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Development of microfluidic devices for experimental study of cell migration activity, use of numerical methods

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Abstract. Assessment of the migration potential of tumor cells, as well as cells of the immune system in tumor foci, is relevant due to the need for highly informative and fast methods for diagnosing and predicting cancer. To study the active movement of cells, a two-level migration cell was developed and fabricated by soft photolithography. It consists of fluid supply channels and two chambers ("gradient" and "storage") 50 μm high, which communicate through "migration" channels 10 μm high. A chemoattractant and nutrient medium were supplied to the "gradient" chamber of the cell. Due to diffusion, mass transfer occurs between the two laminar flows of the chemoattractant and the nutrient medium, a concentration gradient of the chemoattractant is formed perpendicular to the direction of flow, stimulating the movement of cells located in the "storage" chamber. The features of the model are smooth transitions at the junctions of channels and in transition zones; sealed containers of the "storage" chamber, degassing of injected liquids. For cell adhesion, the inner walls of the cell were covered with collagen. With the help of numerical simulation in the Comsol Multiphysics program, taking diffusion into account, the distribution of the velocity field in the supply channels and in the "gradient" chamber was found. The velocities in the center of the channels practically coincide with those obtained during the flow of luminescent latex spherules: 180 μm/s in the supply channels and 150 μm/s in the "gradient chamber". The development of microfluidic devices for monitoring cell migration is an important step towards improving the diagnosis and therapy of cancer.

Keywords: cancer cell, microfluidics, migration cell, microchannel, soft photolithography.

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Разработка микрожидкостных устройств для экспериментального изучения миграционной активности клеток, использование численных методов

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Аннотация. Оценка миграционного потенциала клеток опухоли, а также клеток иммунной системы в опухолевые очаги актуальна в связи с потребностью в высокоинформативных и быстрых методах диагностики и прогнозирования онкозаболеваний. Для изучения активного перемещения клеток разработана и изготовлена методом мягкой фотолитографии двухуровневая миграционная ячейка. Она состоит из подводящих жидкость каналов и двух камер («градиентной» и «накопительной») высотой 50 мкм, которые сообщаются через «миграционные» каналы высотой 10 мкм. К «градиентной» камере ячейки подавались хемоаттрактант и питательная среда. За счет диффузии происходит массообмен между двумя ламинарными потоками хемоаттрактанта и питательной среды, формируется градиент концентрации хемоаттрактанта перпендикулярный направлению течения, стимулирующий движение клеток, расположенных в «накопительной» камере. Особенностями модели являются плавные переходы на стыках каналов и в переходных зонах; герметизированные емкости «накопительной» камеры, дегазация вводимых жидкостей. Для адгезии клеток внутренние стенки ячейки покрывались коллагеном. С помощью численного моделирования в программе Comsol Multiphysics, с учетом диффузии, найдено распределение поля скоростей в подводящих каналах и в «градиентной» камере, скорости в центре каналов практически совпадают с полученными при течении люминесцентных латексных шариков: 180 мкм/с в подводящих каналах и 150 мкм/с в «градиентной камере». Разработка микрожидкостных устройств для наблюдения за миграцией клеток является важным шагом на пути улучшения диагностики и терапии онкологических заболеваний.

Ключевые слова: раковая клетка, микрогидродинамика, миграционная ячейка, микроканал, мягкая фотолитография

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Introduction

Currently, microfluidics is widely used in various fields of biology and medicine. Microfluidic devices are used in foreign studies as an effective tool for creating micromodels of cancer cell extravasation [1], studying intercellular interactions, including at the level of the tumor microenvironment [2], and properties of single cells [3]. Despite progress in the development of new drugs and treatments, metastatic cancer remains an incurable disease and one of the leading causes of death in the world [4]. A hallmark of the process of metastasis is the ability of cancer cells to migrate from the primary tumor to distant sites in the body. Currently, there are no effective methods for assessing the metastatic potential of cancer cells, which leads to delayed diagnosis of aggressive forms of cancer or the choice of suboptimal treatment methods [5].

Materials and Methods
Development of a migration cell

To study the features of the movement of cancer cells, a two-level migration chamber was made, the prototype of which was the microfluidic cell in [6]. Its schematic view is shown in Fig. 1, and is also given in our previous work [7].

It consists of fluid supply channels, two chambers ("gradient" and "storage") 50 μm high, which communicate through "migration" channels 10 μm high, different widths from 10 to 50 μm and 200 μm long, and cylindrical holes in polydimethylsiloxane (PDMS) with a depth of 5 mm and a diameter of 3 mm, the hole diameter is 6 mm (Fig. 1). Chemoattractant solutions and nutrient medium were supplied to the "gradient" chamber of the cell by hydrostatic pumps. Due to diffusion, mass transfer occurs between the two laminar flows of the chemoattractant and the nutrient medium, a concentration gradient of the chemoattractant is formed perpendicular to the direction of flow, stimulating the movement of cells located in the "storage" chamber.

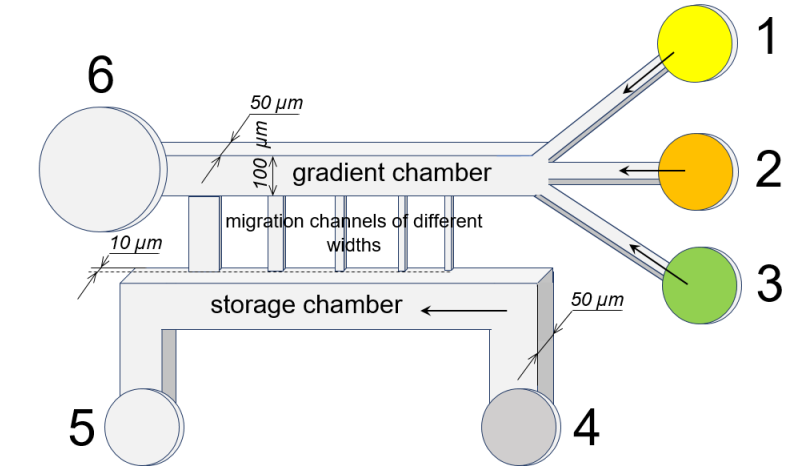

Fig. 1. Schematic representation of a migration cell. 1, 2, 3, 4 are inlets for chemoattractant (1), nutrient medium (2, 3) and cancer cells (4); 5, 6 are outlet holes

When conducting experiments with cells, a number of methodological difficulties were discovered related to the action of capillary forces, the release of gas bubbles from supplied liquid media, and the effect of dynamic blocking [8]. The flow through the stepwise constriction of a suspension containing cancer cells in a nutrient medium, when passing from the inlet container to the microchannel, led to the accumulation of cells at the inlet before the constriction, which practically did not allow the cells to reach the accumulation area in front of the "ladder" being the system of migration channels. The capillary pressure of liquids in the reagent inlet holes with a diameter of 3 mm exceeds the hydrostatic pressure (5 mm w.c.). This prompted us to change slightly the design of the migration cell.

Our two-level migration chamber was made using 2 masks. On one mask there are migration channels through which it is planned to study cell migration, 10 μm high and of various widths, and on the other mask there are supply channels with gradient and storage chambers 50 μm high. For the manufacture of the mask, a vector drawing was developed for printing on a high-resolution printer. The migration chamber mask pattern used in previous experiments [7] has been slightly modified. To prevent the accumulation of microbubbles and cancer cells at the microchannel inlet, the cylindrical containers in the photoresist layer were replaced with containers with a smooth transition, all holes have a smooth expansion, and the junction of the supply channels is rounded. In the middle of storage chamber, an expanded container was made (Fig. 2), due to which the speed drops and the cells are better fixed. Fig. 2 shows the mask alignment area, where it was necessary to combine the second mask with migration channels under the microscope with the first mask at the manufacturing stage. For the convenience of combining the masks, marks were applied, which are clearly visible under a microscope.

Fig. 2. Place where the storage chamber is combined with the mask of migration channels, 3 pieces of the same width (in microns) from left to right: 10, 15, 25, 50, 25, 15, 10

Migration cell fabrication technique

Migration cells are designed and manufactured by soft photolithography. According to it, a photoresist was applied to the glass, the layer thickness of which determined the depth of the resulting microchannels, and the photoresist was exposed through the fabricated photomask. The exposed areas became insoluble (negative photoresist) and were used to replicate microchannels in polydimethylsiloxane (PDMS). Since, in our case, the microfluidic device is two-level: the thickness of narrow microchannels ("ladders") is 10 ± 2 μm, and the thickness of the rest of the cell (supply channels) is 50 ± 5 μm, the fabrication was carried out in 2 stages. At the first stage, using a mask of migration channels, a photomask was made on conductive ITO glass (glasses with good adhesion). After obtaining the photomask, it was heated on a tile at 250 °C for 10 minutes with slow cooling for better fixation to the glass, and also so that the developed protrusions serving as a photomask for migration channels darkened and were visible through the photoresist layer during further manufacturing. Further, at the second stage, a photoresist was poured onto the photomask from above, and using the mask for the supply channels, ledges were made on the glass with a thickness of 50 μm. Due to the dark color of the channels, visible through the photoresist layer and the applied marks, it was convenient under a microscope to combine the place of the mask with the expansion of the storage chamber with the protrusions of the migration channels revealed at the first stage (Fig. 2). When washing, both illuminated areas with protrusions of different heights were preserved. Further, using the method of soft photolithography, based on a two-level photomask, a replica was obtained from the solidified PDMS with channels of correspondingly different depths: 10 μm for migration channels and 50 μm for the rest of the cell. After that, holes 3 mm in diameter were made in PDMS with a thickness of 5 mm for containers, and then a replica of PDMS was attached to a glass slide, the microcell was ready. In microfluidic devices, glass and PDMS with replica channels are transparent, which allows the use of imaging techniques. To create conditions for cell adhesion, the channel walls were covered with collagen, which serves as an extracellular matrix.

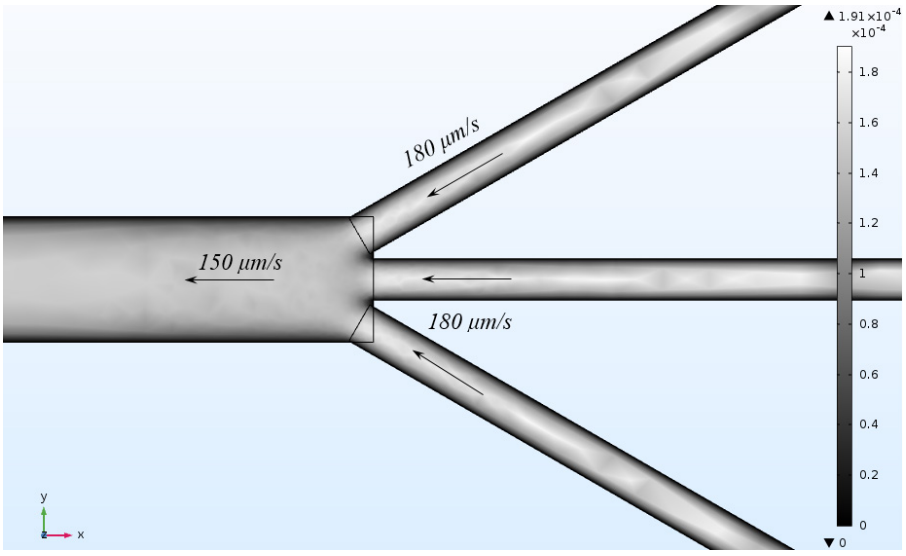

Fig. 3. The distribution of the velocity field in numerical simulation in the Comsol Multiphysics program

Results and Discussion

Preparation for the experiment in the migration cell

Cell migration in the cell was studied under a microscope on a thermostatic table with a temperature of 37 °C; under such conditions, the supplied liquid from the input containers and the output container evaporated for 3 hours. An attempt to manufacture chambers for the cell, even with a polycarbonate thermal pack, was not successful, since liquid condensed on the upper surface. Therefore, cone tips from a 1 ml dispenser with a liquid volume of 0.3 ml were used, one of which was filled with DMEM (or RPMI 1640) medium with a chemoattractant, and the other with only a nutrient medium, a cone was also fixed into the outlet. To reduce evaporation, the cone was covered with a "parafilm" film, in which a 500-micron hole was made to equalize the pressure with atmospheric pressure. After filling the storage chamber containers with cells, they were filled with a nutrient solution; the cell container was sealed with a "parafilm" film pressed against a glass plate. Since there is a rather significant difference between the temperature of the liquids in the cones (25 °C) and in the microchannels of the cell (37 °C), gas bubbles were released in the microchannels during heating. In order to avoid bubbling, the inlet liquids were degassed.

Migration cells were tested with distilled water, water with dye or with latex micron spherules. The liquid was supplied due to the pressure difference between the liquid levels in the inlet and outlet containers, which are a hydrostatic pump 5 mm of water column (50 Pa). To test the flow and determine the diffusion erosion of the boundary between tinted and pure water, a blue dye and luminescent latex spherules 1 μm in diameter were used. The boundary between the colored and uncolored laminar jets was blurred due to diffusion in the area in front of the migration channels ("ladder"). During the passage from the confluence of liquids from three supply channels to the beginning of the "ladder" (12 seconds), diffusion mixing led to the formation of a concentration gradient.

The results of numerical simulations obtained in the Comsol Multiphysics program (Fig. 3) showed that the flow velocities corresponded to 180 μm/s at the point where the nutrient medium and chemoattractant were supplied (three supply channels) and 150 μm/s in the widest part of the microchannel. An analysis of the measurement of particle velocities according to video recording using a Canon XM-2 camera mounted on a Zeiss Stemi 2000C microscope give similar values for the flow velocity of liquids.

Experimental study of the migration activity of cancer cells in a two-level cell

The procedure for preparing the migration cell for the experiment on cell migration was similar to that presented earlier in our work [][7] where we used DU145 cell lines. In this work we used cell lines PC-3 (obtained from bone metastasis of a patient with stage IV prostate adenocarcinoma) and HGF (hepatocyte growth factor) chemoattractant. In the presence of HGF the cell lines in the Boyden chamber showed greater migratory activity than in the presence of IL8 (interleukin 8) [7].

Cells in the microfluidic migration system were observed using an AxioObserver D1 inverted microscope (Zeiss, Germany) with A-Plan ×20 and ×10 objectives using the phase contrast method. Microphotography was carried out using an AxioCam MRc5 digital camera (Zeiss, Germany) with ZEN software (Zeiss, Germany). To describe the nature of movements, frame-by-frame shooting of cells was carried out for 2–8 hours with an interval of 5 minutes with ×10 or ×20 lenses. An experiment was carried out on the movement of the PC-3 cancer line. Before the start of the experiment, the cells were located at the entrance to the migration channels.

Fig. 4 shows the displacement of one of the PC-3 cells depending on time. The photographs show that the cell moves at different speeds, and accelerates closer to the exit from the migration channel, which is most likely due to a higher concentration of the chemoattractant at the exit. First, after entering a narrow 15 μm channel, the cell spreads out, the speed in the time interval of 120-180 minutes (Fig. 4) was 16 μm/hour. Then, in the area of 180–240 minutes, the cell speed was 30 microns/hour. In the last section of 250–255 minutes, the speed was 950 microns/hour. The average speed in the area of 120-255 minutes was about 70 microns/hour.

An analysis of the movement of other cells showed that they move at different speeds and also accelerate closer to the exit from the migration channel. The uniqueness of the results obtained is due to the use of the developed new method, which has the ability to study not only the migration characteristics of cells, but also their deformation, which affects movement in spatially constrained conditions under the influence of various types of chemical agents.

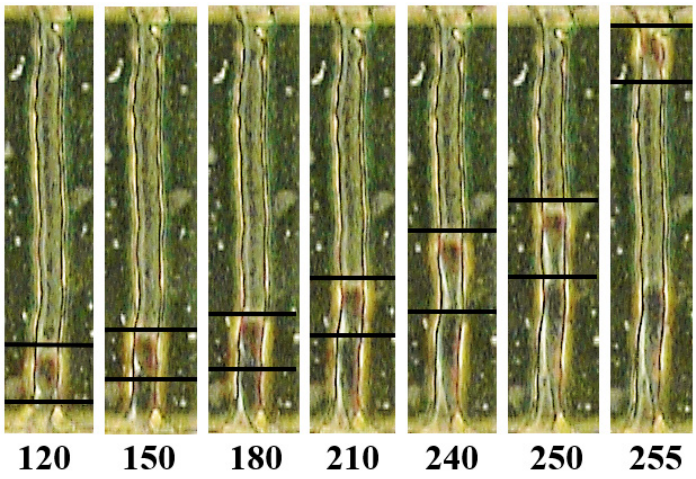

Fig. 4. Microimages of PC-3 cells in the same microchannel 15 μm wide, front and back parts of the examined cell are marked with black lines, the time in minutes from the start of the experiment is shown below the images

Conclusion

To study the migration potential of cells, a two-level migration cell was developed and fabricated by soft photolithography. The device has been improved compared to the previous one [7], by creating smooth transitions at the channel junctions and transition zones to prevent the dynamic blocking effect associated with the accumulation of microbubbles or cells in the constriction. To reduce the effect of pressure drop when filling the storage chamber with cells, the chamber containers after filling with cells were sealed with a "parafilm" film pressed against a glass plate. To prevent the chemoattractant from getting into other containers and to reduce the effect of capillary forces, cone tips were inserted into the inlet and outlet holes; to reduce evaporation, the cone was covered with a "parafilm" film, in which microscopic holes were made to equalize pressure with atmospheric pressure.

With the help of numerical simulation in Comsol Multiphysics program and taking into account diffusion, velocity field distribution in supply channels and in "gradient" chamber was characterized, velocities in the centre of the channels practically coincide with those obtained by flowing over spherules: 180 μm/s in supply channels and 150 μm/s in the "gradient" chamber.

The ability of PC-3 cancer cells to migrate in the migration microfluidic system developed by us has been experimentally demonstrated. The development of this device is carried out to create a method for accelerated analysis of the migration potential of cancer cells, the differences in the movement of tumor cells compared to normal cells will also allow differentiating healthy and cancer cells. The creation of a device and a method for studying cell migration is an important step towards improving the diagnosis and therapy of oncological diseases.

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