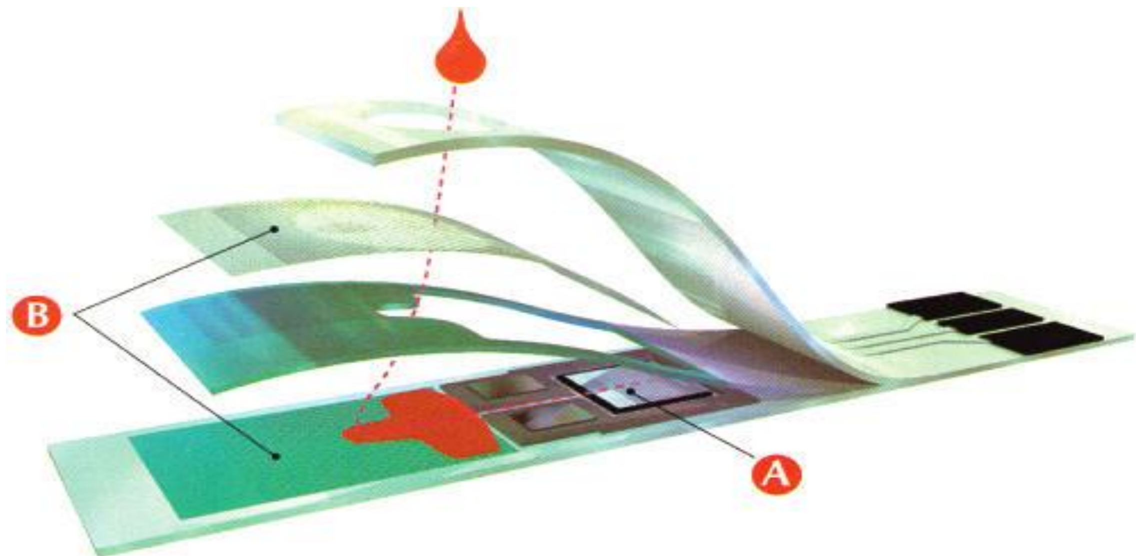


Figure 1.1.1.1: An example of a glucose measurement sensor (A) sensing part; (B) detecting part (drawing the blood). [4]

1.2 Blood Malignancies

Blood stem cells are produced in the bone marrow where they also grow and develop into three types of blood cells: red blood cells, white blood cells, and platelets. If a problem occurs during this process, the period is interrupted by the overproduction of the blood cells. As a result of that, blood malignancies come up with abnormal production and inadequate function of blood cells [5],[6].

There are four main types of hematologic malignancies which are leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, and myeloma. According to the Hematological Malignancy Research Network's data, blood malignancies appear around 9% of all cancers in developed countries and blood cancers are the fourth common malignancy for men (after prostate, lung, and colorectum) and for women (after breast, lung, and colorectum) [7].

1.3 Leukemia

The most well-known blood malignancy is leukemia. In the 1800s, it was named by Rudolf Virchow as it means white blood, when Paul Erlich performed cell staining, Erlich improved about stains which include how to characterize the abnormal white cells. During the 19th and 20th centuries, researches working on leukemia showed that different types of leukemia originated from the different cell lineage [5].

Leukemia is a cancer of blood cells and it begins in the bone marrow. When a marrow cell transforms into leukemic cells, other cells are no longer survive rather than leukemic cells and they are outproduced. These cells grow outrageously and also, they prevent the development of normal cells.

Considering the patient's condition, if the disease gets worse rapidly, it is considered as acute leukemia. On the other side, if the disease has a slow impact, it is considered as chronic leukemia [5].

"Lymphoblastic cell" and "myeloblastic cell" are two types of blood cells that are produced from blood stem cells and they belong to the either lymphoid or myeloid lineage. Lymphoid lineage cells involve T, B, and natural killer (NK) cells, while megakaryocytes and erythrocytes and also granulocytes and macrophages are members of the myeloid lineage [8].

Acute Myeloid Leukemia (AML)

"Acute" means quick, so this type of leukemia can grow quickly. Acute Myeloid Leukemia (AML) refers to increases in the number of myeloid cells in the bone marrow. Sometimes doctors thought this situation is the more aggressive version of Chronic Myeloid Leukemia (CML) but AML has different symptoms and clinical signs [9].

In nearly all cases, AML is a primary disease that is derived from a genetic mutation in a stem or precursor cell [5].

Generally, AML begins in bone marrow but according to leukemic infiltration of various tissues, it can spread to different parts of the body such as the spleen, liver, lymph nodes, skin, bone, and central nervous system [9].

Chronic Myeloid Leukemia (CML)

Chronic Myeloid Leukemia (CML) begins in specific blood-forming cells of the bone marrow. It is a type of cancer that there is a genetic variance that comes up in early-type (immature) of myeloid cells as an increase in not only myeloid cells but also red blood cells, platelets, and most type of white blood cells (except lymphocytes). There is an abnormal gene, called BCR-ABL which is responsible for this change. CML is a quite slow-growing leukemia type; it has 3 to 5 years between the benign phase and the fatal phase. However, during the benign phase, it can be changed into acute leukemia because of the blast crisis [10].

Chronic Lymphocytic Leukemia (CLL)

Chronic Lymphocytic Leukemia (CLL) is a disease that is the most continual form of leukemia in the Western countries and it is the result of the accumulation of mature B lymphocytes in the blood, bone marrow, lymph nodes, and spleen. Based on the similarity between leukemia and lymphoma, these diseases are generally classified together. The outcomes during the disease are different. Considering the natural progress of the disease, some patients have a normal life span even they have CLL. On the other hand, some patients die within five years after the diagnosis [5], [11].

Acute Lymphoblastic Leukemia (ALL)

Acute Lymphoblastic Leukemia is also called "Acute Lymphocytic Leukemia". This leukemia type can proceed quickly, and if not treated immediately, it would probably be fatal in a few months. ALL starts from an immature type of lymphocytes in the bone marrow [12].

In our research, we are interested in B-ALL cells and we developed a biochip based on microfluidics.

1.4 Acute Lymphoblastic Leukemia

Cancer means that the cells in the body start to grow in an uncontrollable state. The body can't prevent making new cells so that the systems start to lose their functionalities. Acute Lymphoblastic Leukemia means cancer begins from early lymphocytes in the bone marrow. This type of cancer grows rapidly and sometimes, it may spread to other parts of the body such as the liver, spleen, central nervous system (brain and spinal cord), lymph nodes, and testicles (in males). ALL primarily affect the bone marrow and the blood.

On the other hand, lymphomas primarily affect lymph nodes and other organs (may also include the bone marrow). Because of that, it can be difficult to decide if there is cancer that was originated from leukemia or lymphoma. [12], [13]

Acute Lymphoblastic Leukemia is the most common type of cancer in childhood. However, it can also occur in adults. When the patients get older, the chance of a successful treatment is getting lower [14].

The bone marrow is the soft inner part of the bones. It consists of blood-forming cells, fat cells, blood stem cells which are formed a few amount of blood-forming cells, supporting tissues, and other elements for maturing cells. Blood stem cells begin to differentiate as a result of that, red blood cells, platelets, and white blood cells are produced in the bone marrow.

Red blood cells (RBCs) are responsible for oxygen and carbon dioxide transfer between the lungs and other parts of the body. Platelets are kind of cell fragments made by a megakaryocyte that means a version of a bone marrow cell. Platelets are responsible for occluding the defects in the blood vessels caused by cuts or injuries. White blood cells are responsible for body protection against infections [12].

The significant part of the immune system is lymphoid tissue, which consists of Lymphocytes. Lymphocytes come out of lymphoblasts. They are divided into 2 different types which are called B (Figure 1.4.1) and T cell according to their lineage stem cells.

B lymphocytes are responsible for protecting the body against germs, bacteria, viruses, and fungi by producing proteins called antibodies. T lymphocytes are responsible for different functions like destroying infections or boosting or slowing operations of other immune system cells.

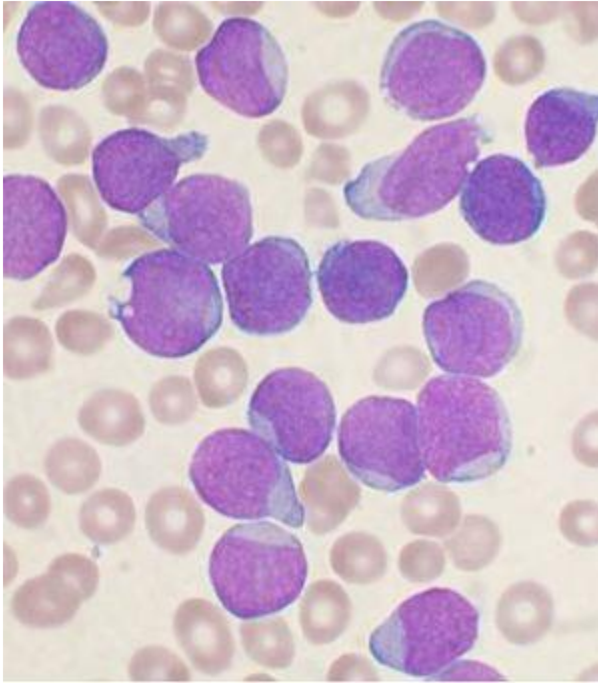


Figure 1.4.1: Stained lymphoblasts of B-cell acute lymphoblastic leukemia [15].

1.5 Classification of Acute Lymphoblastic Leukemia

The first attempt for classifying the ALL was the French American British (FAB) morphological classification. They classified ALL into 3 different types (L1, L2, and L3 subtypes) based on cell size, cytoplasm, nucleoli, vacuolation, and basophilia [16]. In 1997, the World Health Organization recommended a complex classification for defining leukemic blasts' morphology and cytogenetic profile that involve three types of ALL like B lymphoblastic, T lymphoblastic, and Burkitt-cell Leukemia [17]. In 2008, Burrkit-cell Leukemia was eliminated and it was combined with Burkitt Lymphoma. As a result of that, B-lymphoblastic leukemia was divided into two subclasses like B-ALL with recurrent genetic abnormalities and B-ALL not otherwise specified [17], [18].

1.5.1 The French-American-British (FAB) Classification

In 1976, the French-American-British (FAB) defined classification of acute lymphoblastic leukemia based on morphology. They introduced three versions of blast cells in ALL. L1 means leukemia with a homogeneous structure. Leukemia blasts are homogeneous, the nucleus is regular, insufficient cytoplasm, regular blast cells with a high nucleocytoplasmic ratio, and small or no nuclei. L2 means leukemia with a multifarious structure. In this version, there are larger blast cells with irregular nuclei, protruding nucleoli, and a more plentiful cytoplasm. L3 means leukemia with a basophilic cytoplasm. There are prominent cytoplasmic vacuoles and large nucleoli. The L3 type involved most B-ALL cases [19], [20], [21].

1.5.2 Immunophenotype Classification

In 2008, FAB classification was exchanged with immunophenotype based tests such as cytogenetics and flow cytometry. The new classification was called the WHO (World Health Organization) classification of lymphoid neoplasm [18]. The FAB classification was clinically practical, but now the WHO classification has been used instead of it. In this classification, there are two main subclasses. The first one represents where the neoplasm is acquired from B and T-lineage. The other one is to represent the leukemic cells maturation phase of blast cells obtained from B, T, or NK cells [22]. Immunophenotype classification became a primary diagnostic tool with morphology, because of its fast and certainty recognition process. Thus treatability rate is increased [21].

Immunophenotyping via multi-channel flow cytometry (MFC) has become a normal procedure for recognition and subclassification of ALL. Also, it was acquired as a beneficial tool for the monitoring and detection of minimal residual disease (MRD).

The European Group for Immunophenotyping of Leukemia (EGIL) suggested in 1995, to classify B-lineage ALL into four subclasses which are B-I (or pro-pro-B), B-II (pro-B or common-B), B-III (or pre-B) and B-IV (or mature B) [23]. Also, EGIL suggested a

classification of T-ALL based on maturation sequence which are T-I (pro-T), T-II (or early-T), T-III, and T-IV.

1.6 Detection of Acute Lymphoblastic Leukemia

1.6.1 Detection Techniques

In molecular genetics, some general tests are used for the detection of Acute Lymphoblastic Leukemia. However, there are a few special tests that were developed using peripheral blood and bone marrow [5].

A huge amount of white cells and the existence of unusual cells (generally blasts) characterize any kind of leukemias. In the initial stages of malignancies, it is difficult to observe unusual peripheral blood leukocyte, but in the advanced levels, it is easy to detect different cells in the blood. There are four types of examinations used for further investigations [5].

Morphology

Morphology is used for identifying the type of cells in leukemia using Romanowsky staining via a light microscope [5]. Romanovsky dyes are used for the regular examination of blood films and involve different types of stains. They are combined with methylene blue (the main dye) and eosin (an acidic dye). During the staining operation, the main dyes react to acidic structures in the cells. Stains and staining techniques may differ among the hematologist and pathologist. Depend on the biological samples, when the stain reacts with the cells then the colors are generated which can be blue, purple, or violet for basophilic and red or orange for acidophilic or eosinophilic [24].

Flow Cytometry

Flow cytometry is a technique used to recognize and analyzes single cells or particles as they flow. This technology uses single or multiple lasers while particles are stained in a buffer solution. Every particle is measured using light scattering and one or multiple

fluorescence parameters [25]. When the samples are introduced to the flow cytometer, each particle is organized as a single line based on their diameters. After forming a continuous line, the detection system evaluates them one by one [26]. This system is not only used for detecting single or population of the cell (figure 1.6.1.1) but also used for measuring small molecules like DNA nuclei or chromosomes or cell surface markers like CD molecules [5]. Flow cytometry has various applications in multiple areas such as immunology, virology, molecular biology, cancer biology, and infectious disease monitoring. Using this system, synchronous recognition of complex populations of cells from bone marrow and blood can be performed. Also, it can be used for analyzing solid tissues that can be spread into single cells such as lymph nodes, spleen, mucosal tissues, solid tumors, etc [25].

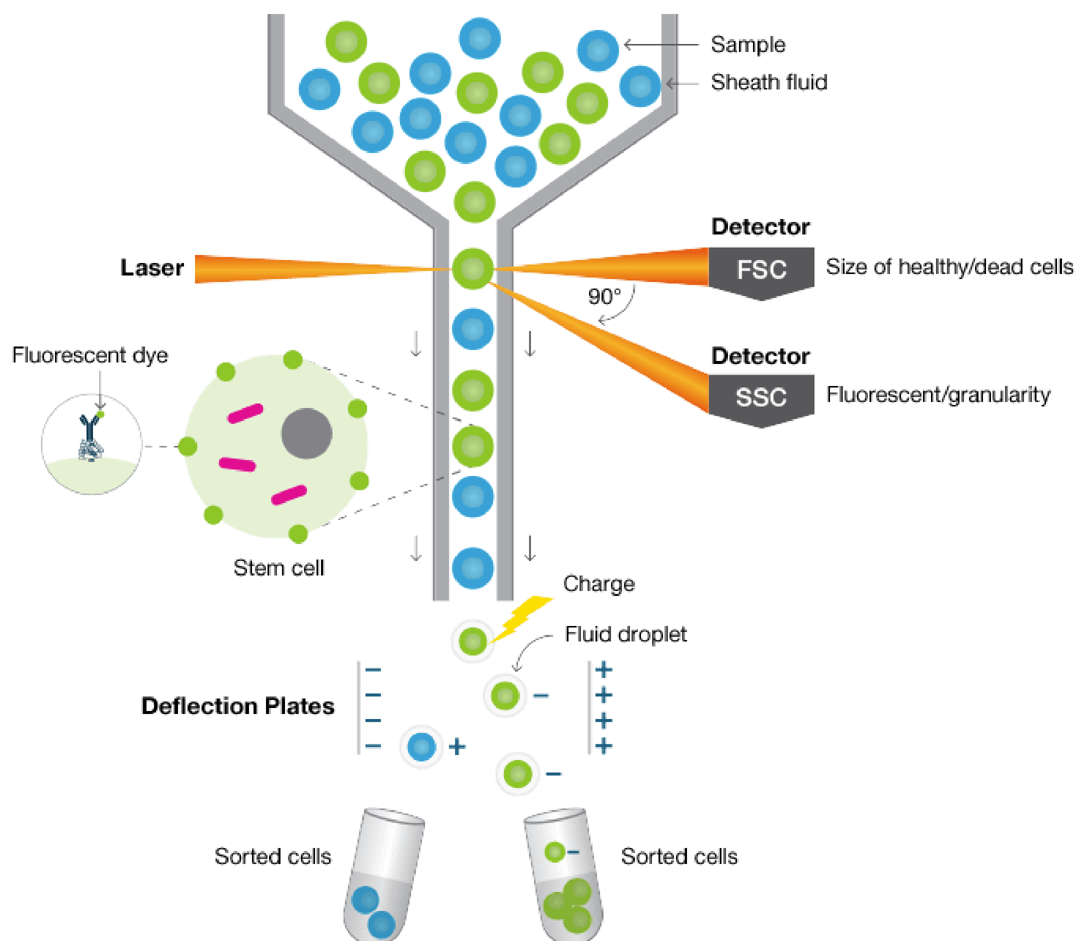


Figure 1.6.1.1: An example of a flow cytometry application that is cell sorting [26].

Cytochemistry

The dyeing of cells of the myeloid lineage depends on the enzymes in their cytoplasm and their granules. The most regularly used dyes/enzymes appointed are chloro-acetate esterase, acid phosphatase, alpha-naphthyl acetate esterase, myeloperoxidase, periodic acid Schiff and Sudan black B [5]. The periodic acid-Schiff (PAS) reaction is so practical in recognizing lymphoblasts, which are represent blocks of positivity. Sudan black B is insubstantially positive. ALL with mature cells that are nearly indistinguishable from mature lymphoid neoplasms and require expert observers for accurate morphological identification [21].

DNA Analysis and Cytogenetics

This method is used for determining the abnormalities and gene mutations in the whole cell or perform an analysis of DNA from the cell [5]. For defining the repeated translocations, conventional chromosome analysis can be useful, also gain and loss of gross chromosomal material is useful. Some methods are used for defining and visualize chromosomal abnormalities in ALL, such as the fluorescence in situ hybridization (FISH) technique with a sensitivity of around 99%. Moreover, array-comparative genomic hybridization (array-CGH, a-CGH) and single nucleotide polymorphisms (SNP) arrays can allow us to detect genetic structures in leukemia [21], [27].

According to where leukemia and myeloma start, bone marrow is the place that we should know. Aspirate and biopsy methods are used to detect leukemic cells from bone marrow [5].

During the aspiration method, a needle is put into the bone and obtains some bone marrow fluid and cells [28].

1.7 Treatment of Acute Lymphoblastic Leukemia

80% of the Acute Lymphoblastic Leukemia (ALL) occurs around in children, but when it happens in adults, it can be a fatal disease [14]. Treatment of acute lymphoblastic leukemia is generally done in 3 parts which are summarized below [29].

Part 1 is remission induction. The aim of this process is to destroy leukemia cells from bone marrow and maintain the number of cells in the blood.

Part 2 is consolidation. The aim of this process is to destroy any remaining leukemia cells.

Part 3 is maintenance. This process aims to take normal doses of chemotherapy medicines to prevent any relapses of leukemia [29].

There are some main treatment methods such as chemotherapy, radiation therapy, stem cell transplantation, and targeted therapy. These treatments are given to patients depending on the lymphocyte types, age of the children, level of risks, or existence of chromosome changes [30].

1.7.1 Chemotherapy

Anticancer chemicals or drugs interfere with nucleic acid and protein synthesis in proliferating leukemia cells, which is called chemotherapy. The main aim of the chemotherapy treatment is to find out the correct biochemical anomaly or chemical agent responsible for the transmutation of the normal cell into a cancer cell [31]. There are many types of drugs used to treat cancer but every drugs or medicines are not used for the treatment of cancer likewise [32]. Chemotherapy treatment has three primary aims in cancer treatment which are the cure, control, and palliation. Cure means to remove all cancer cells if it is possible. If a cure does not happen, the aims of the treatment change into the control of the disease. During this process, the main aim is to reduce and prevent

cancer from growing and metastasis. Most of the time, the cancer is not completely removed, however, it may be controlled like a chronic disease such as heart disease or diabetes. On the other hand, cancer may move away for a while, however, it is most probably to return. Chemotherapy treatment can also be used to relieve symptoms that are called palliation, palliative chemotherapy, or treatment with palliative intent. If the cancer is in the advanced stage, likely it cannot be controlled. When the patient is into this stage, chemotherapy can be used to reduce the pain and improve the quality of life [32].

A single drug can be used for the treatment of cancer, however, frequently lots of drugs are used together. The mixing drugs like that are called combination chemotherapy. This technique may be used to prevent that if cancer may occur resistive to any one of the drugs [32].

Sometimes, chemotherapy is applied to other therapy like targeted therapy, hormone therapy, or immunotherapy. If chemotherapy is used to minimize a tumor before surgery or radiation therapy that way is called “neoadjuvant” therapy. If the chemotherapy is used to remove any remaining cancer cells after surgery or radiation therapy called “adjuvant” therapy [32].

Types and doses of the drugs are determined by the doctors to depend on several variances such as body weight, body surface area, age, etc. Chemotherapy treatment has various side-effects like blood-forming cells in the bone marrow damaged, hair follicles most likely damaged, the cell in the mouth and the reproductive system can be damaged [34].

1.7. 2 Radiation Therapy

Radiation is a physical event, which is used to kill cancer cells. Radiation form is an ion (electrically charged particles) and when external radiation is applied to tissues, it accumulates energy in the cells. Also, this accumulated energy can destroy cancer cells or induce some genetic transformations in cancer cell death [36].

When a genetic material (such as deoxyribonucleic acid, DNA) of cells exposed to high-energy radiation, it takes some damages [37]. As a result of that, the ability to divide the cells and proliferate is prevented [38]. However, normal cells are also suffered from radiation damages like cancer cells; the radiation therapy aims to remove the maximum amount of abnormal cancer cells while minimizing exposure to normal cells, which are neighbors to the cancer cell or in the way of radiation. Generally, normal cells can fix themselves easily and faster than cancer cells. They can also get back to their function rapidly. On the other hand, cancer cells are not as good as normal cells in fixing the damage that comes from radiation treatment [39].

Radiation therapy can not kill cancer cells instantly. This process continues days or weeks when an adequate number of cancer cells die because DNA is damaged. After radiation therapy, cancer cells continue dying during weeks or months.

Radiation therapy has two primary subclasses which are internal and external beam therapy. In external radiation therapy, a big and noisy machine sends radiation on a specific location of cancer region from different ways with a touch less way. This method can be used for local treatment, not to the whole body. In internal radiation therapy, there is a need for an item which is a source of radiation that is put inside the patient's body. Depend on the radiation source, solid or liquid, two different branches of the internal radiation therapy come out.

One type of therapy that uses a solid source is called "brachytherapy". When this type of treatment is applied, some various radiation sources are put into the patient body, in or near the tumor such as seeds, ribbons, or capsules. Brachytherapy is a local treatment like external beam radiation therapy.

Another type of therapy that uses a liquid source is called "systemic therapy". During this therapy, the treatment journeys in the blood to tissues and looking for and destroy the cancer cell. You take systemic radiation therapy by swallowing, through a vein via an IV line (a way that gives any substances or drugs via a needle or tube that is inserted into a vein), or through an injection.

Sometimes, radiotherapy is applied with other therapy like chemotherapy, surgery, or immunotherapy. If radiation therapy is used to minimize a tumor before surgery or radiation therapy that way is called “neoadjuvant” therapy. If radiation therapy is used to remove to any remaining microscopic tumor cells after surgery that is called “adjuvant” therapy [36], [40].

1.7.3 Stem Cell Transplantation

Blood-forming stem cells are very important because they make various kinds of blood cells. After people exposed to the very high doses of chemotherapy or radiation therapy, their blood-forming stem cells are dead. As a result of that, healthy blood-forming stem cells are taken from donors and give to the patients via a needle in their vein. After injection, the stem cells travel to the bone marrow and replace the cells that were removed by treatment, these cells provide grow and renew blood cells [41].

1.7.4 Targeted Therapy

Targeted therapy is the basis of precision medicine. In this treatment type, some proteins that control cancer cell functionalities, are targeted. The treatment can attack cancer cells directly even without causing any damaged to healthy cell. This method treats cancer in different ways such as support the immune system to remove cancer cells, prevent to cancer cells grow, prevent to make new blood vessels, etc.

High numbers of targeted therapies are small-molecule drugs or monoclonal antibodies. When the target is inside the cell, small-molecule drugs are used, because they are adequate to go into cells easily. Monoclonal antibodies are also called therapeutic antibodies, which are specific proteins that are produced in the lab. Their job is to detect or mark cancer cells for the immune system [42].

Chapter 2

Minimal Residual Disease

Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood and it can also occur in adulthood. The disease can be seen under 3 years old children and people are over the age of 50 [43]. Besides, 60% of the patients are under the age of 20 [44].

Minimal residual (remaining) disease (MRD) is a term that means a small number of cancer cells still remain in the body after chemotherapy or radiation therapy. Due to this little amount of cancer cells, doctors cannot observe any physical or other symptoms. Generally, this disease cannot be detected with traditional methods such as observing cells under a microscope and by following unnatural serum proteins in the blood [45], however, it can be detected with more sensitive tests such as flow cytometry or qualitative real-time polymerase chain reaction (QR-PCR) [46], [47], [48].

Cells have different markers. Also, the percentage of existing cell markers can be varied after relapse and we can observe these changes with MRD monitoring techniques (Figure 2.1).

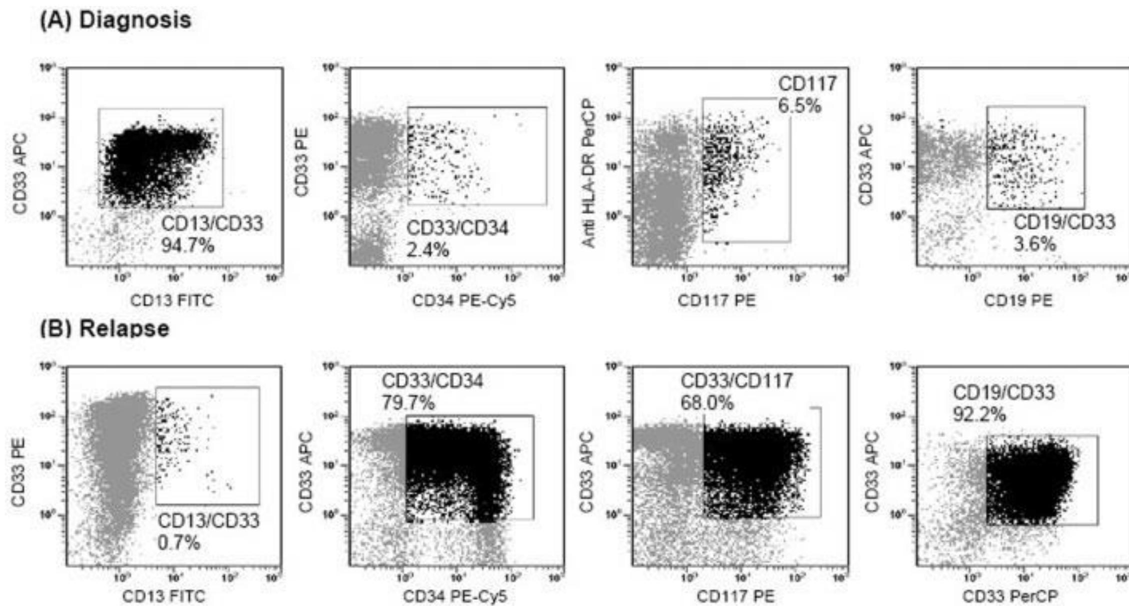


Figure 2.1: Samples are taken from the bone marrow of a child. (a) Different cell markers condition during diagnosis. (b) Changing cell markers depend on relapse [49].

2.1 Detection of Minimal Residual Disease

The first MRD detection was in 1980 by Janossy and his co-workers. They applied an antibody that was used for staining the terminal deoxynucleotidyl transferase (TdT) to identify the leukemic lymphoblasts from normal lymphocytes in the cerebrospinal fluid of ALL patients [50]. After a year passed, they reported research about T-ALL patients where they used a combination of anti-TdT with an anti-T cell antibody, and they were able to recognize MRD in the bone marrow of patients [51]. Developments of flow cytometers take lead to improve the use of antibodies to recognize leukemic cells [52]. Later, polymerase chain reaction (PCR) – based methods that involve PCR amplification of antigen-receptor genes to track ALL cells was reported [53].

MRD studies showed that even a lot of patients achieved a morphological reduction of blast cells there is a higher risk of relapse occurrence [54], [55].

According to cytomorphological definitions of complete MRD response, MRD persistence and MRD relapse seem reasonable (Figure 2.1.1) [56].

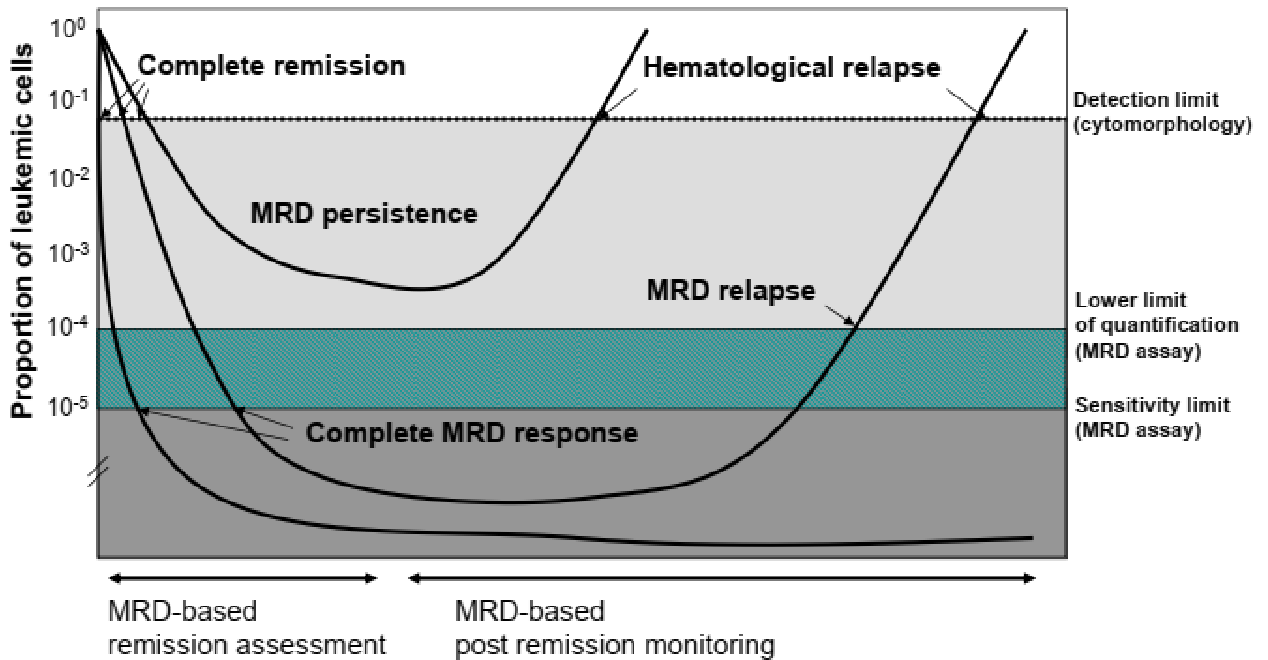


Figure 2.1.1: Suggestions for the definition of MRD terms in ALL [56].

2.1.1 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a technique based on the exponential amplification of nucleic acids by the thermostable “Thermus aquaticus (Taq)” polymerase [57]. This technique is used with two different types of molecular targets. One of them is retaliated to V and J junction regions in the Immunoglobulin (IG) and T-cell Receptor (TR) genes. Those regions are unique to each B or T lymphocytes. Gene fusions are the other molecular targets that are used to identify MRD like BCR-AML1, MLL-AFF1, and their abnormal mRNA results [58].

PCR is used in the antigen-receptor rearrangement process. As a result of this approach, a small number of ALL cells can be detected among the large number of normal lymphoid cells which means this approach has become the most sensitive method [59].

Clonal PCR fragments achieve supplementary allele-specific oligonucleotide (ASO) primers for MRD monitoring (Figure 2.1.1.1) that is usually performed by real-time quantitative PCR (RT-PCR). For each ASO-primer, amplification conditions and sensitivity tests are set on the diagnostic material. This protocol is used with fluorescently labeled probes that can make detection of up to 1 leukemic cell in 100,000 normal lymphoid cells. Thus, during the treatment process, PCR is used to measure MRD in bone marrow samples. However, ASO-PCR techniques require the development of reagents and assay conditions for each patient, which is labor-intensive, expensive, and time-consuming [60].

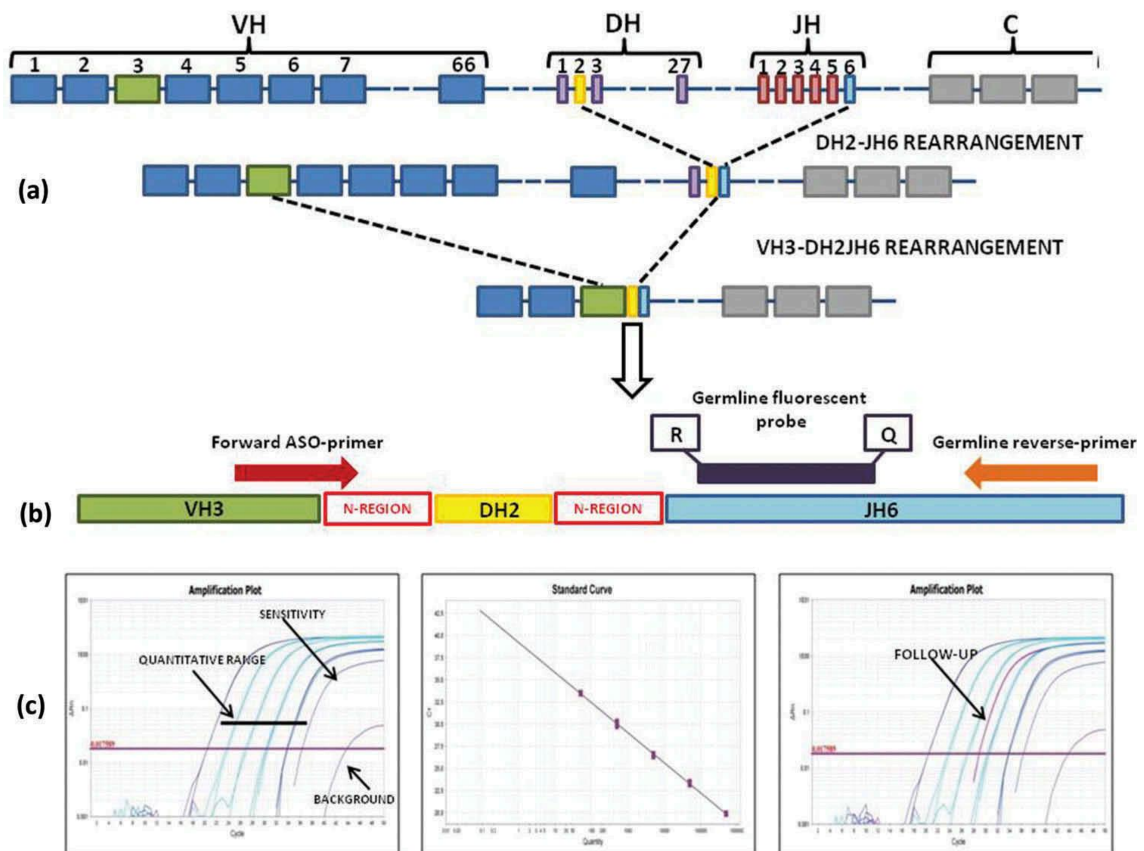


Figure 2.1.1.1: ASO primers for MRD monitoring. (a) Gene rearrangement schematic diagram. (b) ASO-primer for detection of MRD. (c) Repeatable results [61].

2.1.2 Flow Cytometry

Cytometry means quantifying cell characteristics, which include cell size, cell count, cell cycle, etc. When applied to measure cells (Figure 2.1.2.1), a laser beam is directed on the cell, and scattered light collected to obtain some information [62]. Flow cytometry can recognize and define the immunophenotypic characteristics of leukemia cells. The

immunophenotypic proteins can be stained with fluorescent dye-labeled antibodies and detected using MCFC (Multicolor Flow Cytometry) [63].

MRD analysis by MFC is often done at the post-induction part of the treatment. The detection sensitivity of MFC is 1 leukemic cell among 10,000 normal cells of bone marrow, however in PCR application detection of sensitivity is 1 leukemic cell among 100,000 normal cells in bone marrow (Table 2.1.2.1) [64].

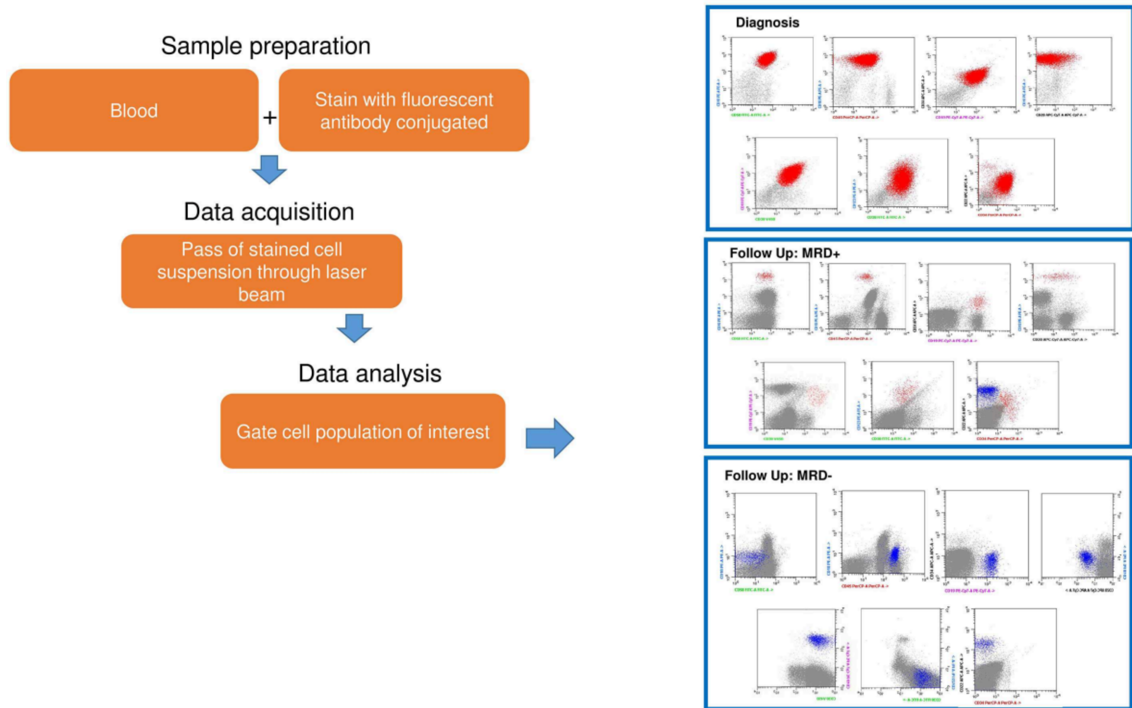


Figure 2.1.2.1: Standard working flow of flow cytometry during the MRD detection process [65].

MRD technique	Conventional flow cytometry	RQ-PCR of IG/TCR genes	RQ-PCR of fusion transcripts and other aberrances
Estimated sensitivity	3–4 colors: 10^{-3}–10^{-4} 6–8 colors: 10^{-4}	10^{-4}–10^{-5}	10^{-4}–10^{-6}
Applicability	BCP-ALL: .90% T-ALL: .90%	BCP-ALL: 95% T-ALL: 90–95%	BCP-ALL: 25–40% (age dependent) T-ALL: 10–15%
Advantages	Fast analysis at cell population level or single cell level Easy storage of data Information about the whole sample cellularity	Applicable in virtually all BCP-ALL and T-ALL Sensitive Well standardized + regular international QA rounds	Relatively easy Sensitive Applicable for specific leukemia subgroups, such as BCR-ABL or MLL-AF4
Disadvantages	Variable sensitivity, because of similarities between normal (regenerating) cells and malignant cells Limited standardization, no QA results	Time-consuming Expensive Requires extensive experience and knowledge	Limited standardization (only harmonization) Limited QA rounds (with conversion factors) Limited applicability in ALL (absence of targets in 50% of cases) Risk of contamination

Adapted from van Dongen et al. Blood. 2015;125(26):3996–4009.
BCP-ALL, B cell phenotype acute lymphoblastic leukemia; T-ALL, T cell phenotype acute lymphoblastic leukemia; QA, quality assurance.

Table 2.1.2: A comparison chart of the MRD detection techniques [66].

Nowadays, immune-based methods depend on analyzing specific combinations of the cell surface proteins (LAIP, leukemia-associated immunophenotype) during diagnosis or relapse periods. Thus, for MRD monitoring antibody combinations (Table 2.1.2.2) are preferred as a standardized panel [66].

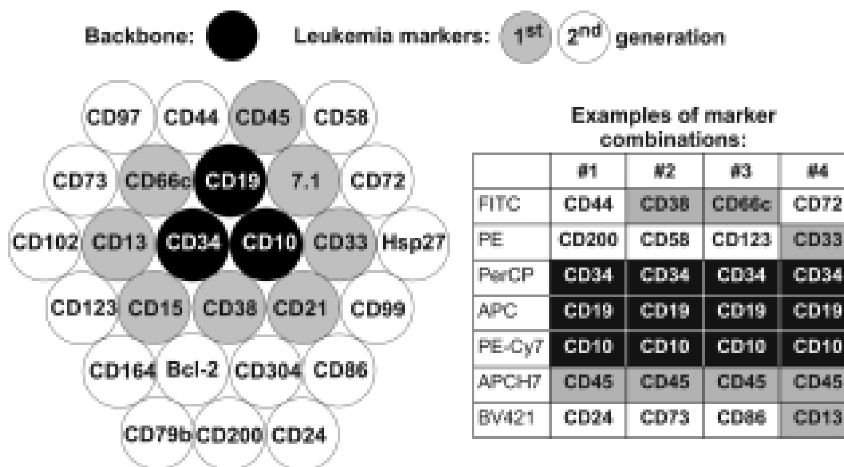
ALL lineage	Type of phenotypic abnormality	Markers	Frequency (%)[a]
B	Overexpression or underexpression of markers also expressed in normal B-cell progenitors	CD19/CD34/CD10/TdT	30–50
		CD19/CD34/CD10/CD22	20–30
		CD19/CD34/CD10/CD38	30–50
		CD19/CD34/CD10/CD45	30–50
		CD19/CD34/CD10/CD58	40–60
	Expression of markers not expressed in normal B-cell progenitors (aberrant marker)	CD19/CD34/CD10/CD13	10–20
		CD19/CD34/CD10/CD15	5–10
		CD19/CD34/CD10/CD33	5–10
		CD19/CD34CD34/CD10/CD65	5–10
		CD19/CD34/CD10/CD56	5–10
		CD19/CD34/CD10/CD66c	10–20
		CD19/CD34/CD10/7.1	3–5
	Expression of markers expressed at different stages of normal B-cell maturation	CD19/CD34/CD10/CD21	5–10
		CD19/CD34/TdT/cytoplasmic heavy chain μ	10–20
T	Phenotypes normally confined to the thymus	TdT/CD3	90–95
		CD34/CD3	30–50

Adapted from Dario Campana. *Leukemia and Lymphoma: Detection of Minimal Residual Disease*. 2nd ed. 2003. p. 21–36.

[a] Proportion of childhood ALL cases in which 1 leukemic cell in 10^4 normal bone marrow cells can be detected with the listed immunophenotypic combination. Most cases express more than one combination useful for MRD studies.

Table 2.1.2.2: Immunophenotypic markers used to study MRD in children with ALL [66].

Also, we have to consider combinations of monoclonal antibodies that are used to detect MRD in flow cytometry (Figure 2.1.2.2).



	#1	#2	#3	#4
FITC	CD44	CD38	CD66c	CD72
PE	CD200	CD58	CD123	CD33
PerCP	CD34	CD34	CD34	CD34
APC	CD19	CD19	CD19	CD19
PE-Cy7	CD10	CD10	CD10	CD10
APCH7	CD45	CD45	CD45	CD45
BV421	CD24	CD73	CD86	CD13

Figure 2.1.2.2: Combinations of B precursor leukemia antibodies [67].

Chapter 3

Magnetic Beads

Nowadays, novel technologies are leading to improve laboratory productivity, rapid and accurate analysis, and sensitivity of detection methods. As a result of these advancements, the detection of different biomolecules such as proteins, DNA, and cells have been developed [68]. Miniaturization efforts resulted in the development of a new generation of point-of-care devices which provides quick tests and portability [69]. Magnetic beads (MBs), quantum dots, and carbon nanotubes have important advantages when incorporated in point-of-care devices [70]. Also, nanomaterials provide huge possibilities for developing rapid and highly sensitive detection systems [71]. MBs have interesting features among the nanomaterials due to their specific magnetic properties. MBs's magnetic cores can be surrounded by a nonmagnetic polymer for the attachment of biomolecules (Figure 3.1). Another great feature of magnetic is that they can be manipulated with a simple, inexpensive magnet. This feature is very useful for isolating and collecting biological samples [72], [73].

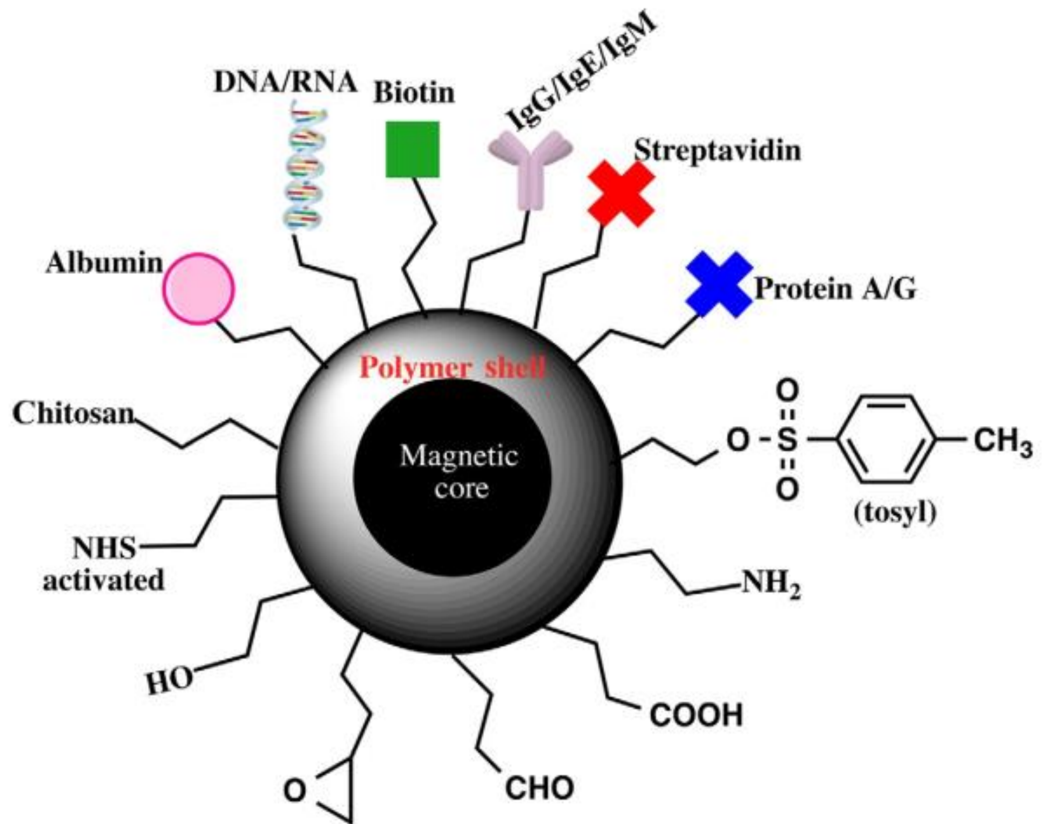


Figure 3.1: A functionalized magnetic bead with various biomolecules [74].

3.1 Applications of Magnetic Beads

3.1.1 Magnetic Separation

Especially in biomedicine, magnetic separation is used to separate target biological samples from their native environment and then to perform further subsequent analysis. Magnetic separation has two steps (Figure 3.1.1.1) which are labeling and collecting. Firstly, a label or tag of the desired biological sample is linked with the magnetic material. Then, to collect and separate these labeled biological samples via a fluid-based system an external magnetic field is applied to labeled particles. Finally, the magnetic field is removed and desired samples are collected [75].

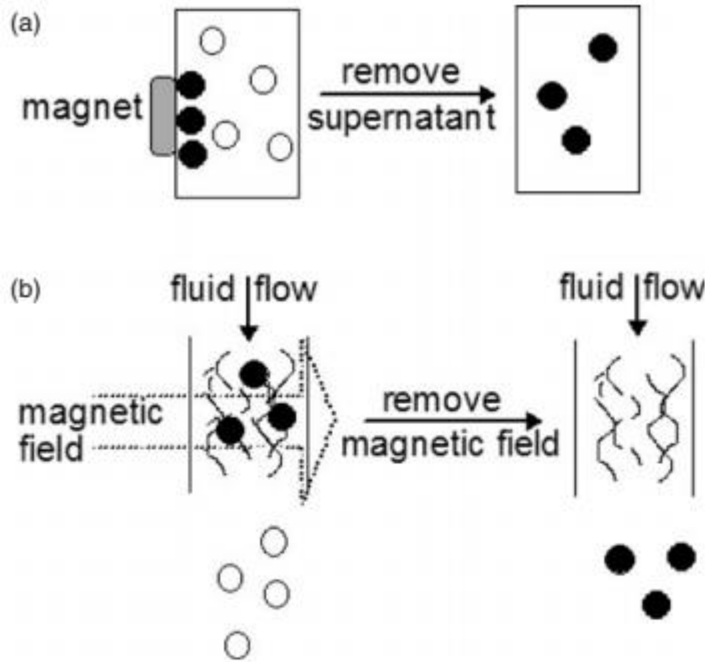


Figure 3.1.1.1: The standard methods of magnetic separation. (a) Magnet is captured labeled material, removed supernatant, and collected labeled samples. (b) A strong magnetic field applied while flows continuously passed through, labeled particles recovered by removing the field and collected with water flow [75].

3.1.2 Drug Delivery

Chemotherapy has a considerable disadvantage that the drugs are non-specific. Thus, they may reach to healthy cells and damage them instead of killing tumor cells. In the 1970s researchers had two main aims which are reducing the related side-effects that depend on the amount of the systemic distribution of the cytotoxic drug, and localized target of drug with reducing the required dosage. Biocompatible magnetic nanoparticles are attached to a drug that makes them as a drug carrier. Drug carriers are injected into the bloodstream and a magnetic field applied in a specific region (cancer region) into the body (Figure 3.1.2.1). According to the enzymatic activity or changes of physiological conditions like pH or temperature, chemicals are released from carriers, and the chemicals enter the tumor cells [76].

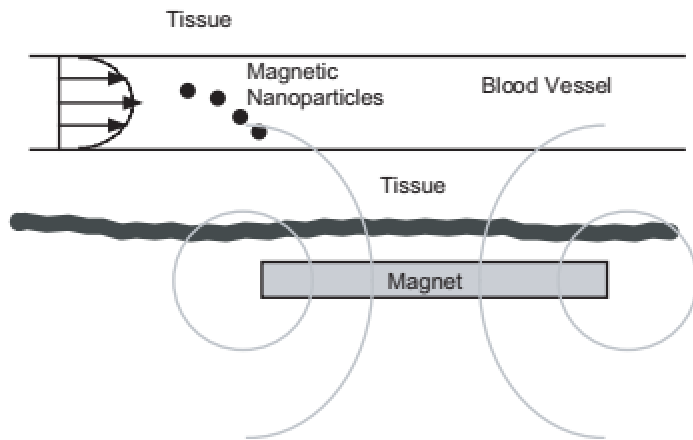


Figure 3.1.2.1: Cross-section of a hypothetical magnetic system [75].

3.1.3 Hyperthermia

Hyperthermia (also called thermotherapy) is one of the cancer treatment techniques. Because of the magnetic field, the tumor region is exposed to high temperatures, and as a result cancer cells are killed (Figure 3.1.3.1). In this treatment, magnetic particles are used and moved to target tissue then applying a magnetic field increase the temperature of magnetic beads. Cancer cells are killed and normal tissues take minimal injury [77].

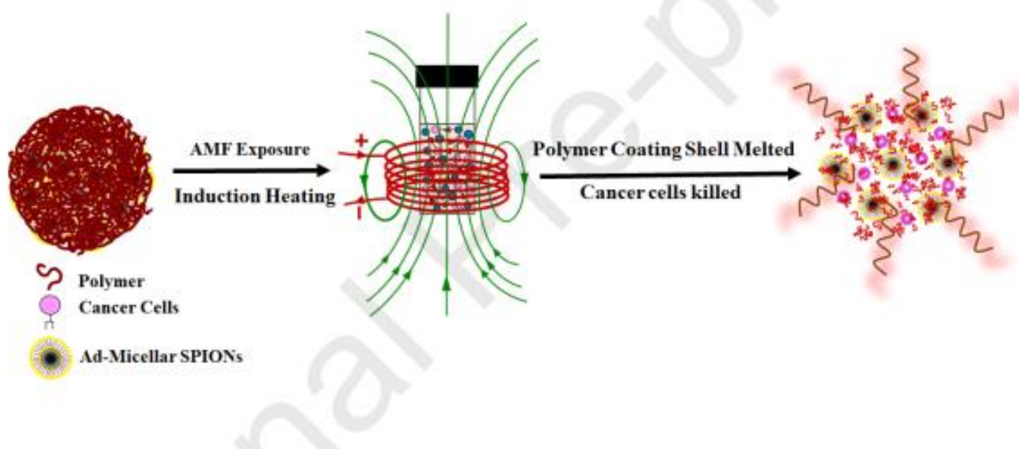


Figure 3.1.3.1: A general example of hyperthermia treatment [78].

Chapter 4

Immunomagnetic Separation

Immunomagnetic Separation (IMS) is used for isolating specific cells out of body fluid or cultured cells. In this method, antibody-coated magnetic beads are used [79].

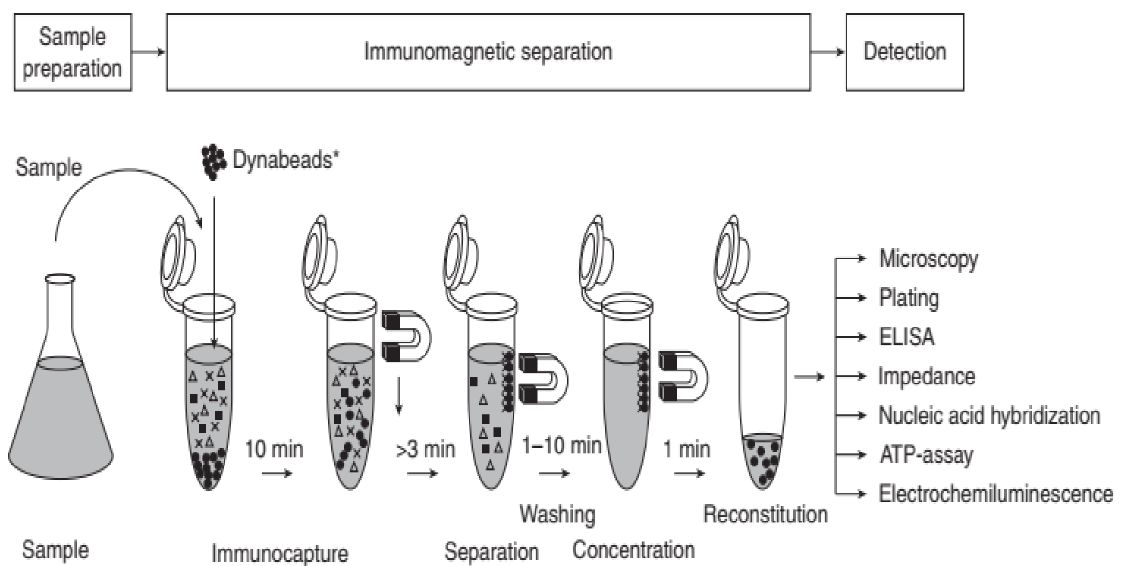


Figure 4.1: An example of ımmunomagnetic separation [79].

In our research, we used the IMS technique with double sorting that means two different antibody-coated magnetic particles are used. We used two different size magnetic beads which are nano-size beads (Microbeads) and micro-size beads (Dynabeads). Also, we prepared a functionalized surface which is coated with another antibody (Figure 4.2).

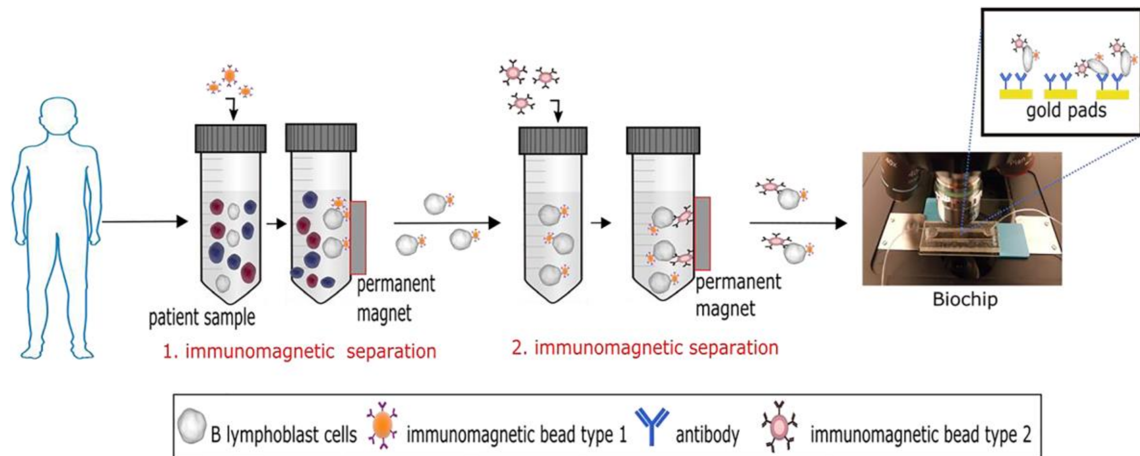


Figure 4.2: Detection strategy of the project [80].

4.1 A Method of Immunomagnetic Separation with Nano-size Beads

4.1.1 Buffer Preparation

Firstly, we prepared the AutoMACS buffer which includes DPBS (Dulbecco's Phosphate Buffered Saline) in 7,2 pH and 2 mM EDTA (Ethylenediaminetetraacetic Acid). Then into 10 mL of our buffer, we added 0,5% BSA (Bovine Serum Albumin) for labeling.

4.1.2 Cell Preparation

Firstly, cell culture (CCRF-SB cell line from ATCC) in the flask was mixed gently to divide cell clusters by pumping the pipette. Then one piece of falcon tube which had a volume of 15 mL was prepared, and put 5 ml of cell culture which was taken from the flask and was centrifuged in 350 xg 5 mins. The next step was removed the supernatant and was poured 5 ml of DPBS for rinse and counting cell numbers by Thoma Glass.

4.1.3 Labeling Process

Microbeads (The brand of Miltenyl and bead size was 120 nm) was used for labeling. $5x10^5$ cells/ml was obtained from the mixed solution and it was centrifuged in 300 xg 10 mins. The supernatant was removed and the pellet was introduced with 80 ul of the buffer. Then 20 µl of CD10 Microbeads were added and mixed by a rotor in 4^oC in 15 mins. After that, 1-2 mL of buffer was added for washing and then was centrifuged 300 xg 10 mins. After the supernatant was removed, the pellet was resuspended with 500 µL of the buffer.

4.1.4 Magnetic Separation

MiniMACS separator was put on the MACS stand. The column was put into the rotor chamber. The column was rinsed with 500 µL of buffer for preparation. This cell suspension was met with the column. The column was washed with 500 µL of buffer and this process was done by 4 times. And as a result of that unlabeled cells were collected into 6 well plates. Then the column was removed and placed on a proper collection tube and 1 mL of the buffer was pipetted onto the column. Labeled cells were flushed out by pushing the plunger into a column.

4.2 A Method of Immunomagnetic Separation with Micro-size Beads

4.2.1 Buffer Preparation

The buffer name was Buffer-I and it was included 1x DPBS with Calcium and Magnesium free in pH 7,4. 2mM EDTA and 0,1% BSA were added into it.

4.2.2 Bead Preparation

CD19 Dynabeads (This is the Invitrogen brand and bead size was 4,5 μm) was put into 50 mL of a falcon to homogenize beads for 5 mins at room temperature. 1 mL of Buffer-I was put into 2 mL of Eppendorf tube. Then 25 μL of CD19 Dynabeads was poured into the Eppendorf and was shaken for a while. Then this solution was put on the separator which was used the magnetic force as opened. The supernatant was removed after 1.30 mins passed and this step was repeated one more time. Finally, beads were ready for the next step.

4.2.3 Cell Preparation

Firstly, cell culture (CCRF-SB cell line is used) in the flask was mixed gently to divide cell clusters by pumping pipette. Then 5 ml of culture was taken from the flask and was added into a 15 ml type of falcon tube and centrifuged it in 350 xg 5 mins. The next step was removed the supernatant and was added 5 ml of DPBS for washing the cells and counting cell numbers by Thoma Glass.

4.2.4 Labeling Process

Dynabeads (The brand of Invitrogen and bead size was 4,5 μm) was used for labeling. $5x10^5$ cells/ml of the cells were divided for mixing with the solution and it was centrifuged in 300 xg for 10 mins. The supernatant was removed and the pellet was introduced with 1 mL of buffer. Prepared Dynabeads and cells were combined and mixed by the rotor in 4^oC for 20 mins. After incubation, cells were ready to separate.

4.2.5 Magnetic Separation

This solution which included cells was put on the 6-tube magnetic separation rack. The magnets were applied magnetics power on labeled cells and they were attracted to the

tube wall. 1 mL of the buffer was removed after 2 mins passed and the tube was separated from the rack, then 1 mL of the buffer was poured and Eppendorf was shaken for a while. This step was repeated 3 more times. When the final volume of buffer was added as 1 mL, labeled cells were collected in the tube.

4.3 Double Sorting

Double sorting means two different antibody-coated magnetic beads are used for capturing. Microbeads (nano-size) beads were applied first; however, if Dynabeads (micro-size) applied first, the second labeling process could not suitable because separation columns might be choked. After the Microbeads process was done, the Dynabeads process was started with CD10 labeled cells. As a result of that, we collected two times labeled leukemic cells (Figure 4.3.1).

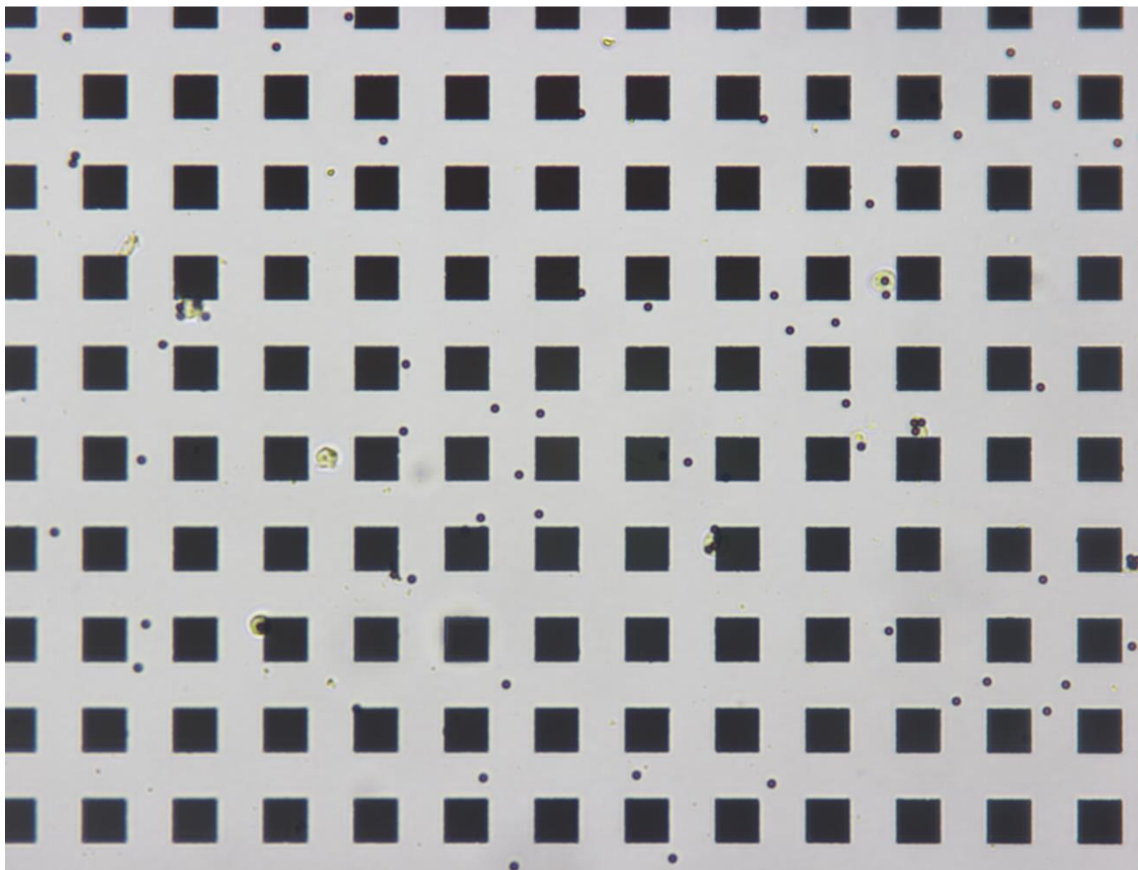


Figure 4.3.1: Captured leukemia cells. Leukemia cells were labeled by 2 different antibody-coated magnetic beads which were CD10 and CD19. This image was recorded with a bright-field optical microscope (Nikon) equipped with a charge-coupled (CCD) camera.

Chapter 5

Microfluidics

Microfluidic is the engineering of microdevices that manipulates and controls a small amount of fluids (usually in the range of microliters (10^{-6}) to picoliters (10^{-12})) by using channels smaller than 1 mm in at least one dimension. Starting from the 1990s, this cutting-edge technology has started to grow exponentially [81]. Attractive features of the microfluidic technology are reduced consumption of reagents, allowing precise control and manipulation of particles, automating multiple analyses simultaneously (also called lab-on-chip), and allowing easy to use imaging and tracking process. Nowadays, microfluidics is used in different fields ranging from biology and chemistry to information technology and optics [82]. Simple microfluidics involves three layers which have one inlet port, one outlet port, and a channel (Figure 5.1).

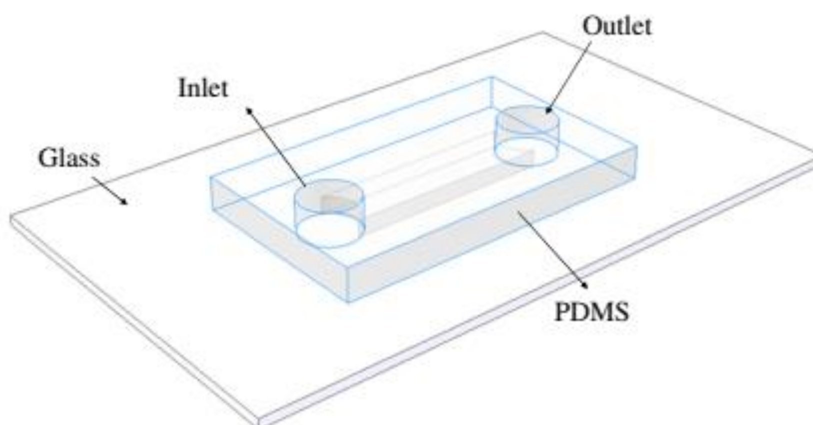


Figure 5.1: Illustration of a simple microfluidic chip design [83].

5.1 Microfluidic Chip Fabrication Process

In our research, we designed a microfluidic system for detecting leukemia cells which were captured by two different antibody-coated beads. In the microfluidic chip, we immobilized another antibody on gold pads as a result of this structure; we made a sandwich assay for capturing leukemia cells.

5.1.1 Design

The microfluidic device consists of four components which were standard microscope glass slide covered bottom side, a middle channel layer, and the top cover is Polymethyl methacrylate (PMMA). PMMA is a standard material that was used in microfluidic design due to its great features such as being elastic, biocompatible, transparent, resistant to UV radiation, good rigidity, lightweight, cheap, and good mechanical properties [84], [85].

The software CorelDRAW (X7) was used for sketching microfluidic chip design and after that, a laser cutter (Epilog Zing Laser Series) was used to identify channels and the inlet, outlet ports on the top cover.

The gold pads were fabricated on a 6-inc glass wafer using a standard lithography process. These gold pads were fabricated with different size arrays ranging from 15 µm x 15µm to 35 µm x 35 µm. The glass wafer size was 7 mm x 10 mm and it was placed into the microfluidic system (Figure 5.1.1.1). The gold pads were used for the functionalization of specific antibodies in the next step.

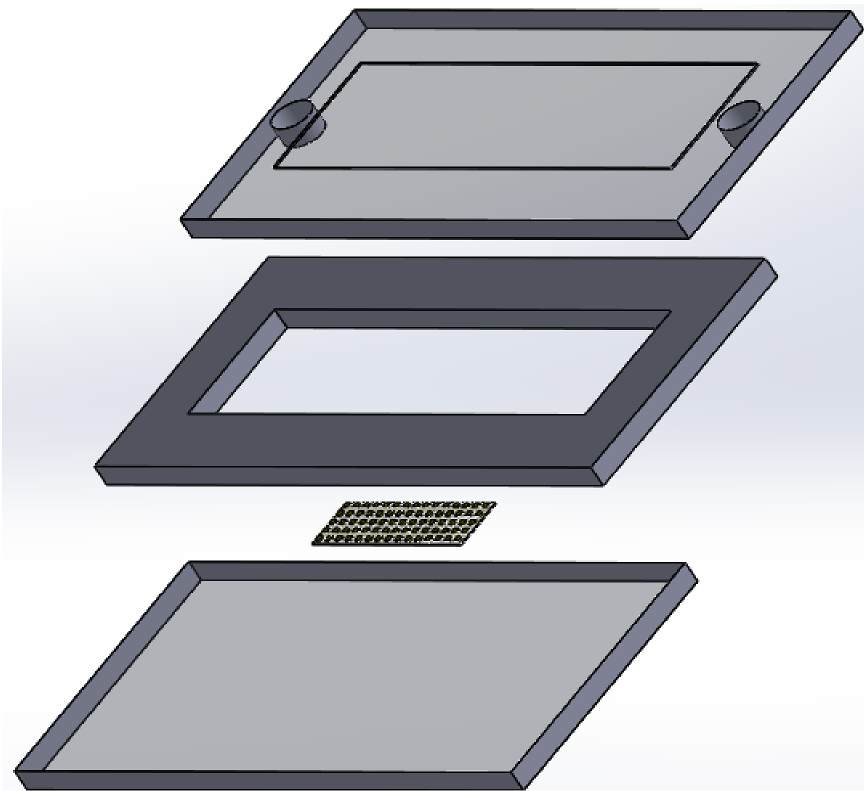


Figure 5.1.1.1: An illustration of the microfluidic system was made with SolidWorks software. The top cover and middle channel layer were made from PMMA (Polymethyl Methacrylate), gold-glass surface was put into the chamber, and the bottom cover was a conventional microscope glass slide.

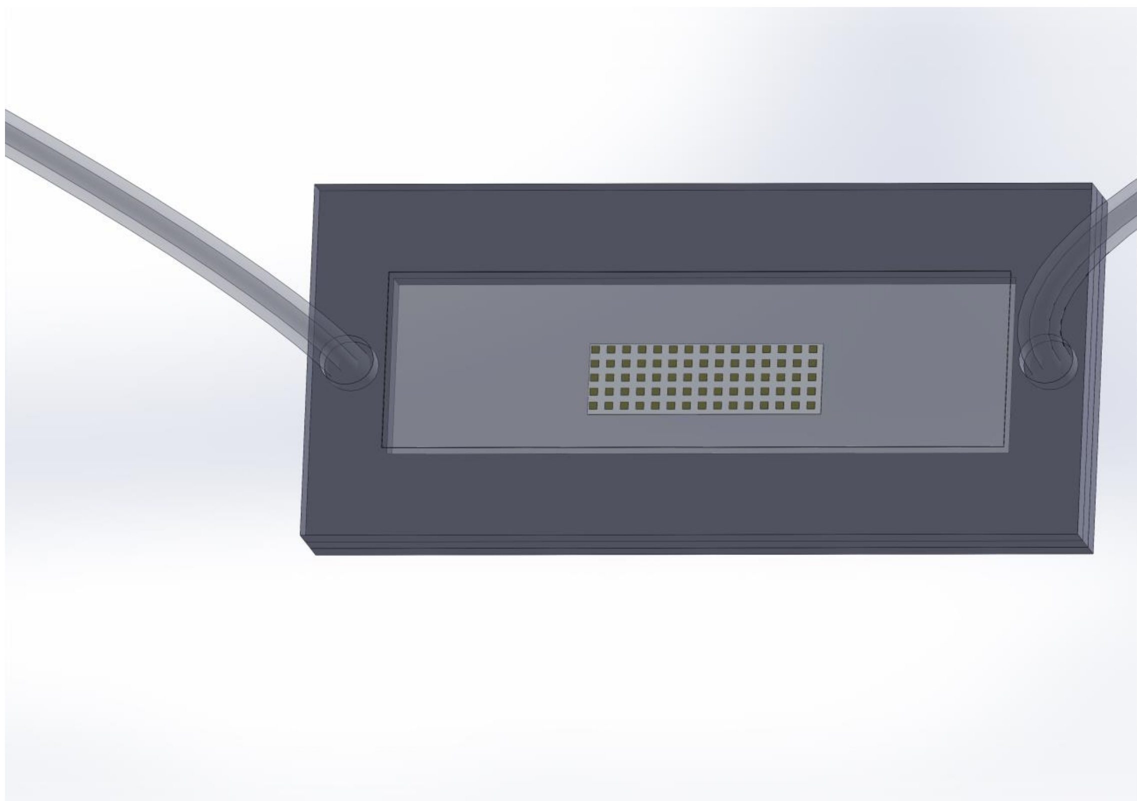


Figure 5.1.1.2: Illustration of a closed microfluidic system.

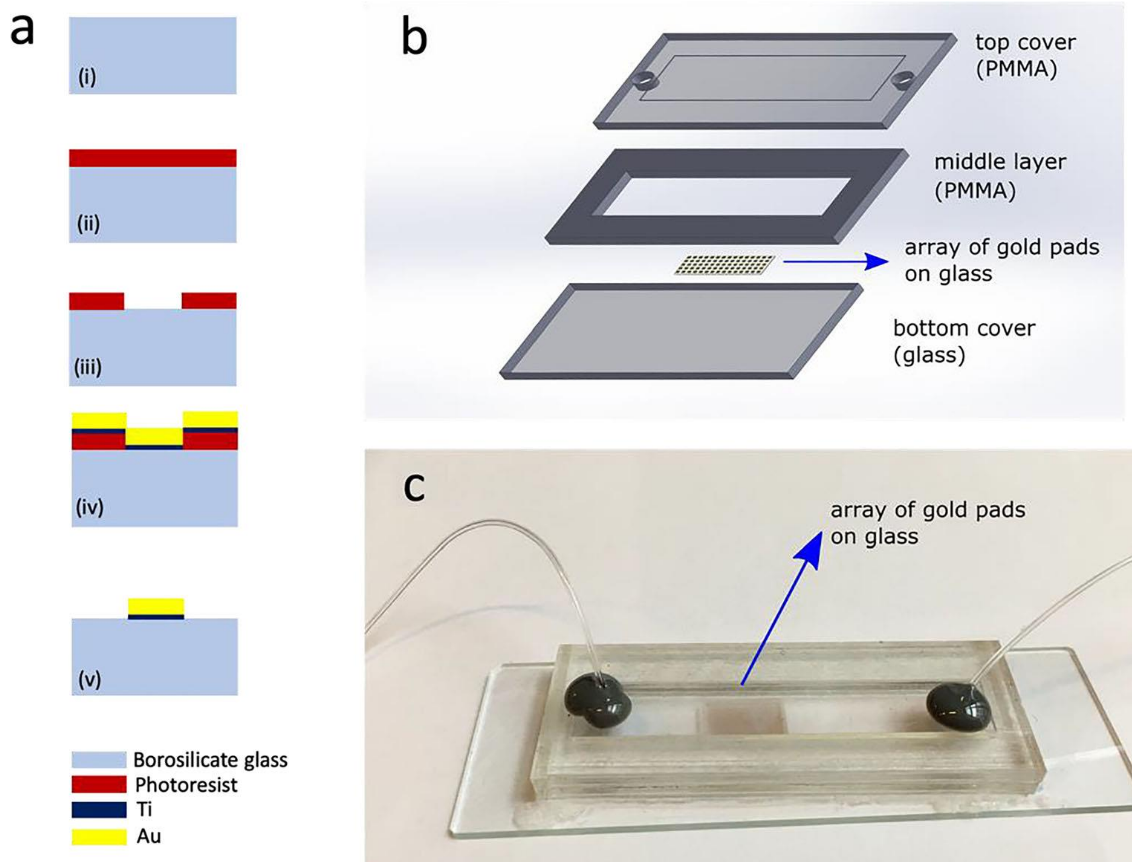


Figure 5.1.1.3: Fabrication process of the system (a) Microfabrication process flow for the array of gold pads: i) borosilicate glass wafer cleaning, (ii) spin coating of photoresist, (iii) patterning photoresist, (iv) titanium and gold deposition (v) lift-off process (b) An illustration of the microfluidic chip (c) The assembled device [80].

5.1.2 Sealing

During the sealing process, the double-sided tape was used for sealing the whole system and inlet-outlet tubes were sealed with epoxy at room temperature. The designed microfluidic chip's chamber size is 2mm x 8mm x 40mm.

5.2 Chip Surface Functionalization

The gold pads were functionalized for capturing leukemia cells with different chemicals [86]. During this process, some control experiments were performed between functionalizing steps. Firstly, the glass slide and gold pads were cleaned with deionized water and absolute ethanol and then dried under a nitrogen stream. After that, the chip

surface was modified with 1mM 11-Mercaptoundecanoic acid (MUA) in ethanol for 24 hours. We performed experiments for identifying the best MUA exposed time (Figure 5.2.1). According to the amount of captured leukemic cells, we applied MUA on the gold surface for 24 hours.

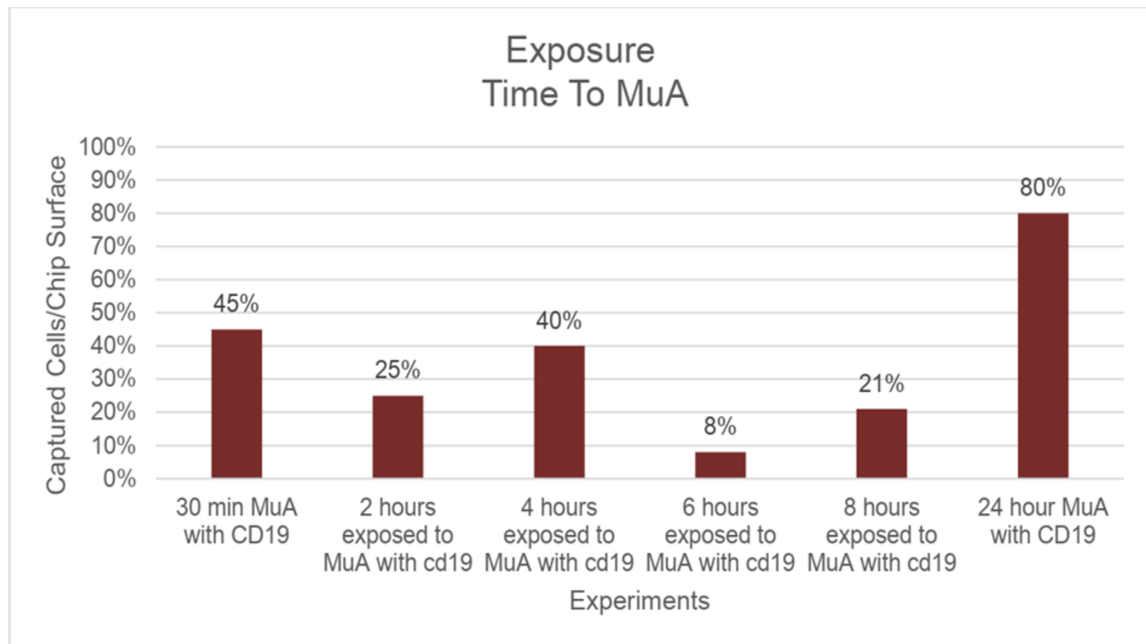


Figure 5.2.1: Captured leukemic cells on functionalized chip surface with applied different exposure times to MUA. Best captured efficiency was obtained when surface exposed MUA for 24 hours [80].

After exposed to MUA, the gold pads were rinsed with absolute ethanol. Then the N-Ethyl-N'- (3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC)/ N-hydroxysulfosuccinimide (NHS) was applied as a cross-linker. 5 mg EDC and 5 mg NHS were put in 1mL deionized water and poured on the surface and wait for 25 minutes, and the surface was rinsed with deionized water for 2 minutes. The next step was adding the protein G solution; 800 µL protein G (5% protein G solution in deionized water) in 5.4 mL Phosphate Buffered Saline (PBS) on the chip surface for 25 minutes, and then the surface was rinsed with PBS for 2 minutes. The 510 µL of stock solution of CD45 (20 µL CD45 antibody in 1 mL PBS) was diluted in 5.4 mL PBS and after that, the solution of CD45 was put on the surface for 25 minutes. Another control experiment was performed during this process. We needed to verify the leukemic cells could not bind to the surface without antibody (Figure 5.2.2).

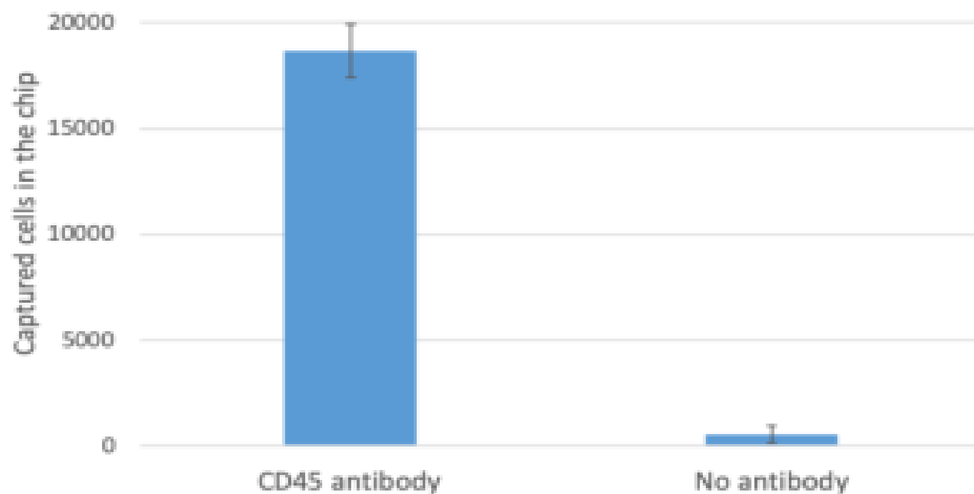


Figure 5.2.2: Antibody control experiments.

The final step was applying of Bovine Serum Albumin (BSA) blocking where 5% BSA solution (0.5 mg BSA in 10mL PBS) was added on the surface for 120 minutes. BSA is provided as a door which prevents non-specific bindings [86].

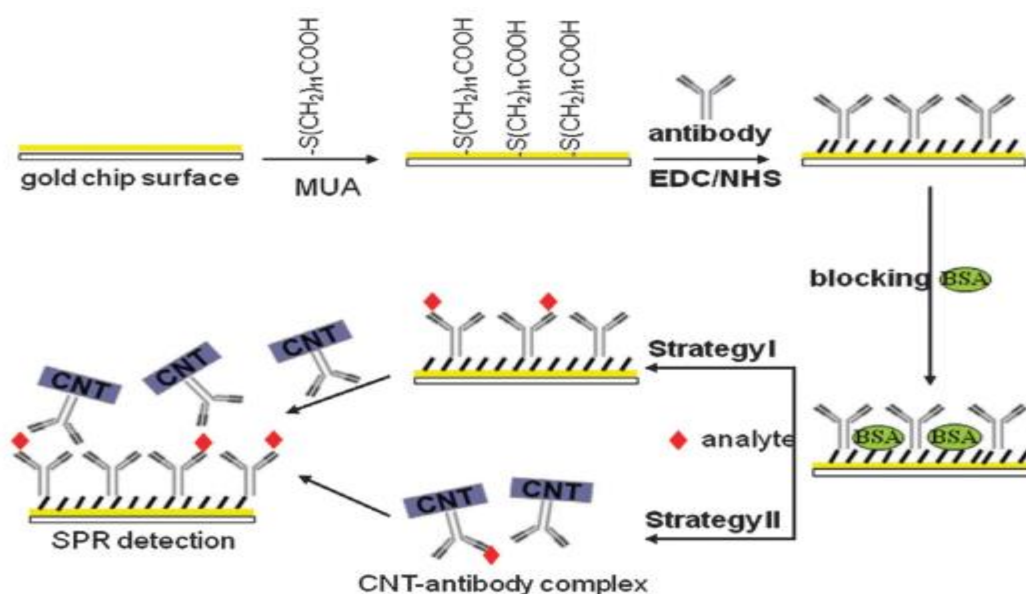


Figure 5.2.3: Illustration immobilization biomolecules on the gold surface. MUA, EDC/NHS, and BSA blocking are shown [87].

5.3 Beads Preparation

Beads preparation techniques are already described in Chapter 4 [88]. We applied the same techniques on culture cells and patient samples.

5.4 Cell Culture Experiments

The cell line name was CCRF-SB that was mentioned in the previous chapter which was used in our research. According to flow cytometry results, approximately10% amount of these cell lines had CD10 antigens and 99% of the cells had CD19 and CD45 antigens. As a result of that, we just performed experiments with only two types of antibodies. Moreover, we examined a bunch of experiments that was about the cell capture ratio of the microfluidic chip (Figure 5.4.1).

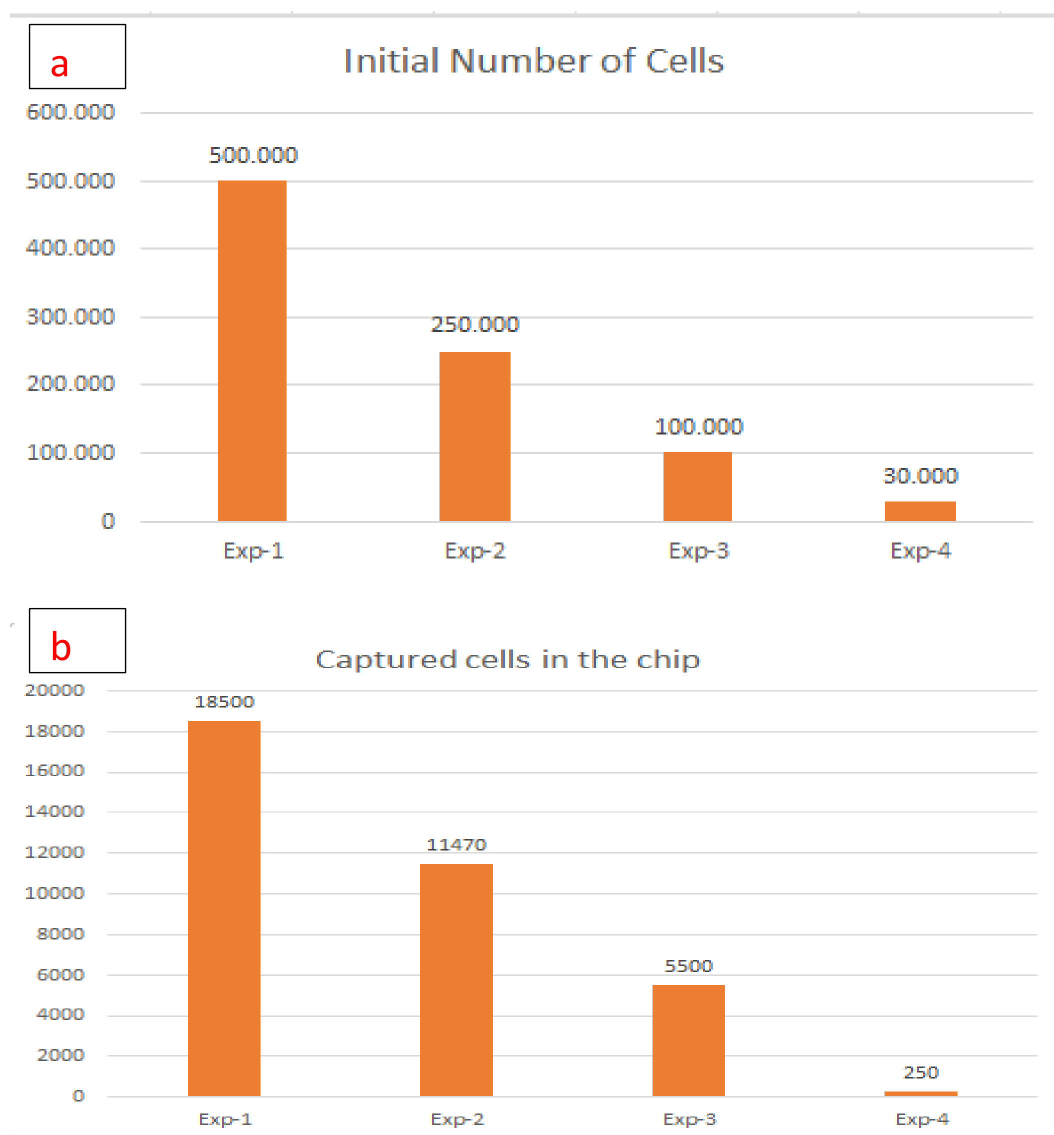


Figure 5.4.1: Cell capture ratio of the microfluidic chip. (a) The numbers of initial cells and (b) the number of captured cells. These experiments were done by multiple times.

5.5 Patient Samples Experiments

We collected 12 samples from 4 different patients who were suffering from ALL and performed experiments with a triple antibody capturing process in the microfluidic system. The average cell size of samples was around 10 um, however, the average cell size of cell cultures was around 25 um. As a result of that, we performed our experiments with different sizes of gold pads. We observed that the cell size of the samples from patients could be various.

5.6 Results

We made a low-cost microfluidic system for the detection of leukemia cells, especially for MRD. We used two different pumps; the first one was a peristaltic pump which was used for delivered our samples to the inlet of the microfluidic chip, and the other one was a syringe pump which was used for collecting nonbinding cells from the outlet of the chip. These pumps had different speeds like 10 µl/min and 125 µl/h orderly. These speed differences belong to some features of our system. The first one was due to the experiments' time. Magnetically captured cells should not wait too much, if they do there might be a blockade occur because of the gravity during flow. On the other hand, we used a syringe pump at the end of the chip with 5ml syringes. Thanks to that pump we collected unbounded cells easily. When the syringe pump's speed scaled; syringe volume, the inner diameter of the syringe, and the outer diameter of the syringe were considered. That's why our pumps' speeds were different. Pumps' speeds can seem different if we just considered the rates of pumps however; if we measure injection volume and withdrawing volume during a certain time, we can observe those volumes are equal.

To observe the designed system, cultured cells were applied to the whole system firstly. Cultured cells were counted just before delivered to the microfluidic chip. Then we

counted again at the end of the experiments. We observed that there was a positive interrelation between the delivered number of cells and binding cells (Figure 5.4.1). Also, we had to find a chance to observe binding cells under the scanning electron microscopy (Figure 5.6.1).

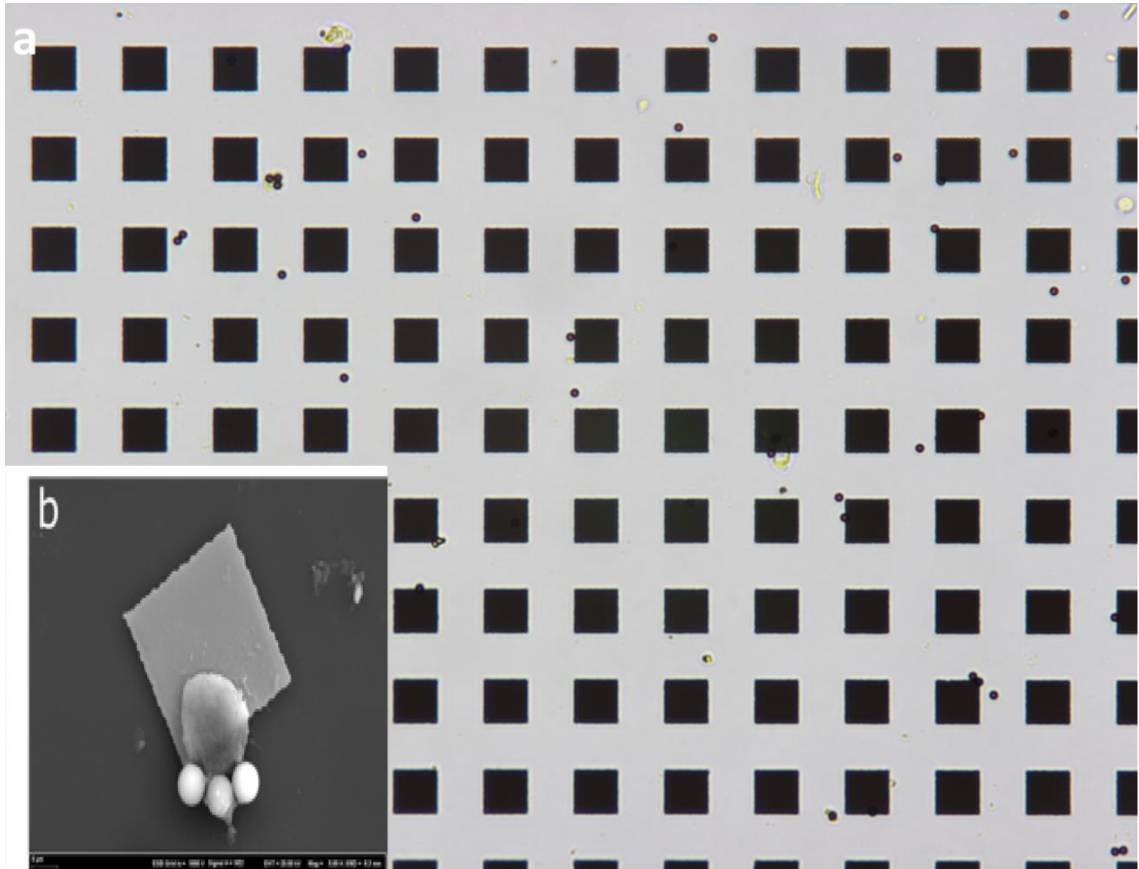


Figure 5.6.1: A captured cell was observed 2 different microscopes. (a) Captured leukemia cells with immunomagnetic beads on gold pads that image was captured from a bright-field optical microscope. (b) A captured leukemia cell with magnetic beads that image captured from a scanning electron microscope.

Besides, we tested a designed low-cost microfluidic system with an aspiration of patients with ALL that was took from bone marrows. The numbers of delivered cells were different. During these experiments, we applied 3 different antibody-coated magnetic beads. The first two magnetic beads were used in the separation process and the third one was already put into the microfluidic chip. Thus, samples were delivered immediately to the microfluidic system after captured from other antibodies. These samples were taken from patients during a chemotherapy treatment on different days. After the experiments, we counted captured cells on gold pads via an optical microscope (Figure 5.6.3).

Captured leukemia cells were counted manually firstly and then an automated cell counting software developed by our research group (Figure 5.6.4). As a result of that, we compared the numbers which came from manual counting and automated counting and we noticed that these two counting methods are statistically matching [89].

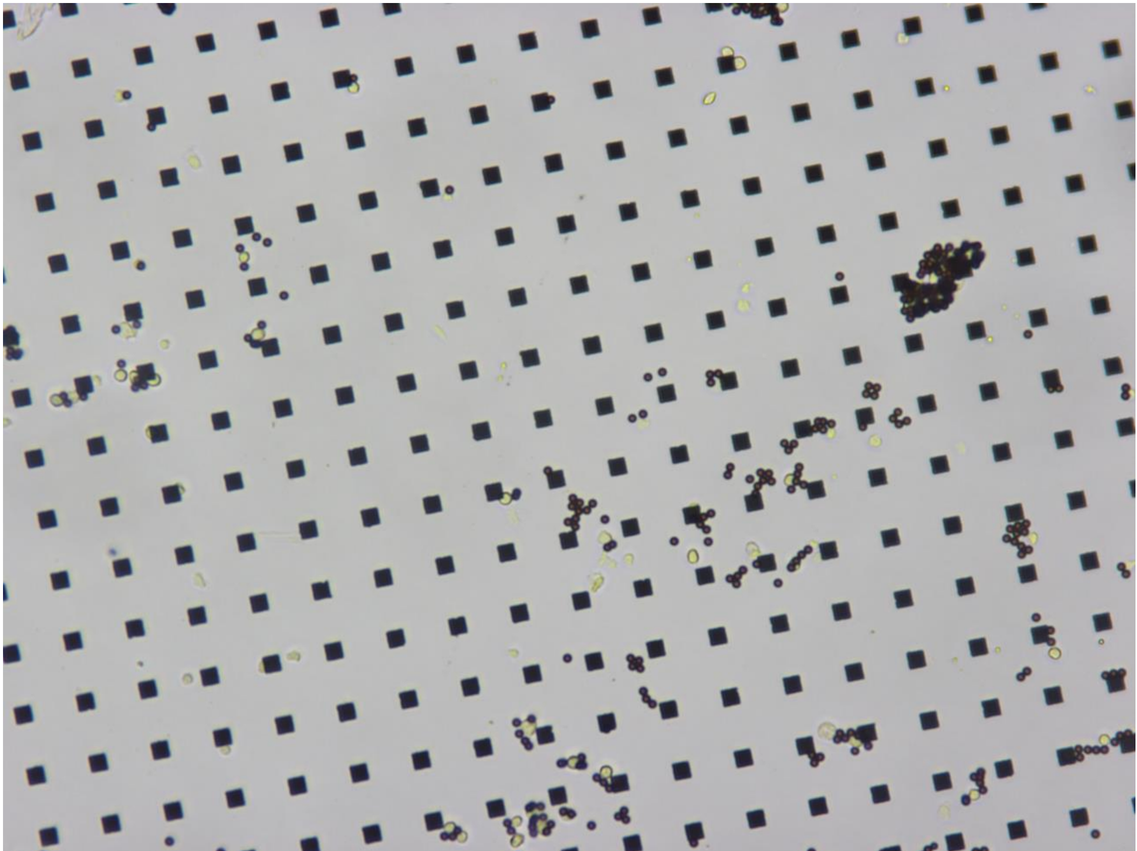


Figure 5.6.2: Captured leukemic cells with 3 different antibodies. The sample was taken from the patient at diagnosis. The picture was recorded by an optical microscope using a 20X objective.

To compare the results of our method and flow cytometry for 8 samples from 4 patients, 0.01 was considered as the statistical significance level. Mann-Whitney Test Statistics Asymp. Sig. (2 -tailed) $P = 0.564 > 0.01$ showed that both methods were 99% agreement in detecting the decrease of cells between the initial diagnosis time and the 15th day of chemotherapy. These results show the potential of the platform and it can be used to detect the patient's YES(+)/NO(−) response to the applied therapy (Figure 5.6.5) [80].

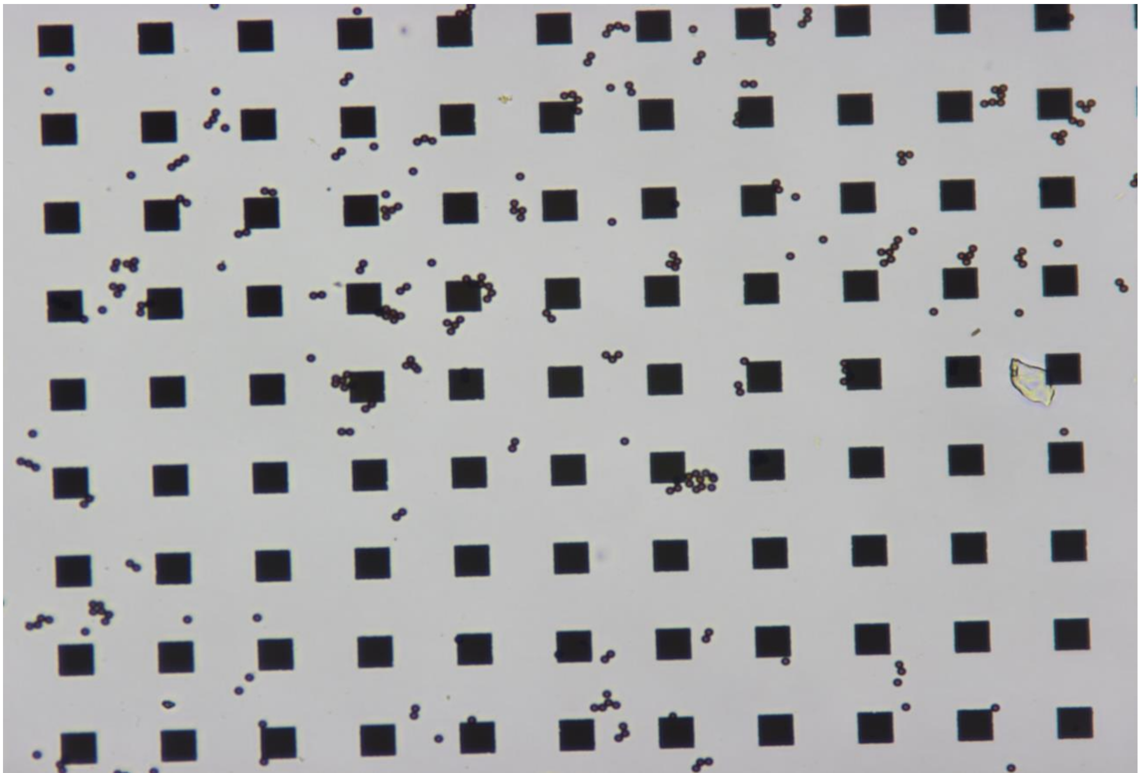


Figure 5.6.3: Captured leukemic cells with 3 different antibodies. The sample was taken from the same patient on the 150th day of chemotherapy treatment. The picture was recorded by an optical microscope using a 20X objective.

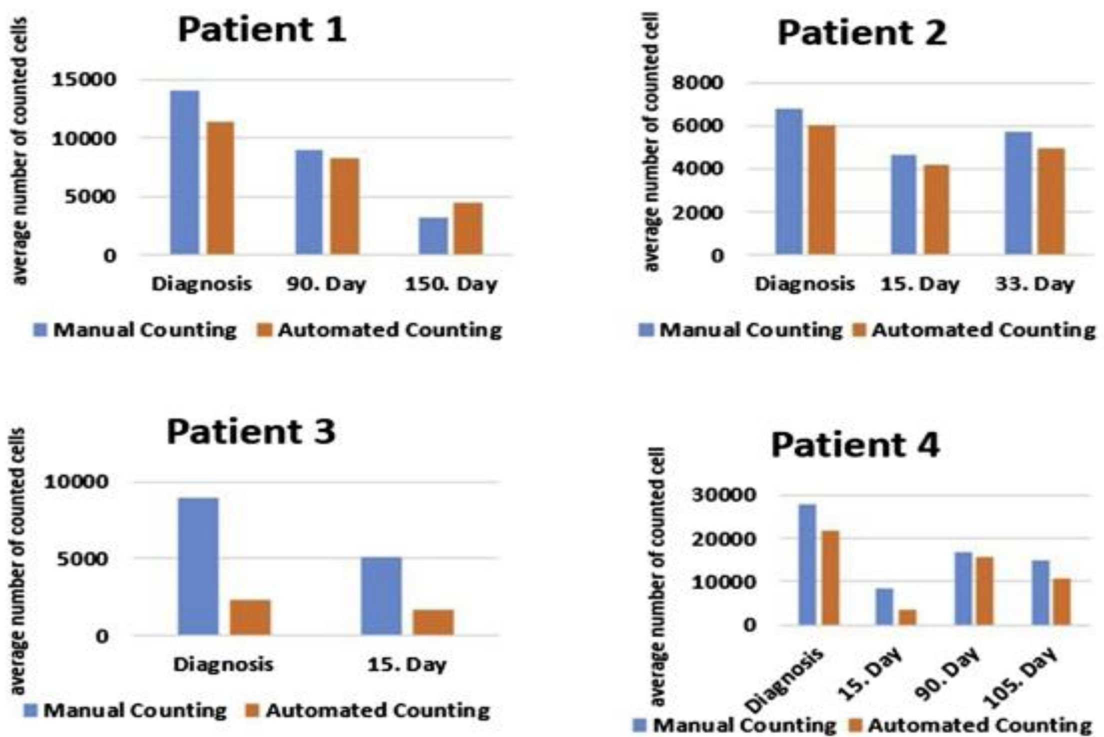


Figure 5.6.4: Comparison of graphs of manual counting and automated counting for different patients' samples [89].

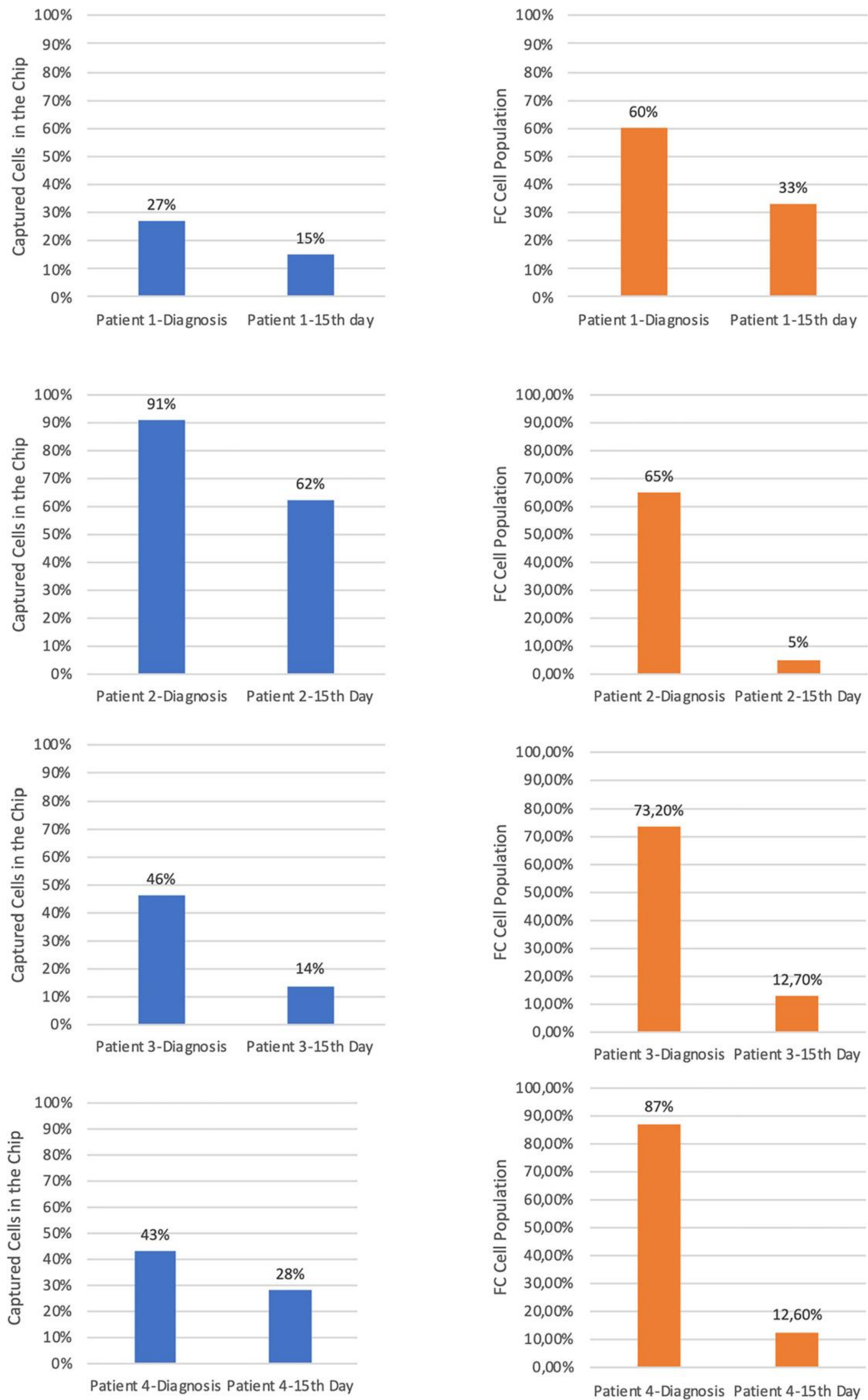


Figure 5.6.5: The analysis of the patients' examples with the developed microfluidic chip and flow cytometry [80].

Chapter 6

Conclusions and Future Prospects

6.1 Conclusions

We tested the low-cost microfluidic system with cultured cells and samples from patients. According to the observations, a low-cost and time-efficient microfluidic chip can be for the monitor patient response to applied chemotherapy during the treatment process. We targeted three different surface markers for capturing target blast cells. The system was able to capture 250 leukemia cells from 3000 cells and the detection method requires just an optical microscope that can be found in every lab.

Also, the size of the gold pads is an important parameter due to observations. We saw that we could not identify cells on the larger gold pads because our sample sizes were various. Our total experiment time is around 2 hours which involves immunomagnetic separation and fluid flow in the microfluidic system.

6.2 Global Sustainability

According to the recent developments of the biosensors, we need low-cost and user-friendly systems. Our study indicated there is no significant difference between our results and flow cytometry. Thus, our system can be an alternative method for observing the patient's response to applied therapy.

6.3 Future Prospects

We had some limitations caused by the design of the microfluidic system. For improving capture efficiency, we would decrease the height and length of the chamber. Our future aim will be to improve the sensitivity of the system to increase the detection ratio up to the flow cytometry detection limit.

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