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Summary

Cellular forces - adhering, shaping, sensing and dividing

A cell takes many shapes and sizes in the wide variety of functions it performs. Cellular function is often directly linked to an environment that is actively deformed. In many other cases, cellular function relies on the cell’s ability to deform and exert forces on its environment. Endothelial cells, for instance, are deformed when blood is pumped through the vasculature and its flow exerts an outward pressure onto these cells that line the inside of blood vessels. As muscle cells contract, they need to exert a physical force from the inside to the outside of the cell to perform their function. While muscle cells work in a multi-cellular sarcomeric unit, the ability to exert forces for individual cells is also vital. Neutrophils, for instance, need to adhere to the endothelium to exit the blood and migrate to an inflammation site. After this physical attachment as a primary immune response, they need to actively deform their membrane to engulf bacteria. Similarly, many other cell types rely on such deformations and forces for their function. Cell mechanics describes how cellular function relates to physical deformations and forces. And as such, forces, deformations and stiffnesses are ubiquitous in the functioning of cells.

Cell mechanics can roughly be divided into two types of processes. A cell can undergo force or deformation (termed outside-in, probed by active techniques) and have a biological response to that action. On the other hand, cells themselves often exert forces on their extracellular environment (often denoted by extracellular matrix, ECM) and as such push or pull on their environment (termed inside-out, probed by passive techniques). These mechanical processes take place on a stiffness that varies over five orders of magnitude throughout a (human) body.
Both inside-out and outside-in coupling between cell mechanics and biology is thus important for a multitude of biological functions. Furthermore, biological behavior changes depending on stiffness. In this thesis, I focus on understanding how cells deform their environment in relation to adhesion, cell shape, biological-mechanical adaptation through the protein p130Cas and cell division.

Chapter 2 demonstrates a new approach to probing cell mechanics. While measuring how a cell deforms its environment, high-resolution optical microscopy is crucial to understanding fundamental processes in cell mechanics. For any high-resolution optical microscopy, the use of a high-NA objective with a short working distance is essential. In particular, to enable super-resolution microscopy, single molecules need to be detected with as many photons as possible. Inverted micropillar arrays flanked by 50 µm high spacers placed micropillars and cells within the short working distance. With inverted micropillars I showed the ability to perform both live- and fixed cell measurements that quantify extracellular force exertion with simultaneous imaging of the actin cytoskeleton and focal adhesions (FAs). Further, I demonstrated the ability to perform super-resolution imaging by quantification of the nanostructure of force-bearing FAs. I showed that the stress accumulation was approximately one order of magnitude higher with the resolved sub-diffraction limited structure as compared to previous measurements.

In further experiments, I investigated specific biological processes. Chapter 3 describes live-cell experiments of fibroblasts that express LifeAct-mCherry, a fluorophore that attaches to the actin cytoskeleton. Through direct correlation, I observed that local force exertion directs along the orientation of the actin cytoskeleton, while I also observed circular curvature along the actin cortex. Active Solid Theory was developed to describe a homogeneous contractile solid and I further expanded the concept of a local mechanical equilibrium along the cell cortex. I included the effect of cytoskeletal orientation attached to the circular cortex and found that this orientation influenced curvature and force exertion at the extremities of the circular arcs. The theory of a contractile solid expanded by force guidance through an oriented actin cytoskeleton corresponded well to measurements of cortex curvature and force exertion.
In Chapter 4 I report on the mechanosensing properties of the FA scaffolding protein p130Cas. It was previously reported that stretching of p130Cas enhances phosphorylation and influences cell migration and actin dynamics. It was never described, however, whether the mechanosensory function of p130Cas would be apparent in physiologically relevant stiffnesses nor what its effect on force exertion dynamics would be. I performed experiments on PolyAcrylamide (PA) gels and micropillars, both of varying extracellular stiffness, with cells that either expressed endogenous levels of p130Cas (Cas WT), a double mutant that did not localize to FAs (Cas ΔSH3/ΔCCH) or lacked p130Cas (Cas -/-). It became apparent that p130Cas indeed effected FA formation and localized to FAs only when the global extracellular stiffness was larger than 47.2 kPa. I further characterized the effect of stiffness-dependent localization to FAs and found that p130Cas increases extracellular force exertion and decreases the rate of force exertion. Quantification of this mechanical-biological-mechanical coupling provided insights in the function of p130Cas as a mechanosensor, which provided further clues into the physics of cancer.

Chapter 5 shows how the extracellular environment is deformed throughout cell division. In previous work, extracellular force exertion was quantified in interphase, while I observed massive cellular reorganization and a build-up of outward pushing forces through mitosis. I changed the extracellular stiffness and observed that - similar to what was observed for inward pulling forces - outward pushing increased with increasing stiffness. Radial forces relative to the cell center were predominant and amounted to 100-150 nN and 400-500 nN on micropillar arrays with a bending stiffness of 16.7 nN/µm and 70.9 nN/µm, respectively. Pulling forces towards the nucleus were released before the start of prophase and outward pushing successively increased upon chromosome alignment in metaphase. After a characteristic plateau, a peak increase in outward pushing forces coincided with telophase, after which the daughter cells pinched off and proceeded into interphase. Interestingly, outward pushing proved essential for bipolar spindle formation and ultimate division into two cells. Such a force balance likely has a direct relation to kinetochore checkpoints that can be bypassed when force exertion is absent. Furthermore, the force exertion hallmarks preceded phenotypic observations, indicating a vital role for extracellular force exertion throughout multiple stages in cell division.
Overall, in this thesis I have shown that cell mechanics plays an important role in multiple biological processes. The interplay between deformation and force exertion that is guided through extracellular stiffness impacts adhesions, cytoskeletal orientation, p130Cas localization and cell division.