IMPACT OF THE EXTRACELLULAR ENVIRONMENT AND ANTI-CANCER TREATMENT ON SPATIO-TEMPORAL DYNAMICS OF THE PLASMA MEMBRANE

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The extracellular matrix (ECM) is more than a mechanical backbone that facilitates the anchorage of cells. Via the continuous communication of a cell with its environment through plasma membrane located signaling processes, the ECM can bear an influence on important cellular processes, including proliferation, migration, and survival [1]. Thus, unsurprisingly, cellular communication with the environment also is tightly involved in tumour development, metastasis and response to anti-cancer treatment [2]. As plasma membrane located signaling processes, in turn, directly depend on the spatiotemporal dynamics properties of the plasma membrane [3], we are interested in the characterization of these dynamics in relation to cellular adhesion in a 3D environment. However, despite the fact that the importance of the ECM is well accepted, high resolution studies on membrane dynamics have so far been performed on cells cultured on flat 2D substrate only. Clearly, while 3D based cell models are mimicking a physiological relevant environment and create a link between monolayer cultures and in vivo experiments, they present a much higher complexity in application for high resolution microscopy. We therefore optimized a 3D cell culture systems based on collagen type I and gelatin suited for long term cell culture for the application of single molecule microscopy. Fluorescent lipophilic tracers were used as membrane markers and imaged using inclined light sheet illumination at frame rates up to 100 Hz. Selection and sub-diffractive localization of single molecules was accomplished by custom-written software designed to accommodate high background fluorescence data typical for 3D cell arrangements, which is based on median filtering, correlation and successive deflation. The use of a cylindrical lens array allowed for 3D tracking and determination of 2D and 3D diffusion coefficients.

Our first results indicate marked differences in the diffusional properties of fluorescent lipid tracers in dependence of the culture conditions. Cells in 3D exhibit a higher membrane fluidity which, in contrast to cells growing in monolayers, is homogeneous across the whole cell body. Ionizing radiation, in turn, seems to affect membrane fluidity differentially in 2D/3D conditions. In conclusion, our optimized 3D culture system and single molecule analysis allows for high resolution investigations of various adhesion proteins and drug/receptor interactions, and their role in response to anti-cancer treatment in a near native environment.