DETECTION OF MODIFIED CELLS USING "LAB-ON-A-CHIP (LOC)" MICROFLUIDIC DEVICES

Marinescu Maria-Roxana[1], Ghiculescu Liviu-Daniel[2]
[1] Phd. Student, M.Sc. Ing., National Institute for Research and Development in Microtechnologies - IMT Bucharest, roxana.marinescu@imt.ro
[2] Professor, PhD., Eng, "Politehnica" University of Bucharest, daniel.ghiculescu@upb.ro

ABSTRACT: Biological medicine is also called "the medicine of the future" because of its performances to detect in time different diseases which the world population is facing. Sometimes, it can be the only chance to survive or to recover. It is wanted to create efficient and precise analytic instruments needed in medical diagnoses. Biochips (lab-on-a-chip systems) are part of the current concerns of the micro-nanobiotechnologies and nanomedicine, both nationally and internationally. Biochips have applicability in medical field, in clinical research due to its complexity and the importance of the information provided for rapid diagnosis and therapy, with implications in lowering the risk of relapse, and due to the reduced costs compared with the current methods.
This paper presents devices that detect, capture or separate different cells using different cell properties (size, surface markers, electric properties, deformability, etc.). In this kind of system, a low-cost sensor is the main piece of interest.
KEYWORDS: biomedicine, nanotechnologies, "lab-on-a-chip" systems, microfluidics, cells.

1. I NTRODUCTION

Detection, quantification and biological molecule modelling has become a very important aspect of daily life in: medicine, industry and environment. The research in based on developing devices that can detect the modified cells in the blood. These changes are representative for various diseases. Detected in time, many lives can be saved.

Worldwide, cancer is one of the main causes of death. If from the beginning there can be found solutions to detect the modified cells, there is a considerable chance of their healing [1].

„Lab-on-a-Chip" devices (LOC) can be used where the existing technologies don't answer to some specific needs of the clients. With their help the type of cells or their number can be detrmined. These devices can be adapted in order to make diagnosis or can be used to select and use immune cells in order to treat other diseases. These devices can adapt to make diagnosis or can be used to select and use immune cells to treat other diseases [2].

The cell is the basic functional unit of the human body, the number of cells in the body being in order of billions. Blood contains three cell types: red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (Figure 1).

The optimal number of each cell group to be found in the human body is shown in Table 1 [3].

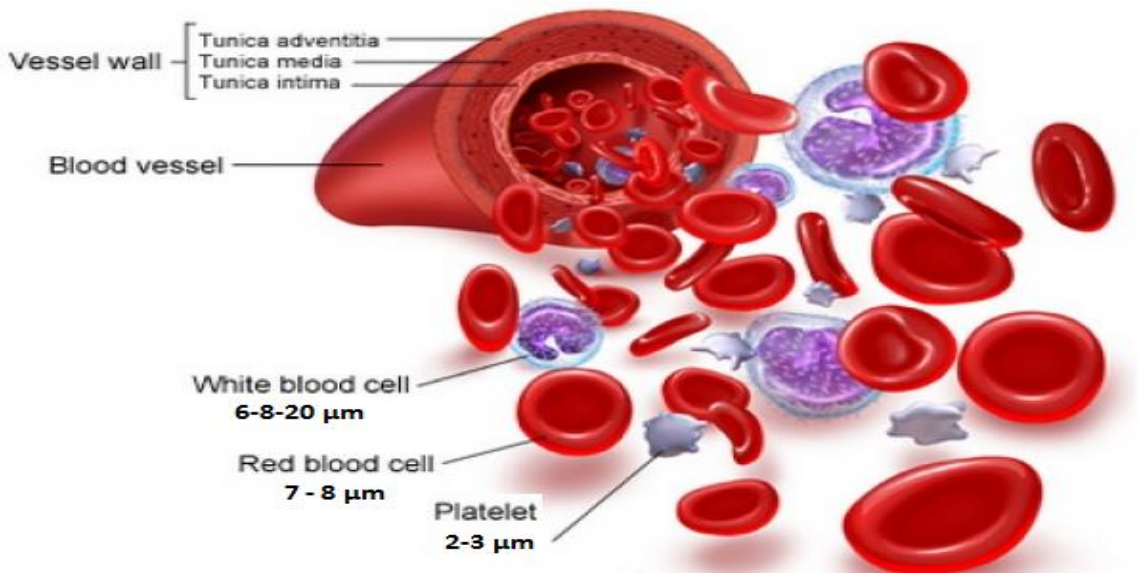

Figure 1. Blood cells [4]

Tabel 1. Components of human blood

Name		Number of cells in blood - µl (mm^3)
1. Erythrocytes		4,5 – 5,0 mil. on women 5,0 – 5,5 mil. on men
2. Leukocytes		6.000 – 8.000
Granulocytes	2.1. Neutrophils	2.500 – 7.500
	2.2. Eosinophils	40 – 400
	2.3. Basophils	10 – 100
2.4. Lymphocytes		1.500 – 3.500
2.5. Monocytes		200 – 800
3. Platelets		300.000

Processing methods of microliter cell samples are required for various applications, such as: biomedical research, medical diagnosis, anti-terrorism, environmental analysis, biotechnology etc.

Developing a quantitative and qualitative cell analysis requires the identification and isolation of certain cells from a complex mixture. In addition, in order to obtain reproducible informations, a reliable and non-destructive purification method is required.

Right in the moment of separating the cells, many difficulties appear, because human cells are very fragile compared to other cells, so they are much more sensitive to changes in the environment.

On macroscopic scale, efficient and rapid systems for cell separation were made using different techniques: cytometry, centrifugation and filtration. These conventional methods have major disadvantages, the sorted cells being diluted and dispersed. The efforts regarding the reduction of the volumes and the energy required for separation have been materialized by a number of miniaturized devices that use the microfluidics and micro-manufacturing techniques advantages.

These advantages given by the microfluidic sorting and separation techniques include: a precise control over cellular media; a small volume of reagent and much lower reaction times in biochemical analyzes. This allows a reduction of the required space but also a significant reduction of the costs: monocells or rare cells that can be handled non-invasively; allows parallel processing of multiple reactions; enables easy process automatization [3].

Microfluidics was considered a technology of miniaturization [5]. It deals with the science and the technology of fluids on micrometric or submicrometric scale, referring to the operation, precise control and manipulation of small volumes of liquids or gases by miniaturization of various geometric shapes and practical functionalities [6].

Intelligent microfluidic systems / lab-on-chip / biochips are used for modeling the mechanisms of the molecular transport in biological fluids, such as: blood, cerebrospinal fluid, amniotic fluid etc. At the time of manufacture of such bio-devices, the development of methods for fluid flow control - monitoring, dosing and controlled release of biological fluids to the elements detection is being pursued.

These microfluidic devices allow the development of innovative and non-invasive methods for early diagnosis of many diseases, including: hypercoagulability to vascular patients, various malignant tumors and other biomolecule circulatory abnormalities. By correlating with a database of health and disease profiles, correct diagnosis can be made and treatment schemes can be described [3].

2. BIODEVICES – APPLICATIONS AND CARACTERISTICS

Biosensitivity systems (biosdevices) include a set of resources: biological (tissues, enzymes, acids), electrical, electronical, mechanical, photonical, and interconnecting elements such as: capsules, terminals, bioliquid (that make the connection with the external environment). They are used to produce detectable signals for monitoring or identifying biological phenomena [7], [8].

The complexity of biosensitive technologies is also helped by other technologies: integrated circuit manufacturing methods; photonics, optic fiber and biotechnology, in particular genetic engineering [8].

Biodevices present different complications, which should be tracked and excluded as much as possible. Any device made to last for freeware applications must meet certain biocompatibility and biostability requirements. One of the most important conditions is that it should not cause toxicity to the surrounding tissues, and should not damage the local tissue by introducing mechanical stress [7].

The specific technical issues to be addressed for "lab-on-a-chip" devices are:
• nucleic acid sensors;
• organism- and cell-based biosensors;
• bioelectronics and biometrics;
• biointerfaces and biomaterials,
• biocompatibility;
• integrated, multimodality sensors and sensor networks;
• system issues, including signal transduction, data interpretation, and validation;
• new sensing algorithms;
• related issues in bio-MEMS and bio-NEMS (microelectromechanical and nanoelectromechanical systems) [8].

2.1 Biosensors

Using the term **"biosensor"** refers to a portable device that can be placed in an environment of interest (a liquid sample) to measure a specific chemical substance at the scene.

Biosensors are sensitive devices to a physical or chemical impulse (heat, acidity, metabolism) that transmit information about the vital processes.

They have a **detection** capacity and a **biological recognition capacity** (biochemical receptor) shown in Figure 2. After detecting these physiological signals, they are transformed into standardized "technical" signals, often electrical, for being transformed from analogue to digital [9], [1].

Due to their ability of being calibrated repeatedly, it is recommended for a biosensor to be clearly delimited by a bioanalytical system that requires additional processing steps, such as adding the reactive. If a device can't be monitored continuously or does not have a rapid and reproducible regeneration after a single use, it will be classified as a disposable biosensor.

The biosensors can be classified by the analyzes or by the reactions they are monitoring.

By directly monitoring the concentration or the reactions that produce or consume such an analyte, alternatively, indirect monitoring of an inhibitor or an activator of the biological recognition element (biochemical receptor) can be obtained [9].

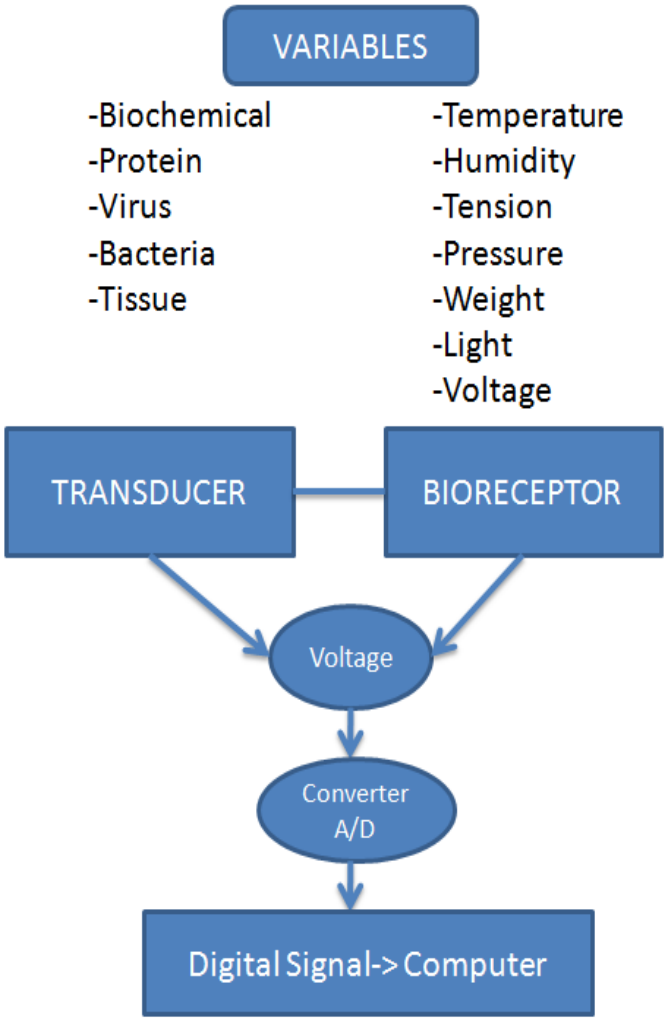

Figure 2. Parts of a biosensor

2.2 Bioreceptors

Bioreceptors are the second component of a bio-device. The bioreceptor is a biological material that includes: a specific protein, antibodies, enzymes, nucleic acid, viruses, bacterias, tissues, etc.

A bioreceptor makes a transducer to generate a voltage signal. There is a specific process between the bioreactors and the biomolecules. These variables can't be determined with the conventional sensors (temperature, pressure, light).

Antibodies and nucleic acids (DNA and RNA) are the most common; and the most commonly used transducers for biosensors are: electrochemical (by measuring the voltage or the current), optical, thermal (by temperature measurement), and piezoelectric.

An antibody is a protein molecule that represents the base of the immune system. When a foreign molecule invades the body, the immune system

recognizes and memorizes the shape of the molecule. It then creates the antibody in order to start fighting (similar to the blocking mechanism and key of enzyme-substrate binding).

The recognized molecule by the antibody is called antigen, and is generally it is a protein molecule. An antibody may also fix a virus particle or a bacteria by recognizing the proteins from the surface. Therefore, antibodies can be used as excellent bioreactors for a wide variety of protein molecules as well as for viruses and bacteria.

Certain cells can be used as a bioreceptor. There are three other important cells that correspond to the body's immune response: B cell, T cell, and natural killing cell (NK cell). Cell B produces antibodies against foreign fibers and molecules, while T-cell recognizes and attacks foreign molecules on its own. This indicates that T cell can also be used as an excellent bioreceptor [10].

Sensors based on cells and tissues offer many advantages over other sensors:

- they can detect and / or classify unforeseen threats (eg new pathogens);

The mamifer cell rather acts as a robust functional reporter of toxicity rather than relying on a surrogate marker such as the nucleic acid or antibody-based detection. Thus, mutated pathogens or new chemical species will be characterized by their functional impact on cellular physiology.

- can report sensor data to human physiology / pathology (eg toxicity);

Sensors can report the physiologically integrated response to an exogen agent. These responses are often nonlinear, multifactorial and difficult to predict with biomolecular recognition.

- provides increased stability of enzymes, receptors or antibodies in biological systems. As self-renewing machines, the cells recover and permanently repair their biomolecule components.

- are sensitive to the environment - introduce severe constraints on materials, processing, manufacturing, delivery and operation. In particular, the cells must be maintained viable, sterile (without bacterial / fungal growth), stable phenotypic and maintained in a fluid medium. The finite lifespan of living systems requires conservation and storage strategies.

- can give a medical diagnosis;

Cell-based sensors are used to predict the clinical results. Mixed leucocyte reaction is an existing example of cell-based assay that is used to predict immune rejection [8].

To make LOC devices at high potential, researchers need to take in consideration the commercial demand from the initial design stages and ensure that each component is compatible [11].

3. TYPES OF "LAB-ON-A-CHIP" DEVICES

The "lab-on-a-chip" microfluidic systems can contain:
- micropumps / microdispensers for fluid controlled quantities (further they may be subdivided into equal or unequal volumes and then released to desired destinations controlled, either sequentially or simultaneously) or to create pressure sources;
- capillary microviscosimeter, for viscosity determination;
- microchannel transport system;
- microfluidic switches that control the closing or opening of microchannels without the need for large differential pressures;
- microfluidic devices to perform logic operations, similar to logic integrated circuits on silicon [3].

3.1 The glucose sensor

The first chemical sensor was the pH electrode from the glass developed in 1922 and implemented later as a portable device. It lasted almost a third of a century before the next chemical sensor had actually been developed, the oxygen electrode invented by Leland Clark in 1954.

Dr. Clark introduced the biosensor concept in 1962 with his invention: the glucose electrode. This is very important because it is involved in the metabolic process. Diabetic patients do not produce enough insulin in the pancreas to adequately control the level of glucose in the blood. To eliminate this drawback, a dose of insulin is given. Patients should regularly check their level of glucose from the blood. The biosensor for blood glucose determination is the most known sensor used and, as a result, the most studied one [12].

The essential components of this biosensor are the capacity of detection - the oxygen electrode - and the biological recognition capacity - the enzyme layer.

3.2 Devices for catching and counting cells

Measuring the integrated flood is essential in lab-on-chip and microfluidics. Microfluidic cell counters based on the counting principle use a small channel or a small diaphragm with microprocessed electrodes. The impedance on the electrodes increases as a cell passes through the channel and a

voltage peak is obtained, the width being proportional to cell velocity [1], [12].

Circulating Tumor Cells (CTC) can be differentiated and captured from other blood cells by physical properties (density, size), electrical properties and biological properties. There are many devices that can capture CTCs. Most of them are based on epithelial markers because they can differentiate CTC from leukocyte [13], [14].

The microfluidic devices based on counting cells - number of cells in a specific volume - (Figure 3) are characterized by a fluid flow. For example, red blood cells are lysed (dissolved) integrally with a lysis solution or with various reagents. Thus, the residual liquid remains, consisting of leucocytes, which will be transported with different flow rates by measuring diaphragm [15].

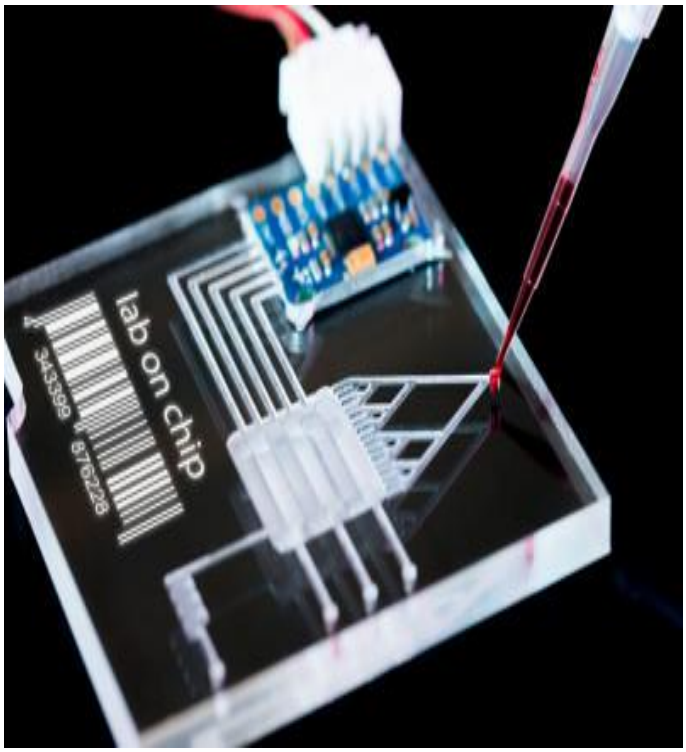

Figure 3. Lab-on-a-chip [16]

The measuring sensors are located before entering and before leaving the capture chamber. Each time a cell passes the measuring sensor, a digital signal (with a 'spike' form) is emitted.

Such an integrated product should be self-sufficient, not require pre-treatment of samples, preparation or amplification. It should be fully automated to reduce errors and facilitate use for operators without microfluidic expertise. Results must be clearly displayed to minimize the need for subjective interpretation of users [15].

3.3 Aspects regarding the design of microfluidic devices

The **sensors** for measuring inputs and outputs wich are located on the **microfluidic channel** are parts of the main components of a microfluidic device. The microfluidic system connects all the channels that drive either blood or other solutions. Each sensor consists of three microelectrodes: the reference electrode, the working electrode and the counting electrode (Figure 4).

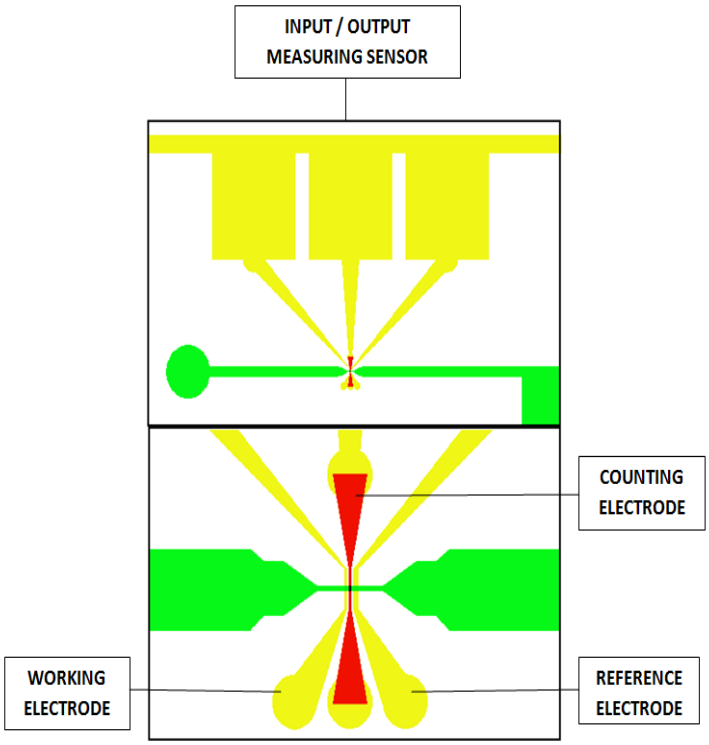

Figure 4. Inputs/Outputs counting sensor

When cells are passing over the microelectroids integrated into the microchannel, the impedance increases and potential spikes appear. Their amplitude is proportional to the cell size, and the pulse width is proportional to the velocity of cell movement in the suspension (Figure 5).

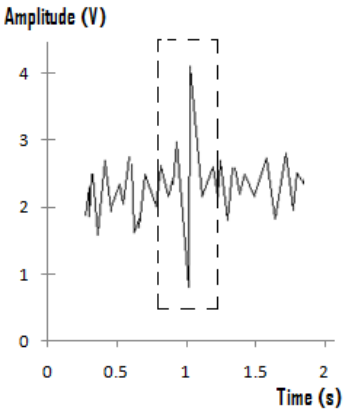

Figure 5. The spikes that appear when a cell passes

The **capture chamber** is the third important component of a biodevice. It contains pillars with the sizes chosen according to the type of cells we want to detect / separate (Figure 6).

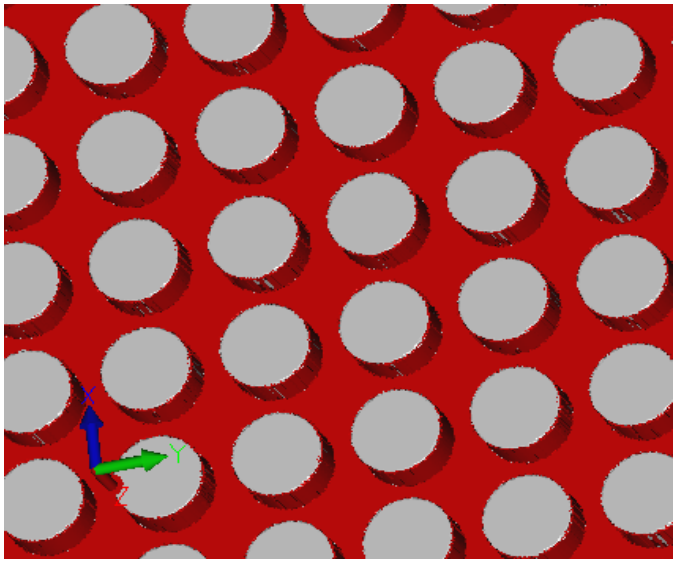

Figure 6. Capture pillars from the chamber

4. CONCLUSIONS

The paper emphasizes the importance of biodevices and their working mode. It was proved that the biodevices are used to perform fast and inexpensive tests of detecting and identifying bacteria, viruses and biological toxins. Very different devices are in used in the state of the art, using various kind of sensors for measuring inputs and outputs, microfluidic systems with channels for solutions, and capture chambers as important parts.

5. REFERENCES

1. Stamatin Ioan, *Nanomateriale aplicatii in biosenzori, surse de energie, medicina, biologie. Elemente de nanotehnologie,* Universitatea din Bucuresti. (2008).
2. Lisa R.Volpatti, Ali K. Yetisen, *Commercialization of microfluidic devices, Trends in biotechnology*, Vol32, No 7., (2014).
3. ***www.imt.ro, accessed at: 20.05.2018.
4. *** "Sel-Sel darah (celulele sanguine)", Available at: http://mahpudeen.blogspot.com/2012/12/sel-sel-darah.html ; accessed at: 14.06.2018
5. Chen, J., Chen, D., Xie, Y. et al. *Progress of Microfluidics for Biology and Medicine*, article, Nano-Micro Lett, 5: 66 (2013).
6. Suman Chakraborty, *Microfluidics and microfabrication*, Springer, (2010).
7. Epure Bianca, *Microsisteme mecanice simple pentru aplicatii medicale*, Universitatea Tehnica "Gheorghe Asachi" din Iasi.
8. Schultz J., Mrksich M., Bhatia S., Brady D., Ricoo A., Walt D., Wilkins C., *Biosensing International Research and Development*, Springer, (2006).
9. Scheper T., *Advances in Biochemical Engineering/Biotechnology*, 2007.
10. Jeong-Yeol Yoon, *Introduction to Biosensors – From Electric Circuits to Immunosensors*, Springer, (2013).
11. Umer Hassan, Nicholas N Watkins, Bobby Reddy Jr, Gregory Damhorst, Rashid Bashir, *Microfluidic differential immunocapture biochip for specific leukocyte counting*, Nature America, Vol 11, No 4. , (2016).
12. Wang Joseph, *Glucose Biosensors: 40 Years of Advances and Challenges*, Electroanalysis No.12, (2001).
13. Gertler R, Rosenberg R, Fuehrer K, Dahm M, Nekarda H, Siewert JR. *Detection of circulating tumor cells in blood using an optimized density gradient centrifugation. In: Allgayer H, Heiss M (eds) Molecular staging of cancer*. Springer, Berlin, 149–155 (2003).
14. Zheng S, Lin H, Liu JQ, et al. *Membrane microfilter device for selective capture, electrolysis and genomic analysis of human circulating tumor cells*. J Chromatogr A, 1162(2):154–161 (2007).
15. U.Hassan, N.N. Watkins, C.Edwards, R.Bashir, *Flow metering characterization within an electrical cell counting microfluidic device* – Royal Society of Chemistry, Lab Chip, (2014).
16. ***"Lab-on-chip", Available at: https://www.shutterstock.com/image-photo/lab-on-chip-device-integrates-several-311155133; accessed at: 1.06.2018.