UDC 004.9

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MODELING OF BIOLOGICAL CELLS DEFORMATION IN MICROFLUIDIC SYSTEMS

Запропоновано методи моделювання деформації біологічних клітин у мікрофлюїдних пристроях. Описані передумови і цілі розроблення механічної моделі живої біологічної клітини для поліпшення моделювання та автоматизованого проектування пристроїв для мікрофлюідних пристроїв біоаналізу та діагностики.

Ключові слова: мікрофлюїдика, біологічна клітина, автоматизоване проектування.

This paper is devoted to the modeling of biological cells deformation in microfluidic systems. The background and purposes of development of the mechanical model of the living biological cell to improve modelling and computer aided design of microfluidic devices for bioanalisys and diagnostics were described.

Key words: microfluidics, biological cell, computed aided design.

Introduction

Nowadays microfluidic devices are widely used in various fields, especially in biotechnology, medicine and pharmaceuticals, as they allow carrying out fast analysis, are highly portable and easily transportable, require fewer amounts of reagents and samples, and provide more accurate results [1].

Biochemical and biophysical markers usually determine state of the cell. Although biochemical markers are used very widely, internal biophysical markers, such as the ability to change the shape under mechanical stress, have some advantages, because it does not require labeling or valuable sample preparation. Nevertheless, modern technics, which are using the mechanical properties of the cells, have currently limited use in clinical trials and studies, which are related to cell biology [2, 5].

One of the newest methods of biological analysis is the analysis, which is based on the mechanical properties of biological cells. Studying of the properties of elasticity allows getting new knowledge about biological cells and causes a clinical interest. Mechanical properties of whole, intact cells are associated with various cellular events, such as movement, differentiation and aging, physiological and electrical activity, as well as the pathology of cells [3].

Formulation of the problem

There are more evidences in favor of that the deformation of cells (i.e., the ability to change shape under the load) is a useful indicator of changes in the cytoskeleton and nuclear organization, and can provide the definition of the state or properties of cells without the use of biomarkers, such as metastatic potential, stage of life cycle of cell, degree of differentiation and activation of leukocytes. Clinically, this measure of malignancies and metastatic potential in tissues or biological fluids may adjust treatment decisions, or measures the degree of differentiation can prevent transplantation of undifferentiated stem cells in regenerative oncogenic therapy. For drugs and personalized medicine, the integrity of the cytoskeleton in simple measure can allow the screening of drugs action or evaluate cytoskeleton drug resistance in biopsy samples. In addition, measures of activation of leukocytes are strong predictors of disease prognosis and response to the treatment on patients with HIV-1 infection or allotransplants rejection [5].

Whereas this subject is extremely topical today, there is a problem of developing of measurement and diagnostic devices that enable efficiently and accurately diagnose and measure, based on the mechanical properties of cells. One possible option is to use of microfluidic devices with optical detection, which shall pass cells through the microchannels and their deformation under load due to one of the used microfluidic techniques. Schematically, the device shown in Fig. To use the most effective way of deformation of cells, which in turn does not destroy them, it is necessary to develop an appropriate structure of microfluidic channels.

Analysis of recent research and publications

The biological cell can be considered as a homogeneous liquid in a thin elastic membrane. In order to develop a mechanical model of the cell and its behavior in microfluidic devices it is necessary to consider the phenomenon of structural mechanics and hydrostatic phenomena that occur in liquid internal environment of the cell.

Today the mechanical properties of the cells are measured by various methods, including atomic power microscopy (AFM), which is based on the use of thin cantilever that is pulled to the surface, while the laser beam is reflected from the head of cantilever, measures its rejection. When cantilever is shifted because of interaction with cell surface, laser reflection point shifts under the surface emitting diode respectively (Fig. 1).

AFM technology has a very high precision and allows the researcher to measure the local elasticity of the cell. This allows measuring the elasticity of the cell membrane. It is assumed that the load and displacement is very small at some distance from the point of application of force, tangential motion along the surface is much smaller than the movement along the normal and shell extends infinitely in all directions, in which case the shell material is flexible and meets Hooke's law (Fig. 3).

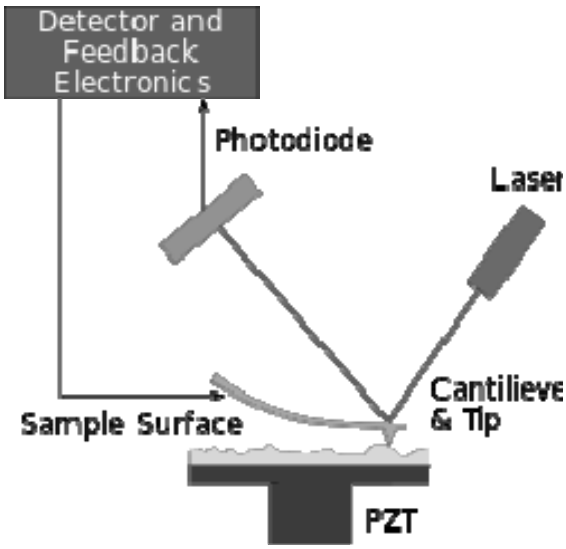

Fig. 1. AFM

Constricted geometry. Advances in microfabrication enable the fabrication of structures on the microfluidic platform to be used for deformation studies on single cell (Figure 2A). Various forms of customized structures for example wedge/funnel shape, vertical gap, long channel, hyperbolic shape and cross road allows for single cell deformability measurement along with parameters to characterized intrinsic cell properties.

Aspiration induced. This particular technique mimicked the concept of micropipette aspiration (MA) (Figure 2B). Generally, by performing cell deformation measurements similar to MA using standard PDMS microchannel surely imposed several challenges due to rectangular cross section. For examples; the

driving fluid might get leaked along the edges, issue on cell conformity since rounded shape of cell cannot accommodate sharp edges of PDMS channel and hard to determine shear force due to presence of additive flow in microfluidic. Nevertheless, the rectangular channels do exhibit several advantages such as ease of fabrication and easier for cell-surface observation under optical microscopy. Compared to other techniques, micropipette aspiration has a number of well-established mathematical models for Young's modulus assessment.

Fluid induced. Another way to deform cell, using microfluidic involves the generation of converging streamlines. This dynamic fluid equilibrium effect produces converging streamlines that able to distinguish, sort and enrich any cells in the fluidic free flow. Specifically, this technique target the cell at the center between two converging streamlines and characterized the deformation index experienced by the cell instead of direct contact with the microstructures (Figure 2C). Deformation index is defined as ratio of both axis of cross sectional area of a deformed cell has been linked with cell deformability and was proved an efficient biophysical marker for cell state

Electrically Induced. Mechanical manipulations involving electrically induced microfluidic has started ever since the Coulter principle was established (Figure 2D). The principle states that any particle moving through an orifice along with electric current should produce a change in impedance. By this means, the impedance changes are due to displacement of electrolytes caused by the particles movement. Vast areas emerged as a result for example electroporation, electrodeformation, electrorotation, dielectrophoresis, microelectrical impedance spectroscopy (μ-EIS) and impedance based flow cytometry (IFC). Electroporation is referred to swelling or expansion in cell size whenever a cell experiences externally applied electric field [4].

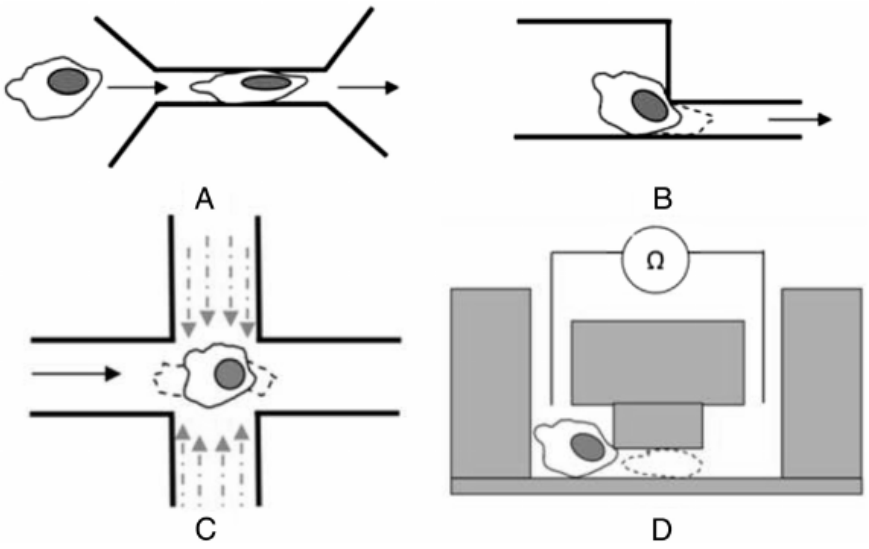

Fig. 2. Microfluidic manipulations (A) Constricted Geometry (B) Aspiration Induced (C) Fluid Induced (D) Electrically Induced

Table 1

Mechanical properties of cells

Cell type	Young's modulus, kPa
Normal erythrocyte	14–18
Erythrocyte in spherocytosis	19–33
Erythrocyte in thalassemia	22–64
Erythrocyte in dehydrogenase	70–110

Formulation of article purpose

The article is an overview and is intended to consider the characteristics of the initial stages of developing of models of mechanical deformation of biological cells in microfluidic devices in order to use these models in the modeling and design of microfluidic devices by which time and costs for prototyping of such devices are reduced.

Presenting of main material

Total simplified block diagram of analyzer is shown in Fig

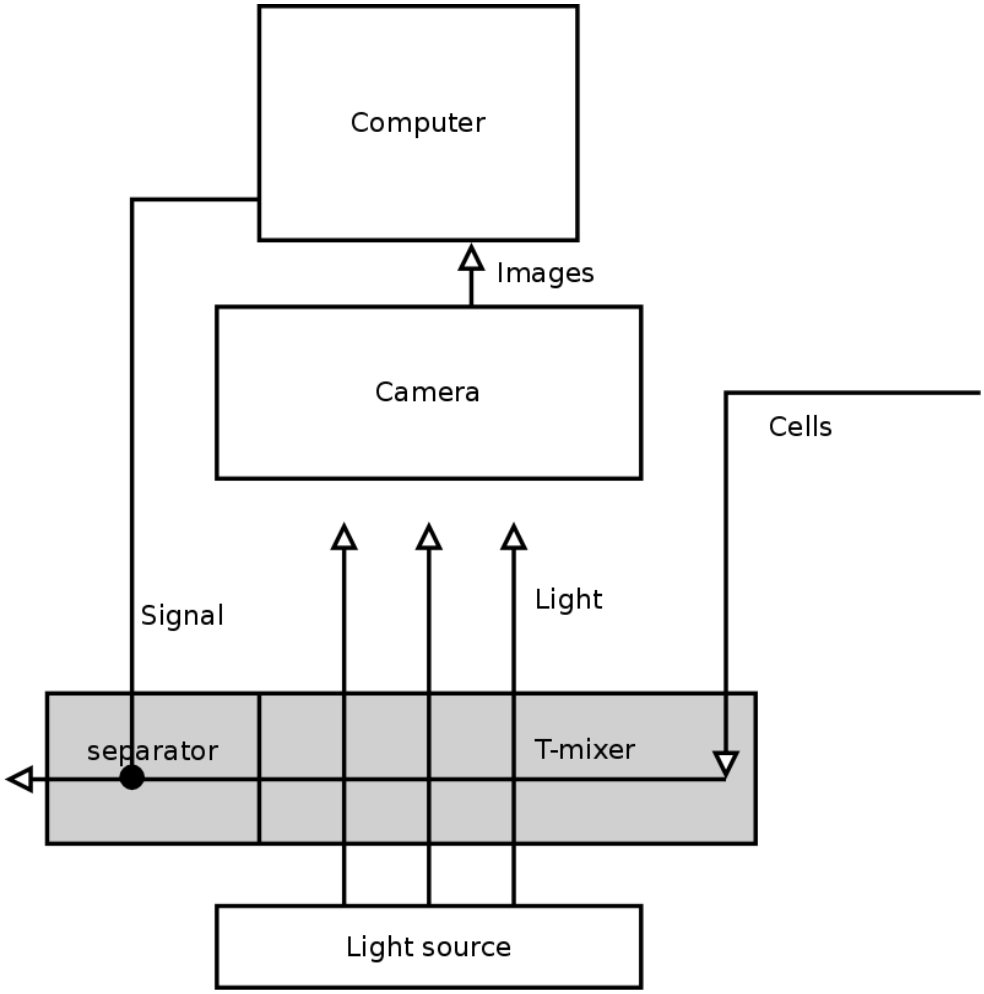

Fig. 3. Analyzer structure

The analyzer consists of the microfluidic lab-on-chip device made of glass, in which the cells are exposed to forces that arise when mixing fluid flows in the T-mixer (Fig. 4).

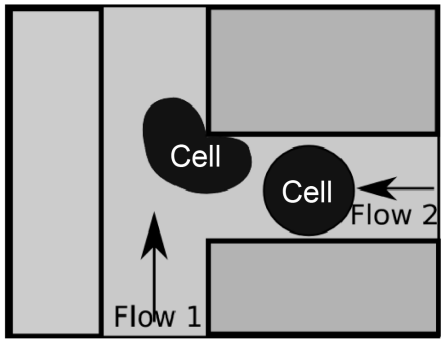

Fig. 4. Cells in T-mixer

Considered design of T-mixer is a combination of approaches of compression by fluid and suction for manipulation of cells and deform them with certain advantages and disadvantages (e.g. cells is touching channel angle by its shell, which can cause membrane rupture under certain conditions).

Under pressure, created by two streams of fluid, cell is bent on the corner of the intersection of channels (Fig. 4). A camera equipped with a magnifying optical system photographs passage of cells through the T-mixer. The resulting images are processed on a computer, using image-processing algorithms that calculate such properties as eccentricity and other changes in cell shape by using contours algorithms detect. These results are then compared with the data previously calculated using statistical methods.

Based on the results of processing, it became possible to make a reference of signal to optional separator unit, which divides the cell into several groups, depending on the investigated properties.

The problem is to find a specific difference in form between healthy and diseased cells and to design a mixer so as to ensure the best possibilities to measure these differences and prevent the destruction of cell walls.

The idea is to develop a mechanical model of bending of cells inside the T-mixer. The simulation results will be used to create a model of contour of curved cell and designing of the T- mixer channels in right order. Images from the camera will be consistent with these models to identify cells with different properties.

During the selection of the approach to the development of cell model, model as an elastic membrane with liquid inside it was chosen. The ratio for determining the bending of membranes is equal:

$$h = \frac{Pl^2}{2\pi D}(-kei(x) - k_R \times \left[(1+\nu)(\frac{\pi}{2} Y_0(c\sqrt{2k_R}) + \ker(x)) + \frac{1}{2} c \ker'(x) \right] + \\ +(\eta - \varepsilon)\ker(x) + \frac{t}{4} c \ker'(x)), \quad (1)$$

where

$$D = \frac{Et^3}{12(1-\nu^2)}; \quad l = \frac{\sqrt{rt}}{\sqrt[4]{12(1-\nu^2)}}; \quad k_R = \frac{l^2}{r^2};$$

$$\varepsilon = \frac{\nu t^2}{10(1-\nu^2)l^2}; \quad \eta = \frac{t^2}{5(1-\nu^2)l^2},$$

where: E – Young's modulus of the sample, Pa; ν – Poisson's ratio of the sample; R – radius of the AFM tip, m; h – the difference between the coordinate tip contact point and the coordinate of separation when the AFM tip withdraw from the surface (moving at material stretching), m; P – applied load, N; r – radius of the shell, m; t – thickness of the shell, c' – the contact area, m^2; l – characteristic size; c – dimensionless quantity, equals to c = c' / l ; kei (c) – Thompson function; k = l^2 / R^2 – coefficient characterizing the thickness of the shell [1].

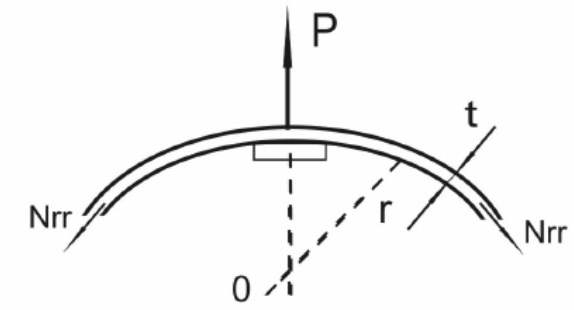

Fig. 5. The spherical shell with normal concentrated force: t-membrane thickness, r-radius of shells

An initial simulation of bending of an empty shell with given parameters under the action of load in two-dimensional space through a system of COMSOL Multiphysics was performed (Fig. 6).

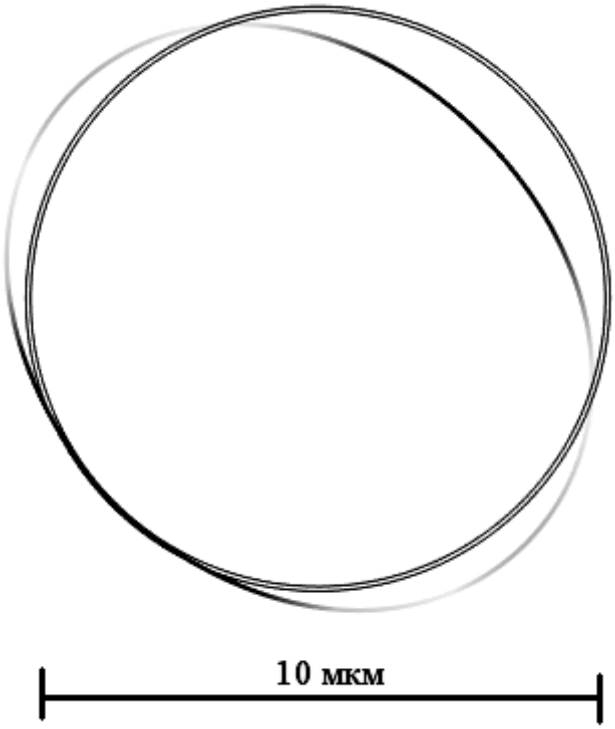

Fig. 6. Bending of the thin membrane under the action of mechanical load. Shell thickness 80 nm, the power $1 \cdot 10^{-3}$ N

Simulation of cells as fluid in the shell also has one important advantage – it allows to check whether the cell membrane is damaged. In real experiments, remains of damaged cells clog the channels and can cause a variety of measurement error.

To implement the model of behavior of fluid in microchannels, three-dimensional model of mixer with the help of a COMSOL Multiphysics system was created (Fig. 7). To simulate the passage of cells through the mixer should use in further an complicated metaphysical model that will combine in itself the liquid model for flow and structural mechanics to model for the impact on cells stress.

Liquid model is based on Navier-Stokes equation (2), which is solved in the deformed coordinate system.

$$\begin{aligned} &\rho\frac{\partial u}{\partial t} - \nabla \cdot \left[-pI + \eta(\nabla u + (\nabla u)^T) \right] + \rho((u - u_m) \cdot \nabla)u = F \\ &-\nabla \cdot u = 0 \end{aligned} . \qquad (2)$$

In these equations, I – unit diagonal matrix, F – the force that is acting on the fluid. It is assumed that gravity and other forces do not effect on the liquid, so that F = 0. The speed of the coordinate system – u_m.

Mechanical model takes into account the viscous forces and pressures that act on the cell from all sides in the flow of liquid (3).

$$F_T = -n \cdot (-pI + \eta(\nabla u + (\nabla u)^T)) . \qquad (3)$$

Navier-Stokes equations are solved in the deformed finite element mesh, which is the domain of liquid and is freely moving. Deformation of the mesh relative to the original form of the domain is calculated using Winslow smoothing. This default value is used on the verge of interaction "fluid-structure".

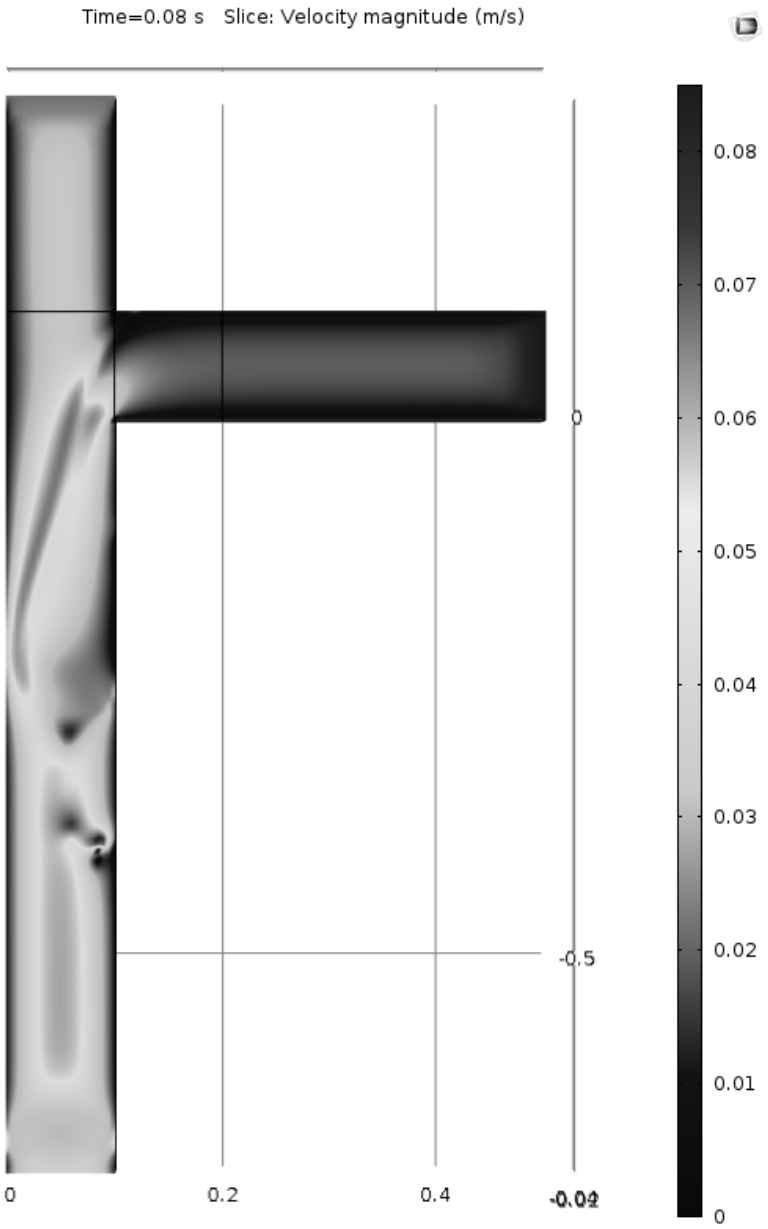

Fig. 7. Distribution of the velocities of fluid in the T-mixer

Conclusions

This article reviewed possible solutions for design of software and mathematical subsystems of device for bio analysis. An approach for modeling the deviations of cells inside of microfluidic systems was elected.

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