Video Article

Study of Cell Migration in Microfabricated Channels

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Abstract

The method described here allows the study of cell migration under confinement in one dimension. It is based on the use of microfabricated channels, which impose a polarized phenotype to cells by physical constraints. Once inside channels, cells have only two possibilities: move forward or backward. This simplified migration in which directionality is restricted facilitates the automatic tracking of cells and the extraction of quantitative parameters to describe cell movement. These parameters include cell velocity, changes in direction, and pauses during motion. Microchannels are also compatible with the use of fluorescent markers and are therefore suitable to study localization of intracellular organelles and structures during cell migration at high resolution. Finally, the surface of the channels can be functionalized with different substrates, allowing the control of the adhesive properties of the channels or the study of haptotaxis. In summary, the system here described is intended to analyze the migration of large cell numbers in conditions in which both the geometry and the biochemical nature of the environment are controlled, facilitating the normalization and reproducibility of independent experiments.

Video Link

The video component of this article can be found at http://www.jove.com/video/51099/

Introduction

Migration is a complex cellular function that is important for many physiological processes in multicellular organisms, including development, immune responses, and tissue regeneration. In addition, certain pathological situations such as tumor invasion and metastasis rely on cell motility[1]. For these reasons, cell migration has become a major field of study in the context of both fundamental and translational research. *In vivo*, most tissues are characterized by a rich extracellular matrix and high cell density. Cell migration therefore, under physiological conditions, occurs in a complex confined environment. Classically, most likely due to historical reasons and technical limits, cell migration has been studied in flat 2D systems that do not reproduce many of the environment properties found in tissues, such as confinement. Moreover, factors as cell adhesion, that are essential for motility in 2D, have been recently showed to not be necessarily required for migration *in vivo* or inside gels, suggesting that the mechanisms that rule cell locomotion in 2D and in other environments are distinct[2]. Several systems have been developed to mimic the complex properties of tissues, the most famous being collagen gels, which aim at recapitulating the properties of the extracellular matrix composition[3]. Here we propose microchannels as a simple complementary method that allows the study cell migration in one dimension under a confined environment.

In this system cells migrate along microchannels into which they enter spontaneously. Migratory cells then acquire the shape of the channels, adopting a tubular geometry that most likely reinforces their polarity. The linear movement of the cells in the channels allows automatic cell tracking and the extraction of quantitative parameters from experiments. From the technical point of view, this system is easy and flexible. The coating of the channel walls can be manipulated, the size and the shape of the channels can be adapted, and a large number of cells can be analyzed in single experiments. This system can be also scaled-up to perform medium range screen analysis of molecules involved in cell motility. The protocol described here has been standardized using dendritic cells (DC) as a cellular model. These cells are key to the immune system as they participate in the initiation and maintenance of specific immune responses[4]. *In vitro*, DCs have been shown to spontaneously migrate in confined environments and are therefore a good model to study cell motility in microchannels[5,6]. Importantly, this system can be extended to analyze migration of any other motile cell type as T lymphocytes, neutrophils, or tumor cells[7-9].

Protocol

Important note: This protocol assumes that the mold containing the shape for the desired microchannels has been already made. Further information on the preparation of the mold has been already published[10]. This protocol also assumes that bone marrow DCs culture is known.

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1. Chip Fabrication

1. Mix PDMS oil and curing agent at a weight ratio 10:1 in a plastic cup. Mix both compounds thoroughly.
2. Cast the mix over the mold bearing microchannels. The total height must be between 0.5-1 cm.
3. Remove air bubbles in a vacuum jar bell during 1 hr.
4. Harden PDMS in the mold by placing the latter in an oven for 2 hr at 65 °C.
5. Once the PDMS is at room temperature, cut a large piece around the structure with a surgical blade and peel it off from the mold (**Figure 1A**).
6. Drill holes where cells will be injected (typically 2 mm) and resize the PDMS with a surgical blade to fit the size to glass-bottom dishes in which migration will be assessed (**Figure 1B**). Before proceeding, remove residual dust from the dish using lens cleaning paper. This favors the binding of PDMS to glass described in step 1.10.
7. Clean the resized PDMS chip containing the channels by sticking and peeling adhesive tape on the structure sides.
8. Sonicate the PDMS pieces 30 sec in 70% ethanol to remove dusts and small PDMS particles. Dry them quickly afterwards by blowing clean air.
9. Activate the PDMS (structures upwards) and culture dishes by air (or oxygen) plasma treatment during 30 sec at 300 mTorr.
10. Place both activated surfaces in contact to permanently stick the PDMS to the substrate. If needed, use metallic forceps to press slightly on top of the PDMS to force the contact between the polymer and the glass of the dish (**Figure 1C**).
11. Incubate the chip in the oven at 65 °C for 1 hr to strengthen the binding.

2. Coating of Microchannels

1. Activate the whole structure by air plasma at 300 mTorr for 1 min. This will promote the entry of liquid into the channels at the next step.
2. Fill rapidly the entry holes of the chip with fibronectin at 10 µg/ml in water. Other substrates such as PEG may be used to modify cell adherence to the channel walls. Pay attention that the liquid spreads throughout the entire structure. This can be easily checked by eye under regular light or using a regular bright field microscope.

   NOTE: In very small structures, in which diffusion is harder, entry of liquid in the channels can be forced by placing the structure in a vacuum jar bell during at least 15 min. To verify the efficiency of the coating fluorescent substrates can be used (**Figure 2**).
3. Incubate 1 hr at room temperature to allow adsorption of the fibronectin or any other coating substrate to the walls of the channels.
4. Wash the structures 3x with PBS to remove the nonbound substrate.
5. After washing, proceed to Protocol 3 or store the chip at 4 °C for a later use (24 hr maximum).

3. Cell Loading

1. Remove the PBS from the plate and fill the microsystem with cell medium. Let incubate for 1 hr to saturate the channels with the medium.

   NOTE: For experiments involving drugs like molecule inhibitors, it is advised to preincubate the channels with a medium containing the drug at the right concentration. Moreover, some drugs tend to solubilize in the PDMS structure which reduces the effective concentration. Some solutions have been proposed to counteract this issue[11-12].
2. Remove floating DCs and recover semi-adherent cells by flushing with culture medium. Count cells using a hemocytometer.

   NOTE : For nucleus imaging, a Hoechst 33342 staining can be achieved beforehand by incubating 2 x 10^6 cells during 30 min with the dye at 200 ng/ml in complete medium. Wash twice by centrifugation to remove excess of dye before continuing the protocol.
3. Centrifuge the cells at 300 x g during 5 min (time and speed can be different depending on the cell type) and discard the medium. Dilute the pellet to reach a concentration of 20 x 10^6 cells/ml.
4. Remove the excess of medium from the plate and empty the entry holes in the PDMS structure using a micropipette. Fill the entries with 5 µl of cell solution to reach an amount of 1 x 10^5 cells in each entry hole.

   NOTE: High cell density is required to favor the contact of the cells with the channels. Low cell density may result in low number of cells inside channels and failure of the experiment.
5. Incubate the microchip for 30 min at 37 °C in the incubator. Add 2 ml of complete medium to the experiment dish.

   NOTE: The whole PDMS structure must be covered in order to avoid drying of the cells during the experiment. If 2 ml is not enough, add more medium to completely cover the PDMS structure.

4. Imaging

1. Clean the external bottom surface of the dishes with lens cleaning tissue before placing the plates under the microscope.
2. To analyze migration in large number of cells, use 10X magnification and wide field illumination in a CO_2 and temperature equipped video-microscope (**Figure 3**). To facilitate cell tracking, Hoechst staining and UV light can be used (see step 3.2). Migration can be also analyzed using confocal microscopy in order to get higher resolution of cellular structures during migration.
3. For time-lapse microscopy at 10X, choose a time frequency according to the expected cell speed (typically 2 min for dendritic cells migrating with a speed of 5 µm/min).

   Note: The protocol described here does not include the analysis of the cell migration parameters. A few points are listed in the discussion to advise the readers on how to proceed to extract information from time-lapse movies.

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Representative Results

In each experiment the surface of the PDMS is coated with a molecule adapted to the interest of the study. **Figure 2** shows channels coated with a fluorescent molecule, PLL-g-PEG, before and after washing (step 2.4). Such an experiment allows the control of the homogeneity of the coating in the channels.

After cell loading, video microscopy can be performed to follow cell migration. **Figure 3A** shows an example of DC migrating in microchannels at a density appropriate to track the cells. Both, phase contrast and Hoechst staining can be used for this purpose (see Discussion). The technique is also compatible with fluorescent confocal microscopy at higher resolution, and can be used to track organelles or cellular structures. **Figure 4B** shows an example of the dynamic of polymerized actin in migrating DCs expressing LifeActGFP.

An example of quantification of cell motility analyzed in migrating DCs is shown in **Figure 5**. **Figure 5A** shows kymographs generated from WT or Y27632 treated DCs. Y27632 is a well characterized inhibitor of cell contractility and migration, and was used to verify the ability of the system to detect changes in speed. The kymographs can be further analyzed to extract position and size of cells as a function of time, allowing the study of different parameters to describe cell migration. **Figure 5B** shows the speed of migration tracked by imaging nucleus positioning using a homemade software[13]. DCs have a mean velocity close to 6 µm/min. Treatment with Y27632 decreases significantly their velocity. This example shows the power of the technique, which allows the quantification of large number of cells in single experiments and the utility of the system to detect differences induced by drug treatments. This can be extended to the use of siRNA and screening of molecules involved in the control of migration.

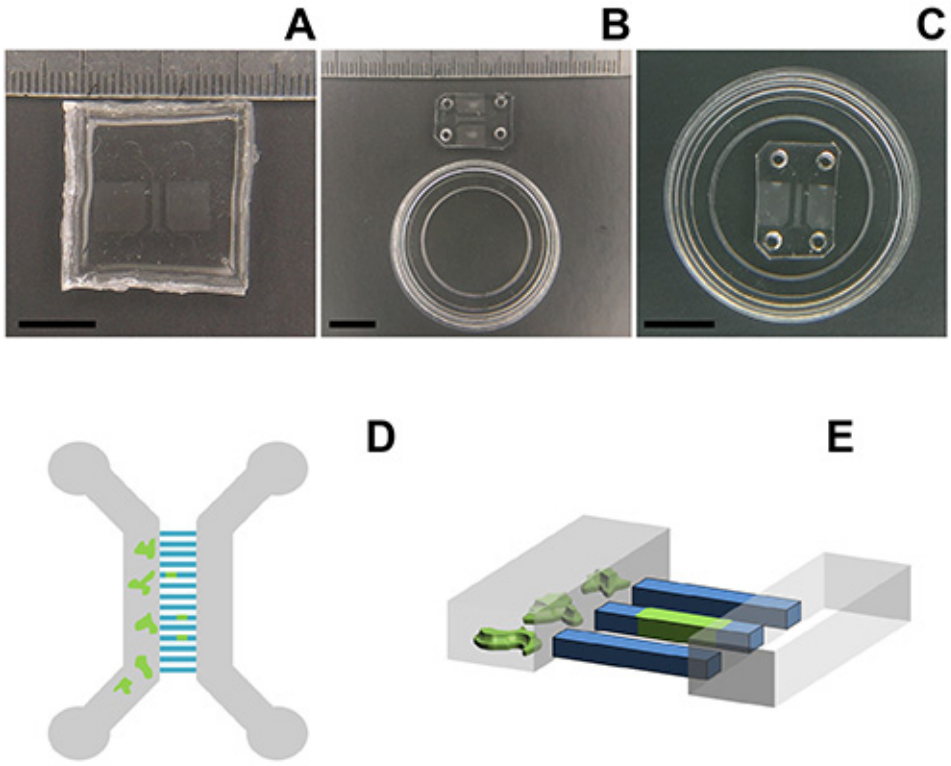

Figure 1. Steps in channels assembly. PDMS chip was replicated from a channel mold. (**A**) The chip containing the channels was extracted directly from the mold. (**B**) The chip resized to fit the experimental dish and a hole was made to allow the entry of the cells. (**C**) The chip was finally pasted on top of the glass dish. (**D** and **E**) Schematic representation of the device. (**D**) Top view. (**E**) Side view. Green objects represent cells in or out channels (blue). Bar 1 cm.

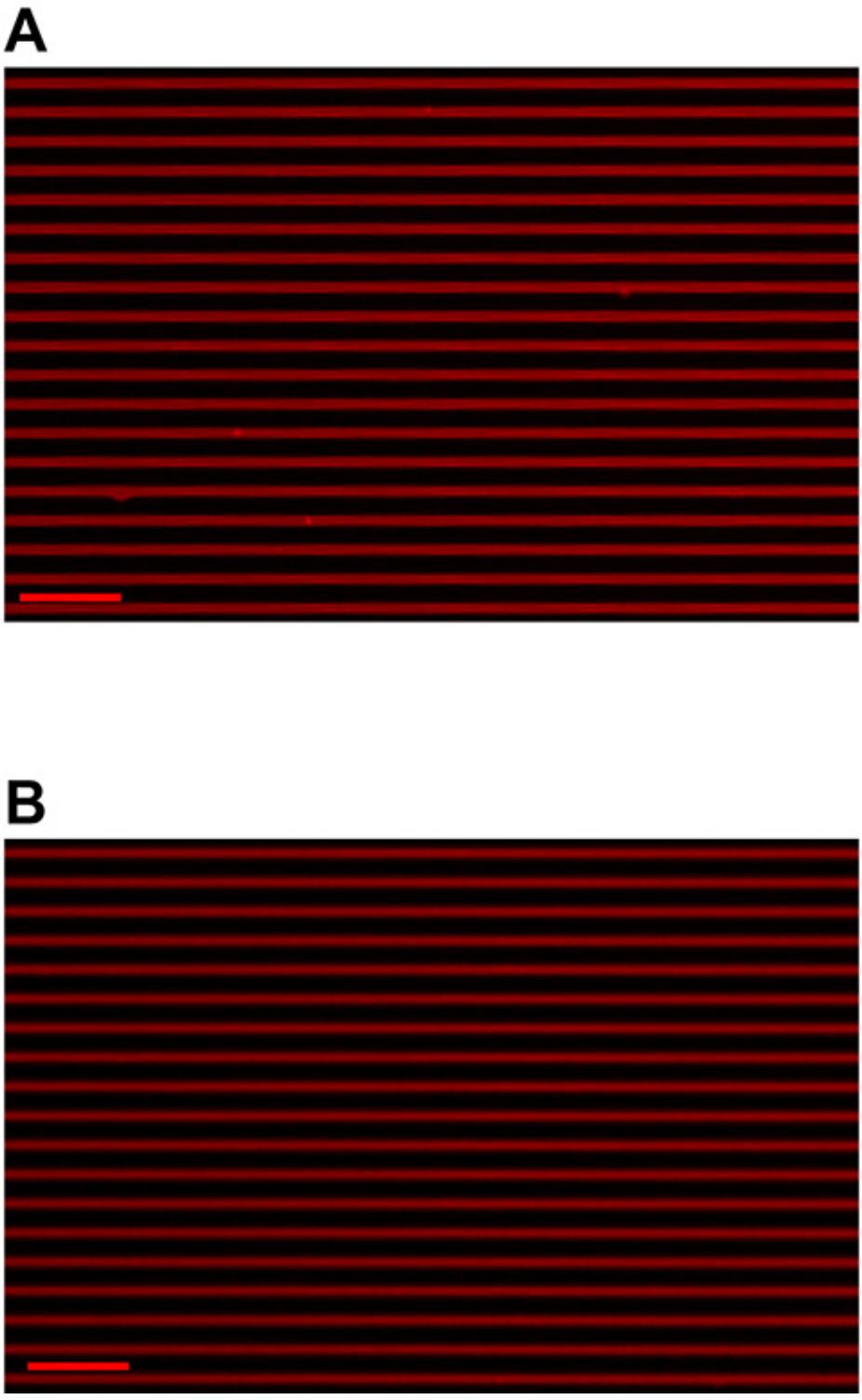

Figure 2. Coating of channels with a fluorescent substrate. (**A**) 5 µm x 5 µm channels incubated with fluorescent PEG at 100 µg/ml during 1 hr. (**B**) Image representative of the remaining fluorescent PEG after PBS washing of the channels. Bar 50 µm.

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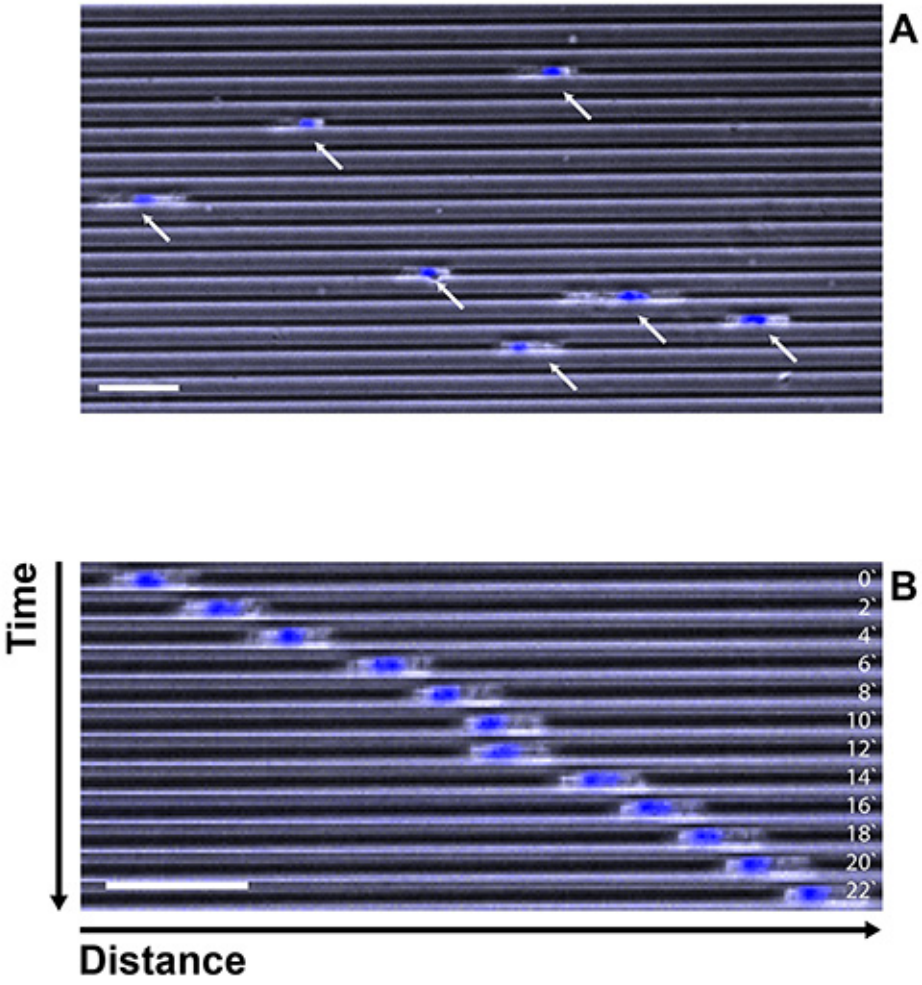

Figure 3. Cells migrating along microchannels. Phase contrast (grey) and nucleus staining (blue) of cells migrating in 5 µm x 5 µm microfabricated channels. (**A**) Single image extracted from a movie of DCs migrating along channels. (**B**) Montage showing the displacement of a selected DC migrating along channels. White arrows indicate migrating cells. Timescale 1 img/2 min. Bar 50 µm.

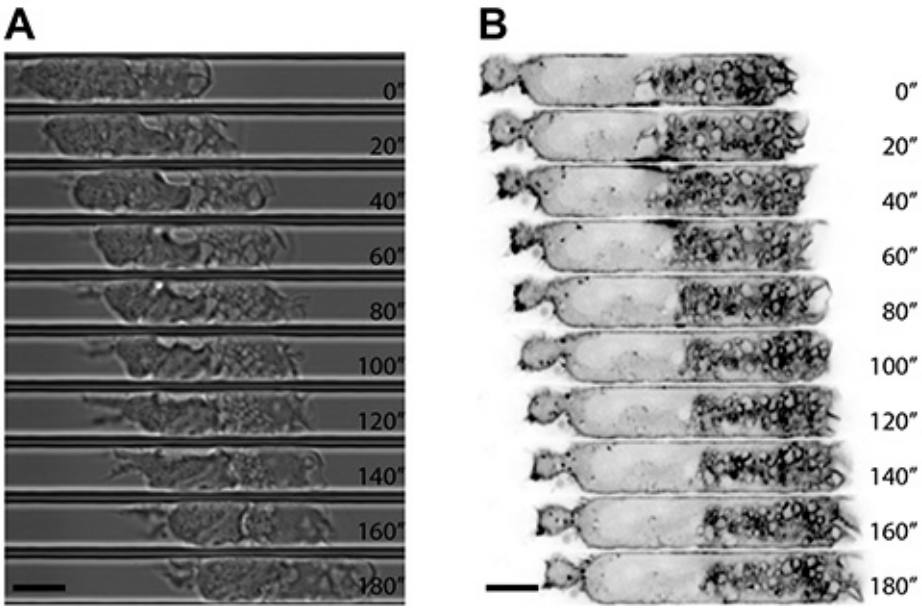

Figure 4. Example of high resolution cells in microchannels. High-resolution imaging of DCs migrating along single microchannels. (**A**) Montage of phase contrast images (1 img/20 sec) of a DC migrating in a microchannel (100X magnification). (**B**) Montage of a DC expressing lifeact (highlights polymerized actin) showing compatibility of the system with fluorescence imaging (1 img/20 sec, 60X magnification, single optical section). Polymerized actin is shown in black. Channel size 10 µm wide x 5 µm high. Bar 10 µm.

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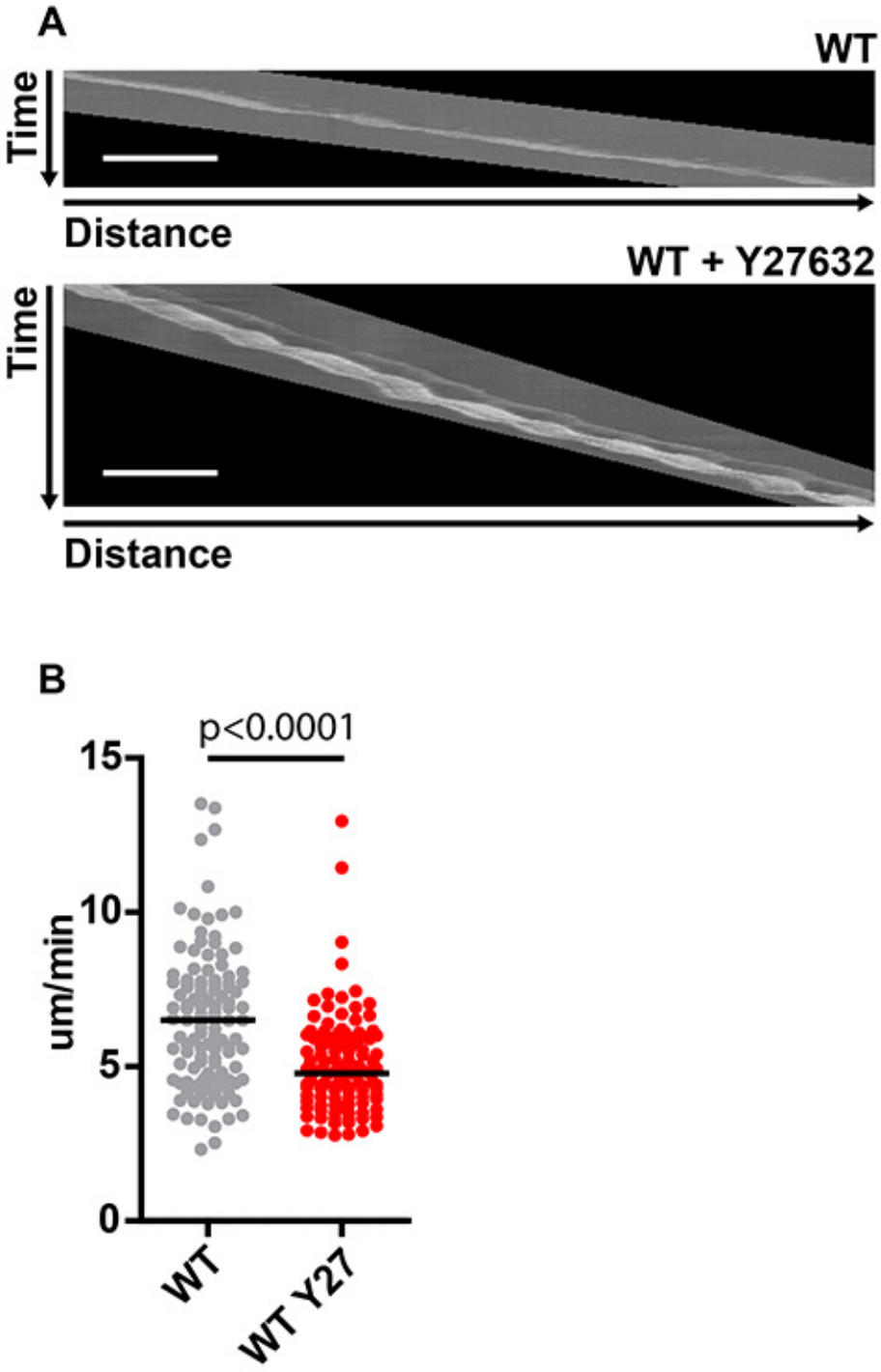

Figure 5. Representative results of DCs migrating along microchannels. This figure illustrates the type of analysis that can be done from movies of cells migrating along microchannels. **Figure 5A** shows kymographs generated from time-lapse phase contrast images of WT or Y27632 treated DCs migrating in microchannels (10X, 1 img/2 min). **Figure 5B** shows an example of quantification of cell velocity obtained after tracking the DC nucleus using Hoechst staining and a homemade software[13]. Data are analyzed using a nonparametric t test (Mann-Whitney test). Bar 100 µm.

Discussion

Here we describe a device composed of microchannels as a method to study the migratory properties of large number of cells in single experiments. This experimental system mimics the confined environmental constraints found in tissues by endogenous migratory cells. However, by forcing migration in a single dimension, it facilitates automatic cell tracking and the extraction of measurables (**Figure 5**). We also show that

our device is compatible with fluorescence microscopy and can therefore be adapted to study organelle positioning during the different phases of cell motility (**Figure 4**).

A critical step within the protocol is the quality of the channels. It is important to control the good sticking of the PDMS on the glass surface. When troubles of sticking appear the first things to check are the cleanliness of both surfaces and the one of the plasma cleaner. Another parameter that may be critical is the density of loaded cells. The spontaneous migration requires close proximity of the cells to the channels to facilitate the entry into the tubes, and the optimal number should be assessed for each cell type.

As an example, we have shown the spontaneous migration of DCs, but the system can be used to analyze the migration of other cell types. In the laboratory, we have tested primary T lymphocytes, macrophages as well as tumor cell lines (data not shown and references[7-9]).

To analyze cell migration in the channels, two main protocols have been tested. The first requires imaging by phase contrast (10X), and is based on the automatic detection and generation of kymographs from migrating cells[3] (**Figure 5A**). Velocity, cell size and persistence are, among others, parameters that can be extracted from kymographs. This system is limited by the quality of the kymographs and to the development of the software to generate and analyze them. A second and easier way to quantify the data is based on the semi-automatized tracking of the nucleus using Hoechst staining (**Figure 3**). The use of fluorescence allows the tracking and analysis of migration with standard imaging software. An example of such strategy has been developed for the first world cell race[12].

Given that the surface of the PDMS can be adsorbed with virtually any protein, microchannels can be used to evaluate the impact of extracellular matrix proteins in cell migration. The geometry of the channels can be also modified, facilitating the study of the impact of physical constrains in cell migration.

Finally, this experimental system can be adapted to acquire several conditions in single experiments. This, together with a semi-automatized analysis, is compatible with medium throughput screenings for the discovery of new actors involved in the migration of cancer cells.

Disclosures

The authors have nothing to disclose.

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