Imaging the membrane tension in lymphocyte migration

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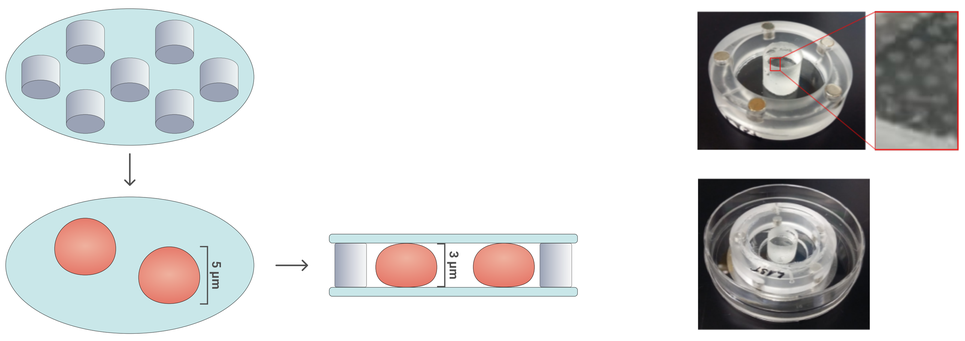
1. Introduction

Leukocyte motility is an essential process for mounting an efficient immune response. A vast amount of research is dedicated to the transmigration of leukocytes from the blood and lymph to the tissue and secondary lymphoid organs and vice versa. But not that much focus is paid to **lymphocyte migration within the lymph node** (LN). Especially, the recognition of surroundings and the signaling essential for continuous scanning of the surroundings for potential antigens.

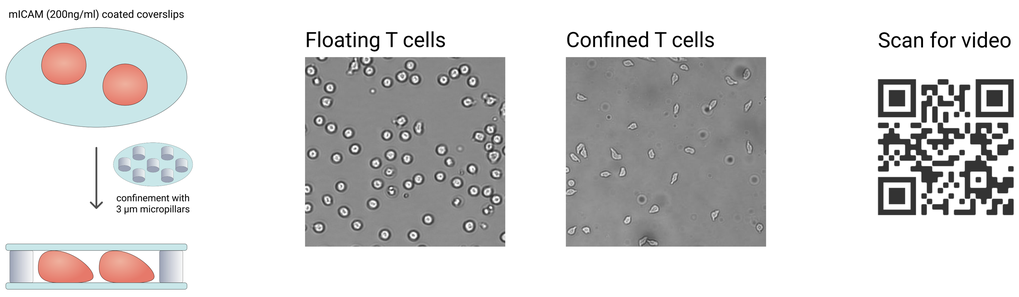
Our research implements a reductionistic model of cell confinement to mimic the situation in the packed environment of the LN. We use PDMS micropillars to confine cells to 3 µm from their original size of 5 µm.



Schematic explanation of the confining system (on the left). The confining system consists of the micropillars (enlarged right in red) on top of the confining PDMS cylinder placed in the middle of the device with magnets (top image). The entire confining device in a petri dish, micropillars facing down. The device is fixed using magnets engaged to a metal ring below the dish (bottom image).

2. Lymphocytes polarize and persistently migrate under confinement

Primary mouse naïve T lymphocytes have an average size of 5 µm. After confinement to 3 µm, T cells spontaneusly polarize and persistently migrate without the involvement of chemokine signalling.



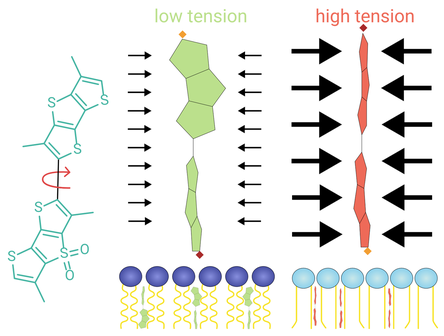
Schematic representation of the confinement method (left). Naïve T cells were seeded on mICAM coated coverslips and imaged (floating) or confined under 3um micropillars and imaged (confined). For the video BF images were taken every 20sec for 15min with Leica DMi8 with 20x objective.

3. Membrane tension and cell migration

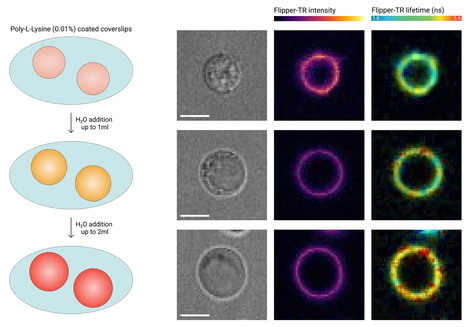
The actin cytoskeleton is one of the driving forces of leukocyte locomotion, and its reorganization generates mechanical forces under the plasma membrane, an overlooked player in cell mechanics. Therefore we focus on the role of **membrane tension** in lymphocyte migration under confinement.

We implement the newly developed fluorescent probe **Flipper-TR**, which inserts itself in the membrane and changes its fluorescent lifetime dependent on the tension (Colom *et al.*, 2018).

As a control, T cells were exposed to a hypotonic environment (H_2O) to see if membrane tension was increased.



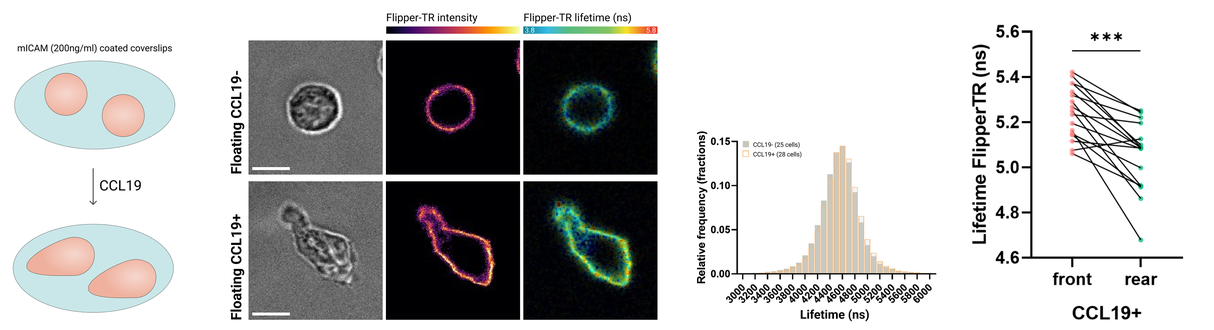
Flipper-TR planarizes in presence of higher membrane tension and increses its flurescent lifetime. Adapted from Colom *et al.*, 2018.



Increase in cell membrane tension in hypotonic solution. Flipper-TR stained T cells were loaded on coverslips coated with Poly-L-Lysine (control cells top) and treated with increasing amounts of water (1ml middle, 2ml bottom).

4. T cells establish a membrane tension gradient when polarized

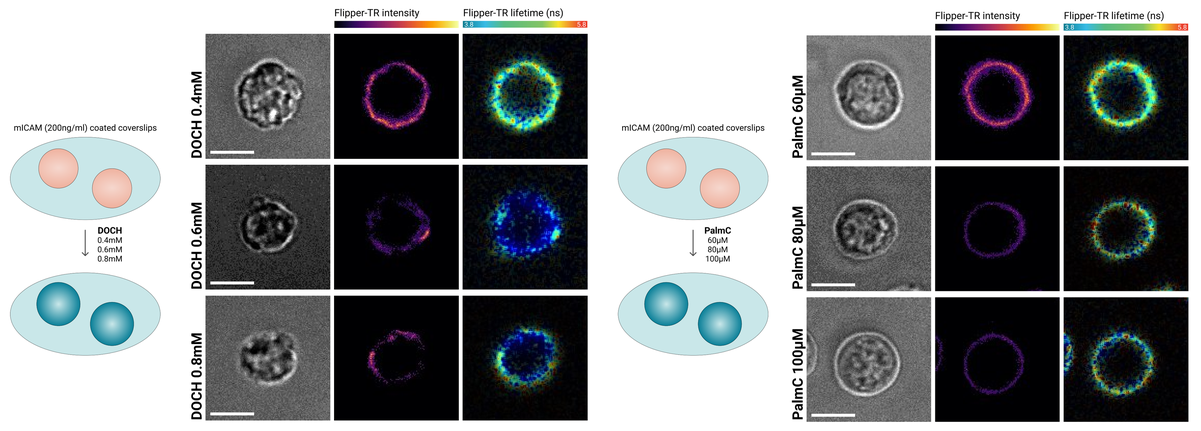
T lymphocytes treated with CCR7 ligand CCL19 establish a polarized phenotype and generate a front-rear membrane tension gradient as previously described in other cell types.



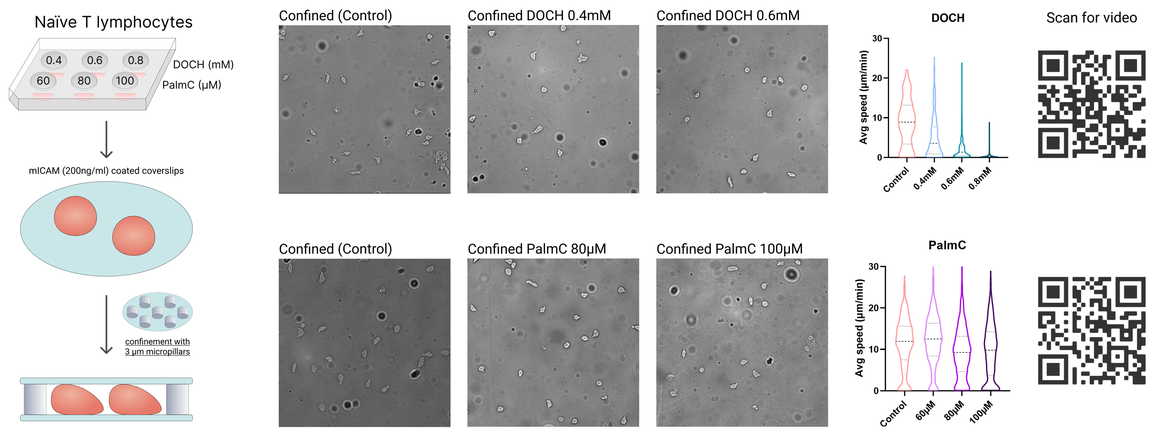
Schematic explenation of the experiment on the left. T cells were treated with CCL19 and loaded with Flipper-TR. The representative cell image shows BF image (left image, white bar indicates 5µm), than the intensity of Flipper-TR is shown (middle image) and the last is the FLIM image (right image). The increase in membrane tension at the leading edge of the polarized cell is visible. Histogram shows the lifetimes of lymphocytes without (25 cells) and with (28 cells) CCL19. The last graph demonstrates the decrease in membrane tension at the cell rear.

5. Membrane tension is reduced in the presence of deoxycholate and palmitoylcarnitin

Deoxycholate (DOCH) and Palmitoylcarnitine (PalmC) substantially decrease the membrane tension in naïve floating T lymphocytes.



Cells were treated with increasing amounts of deoxycholate (DOCH) or palmitoylcarnitine (PalmC), loaded with Flipper-TR and seeded in mICAM coated coverslips. BF image (left image, white bar indicates 5µm), than the intensity of Flipper-TR is shown (middle image) and the last is the FLIM image (right image).



Cells were treated with increasing amounts of deoxycholate (DOCH) or palmitoylcarnitin (PalmC), loaded with Flipper-TR and confined. For the video BF images were taken every 20sec for 15min with Leica DMi8 with 20x objective. Graphs show the average speed of tracked cells, which were untreated or treated with DOCH/PalmC.

6. Conclusion and future plans

We show that primary naïve T lymphocytes can **spontaneously polarize and migrate** under confinement. To study the contribution of membrane tension to this phenomenon, we are able to implement the membrane dye Flipper-TR, which can change its lifetime in correspondence to the change in membrane tension.

Going forward, we want to focus on membrane tension propagation in cells migrating under confinement to see if they establish a similar front-rear membrane tension gradient as seen in cells polarized by CCL19. And how is the confinement-induced migration influenced by membrane tension lowering agents such as deoxycholate of palmitoylcarnitine or other inhibitors showing an effect on this specific type of cell migration.

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