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Microfluidic Chips for the Study of Cell Migration under the Effect of Chemicals

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Abstract—Numerical simulation of the formation of a chemoattractant gradient in reaction chambers of a chip having different geometries enabled the determination of a structure suitable for the study of cell migration, in accordance with which hybrid polymer–glass microfluidic devices were manufactured. Verification of the procedures of alignment of cells in the reaction chamber of the chip by centrifugal force and subsequent culturing of the cells showed that microfluidic chips can be used to study cell migration under the effect of the chemoattractant gradient in vitro.

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Cell migration is a deterministic process that occurs at various interactions between cells, tissues, and their environment [1, 2]. These interactions play a major role in biological processes, including immune responses, morphogenesis, and wound healing [3, 4].

The concentration gradients of chemicals are related to environmental factors affecting the migration of cells [5–7]. Because of the high biological and physiological importance of chemotaxis, the understanding of its mechanisms is one of the problems of cell research. Currently, microfluidic chips (MFCs) are used to study the chemotaxis of cells [8, 9]. The key advantages of MFCs are the possibility to configure fluid flows and to create and stabilize the concentration gradient of chemicals, low consumption in reagents, and setting of high-performance analyzes [10]. The chemoattractant concentrations formed in MFCs can vary from a few tenths to hundreds of nanograms per milliliter [11, 12].

The purpose of this work is to create a universal topology of MFCs suitable to study cell migration under the effect of a chemoattractant gradient, enabling the positioning and culturing of cells in the reaction chamber of the chip and the smooth adjustment of the gradient created in a wide range.

There are three basic topologies of MFCs used to create gradients of chemicals [13]: T/Y, network, and diffusion. We have decided to use a Y topology involving two input channels through which solutions are supplied; one of the channels contains a chemoattractant. The channels are arranged symmetrically opposite each other and mate with a wide channel or a reaction chamber. When flows are in contact, diffusion of chemoattractants occurs; one can create the desired concentration gradient by adjusting the flow rates.

To determine the optimal topology of the reaction chamber, we carried out numerical simulations using the COMSOL Multiphysics software package. We studied the distribution of chemoattractant in the channels and reaction chamber of the MFC at different angles of inclination of its walls, different widths, and various flow rates. A two-dimensional problem in a horizontal cross section of the reaction chamber and channels was considered (Fig. 1a). This approximation can be used, as the convective transport in the vertical direction is absent and the depth of channels of the chip is rather small (a few tens of micrometers), which ensures rapid attainment of diffusion equilibrium. A waterlike liquid at a density of 1000 kg/m^3 and a dynamic viscosity of 0.001 Pa s was considered as a solution.

Width L of the reaction chamber was varied from 300 to 1500 μm with 400-μm step at $\alpha = 45°$ (Fig. 2). The diffusivity of chemoattractant was selected to be $D = 10^{-10}$ m^2/s (typical for proteins in water). Flow rates of the solution with chemoattractant Q_1 and a

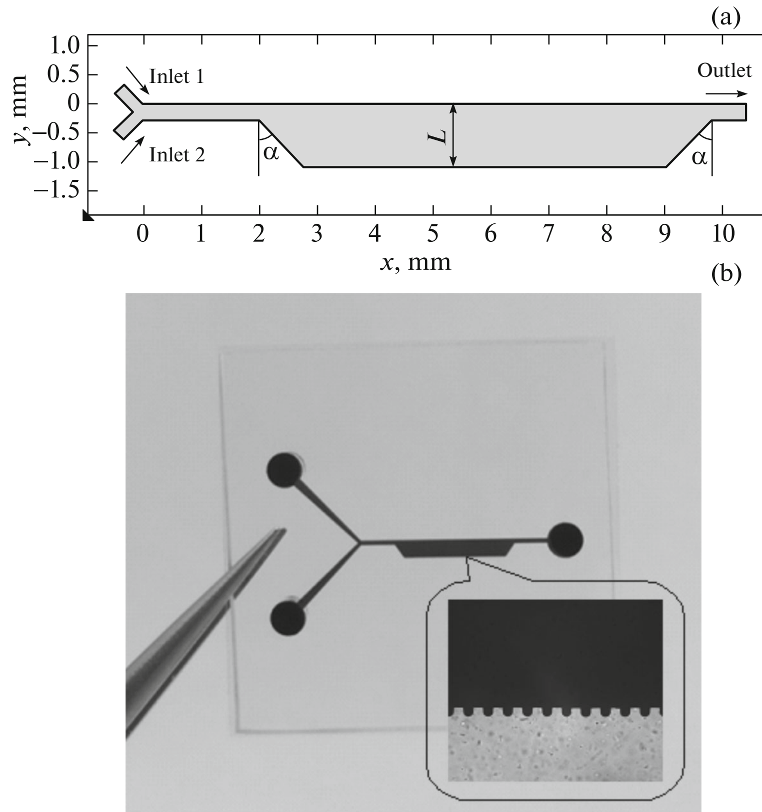

Fig. 1. (a) Simulation region in the study of the distribution of concentrations of chemoattractant in the reaction chamber of the MFC. (b) Prototype of hybrid PDMS–glass MFC. Inset: an enlarged view of the reaction chamber wall equipped with a trap to retain cells.

buffer solution Q_2 varied from 0.01 to 2 μL/min at their equality ($Q_1 = Q_2$). The distribution of concentrations was also studied at a total flow rate of 2 μL/min with the flow rate of the solution with chemoattractant increasing from 1 to 1.8 μL/min.

It was found that, with an increase in the chamber width from 300 to 1500 μm, the maximum gradient decreased by 5.3 times, due to its scaling when the geometry of the chamber changed. It was determined that changing the flow rates from $Q_1 = Q_2 =$ 0.01 μL/min to $Q_1 = Q_2 = 2$ μL/min at a chamber width of 1.1 mm causes a 33-fold change in the gradient, from 260 to 8570 mol/m^4. Whereas large values of the gradient is achieved at high flow rates, the smaller value of the flow rates yields gradients that change more smoothly over the channel width. When changing the flow ratio of $Q_1 : Q_2$ from 1 : 1 to 9 : 1, the concentration front is shifted from the middle of the reaction chamber to the position at four-fifths of its width, wherein the gradient value decreases by 18%. Changing angle α of the chamber walls from 75° to 0° leads to an increase in the gradient value by 33% for $Q_1 = Q_2 =$ 0.01 μL/min and by 6.4% for $Q_1 = Q_2 = 2$ μL/min. The geometry of the reaction chamber with an angle of wall inclination of 45° gives a greater concentration gradient at lower flow rates. We performed calculations for the geometry proposed in [14], where the contact of liquids with a chemoattractant and without it occurs in a straight channel with a width of approximately 300 μm. The simulation results for the straight channel and for the reaction chamber proposed in this work (Fig. 2) showed that, in the former case, the concentration profile changes rather sharply, which reduces the possibility of studies; in contrast, in the latter case, the gradients can be adjusted in a broader range and smoother changes in the concentration profile can be attained, which shows the versatility of the proposed reaction chamber. Eventually, a reaction chamber with a width of 1100 μm and an angle of wall inclination of 45° was selected, as this configuration yields a greater concentration gradient of chemoattractant at a lower consumption of solutions.

Given the simulation results, an MFC topology was developed featuring an array of 78 traps (the trap dimensions are 40 × 40 μm with a radius of curvature of 20 μm), arranged along the entire length of one of the walls of the reaction chamber (Fig. 1b). The depth of all structures was 30 μm. The traps are designed to keep the cells captured in them during centrifugation and to align them along the wall, that is, to create a common "start" position (baseline), which opens up

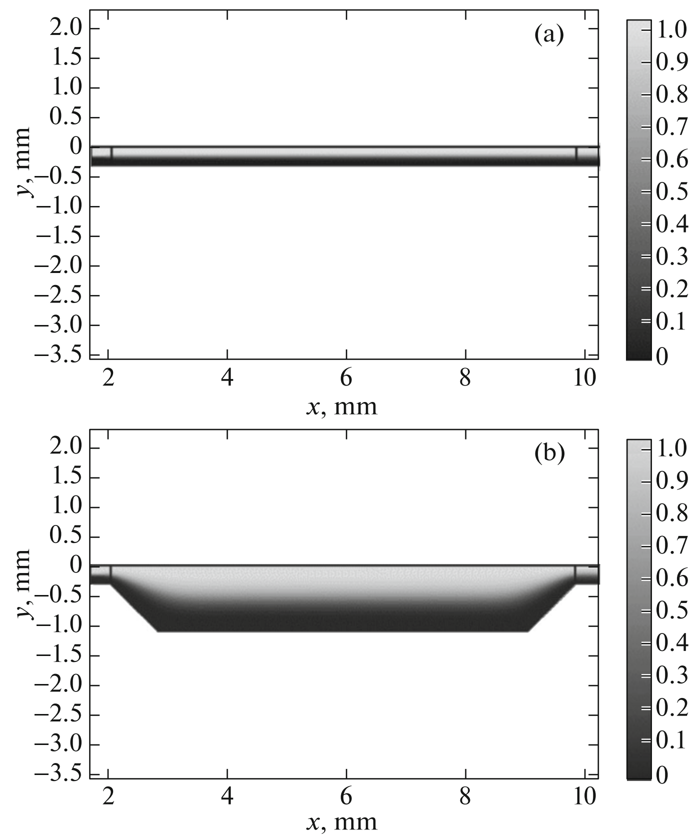

Fig. 2. Distribution of concentrations of chemoattractant in the reaction chamber of the MFC at the flow rates of $Q_1 = Q_2 =$ 1 μL/min and its width of (a) 300 and (b) 1100 μm; the width of the side channels is 300 μm.

new possibilities in the study of heterogeneity of cell population.

Based on the developed MFC topology, master molds were made in order to produce polydimethylsiloxane (PDMS) replicas by them. The manufacturing of master molds made of silicon coated with a layer of SU-8 photoresist (MicroChem, United States) was performed by the conventional method [15]. The replicas of microstructures were produced by casting Sylgard 184 PDMS material (Dow Corning, United States) by the manufactured master molds in accordance with the known protocol [15]. Sealing was carried out by pressing a PDMS replica and a glass plate after the activation of their surfaces in an oxygen plasma for 1.5 min in Plasma Cleaner ZEPTO (Diener Electronic, Germany). The image of the produced hybrid PDMS–glass MFC is shown in Fig. 1b.

The procedure of alignment and subsequent culturing of cells in the reaction chamber of the MFC was developed using cells of CHO cell line from Chinese hamster ovary. The procedure of alignment consisted of the stages of loading 20 μL of sample with a desired concentration of cells into the MFC and alignment of cells in the MFC by centrifugation for 15 s at 2000 rpm (Fig. 3a). After alignment of the cells in the reaction chamber, the chip was placed in a Petri dish and filled with DMEM medium containing 10% calf serum. The Petri dish with the chip was then placed in an incubator maintaining the temperature at 37°C and the CO_2 concentration at 5% and kept there for 20 h to allow cells aligned in the reaction chamber to spread and start cell division cycle (Fig. 3b).

The procedure for aligning and culturing cells in the reaction chamber of the MFC allows preparation of conditions for the experiment to study cell migration. The procedure of alignment of cells (centrifugation) may be omitted if there is no need to obtain a uniform “start” position.

Experiments were carried out confirming the functional suitability of the developed device for studying migration by the example of HepG2 cell line derived from a human liver tumor. The response of these cells to the presence of a gradient of epidermal growth factor in the reaction chamber of the device was studied as a test reaction. It is known that HepG2 cells have receptors capable of reacting with the growth factor, which leads to the migration and proliferation of cells in the direction of its increasing concentration, while, in the absence of growth factor in the medium, these processes have no strictly determined direction [16]. These cells grow in the form of colonies, so it was decided to abandon the alignment procedure because

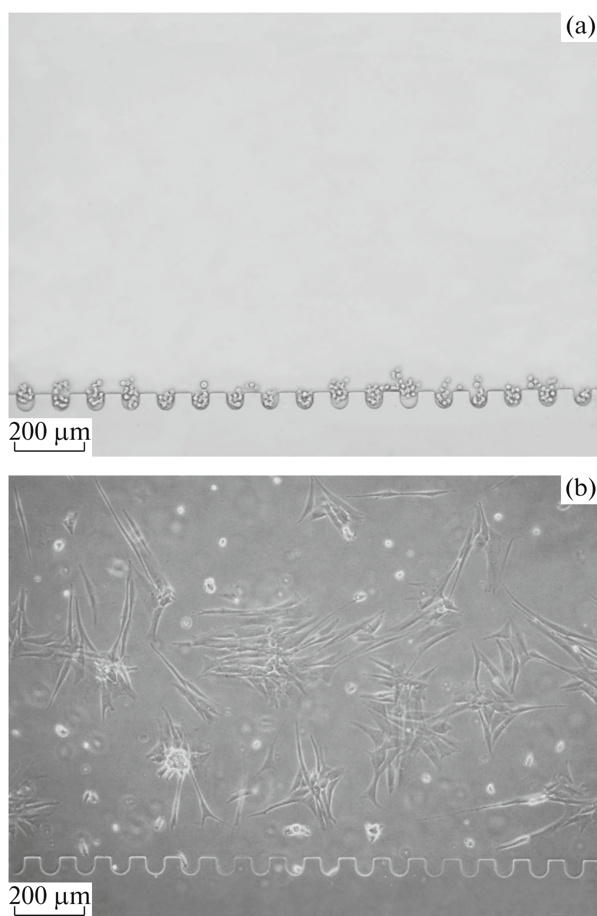

Fig. 3. Distribution of CHO cells in the reaction chamber of the MFC (a) after centrifugation and (b) after culturing on the chip for 20 h.

each cell colony has a defined boundary, which can be considered as the baseline. Colonies were located at the center of the reaction chamber, which ensured liquid circulation from all sides and prevented the possible effect of the distribution of matter on the direction of cell migration without growth factor. Thus, only the procedure of cultivation of cells was executed after loading them into the chip. Then, the Petri dish with the MFC was filled with the liquid that was transferred from the incubator to the objective table of an Axio Observer.Z1 microscope (Carl Zeiss, Germany). Capillaries were connected to the chip, through which DMEM solution with growth factor and without it was supplied. The solution flow rate was controlled using a 70-2209 syringe pump (Harvard Apparatus, United States). The consumption of the solutions was set to 0.25 μL/min. The microscope was equipped with system to control temperature, humidity, and CO_2 level; this ensured the maintenance of temperature at 37°C and the CO_2 level at 5% and prevented the evaporation of the liquid. The experiment was carried out for 10 h with recording of the processes occurring in the reaction chamber of the MFC every 5 min. The results obtained in the experiment showed that the colonies of HepG2 cells grew by ~10% mainly in the direction of increasing the concentration of the growth factor, while, in the opposite direction, the colony growth was only ~1%.

Thus, based on the results of numerical modeling of chemoattractant gradients in various geometries of the reaction chambers, we have proposed an original MFC topology suitable to study the effect of the concentration of chemicals on cells. Using this topology, microfluidic chips of PDMS and glass were promptly made, which made possible the use of optical microscopy for observation of cells. The procedure of alignment of CHO cells in the MFC by centrifugal force was worked out in order to create a baseline for the study of migration process. The efficiency of the device during a prolonged experiment (up to 10 h) was confirmed by means of observation of HepG2 cells. Thus, MFCs can be a valuable tool for solving problems on the effect of chemical gradients on the living cells, which is one of the most important issues of modern biology and ecology.

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