# IN-FLOW CELL DEFORMATION TO STUDY MECHANOBIOLOGY AT SINGLE CELL LEVEL.

**Maria Isabella Maremonti** – Advisor: Prof. Filippo Causa and Prof. Paolo A. Netti

Curriculum: Ingegneria dei Materiali e delle Strutture

As label-free biomarker for cell phenotyping, single cell mechanical properties gained an increased importance over the last years. Particularly, cell deformability provides information about cell state, as healthy or pathological. The hereby imposed stresses can alter cell migration, differentiation or growth, caused by cytoskeletal and nuclear alterations. In the last years, analysis of intrinsic cell properties emerges as an interesting complementary approach to the well-known classical molecular biology analysis. In general, cells show a combined process of mechanosensing and mechanotransduction, defined as the cell ability to generate a cascade of biochemical signals up to the nucleus level and to physically deform under the action of the external forces, respectively. Since cells are highly heterogeneous, the magnitude of force as well as the way of application, strongly influence the final mechanotype outcome. In fact, depending on whether forces apply local deformation, stretching or compression, cells respond mechanically in distinct ways, since the internal structures are stressed differently. For instance, suspended cells show how the nucleus plays a dominant role in cellular deformation responses, being stiffer than the surrounding cytoplasmic region. Particularly, it has been demonstrated that for small deformation (~pN) and long times of stress applications (~min), the major contribution in suspended cell response is caused by the actin cortex that, underlying the plasma membrane, shows a passive elastic behavior. On the contrary, at large deformation and relatively low time scales (from ms to s), actin cortex components do not play a relevant role, since the microtubule network and the nucleus contribution become dominant. Moreover, the relative nucleus size, nuclear structural components and chromatin condensation degree are dominant factors in possible molecular changes or in the activation of possible trafficking phenomena from cytoplasm to nucleus and *vice-versa*. In recent years, it has been demonstrated how adherent cells in quasi-suspended condition, are able to express nuclear levels of yes-associated protein (YAP) regardless of the formation of a mature cytoskeleton structure. A direct conversion of the applied force to a nuclear YAP import has been addressed as a novel mechanosensing mechanism. In fact, a nuclear flattening leads to an increased nuclear entry of transcriptional factors, due to an enhanced permeability may be caused by nuclear pore stretching or protein re-assembly at the nuclear envelope level. However, a precise description of how different force entities could induce such kind of responses, is still missing.

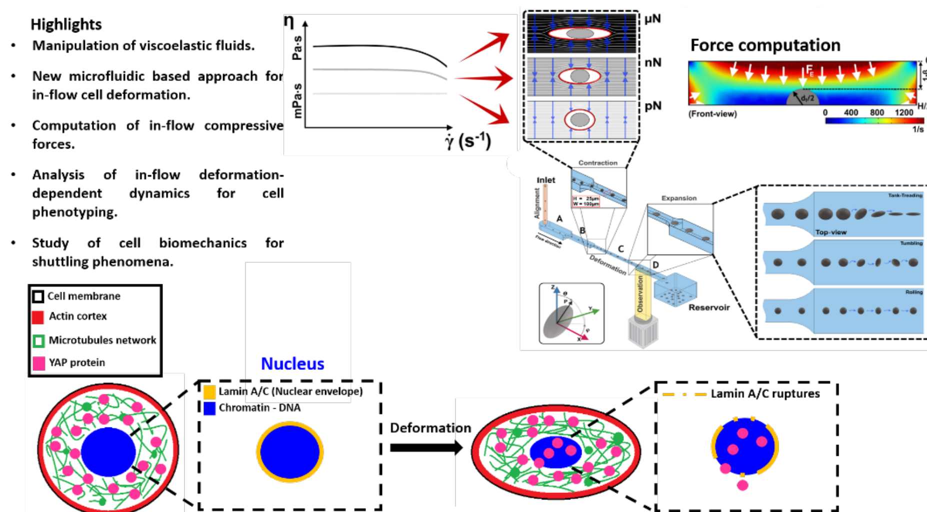
Nowadays, different techniques are employed to induce cell deformation, reducing the costs and yielding improved repeatability for classifying cells. Microfluidic approaches allow a totally new perspective in the study of cell mechanics, compared to traditional methods such as atomic force microscopy (AFM) or micropipette aspiration. For instance, microfluidics can solve the problem of cell throughput rates (100-10000 cells/sec) as well as enable the possibility to deform cells in hydrodynamic conditions, involving the whole cell structure. However, currently available approaches still need a proper calibration and variation of the hydrodynamic force, which strongly influence the final cell phenotype outcome.

An interesting possibility to obtain a good calibration and wide application range of deformative forces according to the chosen flowing fluid and the used channel geometry is given by viscoelastic fluids. When cells move in such a kind of viscoelastic solutions, they are influenced by an elastic force that guides the migration, and then the centreline alignment, thanks to an unbalance of both first and second normal stresses. Moreover, in rectangular channel sections, the initial orientations of cells could result to strongly influence their final trajectories, clearly distinguishing different shape-dependent equilibrium positions in-flow. Experimental and simulative results in a simple shear flow revealed that, the initial orientations of spheroidal objects strongly influence their dynamic behaviours and trajectories. For instance, when the revolution axis of the spheroidal object is in the shear plane, the object can rotate as a *tumbling* motion. Differently, when the long axis of the object oscillates around a mean orientation in the shear plane and the membrane rotates about the spheroidal shape, the object takes a *tank-treading* motion. Another in-flow dynamics is found when the object revolution axis is perpendicular to the shear plane, taking a *rolling* motion.

Governing factors for such cell motion regimes are i) the imposed velocity field, ii) the chosen fluid rheology, iii) the initial position of the object and iv) its own shape, defined as aspect ratio and, eventually, as cell deformation. As result,

when objects of different shapes move in a microfluidic chip at the same velocity condition and start from the same initial position, the only significant parameter for the variation of the in-flow dynamics is the shape *per se*. These findings could be used as new microfluidic tool for the measurement of single-cell mechanical properties.
In this work, we propose a microfluidic chip coupled with a high-speed camera system to allow a direct and rapid measurement of cell morphology and deformability. During the first year, we aimed to manipulate viscoelastic fluids in order to conceive a new deformation approach based on variable in-flow forces, able to induce different levels of deformation on suspended single cells. Such forces have been estimated by computations and simulations based on the established fluid-flow condition. The resulting microfluidic chip would offer the unique opportunity to directly measure and detect dynamics and trajectories of such deformed cells, representing an interesting research tool for a label-free single-cell phenotyping.
During the second year, we collected interesting data about deformation-induced cell trajectories, directly analysing cell *rolling*, *tumbling* and *tank-treading*. We observed that different deformation degrees correspond to different in-flow motions, allowing us to phenotype cells depending on the induced deformation and relative in-flow trajectory. However, for suspended cells, phenomena like mechanosensing and mechanotransduction are still unclear. For this reason, we further focused on the investigation of the effects of cell deformation up to the nucleus level, interrogating the inner cell compartments, generally involved in the mechanical response. The idea was to analyse cells before and after the deformation, in order to understand if there is a compartment that covers a major role in the outcome and if a direct conversion of the applied force into molecular responses would occur.

Then, our target for the third year was to investigate if there are variations of the actin cortex and microtubule networks as well as of the nucleo-cytoplasmic barrier, made up by Lamin A/C, after deformation. In particular, we investigated if possible alterations as well as different degrees of compaction of such structures would be associated to observations of enhanced trafficking of molecules inside the nucleus. Final results show that shuttling molecules (such as YAP, Hoechst and Cgas), from the cytoplasm to the nucleus, can be actually observed after the applied deformation. Moreover, such process, can be also tightly controlled thanks to the variability of the imposed in-flow viscoelastic forces.




*References:*

*D. Di Carlo, "A mechanical biomarker of cell state in medicine",Journal of Laboratory Automation, 17(1):32–42, 2012. PMID: 22357606. DOI: 10.1177/2211068211431630.*

*A. Lomakin et al., "Forcing Entry into the Nucleus", Developmental Cell, 43(5):547 – 548, 2017. DOI: 10.1016/j.devcel.2017.11.015.*

*X. Lu et al., "Particle manipulations in non-Newtonian microfluidics: A review", Journal of Colloid and Interface Science, Volume 500, 15 August 2017, Pages 182-201. DOI: https://doi.org/10.1016/j.jcis.2017.04.019.*

*G. D'Avino et al., "Numerical simulations on the dynamics of a spheroid in a viscoelastic liquid in a wide-slit microchannel", J. Non-Newtonian Fluid Mech., 2019, 263, 33-41.*

*W. Jung et al., "Nonlinear Elastic and Inelastic Properties of Cells", Journal of Biomechanical Engineering, Vol. 142 / 100806-1, 2020. DOI: 10.1115/1.4046863.*

*C. Rianna et al., "Direct evidence that tumor cells soften when navigating confined spaces", Mol. Biol. Cell, 2020, mbc-E19. DOI: https://doi.org/10.1091/mbc.E19-10-0588.*

**Maria Isabella Maremonti, PhD student XXXIV cycle, May 2021**