Biophysical studies of intracellular and cellular motility

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Summary

Motile behavior, encountered in living systems at length scales from the single-cellular level, such as bacteria, to complex multicellular systems, like plants and animals, is crucial for various essential functions of these organisms, and a fundamental aspect of life, as it enables interaction with the surrounding environment, adaptation to altering conditions, and survival. At the cellular level, single-cell or collective motion in complex multicellular organisms is essential for vital biological processes, including embryogenesis, tissue formation, wound healing, and immune responses. To maintain the functionality and homeostasis at the cellular level, the cell largely relies on intracellular motion, among other processes, which entails the transport of molecules and organelles within the cellular interior in response to intra- and extra- cellular cues. Understanding the principles that govern motile behavior in living organisms is essential not only for advancing the knowledge in biological sciences by deciphering the underlying mechanisms, but also for fostering progress in biotechnology and medical applications, for instance for the development of organs-on-chip, drug delivery systems, or treatments for diseases like cancer, where cell migration plays a key role in metastasis.

Utilizing tools like fluorescence microscopy, single-particle tracking, and micro-/nano- fabrication, in combination with theoretical frameworks and computational approaches, biophysical studies contribute significantly to revealing and comprehending the dynamic nature of (intra-) cellular motility. Fluorescent markers, specifically tagging molecules or organelles, in combination with stage incubators which maintain the temperature and CO_2 levels required for living cells, and technological advancements in optical microscopes such as automated time-lapse acquisition and super-resolution microscopy, have enabled the observation of (intra-) cellular dynamics in real time and at exceptional level of detail. Defined microenvironments, designed to mimic the complex conditions that cells experience *in vivo*, allow researchers to dissect intricate interactions between cells and their surroundings in a controlled setting. Such environments can be created in a variety of ways, to allow the observation of specific processes. For instance, synthetic or natural hydrogels or nanotopographical features are used to mimic the extracellular matrix (ECM) and allow to gain insights in the cells' adhesion, morphology and migration in response to varying physical cues

of these environments. Application of mechanical forces to cells is employed to understand cellular responses to mechanical stimuli. Microfluidics allow manipulation of fluid flow and facilitate gradients within the microenvironment, thereby providing a platform to study how cells navigate and respond to biochemical signals. Overall , via bridging the gap between physics and biology, biophysics elucidates intricate mechanisms that drive life at the (intra-) cellular level.

In this thesis, two types of topographically defined microenvironments were used to characterize either intracellular or cellular motion using high-resolution fluorescence microscopy. For the first part, comprising Chapters 2-4, chemical surface patterning (microscale plasma-initiated patterning (μPIP)) was used for the neurites of neuron-like cells to be guided along lines. As the intracellular transport can be affected by the organization of the cytoskeleton, which is in turn influenced by the cell morphology, this approach enabled the characterization of intracellular organelle motility in two conditions, namely when the neurite morphology was one-dimensional, versus when the neurites were allowed to adopt any random morphology on the two-dimensional surface.

After establishing the experimental setup by confirming the differentiation efficiency of PC12 cells, the functionality of the micropatterning technique, and successful neurite alignment in Chapter 2, we set out to monitor and quantitatively characterize lysosomal motion inside the two neurite morphologies using time-resolved analysis of the (local) mean-squared displacement ((l)MSD). In Chapter 3, it was observed that neurite alignment resulted in faster diffusive and super-diffusive lysosomal motion. Subsequently, we mimicked a pathological cellular phenotype, by introducing a perturbation in the cellular environment via sucrose accumulation and induced lysosome swelling. It was then evident that the swelling-induced mobility change affected each of the (sub-/super-) diffusive motion modes differently and depended on the alignment configuration of the neurites, with larger effect when the motion occurred inside randomly oriented neurites. As a subsequent step, we sought to explore the interplay between organelle transport and the same two neurite geometries with a disease-associated homeostasis disruption. A mutation linked with neurodegenerative diseases was selected that, according to research, is associated with disrupted axonal trafficking. To this end, in Chapter 4 we focused on the G_4C_2 hexanucleotide repeat expansion (HRE) and transfected PC12 cells such as to produce two non-functional dipeptide-repeat (DPR) proteins namely $(GR)_{20}$ or $(GA)_{39}$. Our analysis confirmed that lysosomal motion inside neurites was affected by the HRE presence, and this was more eminent when the neurites were aligned.

Moving on from intracellular to cellular motion, in the last part of the thesis, we used a microenvironment comprising topographical features in the form of micropillars to quantitatively compare two types of single-cell motility, namely amoeboid and mesenchymal. The size of each pillar was comparable to the respective average cell diameter and the free space among the pillars

was accordingly tuned to create three different crowding regimes, namely with available space that was smaller, approximately equal to, or larger than the cell size. The results, presented in Chapter 5, confirmed that the cell trajectories were persistently-random in all crowding regimes, and exhibited an anti-correlation between speed and persistence time, which was larger as the available space increased. Moreover, it was found that increased crowding enhanced the amoeboid motility but impeded the mesenchymal movement. Lastly, in this chapter, computational simulations were used, which reproduced the experimental results, and demonstrated how computational modeling results can resemble live-cell experimental data.

Overall, the work presented in this thesis provided novel insights into how organelle motion is affected in response to altered neurite morphology, and in combination with disrupted intracellular homeostasis, as well as a direct comparison for the first time of amoeboid and mesenchymal motility in defined microenvironments with varying crowding regimes. The approaches used in this work are versatile and can not only be adapted for other cell types, but also have the potential either to be expanded to other surface patterning geometries or microenvironments, or to be combined together or with other techniques to create more complex defined microenvironments for (intra-) cellular transport studies.