The effect of intermediate filament, microtubule and microfilament polymerization on the mechanical properties of cells

Place of work/: Atomic Force Microscopy and Related Techniques Lab & Cell Structure and Dynamics Lab, FCUL, Lisbon

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WORKPLAN

The effects of mechanical interactions are determinant in a number of cell aspects, like mechanotransduction, morphogenesis, disease progression, metastasis, drug cell interactions, etc. However, establishing the link between said properties tends to be highly complex and despite some success, the links are still poorly understood. With a clear strategy for measuring cell viscoelasticity, we plan to pharmacologically inhibit intermediate filament, microtubule and microfilament polymerization in glioma cells from the nervous tissue. This can be achieved by using chemical inhibitors such as withaferin, nocodazole or latrunculin, respectively or by genetically removing specific intermediate filaments or their organizers (ex: sacsin or plectin). The respective cell culture and modification will be carried out at F. Herrera' biochemistry laboratory under the supervision of F. Herrera's team. The alterations of the cytoskeleton microtubules and actin filaments will be characterized by confocal fluorescence imaging. This will permit the estimation of the density of each type of fibre, arrangement and shape, cell nucleus size, presence/absence of cytoskeleton-binding proteins, and, through combination with membrane markers, the link between the cytoskeleton and the membrane. The mechanical properties of the cells will then be measured with atomic force microscopy. The main goal is to establish correlations between cytoskeleton morphology and the viscoelastic properties of the cell by using fluorescence microscopy and both conventional and nonconventional AFM strategies to measure the differences in the mechanical properties as a consequence of aforementioned cell alterations.

This work is in line and integrated with the FCT project: Viscoelastic Cells - new experimental approaches based on atomic force microscopy PTDC/FIS-MAC/2741/2021