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# Study of dendritic cell migration using micro-fabrication

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ABSTRACT

Cell migration is a hallmark of dendritic cells (DCs) function. It is needed for DCs to scan their environment in search for antigens as well as to reach lymphatic organs in order to trigger T lymphocyte's activation. Such interaction leads to tolerance in the case of DCs migrating under homeostatic conditions or to immunity in the case of DCs migrating upon encounter with pathogen-associated molecular patterns. Cell migration is therefore essential for DCs to transfer information from peripheral tissues to lymphoid organs, thereby linking innate to adaptive immunity. This stresses the need to unravel the molecular mechanisms involved. However, the tremendous complexity of the tissue microenvironment as well as the limited spatio-temporal resolution of in vivo imaging techniques has made this task difficult. To bypass this problem, we have developed microfabrication-based experimental tools that are compatible with high-resolution imaging. Here, we will discuss how such devices can be used to study DC migration under controlled conditions that mimic their physiological environment in a robust quantitative manner.

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## 1. Introduction

Cell migration plays a key role in immune responses. At early stages of infection it allows pathogen clearance by promoting the local concentration of phagocytes at the site of inflammation (Soehnlein and Lindbom, 2010). Later, during the adaptive immunity response, a more complex migration program between distant locations is established. This program is characterized by exchanges of cells between infected tissues and lymphoid organs, a migration game intended to promote communication between distant compartments to ensure both tolerance to self and elimination of infectious agents. In that context, DCs play a key role in transporting antigens to lymph nodes for presentation to T lymphocytes either under homeostatic conditions for establishment of tolerance, or during infection for the onset of the immune response (Baratin et al., 2015).

DC migration from peripheral tissues to draining lymph nodes occurs through lymphatic vessels (LVs) and requires the upregulation of the chemokine receptor CCR7, which binds both CCL19 and CCL21 upon DC activation. In the mouse skin, CCL21 produced by lymphatic endothelial cells forms an immobilized gradient that is followed by activated DCs and is required for their recruitment to LVs (Weber et al., 2013). To enter these lymphatics DCs deform and expand preexisting portals localized at their surface (Pflicke and Sixt, 2009). At this stage, the expression of Podoplanin at the surface of endothelial cells helps recruiting mature DCs into LVs (Acton et al., 2012). In lymph nodes, CCL21 is produced by fibroblastic reticular cells (Link et al., 2007) at the subcapsular sinus and locally scavenged by its non-signaling receptor CCRL1 (Ulvmar et al., 2014). This allows gradient formation and penetration of DCs to the lymph node T cell zone. The ability of tissue-resident immature DCs to explore their environment searching for harmful agents was shown to be associated to active motility in both the gut and the skin (Farache et al., 2013; Ng et al., 2008). Although this occurs independently of CCR7, it has been shown to involve the chemokine receptor CX3CR1 and its ligand Fractalkine or CX3CL1 in the gut, which is produced by epithelial cells (Niess et al., 2005; Kim et al., 2011). In addition, chemokine receptors such as CCR5 were shown to be down-regulated at the surface of DCs upon activation by inflammatory stimuli (Le et al., 2001).

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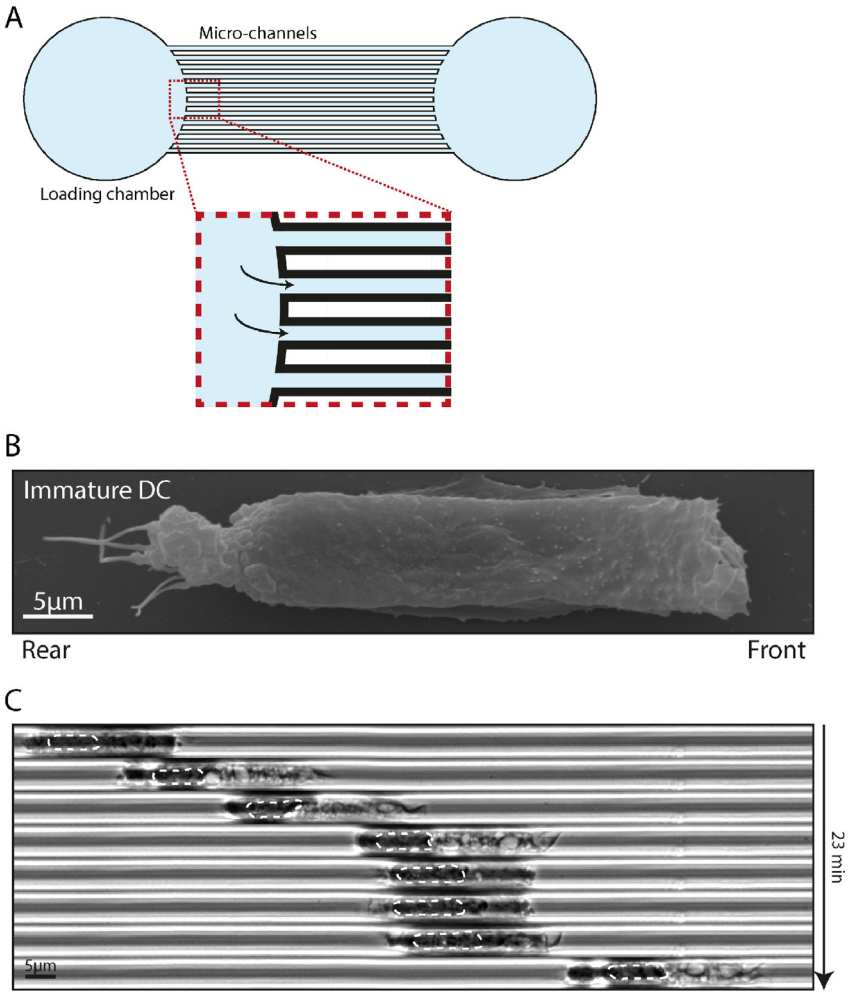


**Fig. 1.** DCs migration in micro-fabricated channels A. Scheme of a micro-fabricated device used to evaluate cell speed. DCs are loaded in the "loading chamber" and then spontaneously migrate in the micro-channels (inset). B. Scanning electron microscopy of an immature DC in a micro-channel. C. Time lapse of an immature DC migrating in a micro-channel. Fast and slow motility phases can be observed.

However, whether the corresponding ligands form gradients in vivo and thereby influence environment patrolling by immature DCs remains to be established. Furthermore, the molecular mechanisms by which DCs sense and respond to chemokine gradients by modifying their migration capacity are far from being fully understood. This results at least in part by the tremendous complexity of tissues as well as by the limited resolution of in vivo imaging techniques.

In addition to rely on the external biochemical cues described above, DC migration is strongly dependent on physical properties of their environment. In particular, their motility is highly sensitive to external geometry as illustrated by their capacity to move in 3- but not 2-dimensional environments in the absence of integrins or to migrate significantly faster when confined (Lammermann et al., 2008; Heuzé et al., 2013). This stresses the need to develop experimental systems that are compatible with high-resolution microscopy but can also be used to isolate specific biochemical or physical properties of tissues in order to define their impact on the migratory capacity of DCs. Here, we review the use of confined micro-fabricated channels to study, in a controlled environment, different aspects of DC migration and dissect the underlying molecular mechanisms.

## 2. Generation of micro-fabricated channels to study spontaneous DC migration

In general, when migrating in tissues, cells are immersed in a complex landscape composed of extracellular matrix and other cells. This configuration can vary depending on the organ, but retains a common property: cells migrate in constrained environments. To mimic this property of tissues we use micro-channels, tiny tunnels in which cells migrate under confinement. To make these channels, PDMS, a polymer permeable to gas that allows sample oxygenation, is poured into micro-fabricated molds. These are used to generate large sets of chips with identical geometries. Starting from a mold, preparation of micro-channels is fast, simple and inexpensive (Vargas et al., 2014). Channels exhibit a square or rectangular section and their size and shape can be defined based on which geometrical property of the cell environment one wishes to isolate (see Section 3). Three channel walls made of PDMS are stuck on a glass coverslip, making these disposable devices compatible with high-resolution imaging (Fig. 1A). They can be coated with extracellular matrix protein such as fibronectin or collagen. Cells are loaded into a central chamber communicated with multiple micro-channels that can be analyzed simultaneously by microscopy (Fig. 1A). This allows the generation of large datasets from each experiment from which key migration parameters such as cell speed and directionality are automatically quantified. This is achieved either by using state-of-the-art software to track the nucleus of Hoechst-labeled cells, or by generating kymographs from processed phase contrast images (Vargas et al., 2014). Importantly, each migration experiment can be done with few thousands of cells, making it compatible with mouse or human primary samples (Fernandez et al., 2011).

As mentioned above, tissue scanning by immature DCs can be associated to active cell migration. Because immature DCs spontaneously

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