THE PECULIARITIES OF THE CELLS METABOLISM DUE TO THE FLOW OF LIQUID THROW CELL MEMBRANE

O.I. Krivosheina, I.V. Zapuskalov,Y.I Khoroshikh, O.B.Zapuskalova
Siberian State Medical University, Tomsk, Russia
E-mail: oikr@yandex.ru

Abstract: A device is designed for *in vitro* modeling of the directed flow of a nutrient medium similar to the fluid flow in the eyeball. The primary culture of human fibroblasts was cultivated in the permanent directed flow of the medium for 24 and 48 h. Under dynamic conditions, an increase in the intracellular fermentative activity of cells of the fibroblastic population and the acceleration of the process of their differentiation into mature forms were observed.

Keywords: culture of human fibroblasts, the fluid flow, the intracellular fermentative activity, the process of the differentiation/

Implementation of morphofunctional capabilities of cells intermediating the initiation, development, and outcome of any pathological process depends significantly on the modulating influence of microenvironment factors. In the eyeball, the microenvironment consists of the interacting system of anatomico-physiological features and extrastromal regulation components. Anatomico-physiological features are determined by the presence of the directed flow of the intraocular fluid and by the fibrillar structure of the vitreous body. Extrastromal components are represented by cellular elements migrating into the vitreal cavity (cells of the retinal pigment epithelium, monocytes/macrophages, lymphocytes, etc.) and by humoral factors (cytokines, growth factors). Of particular interest, in our opinion, is the directed flow of fluid in the eyeball induced by the pressure gradient.

The aim of this work was to study the influence of the directed fluid flow on the morphofunctional state of human fibroblasts.

A device has been designed for the *in vitro* modeling of the flow of a nutrient medium similar to the fluid flow in the eyeball. The device is a closed system with a chamber equipped with a semipermeable filter. The system was first filled with the nutrient medium with the aid of a vessel. The nutrient medium contained 200.0 ml of the DMEM nutrient medium in the Iscove modification and the 4% gentamicin solution (0.02 ml gentamicin per 10.0 ml of

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the nutrient medium). For the study, we used the fibroblast culture of human lung after 3 to 4 passages in a concentration of $5 \cdot 10^4$ cells/ml.

The cellular material came to the chamber through a valve hole. The chamber was connected to the vessel containing the nutrient medium through a roller pump equipped with a maintaining valve.

The roller pump generated the uniform directed flow of the nutrient medium with a rate of 2.1-2.4 mm^3/min. The primary culture was incubated in the permanent flow of the nutrient medium under the cultivation conditions kept unchanged for 24 and 48 h. For control purposes, fibroblasts were cultivated on a semipermeable filter placed in a Petri dish with the nutrient medium at the strict observance of temperature conditions (37° C), $_2$ content (5-7%), and the humidity level (100%).

The cellular material was examined by cytochemical methods.

At the flow cultivation of fibroblasts, the following results were obtained.

Twenty four hours after the beginning of the experiment, the cytochemical analysis revealed the moderate activity of -naphtylacetatesterase (22.56+/-0.90) and alkaline phosphatase (10.23+/-1.05) in cultivated cells. The area of the cell surface averaged 238.94+/-5.36.

Forty eight hours later, the activity of the both ferments in the described cells increased compared to the initial indices and to cells cultivated under standard conditions ($p_Z<0.01$). In this case, the level of -naphtylacetatesterase was 26.98+/-0.87, while that of alkaline phosphatase was 14.67+/-1.21. The area of cell surface of fibroblasts averaged 179.43+/-7.81 ($p_Z<0.001$).

When fibroblasts were cultivated under standard (stationary) conditions, in the entire series of experiments the cytochemical analysis revealed the low activity of -naphtylacetatesterase in cells. This activity increased gradually during the cultivation ($p_Z<0.05$). No alkaline phosphatase was observed in cultivated cells. The area of cell surface was 307.19+/-6.02 24 h later and 211.66+/-5.29 ($p_Z<0.001$) 48 h later.

The utmost discovery of the 19th century – the discovery of a cell in a living organism – stimulated the intense study of various pathologies from the position of the cellular structure of organs and tissues. R. Virchow in his classical paper "Die cellular Pathologie in ihrer Begrundung auf physiologische und pathologische Gewebelehre", systematizing voluminous experimental data, for the first time presented a complex organism as a system of cell or a "cell nation."

However, during the whole era of optical microscopy in morphology, a cell was thought to be a so stable component of a tissue and organ structure that its functional and morphological changes observable in an optical microscope seemed to be not related to the dynamics of cellular structures. The idea of a cell as a versatile and unchangeable unit of tissues and organs dominated.

Only new methods of morphological investigations, first of all, electronic microscopy, changed radically the idea of a cell and dynamics of its changes. The cell culture technique, which allows cells to be studied in their

living state, actual action, and interaction with the microenvironment, has helped significantly in the understanding of the integration and interpenetration of the structure and functions. Intracellular structures and biochemical processes occurring in them, as well as the permanent energy flow in a cell are in a deep and close relation with each other, and together they complete the integral pattern of the united structural-functional system, namely, a cell.

One of the main functions of the cell surface and the plasmatic membrane is the perception and transfer of external regulatory signals into a cell. Just this function is responsible, to a great extent, for the interaction between the function of the cell membrane, its permeability, and the activity of intracellular metabolism processes. Now a significant progress is achieved in the understanding of molecular mechanisms of information reception, processing, and transfer from the plasmalemma to intracellular organelles. It is established that modulating factors of the extracellular medium act as exogenous regulatory signals contacting with receptors of the cell surface. Under the conditions of our experiments, the permanent directed flow of the nutrient medium and the extracellular matrix can be such an exogenous signal for fibroblasts adhesed to the filter.

We can assume that after the interaction of the external signal with cell receptors, a cascade mechanism of certain intracellular processes is initiated. Thus, for example, changes occur in the structure of receptor-related membrane ferments, which catalyze the synthesis of endogenous regulatory molecules. As a result, their concentration changes, and the cell permeability changes too. Variations of the membrane potential also play an important role.

It should be emphasized that the plasmatic membrane not only serves a mechanic barrier, but also regulates the consecutive income of substances to a cell. Diffusion into tissue complies with Fick's law that reads as follows: as soon as differences in concentration of one or another substance appear in the medium, there is a flux of this substance leading to decrease in its concentration, which is proportionate to the concentration gradient.

This equation applies to describe movement of molecules as well as microparticles if their concentration is small.

Liposoluble low-molecular substances, first of all oxygen and carbon dioxide – also penetrate easily through endothelial cells.

All macromolecules, such as proteins, nucleic acids, polysaccharides, and lipoproteid complexes, come to a cell through the vesicle formation and joining process, that is, endocytosis. The higher is the speed of the directed fluid flow through a cell, the more intense is the endocytosis process, and, correspondingly, the greater amount of substances comes into the cell. This, in its turn, determines the degree of the metabolic activity of the cell, which is confirmed by the results of fibroblast cultivation under the flow conditions.

The speed of the movement of water molecules inside the cell is also caused by physical forces: gradients of the osmotic and hydraulic pressures on the both sides of a cell. The higher the gradient, the faster is the intracellular motion of water molecules and, correspondingly, transport vesicles, which transport nonliposoluble substances, moving from one compartment to other.

The directed movement of transport vesicles results in the reconstruction of cellular compartments and the cell surface, as well as the retention or destruction of intercellular units. One can assume that the content and components of the donor compartment would ultimately disappear in the process of transportation and the donor compartment (endoplasmic reticulum in this case) would decrease in size, while the size of the acceptor (Golgi complex) would, correspondingly, increase. However, this does not occur, because in the cell there homeostatic mechanisms, regulating and maintaining the composition of every organelle, for example, with the aid of the membrane return mechanism. As transport vesicles of the endoplasmic reticulum fuse with the acceptor membranes of the Golgi complex, certain proteins return from the Golgi back to the endoplasmic reticulum. This process is known as a retrograde transport. In contrast to it, at the anterograde transport, proteins continue to move along the secretory pathway, namely, intercisterna coated vesicles transport them through cisternae of the Golgi complex.

At the most part of the Golgi trans-network, proteins are sorted, and, leaving this compartment, they are distributed over primary lysosomes, constitutive vesicles, and secretory granules depending on their designation: in the plasma membrane, in the cell, or outside.

In addition, from indices of intracellular metabolism, it is possible to judge the state of cells and the direction and intensity of their activity. Thus, for example, every stage of differentiation is intimately connected with the activation of additional ferment systems and the formation of new biosynthesis mechanisms. The data of cytochemical investigations of fibroblasts cultivated under the flow conditions compared to indices under the stationary conditions indicate that the activity of both specific (alkaline phosphatase) and nonspecific (-naphtylacetatesterase) ferment systems increases, which is indicative of the acceleration of the cell differentiation process. This is confirmed by the more significant (compared to the stationary case) decrease in the area of cell surface of fibroblasts cultivated under the flow conditions as a reflection of the degree of fibroblast mature.

Thus, at the cultivation of human fibroblasts *in vitro* under the conditions of the directed nutrient flow, the increased intracellular fermentative activity of fibroblasts is observed. Under the modulating influence of microenvironment factors (directed fluid flow, extracellular matrix), the process of cell differentiation into mature forms accelerates.

The data obtained extend the idea of the microenvironment influence on the morphofunctional state of cells of a fibroblast population and allow cellular mechanisms of development of fibrovascular proliferation in the eyeball to be studied from new positions.