

Single-cell pharmacology: atomic force microscopy and spectroscopy for multiparametric imaging of drug-induced alterations in vitro

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Since its first construction by Gerd Binnig, Calvin F. Quate, and Christoph Gerber in 1986 [1], it took only a few years to disclose the tremendous potential of atomic force microscopy (AFM) in the research of biological objects [2]. It is mainly because AFM does not need sample modification and labelling. Freedom of selection of conditions, including performing measurements in culture media, control of pH, and temperature combined with large x,y, and z piezo-scanner ranges all have led to the rapid development of live cell imaging. Finally, novel AFM imaging modes, based on a fast acquisition of force-distance curves, such as Quantitative Imaging (QI), PeakForce Tapping, or PinPoint have enabled 4D (3D plus time) imaging of changes in biological samples.

Here, the results of the development of so-called in vitro pharmacology on a single cell will be presented [3-5]. Briefly, the methodology allows tracking the single-cell morphology and its nanomechanical properties over time. Then, a drug is injected into the culture medium and the cell response to the drug is further observed. It allows monitoring of morphological features and the cytoskeleton remodelling, including its effect on alterations in Young's modulus distribution. Depending on the area of interest single frame is collected in the range of minutes to several seconds allowing observation of drug-induced changes in the morpho-mechanics of cells.

The main focus will be devoted to primary murine liver sinusoidal endothelial cells (LSEC) in vitro. LSEC have transcellular pores, called fenestrations, that are indicators of the healthy phenotype of the liver. These nanostructures – 50-350 nm in diameter – participate in the transport of lipoproteins and solutes (e.g. hormones) between the vascular system and the liver parenchyma. AFM remains the exclusive tool allowing monitoring of drug response in living LSEC for up to 6 hours. Fenestration number, diameter, lifespan, migration range, and deformability can be quantified [3]. Moreover, Young's modulus distribution over the whole cell can be calculated [5]. We show that fenestration lifespan varies from minutes to hours. During this time fenestration changes their diameters and migrates within the cell as far as several micrometers. We employed so-called loading force-dependent tomography to assess the deformability of fenestrations. We test the

established methodology in several pharmacological strategies aiming to restore fenestrations in LSEC originating from wild-type animals and genetic knockouts.

We conclude that novel AFM provides multiple parameters for quantifying drug responses in LSECs and their fenestrations in real time. Introducing in vitro pharmacology on LSEC fenestrations will enhance understanding of the mechanisms underlying fenestration formation and function.

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Reference:

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