Experimental study of monocyte migration using the developed microfluidic device

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The paper presents the experiments conducted in a microfluidic device for migration of monocytes in their interaction with a hemoattractant that is a conditioned living medium of prostate cancer cells of the androgen-independent line PC3 or androgen-dependent line LNCaP. The average rate of migration to the medium from LNCaP ($\sim 37\,\mu$m/h) appeared to be higher than that to PC-3 ($\sim 12\,\mu$m/h). The number of monocytes migrating to the conditioned medium from LNCaP for a healthy person was higher than that for patients with prostate cancer; however, average migration rates of monocytes from healthy people and patients were almost the same. Development of the migration devices is an important step towards improving diagnosing and treatment of oncological diseases.

Keywords: microfluidic device, chemoattractant, migration, monocyte, prostate cancer.

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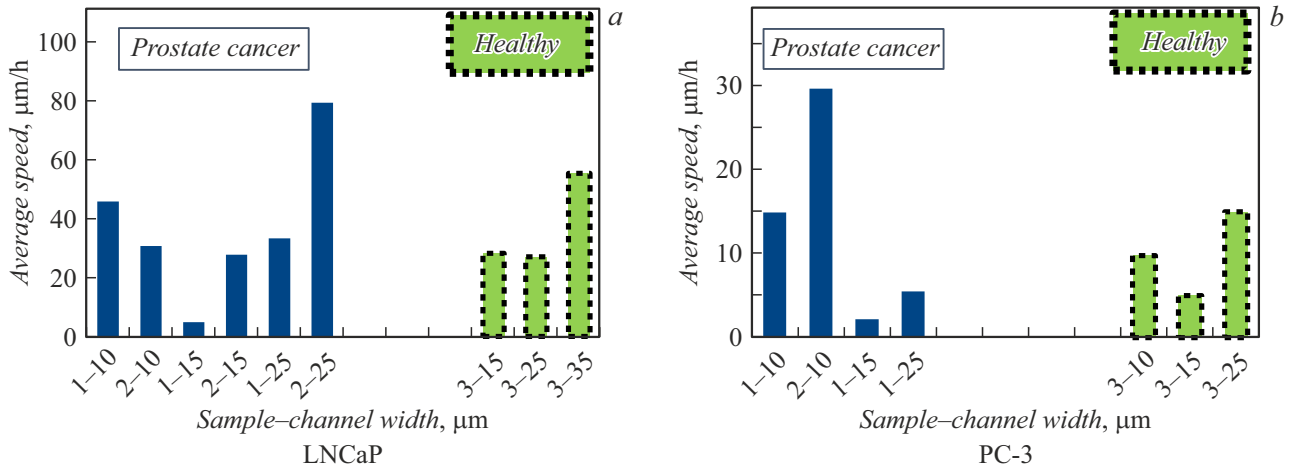
Migration is a key property of living cells. It is responsible not only for the bodyś normal development and immune response, but also for inflammatory processes and tumor metastasis. Monocytes are white blood cells, a kind of leukocyte; they consume microbes and bacteria entering the body and thus make the body rid of them. The relevance of the problem of assessing the migration potential of monocytes in prostate cancer (PCa) of different aggressiveness under 3D conditions simulating the tumor microenvironment is caused by the prevalence of prostate cancer and cancer in general and by immaturity of minimally invasive, highly informative, low-cost and rapid methods for cancer diagnosing and prediction. Various microfluidic 3D devices are currently considered as a convenient, economical and efficient tool for creating micromodels of an entire organ and for studying the mechanisms of intercellular interaction at the level of the tumor microenvironment and properties of single cells [1–4].

Microfluidic devices are fabricated using the soft photolithography technique. The prototype of the microfluidic migration chamber (MMC) for studying cell migration was a microfluidic cell described in [5]. The procedure for manufacturing the device presented in this paper is similar to that described in [6], while the migration cell schematic diagram is similar to that given in [7]. It consists of fluid-supply channels, two chambers („gradient“ and „storage“ones) $50\,\mu$m in height communicating through the „migration“ channels $10\,\mu$m in height and 10, 15, 25, $35\,\mu$m in width, and cylindrical holes in polydimethylsiloxane 5 mm deep; all the holes are 3 mm in diameter except for the outlet one whose diameter is 6 mm. The gradient chamber of the cell was supplied with the chemoattractant solution and nutrient medium by using a hydrostatic pump. This model differs from the previously constructed device [6] in that it has a lower number of channels supplying chemoattractant and nutrient medium (two channels $50\,\mu$m wide each instead of three $33\,\mu$m wide); this was done to reduce the channel manufacturing errors, effect of bubbles, and probability of the dynamic blocking effect. Smooth transitions, i. e. expansions of the holes, were added; all the holes (slots) were gradually expanded, and the junction of two supply channels was rounded. In the storage chamber there was made an expansion (vessel) that decreased the speed and retained the cells inside itself.

Using MMC, experiments with monocytes from a healthy person and PCa patients were carried out (see the Table). As a chemoattractant, conditioned serum-free medium X-VIVO containing waste products of cancer cells of the androgen-dependent line LNCaP or androgen-independent line PC-3 was used. For the experiment, we used new MMCs (created not earlier than ten days before). Prior to starting the experiment, the channel was treated with UV light for 15 min. The system as a whole was placed on the table of inverted microscope AxioObserver D1 (Carl Zeiss) heated to 37°C. The used objective was of the A-Plan × 10 type; the phase-contrast method was applied. Microphotography was carried out using an AxioCam MRc5 digital camera with the ZEN software (Zeiss, Germany). To record the motion of cells, time-lapse photography of cells was continued for 20 h with the interval of 5 min. A series of acquired images was used to observe the cells motion in microchannels.

Before the experiment was begun, the cells were located at the entrance to the migration channels. Non-living cells completely flatten out over the glass surface and get adhered to it. Unlike the dead cell, the living cell nucleus does not flatten out completely. As known, spherical objects reflect light in almost all directions; therefore, living monocytes



Average speed of the monocyte samples motion in the migration channels with the conditioned medium from the LNCaP cells (*a*) and PC-3 cells (*b*). Figures at the abscissa indicate the Nos. of monocyte samples (see the Table) and migration channel widths (in μm).

look glowing in photographs. Just the presence of glowing easily distinguishes the living cells from dead ones. In the experiments, average content of living cells was about 50%. The largest number of migrated cells (14 cells) was observed in the experiment with monocytes from a healthy donor (sample 3) and conditioned medium of LNCaP cells (see the Table). For samples 1 and 2 from PCa patients, the number of migrated monocytes appeared to be similar in the experiments with the conditioned medium from both the LNCaP and PC-3 cells (see the Table).

In the experiments, the camera took pictures of migration channels 10, 15, 25 and 35 μm wide every 5 min; in processing the obtained photos, the average speed was calculated only for those microchannels where active migration of monocytes was detected (see the Figure). Thereat, the average value was calculated over all the sections of microchannels of the same width for all the cells that have passed through them. No influence of the microchannel width on the migration rate was observed for migration channels of comparable widths (10–35 μm) (see the Figure): a monocyte cell can move either faster or slower than a similar cell in a wider channel. In different parts of the microchannel, monocytes moved at different speeds, often accelerating when approaching the exit that is the place with the highest chemoattractant concentration.

Experiments in MMC in which the X-VIVO medium conditioned by LNCaP androgen-dependent tumor cells was used as a chemoattractant showed for monocyte samples № 1–3 (see the Figure) that the average speed of motion through channels of various widths was 36.9 μm/h. The speed of motion towards the MMC gradient chamber containing the conditioned medium from the androgen-independent tumor cell line PC-3 appeared to be significantly lower, namely 11.7 μm/h. Average speeds of the cells motion from the PCa patients and healthy individuals towards the chemoattractants of the LNCaP cell line were almost the same (36.91 and 36.86 μm/h, respectively).

When the conditioned medium from the PC-3 line cells was used as a chemoattractant, monocytes migrating from the PCa patients were shown to move towards the gradient chamber somewhat faster (13.1 μm/h) than monocytes from a healthy person (9.9 μm/h).

The obtained results demonstrate that monocytes move towards the conditioned medium of the androgen-dependent cancer line faster than in the case of the androgen-independent line. A healthy person has more monocytes responding to a threat than PCa patients, though the speeds of migrating cells are almost the same for the same lineage. The observed differences may be a basis for developing an algorithm for assessing the monocyte migration in microchannels in healthy individuals and patients with prostate cancer depending on their clinical characteristics.

Number of migrated cells of the monocyte samples

No. of the monocyte sample (group)	Conditioned medium of the cell line	
	LNCaP	PC-3
1 (PCa)	6	6
2 (PCa)	6	4
3 (healthy donor)	14	7

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Compliance with Ethical Standards

All the in–human trials performed in the framework of this study comply with Ethical Standards of the institutional and/or national Research Ethics Committee, as well as with Declaration of Helsinki (1964) and its subsequent

amendments, or comparative ethical norms. Cells from both the PCa patients and healthy people were taken under the Informed Voluntary Consent for clinical and scientific research. The obtained results are presented confidentially, without indicating personal details.

Conflict of interests

The authors declare that they have no conflict of interests.

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