Forces and symmetries in cells and tissues

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Summary

What feature do whales, snails, humans, flowers, and all other creatures have in common? We are all made up of the same building blocks called cells. For instance, trillions of cells ranging in size from tens of microns arrange themselves in a network to form the organs of the human body and the surrounding structure. They go through their own life cycle, communicate and coordinate to provide the characteristics of our individuality. Just the fact that you were able to open this book and hopefully enjoyed reading this thesis is thanks to your network of cells.

What is the mystery behind this network that makes our whole life possible with such small cells? Simply put, the cell network is not so different from our community. We still tend to distinguish humans by their gender, country of origin, or cultural background, even though we are all the same. Cells are classified into different cell types such as stem cells, skin cells, muscle cells, and lung cells based on their molecular structure and composition. Humans are in contact with each other in their daily lives, queuing at the supermarket, getting squeezed in the metro, crossing bridges with others, or just enjoying a soccer match in a large stadium. We interact with our community, but we can also enjoy our privacy and remain separate. Cells basically do the same thing. They are connected to each other to form small structures, monolayers, and tissues such as vesicles and organs. Cells also interact with their surrounding microenvironment and migrate through it individually or in small colonies, as is the case with cancer metastases. We see that environmental perception, communication, coordination, and collectivity play a major role at different scales. This thesis covered such topics in the fields of cell biology, biological and soft matter physics, and combined experimental observations with theoretical descriptions.

The emergence and role of interactions between cells have been discussed over the past decades and remain an urgent topic of daily research across various research disciplines. While physicists describe cell-cell adhesion in terms of tension, forces, pressure, and stress at the cell interface, biologists study the molecular composition and mechanisms within the cells

involved. Some molecules across the membrane connect cells to each other or the cell to the microenvironment, others form a skeletal structure, the cell's cytoskeleton, and yet others combine all internal parts. In **Chapter 1**, we have reviewed this topic and introduced a definition of cell-cell adhesion that provides a common base for understanding the biological and physical principles of cell-cell adhesion. When cells are in contact with each another, they experience pressure and tension of their neighboring cells, just like humans squeezed on a metro train, bumping into each other at every single turn. Cells are able to respond to forces of their microenvironment and neighboring cells. Any difference in tension experienced starts a signaling pathway within the cell. Molecules are recruited and rearranged throughout the cell to support stability at the cell-cell adhesion interface, for example, and they can lead to changes in the cytoskeleton, resulting in changes in the shape of the cells. In turn, to each action of a cell there is always opposed an equal reaction of other cells. From external point of view, cell-cell adhesion is also associated with shape changes, coordination, and collective behavior of cells.

In **Chapter 2**, we have studied the interaction of individual cells with their microenvironment. The internal structure of individual cells differs from that of connected cells. Individual cells adhere to the substrate solely with the support of adhesion molecules. Adhesion molecules, in turn, connect to the cytoskeleton that supports the cell's shape. This cytoskeleton is able of contracting, creating a pull on the substrate and allowing the whole cell to move, similar to the muscle contraction of a snail. Contraction, and thus the traction force applied, is a property of cells. For example, muscle cells generate high forces to pump blood through the veins. Here, we compared two different cell types based on their traction forces and studied how their shape is related to this. To measure traction forces of cells, we used a specific tool called the micropillar array. Micropillar arrays consist of hundreds of tiny elastic beams, much smaller than the cell. When a cell adheres to a bed of micropillars and applies a force, the beams bend. This bending can be measured with microscopes and software that converts the beam's deflection into a force. In our experiment, we used endothelial and fibroblast cells. Endothelial cells form tightly connective two-dimensional tissues that surround blood vessels. Fibroblasts, in turn, are motile, maintain the integrity of tissues, and are thus involved in tissue repair. In our study, we showed that endothelial cells apply half the traction forces of fibroblasts on their surroundings. This result displays the function of each cell type. Since the cell-cell adhesion is pronounced in endothelial cells for tissue formation, the contraction of the cytoskeleton is stronger at the

cell-cell interface than at the cell-substrate adhesion. Fibroblasts require stronger cell-substrate interactions to remodel the cell's environment. We also have shown that the traction force distribution of endothelial cells is broader and correlate with their round morphology, whereas fibroblasts are more elongated and the traction forces are more localized. We speculate that the correlation of the force distribution-pattern with cell morphology could lead to guide the directionality of cell movements and collective behavior, an insight that may be important for the mechanism of cell and tissue migration.

During dynamic processes such as collective cell migration, cells experience rapid changes of tension at their cell-cell interface. Cell-cell adhesions must be formed to resist detachment forces and maintain tissue integrity. The following questions arise here: what is the maximum force that can be applied to break the cell-cell adhesion, and can it be measured? To answer these questions, we have developed the micropillar array technology further to exactly measure cell-cell detachment forces. In **Chapter 3**, we introduced our novel technology, the Cell-Cell Separation Device or CC-SD for short. The CC-SD consists of closely spaced micropillar array blocks to which cells can adhere and connect across the gap. Ideally, two cells in a doublet configuration attach to the micropillars. Bending of micropillars is again used for cell traction force measurements. The applied forces surrounding the cell doublet should balance to a mechanical equilibrium. When you hold hands with another person, you both feel the tension in your arms, standing steady on your feet without moving. This tension between cells can be measured using micropillars. We have shown that the cell-cell adhesion force in such steady conditions is proportional to the total applied traction force acting on the pillars. In order to apply a strain to the cell-cell contact, we connected the blocks to a thin layer underneath. Stretching the layer separates the blocks and increases tension on the cell-cell adhesion. The tension is mainly localized at the cell-cell contact and less below the cell due to the blocks that prevent deformation of the pillar fields. In our example, when the distance to the other person in front of you increases, the tension in your arms increases too. You also feel more tension in your legs and exert more resistance on the ground. The increase in substrate forces applied by cells can be measured by the CC-SD and provides information about the increased tension between cells and even allows the contact to break. Our novel designed CC-SD opens up possibilities for analyzing cell-cell detachment forces and sheds light on the robustness of cell-cell adhesions during dynamic processes in tissue development.

As we discussed in **Chapter 1**, cells in tissues undergo dynamic pro-

cesses involving cell-substrate and cell-cell adhesions. The pressure and tension from neighboring cells lead to changes in the shape of individual cells and even drive collective migrations within a tissue. In a tightly packed metro train, when you are just close to the door, the train stops, and the door suddenly opens, sometimes you have no chance to stay inside the train. You are forced to follow the stream of the other passengers in a directed way. The collective behavior and directionality of cells are the current state of research and can be found in a wide variety of systems such as wound healing, cancer metastasis, and embryonic development. To explain and study the collectivity of cells during migration, scientists have mainly used a so-called nematic symmetry derived from liquid crystals, i.e. rod-like structures, in soft matter physics. Nematics describes the orientation and elongation of cells on single-cell scale and their alignment on global scale. The overall alignment can be summarized by a single value, the nematic order parameter, that represents how collectively oriented cells are. However, if you take a closer look at a tissue, you will notice that cells are not always elongated. Cells can have a rounded shape and line up with their neighbors in a hexagonal pattern where the distances to all six neighbors are nearly equal. Describing these six preferred directions requires a different kind of symmetry, namely hexatic symmetry. The description of cells in monolayers based on nematic and hexatic order was the subject in **Chapter 4**. Combining experimental observations with numerical simulations, we compared the hexatic and nematic order of cell systems across different length scales. Starting with the single cell level, we showed that cells have indeed a dominant hexatic order. They are more hexagonal-roundish rather than elongated. When we considered the nearest neighbors of the cells, we saw that the cell system switched from hexatic to nematic symmetry as we considered more and more neighboring cells. The results showed that our novel approach identified the coexistence of hexatic and nematic symmetry at different length scales. Knowing the correct symmetry of the system opens the possibilities to study the hierarchical structure of cells in tissues, and to find out how cells coordinate and achieve multicellular organization.

Multicellular organization is important for developmental processes and occurs in cancer metastases. We have already shown in **Chapter 2** that different cell types have different morphological properties due to their traction forces and cell-cell adhesion. The main type of tissue in our human body, covering all surfaces including organs, is epithelial tissue. Epithelia are known for their strong cell-cell adhesion. Under certain circumstances, epithelial cells can lose their cell-cell adhesion, develop stronger adhesions to the substrate, gain mobility, and even become individual. This process

is known as epithelial-to-mesenchymal transition, in which cells transform from epithelial tissue cells to invasive and active mesenchymal cells involved in wound healing, fibrosis, and cancer progression. During these processes, the entire multicellular organization changes. In **Chapter 4**, we have shown that the symmetry of tissues differs across different length scales and is possibly linked to multicellular organization. How does the symmetry change when cells change their adhesion properties and tissues their cellular density? We addressed these questions in **Chapter 5** by comparing epithelial cells with mesenchymal-like cells in which, unlike epithelial cells, only a specific type of cell-cell adhesion molecule has been removed. Removal of adhesion molecules results in a strong formation of the cytoskeleton to increase the cellular stability by the substrate. To follow up on our example from above, when you are on the packed metro train, you keep the balance through the other passengers. There is no space to fall down. However, when there are fewer passengers, your legs must take over and coordinate your balance. In our experiments, we showed that, indeed, mesenchymal-like cells are larger and more spread due to their highly developed cytoskeleton network. When we looked at the symmetry of individual cells in tissues, we found that independent of the existence of the certain type of cell-cell adhesion molecule, and no matter how much they were squeezed, cells always have a dominant hexatic order. They prefer to be hexagonal-roundish rather than elongated and nematic. When considering, again, more and more neighboring cells, the monolayer, again, has a higher and dominant nematic symmetry. This crossover, i.e. the number of cells considered, from hexatic to nematic does depend on the cell-cell adhesion. Removing cell-cell adhesion molecules causes a dominant nematic organization in multicellular systems on global scale. Cells with strong cell-cell contacts keep their hexatic organization for slightly longer length scales. This is also true for monolayers with higher cell density. These results indicate that the interplay between cell-substrate and cell-cell adhesion controls the length scale of the hexatic symmetry.

However, many questions remain. Is it possible to study optical features of cells, such as tissue symmetry, collective migration, and changes in cell shape, and use this information to identify intercellular mechanisms? Can we study the 'symptoms' of cells to get the correct 'diagnosis' of disordered tissue organization? Can we identify a pattern and even predict collective cell behavior and thus developmental processes?

Questions upon questions. If you have not stopped reading or fallen asleep, I hope I have convinced you that this thesis opens a chapter to

answer these questions. We have shown that cell mechanics and geometric properties such as cell and tissue symmetry provide information about intercellular processes and cell-cell interactions at the molecular scale. Combining the expertise from different research disciplines is essential for gapless research and opens up possibilities to put all result-pieces of the research-puzzle together to complete the big picture.

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