MARIE SKLODOWSKA-CURIE ACTIONS

Active response of Red Blood Cells to mechanical stress in splenic filtration

Context

Blood consists in a highly concentrated suspension composed mainly of **red blood cells** (RBCs) and few other blood cells. The efficient and sustainable circulation of RBCs is an outstanding physical tour de force. During their 120-days lifespan, they continuously circulate through our intricate microvascular network composed of slits, capillaries, bifurcations, etc. During such cycles they undergo **very strong deformations**: for example, the RBC passes through blood vessels smaller than the RBC size or through submicron-sized splenic slits. The RBC cannot go through such constrictions if not highly deformable, and highly robust as well. A mechanosensitive ion channel was recently discovered whose activation is triggered by a mechanical stress applied on the RBC membrane. A second ion channel ion is then activated in cascade, leading to water release out of the cell, as a way to control the RBC volume. A new hypothesis thus arised that these ion channels could play an active role in RBC volume changes, in order to rapidly adjust the cell deformability under a mechanical constraint.

Project

The purpose of the thesis project is to understand quantitatively the physical mechanisms of large deformation and the molecular mechanisms of volume regulation **in the case of the passage through the splenic slits**. Recent studies suggest that the spleen senses RBC deformability and spheroidicity thus defining the size and shape of RBCs allowed to remain in the microcirculation. A current hypothesis is that **RBCs have to pass a 'physical fitness test' via the splenic slits** (Fig. 1A) to be allowed to remain in the blood flow. Yet, no known physical mechanisms rationalize this hypothesis as experiments are strongly lacking.

From a physics point of view, the process of splenic filtration raises basic physical questions: what is the link between RBC mechanical parameters and passage/sequestration in splenic slits? Is the passage based on mechanical criterions only, or are indeed additional phenomena needed?

From a clinical point of view, there is a strong need for understanding the clearance process by the spleen. It normally occurs to eliminate older RBCs and renew the RBC pool in the blood stream. However, in various blood diseases, the RBC deformability is altered leading to massive sequestration in the spleen and subsequent severe hemolytic anemia. We thus expect that physical understanding of RBC passage through slits and how the process is affected in RBC genetic disorders will have a major impact in haematology.

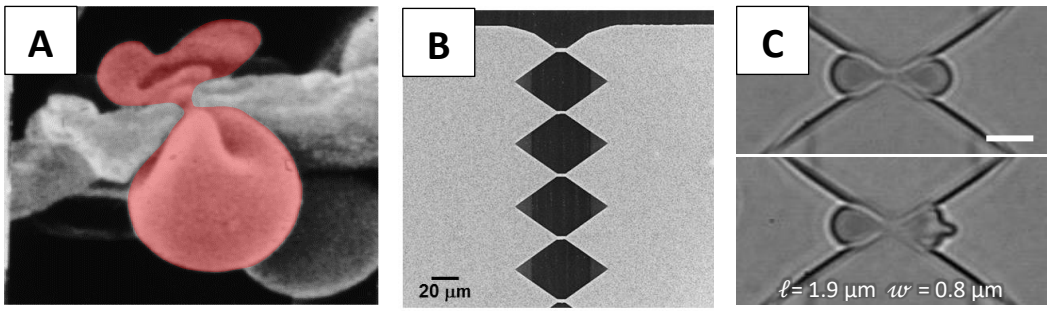

Figure 1. A) An RBC (highlighted in red) squeezing through a splenic slit (≈0.5 x 2 x 5 µm^3). B) SEM image of the silicon master of a typical microfluidic device with a series of slits. C) Optical images of RBCs squeezing through 0.8 x 1.9 x 5 µm^3 biomimetic slits. The bottom one displays a tip while exiting the slit. Scale bar: 5 µm.

The project is based on the recent **technological breakthrough** we made in 2017, by fabricating a **microfluidic device with slits of physiological splenic dimensions** (Fig. 1B). This is the first device that reproduces the dimensions of splenic slits (≈0.5 x 2 x 5 µm^3). The observation of human RBCs passage through these biomimetic slits revealed new modes of deformation due to high confinement (Fig. 1C, bottom). Here, we will study the RBC

behavior as they are submitted to **controlled mechanical stress** (the slit dimensions and the flow pushing the RBCs) and investigate whether their volume actively changes in response to the stress. To do so, we will target the two ion channels which are thought to act together, the mechanosensitive Piezo1 and the Ca^{2+}-sensitive Gardos (Fig. 2A). A recent work by the group of Kaestner studied their interplay using 3-µm wide constrictions, they observed a response even at such small RBC deformation. We thus expect a stronger response by using much thinner slits. The channels' activity and interplay will be modulated via various combinations of known inhibitors and activators (Fig. 2B). Healthy RBCs as wells as RBCs from patients affected with channel disorders with will be assayed in the biomimetic slits, untreated and treated with the biochemical blocking/activating agents.

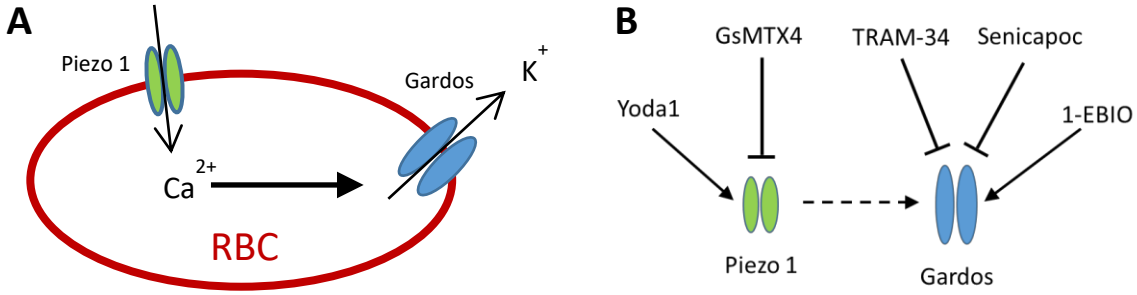

Figure 2. A) Schematics of Piezo 1 and Gardos interplay that controls ion fluxes: mechanical stress, e.g. RBC stretching, activates Piezo1 which become permeable to cations, including calcium. The Ca^{2+} influx activates Gardos leading to K^+ and Cl^- exit concomitantly with water. It results in a decrease in RBC volume, thus in an increase in area-to-volume ratio, and presumably an increase in healthy RBC deformability. B) Activators (arrows) and inhibitors of the two channels that will be used in the study.

Our quantitative results will be combined with 3D computations (from our international collaborator in Notre Dame University, USA) that take into account the RBC dynamics and the channels' activity to derive the physical mechanisms responsible for RBC active response to mechanical stress applied. Our findings will highlight which physical parameters can be used as a new read out to follow disease evolution or treatment effect, and potentially lead to novel therapeutic targets.

Thesis supervision:

Emmanuèle HELFER (helfer@cinam.univ-mrs.fr), Physics and Engineering of Living Systems, Interdisciplinary Nanoscience Centre in Marseille
Catherine BADENS (catherine.badens@univ-amu.fr), Laboratoire de Génétique Moléculaire, Hôpital d'enfants de La Timone, Marseille

Candidate expected profile

The applicant must have a master degree in experimental physics or biology, with a strong interest towards biophysics as she/he will have to interact with physicists, biologists, biophysicists and physicians. A background in optical microscopy and/or basic knowledge in biology, though not required, will be welcome. Skills in image analysis and programming (Image J, Matlab, Python) will be acquired during the PhD period. Fluency in English is strongly valuable.

Application deadline : April 9, 2018

More details on the projet and selection criteria from the COFUND programme are given in the proposal link:
https://doc2amu.univ-amu.fr/en/active-response-of-red-blood-cells-to-mechanical-stress-in-splenic-filtration