

# Part I Introduction

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## 1 Introduction

The center focus of this thesis is epithelial monolayers. From the materialist perspective, these monolayers are endlessly fascinating. They are shape changing, self healing, continuously deform or jam depending on the requirement. They are the simplest system conceptually to understand the wider world of morphogenesis. This introduction is a primer to all the topics relevant to the thesis. First I will give a brief introduction to the epithelial tissue itself and its key components along side a snippet of its role in disease and development. Then I will give a summary of morphogenesis and how we can think of epithelia as an active material and what are the historical ways of modelling it. Finally, I will conclude with the emerging field of bottom up morphogenesis, where researchers are reconstructing the biological systems from scratch.

### 1.1 Epithelial tissue

Epithelial tissues are cell sheets with strong intercellular bonds that form physical barriers for major organs such as the lungs, skin, and intestine. It protects the organs from external physical, chemical, and microbial onslaughts. Besides protection, the main functions of epithelial cells include secretion, selective absorption, transcellular transport, and detection of sensation (Powell, D.W., 1981). It also plays a key role in developmental stages by supporting growth and driving critical shape changes.

Epithelial cells are polarized, i.e., their apical side, faces the lumen of the organ, differs in shape and composition from the basolateral side. Its polar organization is reflected in the vectoral functions like transporting epithelia such as those of the renal tubule, absorptive epithelia of the intestine, and secretory epithelial cells like hepatocytes, which are typical examples of epithelia that create and maintain concentration gradients between the separated compartments (Simons, K. and Fuller, S.D., 1985). In addition, polarized epithelia guide the developmental process by determining the fate of cells (Kim, E.J.Y., Korotkevich, E. and Hiiragi, T., 2018).

Epithelial cells have different shapes and may be arranged in single or multiple layers. They are usually classified according to two features: the number of cell

layers and the shape of the cells. Simple epithelia are single-cell layers where all the cells contact the underlying basal lamina and have an apical free surface. The shape of the cells can be flat (wider than high), cuboidal (as wide as high), or columnar (higher than wide). However, stratified epithelium contains two or more layers of cells.

Figure: Art work of quilling and then showing different epithelial types then showing apical basal polarity of epithelial and monolayer.

Epithelial function primarily depends on the tissue's structure and microenvironment. In essence, it can be described completely in three parts: first, cell structure; second, cell-substrate connection; and lastly, microenvironment.

In general, cell structure helps cells maintain their shape along with providing mechanical support to perform vital functions like division and migration. This structure is known as the cell cytoskeleton. It includes different components, playing various roles together. Eukaryotic cells are constructed out of filamentous proteins to support the cell and its cytoplasmic constituents. There are three major filaments, which differ in size and protein content. Microtubules are the largest type of filament of the protein tubulin, with a diameter of about 25 nm. Actin filaments are the smallest type, with a diameter of only about 6 nm. Finally, intermediate filaments are medium-sized, with a diameter of about 10 nm. Unlike actin filaments and microtubules, intermediate filaments are constructed from several different subunit proteins. These filaments dynamically alter themselves in reaction to signals from microenvironments and cell networks (Alberts, B., et al., 2013; Fletcher, D.A., and Mullins, R.D., 2010s). Mechanically, actin filaments are stiffer than microtubules in extension, but they rupture at lower extension. It is also reported that the intermediate filaments exhibit an intermediate extensional stiffness at lower extensions, but that the intermediate filament can sustain much larger extensions than the other two types of filaments while exhibiting a nonlinear stiffening response (Janmey et al., 1991; Mofrad, M.R., 2009).

In the case of epithelial layers, the actin cytoskeleton and intercellular junctions make cell-cell contacts stronger and provide integrity (Braga V. 2016). The perfect example of these tissue-level structures can be seen in wound healing assays: cells surrounding the wound create a ring of actin to close it (Brugués, A., et al., 2014). One must keep in mind that these structures tend to self-organize as well. It can be seen when cells are confined in a specific shape, like in the case of circular islands of epithelial cells that showed radial patterns in the actin organization (Jalal S., et al., 2019).

Multiple membrane molecules can mediate adhesion between cells. One of these are cadherins, critical for epithelial cell cohesion through the formation of adherens junctions. In these junctions, cadherins are coupled to the cell cytoskeleton enabling force transmission between cells. It is finely regulated by both

internal and external mechanisms. Desmosomes are another type of intercellular junction. They are coupled with intermediate filaments, and the resulting supracellular network confers mechanical resilience on cell layers (Hatzfeld, M., Keil, R., & Magin, T. M. 2017; Latorre, E., et al., 2018). Tight junctions (TJ) perform a barrier function and enable the transport of ions across epithelial layers to be actively regulated. This plays an important role in the control of fluid pressure in tissues. Together, adherens junctions, desmosomes, and tight junctions are the major mediators of epithelial cell-cell adhesion, and their regulation enables emergent behaviors in cell sheets that are not observed in single-cell systems (Treat, X., and Sahai, E., 2018; Ladoux, B., and Mège, R.M., 2017).

Figure: all the cytoskeleton and cell junctions and their mechanics

Extracellular matrix (ECM) is the cell environment or substrate to which cells adhere; it is also known as the matrix or cellular microenvironment. ECM serves many functions: it endows a tissue with strength and thereby maintains its shape; it serves as a biologically active scaffolding on which cells can migrate or adhere; it helps to regulate the phenotype of the cells; it serves as an anchor for many substances, including growth factors, proteases, and inhibitors of such; and finally, it provides an aqueous environment for the diffusion of nutrients, ions, hormones, and metabolites between the cell and the capillary network. On top of that, it is subjected to mechanical forces such as blood flow in endothelia, air flow in respiratory epithelia, or hydrostatic pressure in the mammary gland and bladder (Roca-Cusachs, P., Conte, V., & Treat, X. 2017; Humphrey, J. D., et al., 2015; Waters, C. M., Roan, E., & Navajas, D., 2012; Paszek, M. J., & Weaver, V. M., 2004; Bross, S., et al., 2003). It is shown that the ECM regulates cell shape, orientation, movement, and overall function in response to these forces.

Cells and ECM have a symbiotic relationship with each other from signaling cues to various sensors on the cell surface. These cues are primarily sensed using integrins and focal adhesion complexes in cell-substrate adhesion (Kechagia, J.Z., Ivaska, J. and Roca-Cusachs, P., 2019). Thus, triggering complex molecular processes that are required to maintain homeostasis and strongly affect processes in development or tumorigenesis (DuFort, C. C., Paszek, M. J., & Weaver, V. M. 2011; Northey, J. J., Przybyla, L., & Weaver, V. M. 2017).

ECM is a fibrous network of proteins; from a mechanical perspective, the three primary structural constituents of the ECM are typically collagen (the most abundant protein in the body), elastin (the most elastic and chemically stable protein), and proteoglycans (which often sequester significant water as well as growth factors, proteases, etc.). Due to its water content, the deformation of ECM can produce cracks in epithelial layers. ECM acts as a poroelastic material, soaking up water upon stretching (like a sponge) and releasing it under compression, causing a hydraulic fracture effect (Casares, L., et al., 2015). Moreover, collagen remodels itself under the influence of cells aiding in migration or

under stress (Shields, M. A., et al., 2012; Humphrey, J. D., 2003). Like most cytoskeletal proteins, most extracellular components turnover continuously, albeit some very slowly. For example, collagen in the peridontal ligament appears to have a half-life of a few days, whereas that in the vasculature may have a normal half-life of several months. In response to altered loads, disease, or injury, however, the rates of synthesis and degradation of collagen can increase many folds to have a rapid response (Humphrey J. D., 2003).

Epithelial integrity and homeostasis are of central importance to survival, and mechanisms have evolved to ensure these processes are maintained during growth and in response to damage (Macara, I.G., et al., 2014). For example, epithelial cells have one of the fastest turnover rates in the body. The entire gut cell lining turns over in 3–4 days. This turnover implies constant cell division and death. The excessive rate of division and death may give rise to tumors. It is known that 90% of cancers emerge in simple epithelia (Torras, N. et al. 2018; Eisenhoffer, G.T., and Rosenblatt, J., 2013). Not only this, but it could easily disrupt the barrier function, as no gaps should emerge around dying or dividing cells. There is a range of distinct disease states that all have the effect of compromising epithelial and/or endothelial barrier function.

If the fluid compartmentalization goes awry, it has profound implications for epithelial and stromal homeostasis, fluid and/or electrolyte balance, the generation of inflammatory states, and even the tumor microenvironment. Several bacterial toxins are known to target junctions and cause changes in the TJ protein ZO1, resulting in compromised barrier function and pathologies such as diarrhea and colitis (Fasano, A. et al., 1991). Cancer cells tend to spread and disperse metastatically by using their very high rate of cell motility and a diminished sense of cell adhesion. This elimination and/or reduction of TJ barriers in cancer is essential to allowing metastatic cells to break into and out of blood vessels. The leaky barrier also allows a growing epithelial tumor to access luminal fluids as an additional source of nutrients (Mullin, J.M., et al., 2005).

Epithelia undergo changes in shape with deformation and reorganization from the embryonic to the adult stage. Unsurprisingly, any improper function would lead to damage and disorder. Like in the case of epithelial–mesenchymal transition (EMT), a developmental process when epithelial cells gradually transform into mesenchymal-like cells by losing their epithelial functionality. EMT is involved in the pathogenesis of numerous lung diseases ranging from developmental disorders to fibrotic tissue remodeling to lung cancer. Another example is bronchopulmonary dysplasia (BPD), a chronic lung disease that occurs in very premature infants and is characterized by impaired alveologenesis and vascular development. BPD develops because of injury or infection in a very immature lung (Bartis, D. et al., 2014). Give better and more prominent examples then to conclude the chapter with telling what follows

## 1.2 Morphogenesis

During embryonic development, epithelia forms transient structures, such as the neural tube, somites, and the precardiac epithelium, that serve as progenitors for the development of more complex organs. Different epithelia acquire diverse morphological forms and performs their specific functions, such as the thyroid follicles, the kidney tubules, the interconnected bile canaliculi and sinusoids in the liver, and the complex branching structures found in the lung and salivary glands (Gumbiner, B.M., 1992). Owing to its multifaceted regulation and hierarchical organization, epithelial morphogenesis is a complex phenomenon dependent on factors at multi spatial-temporal scales.

It can be fast at cellular level like the change in cell shape driven by apical constrictions, which is required for epithelial remodeling during tube formation of ventral furrow cells in *Drosophila* gastrulation (Miller CJ, Davidson LA. 2013). Or it could be a slower self-organization at embryo level like a cluster of dissociated mouse embryonic stem cells (ESCs) cultured in vitro spontaneously form an optic cup, exhibiting all layers of the neural retina, when cultured in appropriate medium (Eiraku et al., 2011; Bedzhov, I. & Zernicka-Goetz, M. 2014). This structure underwent similar changes to the in vivo tissue like invaginating to form the characteristic morphology of the optical cup without external scaffolding or original growth environment.

Figure: slow vs fast process; programmed vs self-organizing structure; function and form logic!

At the end of the day, all cells come from cells ('*omnis cellula e cellula*') (Virchow R.L.K., 1858), all tissues come from cells that contain essentially the same genetic information. Nonetheless, every tissue exhibits a distinct architecture and function. One could ask many questions from here, as how form-function work in synchrony? How organization is triggered physically? Reductionists would ask whether function follows form, or it is other way around.

As per the twentieth century architecture principle of "Form Follows Function"; where the organization of a structure should be based upon its intended function. In developmental biology there are many examples of indicating that the same principle is at work in self-assembling systems like intestinal organoids, cancerous spheroids, and functional kidney tissues (Gjorevski, N, et al. 2016; Ishiguro, T, et al. 2017; Morizane, R. and Bonventre, J.V., 2017). Each emerging out of a set of cells in appropriate environment changing and adapting itself to perform the biological function. However, exactly the opposite design principle is at work in numerous in vitro experiments with controlled cellular environment; illustrating geometric constraints drives biological function. For instance, in a micropatterned collagen scaffold (with structures of intestine) a human small intestinal epithelium was generated that replicates key features of the in vivo small intestine: a crypt-villus architecture with appropriate cell-lineage compartmentalization and an accessible luminal surface (Wang, Y et al., 2017). Or

cell reprogramming like in case of fibroblasts turning into induced neurons when supported by specific substrate topography (Kulangara et al. 2014).

Figure: images from D’arcy thompson book and connecting it to the new work of forces.

One could easily reach a conclusion that there are more things involved in understanding dialectics of form and function. This was a subject of D’Arcy Wentworth Thompson’s classical text “On Growth and Form” (Thompson, 1917). Thompson tries to explore biological forms during development and across evolution with considering geometric and physical constraints. (*Here talk more about specific examples from the book*) After more than 100 years of its publishing we can answer more specific questions about shape and function using advances in bioengineering and microscopy.

Structure without function is a corpse, function without structure is a ghost (Wainwright, S.A., 1988.)

It is quite apparent after reading till here that there is a specter is haunting this subject—the specter of force. In last couple of decades, there has been a resurgence of interest in physical forces as regulator of development, homeostasis, and disease (Ingber, D. 2005; Barnes, J.M. et al., 2017). This has led researchers across the disciplines to examine the physical mechanisms of tissue formation and its regulation. Unravelling mechanism of Thompson’s mysteriously generated ‘Diagram of the forces’ which governs biological processes (Thompson, 1917; Heer, N.C. and Martin, A.C., 2017).