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Biophysical analysis of in-flow deformed lymphocytes by static light scattering

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Abstract: The microfluidic based characterization (static light scattering) of morphometric and optical cell properties over time related to an in-flow applied compressive force over time can reveal useful insides in cell-mechanical processes.

1. Introduction

As label-free biomarker for cell phenotyping, the analysis of single cell biophysical properties has emerged as an interesting complementary approach to the well-known classical molecular biology [1]. In particular, cell deformability turned out to provide useful information about the state of a cell [2-3]. Separation and classification of cells is technically challenging, so new strategies are needed [4]. The combination of microfluidic based approaches like deformability cytometry, microfluidic constrictions, real deformability cytometry and hydrodynamic stretching techniques are promising tools to study cell deformation [5-7]. But, as cells are highly heterogeneous, the magnitude of force and the time of application to deform them, strongly influence the final phenotype outcome [8]. In fact, the possibility to read a wide range of biophysical and mechanical single cell properties by properly tuning the applied force, becomes crucial. Therefore, we utilize a simple and non-destructive static light scattering approach to label-free analyze individual lymphocyte properties before and after in-flow applied deformation forces. Such an approach reveals multiple biophysical properties of cells, which can be related to cell mechanical processes.

2. Materials and Methods

2.1. Viscoelastic medium and cell sample

To align and study the deformation of single cells under different flow conditions in microfluidics, we use a highly biocompatible viscoelastic polyethylene oxide (PEO, 4MDa, Sigma Aldrich). Depending on the rheological fluid properties it gets possible to align or deform cells according to highly tunable applied compressive fluid forces. In this work we investigate peripheral blood monocular lymphocyte cells. More detailed information about the fluid force calculations [9] and the lymphocyte sample preparation [10] have been recently published by our working group. However, lymphocytes are diluted in 500 µl of PEO 0.9wt% medium to reach a final cell concentration of circa 50 cells per µl and processed at room temperature directly after sample preparation.

2.2. Deformation chip

The cell deformation setup includes a pressure pump (P-pump, Dolomite Microfluidics), a round shaped flexible fused silica capillary tubing, a fluid connector and an *ad-hoc* designed cell deformation chip [9]. Briefly, the pressure pump pushes the sample volume (PEO 0.9wt%) through the capillary in the chip inlet, the middle section of the chip with a cross-section of 25x100x40000 (HxWxL) deform cells, while at the end of the chip a reservoir collects the deformed cells. More detailed information is shown elsewhere [9].

2.3. Scattering setup and chip

We used a light scattering approach, which is able to obtain precise single cell scattering information in a continuous angular range from 2° up to 30°, with an angular resolution of 0.1°. Such an approach opens the possibility to distinguish morphological cell properties within the sub-micrometric range. However, cells are investigated before and after in-flow deformation with a microfluidic chip described in Fig. 1. Briefly, cells are perfectly aligned and pass a tightly collimated laser beam (λ=632.8 nm) at the indicated measurement position. The scattered light is collected and mapped on a camera sensor and the hereby obtained scattering signatures are processed and matched with a look-up table of previously calculated theoretical scattering profiles. In such a way, biophysical cell properties such as the cytoplasm diameter (d_1), refractive indices (RI_N and RI_C) and diameter ratio (N/C-ratio) are obtained. More detailed information is shown elsewhere [11].

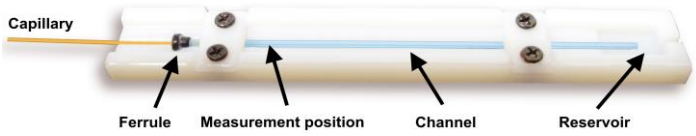

Fig. 1. Microfluidic chip for scattering measurements. Cells are precisely aligned in the capillary before entering the duct channel through a soft ferrule. Such a ferrule allows the capillary (OD=375µm, ID=75µm) to enter into the duct of 500x500x50000µm, while also sealing its entrance.

3. Results and Discussions

Cells were analyzed before (PRE) and after induced deformation (POST) using the light scattering setup and the microfluidic chip of Fig. 1 to obtain biophysical cell properties. Briefly, cells are collected after 10 min of measurement from the deformation chip reservoir, diluted to a final PEO 0.2wt% concentration and analyzed with the microfluidic chip for the scattering experiment (POST). Results are summarized in Fig. 2.

	d_1	*SEM*	N/C-ratio	*SEM*	RI_N	*SEM*	RI_C	*SEM*	Number
PRE	6.78	*0.32*	0.963	*0.013*	1.361	*0.003*	1.401	*0.004*	88
POST	6.86	*0.54*	0.950	*0.024*	1.359	*0.001*	1.395	*0.006*	77

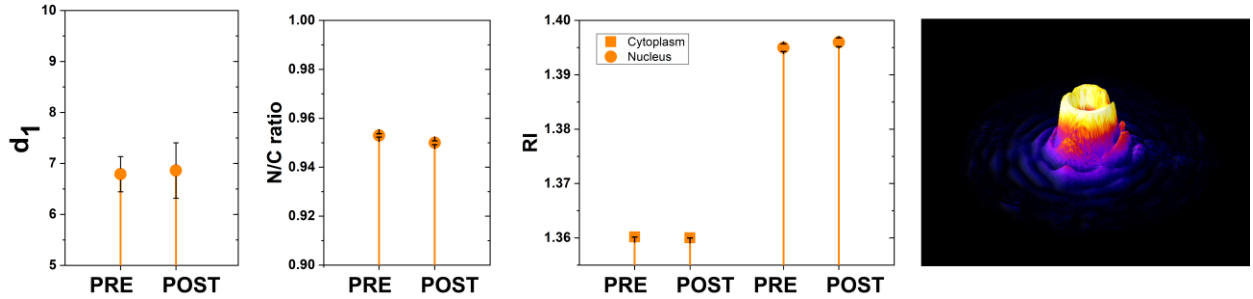

Fig. 2. Biophysical lymphocyte properties before (PRE) and after (POST) in-flow deformation. The right panel illustrate an optical scattering pattern before in-flow deformation.

The deformation chip applies a maximum compressive force of circa 10µN on passing lymphocytes, due to the channel height and cell size as well as rheological fluid properties. The application of the compressive force for 4 seconds leads to (middle section) a cell deformation [9] (CD ~0.04) which can be recognized in the deformation chip by in-flow measurement after the middle section. A complete recover of the deformation occurs after 10 minutes (measured off-chip). Note, CD values are calculated out of in-flow records, taking under consideration only morphological parameters. However, after deformation small changes of N/C-ratio as well RI values can be noticed by the scattering results, which can indicate structural changes of a cell. The increase of RI_N values reveals possible nuclear changes due to the applied forces. Further investigations of chromatin changes will help to better interpret such nuclear changes. The ability of lymphocytes to recover high entities of fluid forces could be due to their morphological structure, having a relatively big nucleus compared to the overall dimension.

4. References

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