Mechanics of inflated epithelia

Nimesh Ramesh Chahare

Contents

[Abstract 2](#_Toc124846968)

[Part I: Introduction 3](#_Toc124846969)

[Epithelial Layers 4](#_Toc124846970)

[Introduction 4](#_Toc124846971)

[Key components 5](#_Toc124846972)

[Role in disease and development 8](#_Toc124846973)

[Forms of epithelia 9](#_Toc124846974)

[The mechanical basis of Morphogenesis 10](#_Toc124846975)

[The complexity of the morphogenesis 10](#_Toc124846976)

[On growth and form 11](#_Toc124846977)

[Mechanobiology 13](#_Toc124846978)

[Active tissue mechanics 21](#_Toc124846979)

[Actin cytoskeleton and force generation 21](#_Toc124846980)

[Modeling active tissue dynamics 24](#_Toc124846981)

[Bottom-up morphogenesis 32](#_Toc124846982)

[Learning by building 32](#_Toc124846983)

[How to build epithelial structures? Epithelial domes 33](#_Toc124846984)

[Mechanical instabilities: Buckling 33](#_Toc124846985)

[What is to be done? 33](#_Toc124846986)

## Abstract

Epithelial sheets form specialized 3D structures suited to their physiological roles, such as branched alveoli in the lungs, tubes in the kidney, and villi in the intestine. To generate and maintain these structures, epithelia must undergo complex 3D deformations across length and time scales. How epithelial shape arises from active stresses, viscoelasticity, and luminal pressure remains poorly understood. To address this question, here we developed a microfluidic setup and a digital twin to engineer 3D epithelial tissues with controlled shape and pressure. In the experimental setup, an epithelial monolayer is grown on a porous surface with circular low adhesion zones (footprint). On applying hydrostatic pressure, the monolayer delaminates into a spherical cap (dome) from the circular footprint. This simple shape allows us to calculate epithelial tension using Laplace’s law. Through this approach, we subject MDCK epithelial cells to a range of lumen pressures at different rates and hence probe the relation between strain and tension in different regimes, while computationally tracking actin dynamics and their mechanical effect at the tissue scale. Slow pressure changes relative to the timescales of actin dynamics allow the tissue to accommodate large strain variations. However, under sudden pressure reductions, the tissue develops buckling patterns and folds with different degrees of symmetry-breaking to store excess tissue area. These insights from experiments and the digital twin allow us to pattern epithelial folds by rationally directed buckling. Our study establishes a new approach for engineering epithelial morphogenetic events.

# Part I: Introduction

The central focus of this thesis is the epithelial tissue monolayer. From the perspective of a mechanical engineer, these monolayers are endlessly fascinating. They are shape-changing, self-healing, and continuously deforming or jamming depending on the requirement (xi2018). They are the most basic system in terms of developing a physical understanding the biological morphogenesis. Epithelia are everywhere: covering all our bodies and lining body cavities and organs. Epithelial monolayers adopt a range of shapes from simple spherical blastocysts to highly branched and folded lungs. These shapes are developed and maintained through constant renewal and adaptation. In this work, we have explored the physical principles behind the epithelial shape by combining theory and experimentation with simple epithelial monolayers.

The chapters in this part are to be a primer for all the topics relevant to my PhD. Starting with a brief introduction to the epithelial tissue itself and its key components. Subsequently, the role of mechanics in morphogenesis, and the approaches to modeling these tissues. Finally, I will conclude with the emerging field of “bottom-up” morphogenesis, where researchers are reconstructing biological systems from scratch.

## Epithelial Layers

### Introduction

Figure: The Anatomy Lesson of Dr. Frederik Ruysch, 1670 by Adriaen Backer

A Dutch botanist, Frederick Ruysch, in the early eighteenth century coined the term ‘epithelia’ to describe the tissue he found while dissecting the lips of a cadaver, combining Greek roots ‘*epi*’ for the top and ‘*thele*’ for a nipple[[1]](#footnote-2). A few decades later, Swiss scientist Albrecht von Haller started to use the term epithelium/epithelia to describe the fiber(s) of the body. The idea of fibers was an old renaissance theory that the body is made of fibers, which was believed to be a fundamental building block of living things.[[2]](#footnote-3) The understanding then was that these fibers/tissues arranged in different arrays gave rise to biological structures (maccord2012, zampieri2014). Not far off as epithelial tissues are ubiquitous and cover most of the organs inside out (contains more than 60% of the cells in a vertebrate’s body) (alberts2015). In twenty-first-century science, epithelial tissues are understood as a type of animal tissue in which cells are packed into sheets. The epithelial cell sheets have strong intercellular bonds that form physical barriers to compartmentalize the organs and maintain homeostasis. It plays a key role in developmental stages by supporting growth and driving shape changes in the organs. It protects the organs from external physical, chemical, and microbial onslaughts (marchiando2010).

Epithelial cells are polarized, i.e., their apical side (faces the lumen of the organ), which differs in shape and composition from the basolateral side. Its polar organization is reflected in the vectorial functions like creating and maintaining concentration gradients between the separated compartments (marchiando2010). Typical examples of these are transporting epithelia such as those of the renal tubule, absorptive epithelia of the intestine, and secretory epithelial cells like hepatocytes (alberts2015). In addition, polarized epithelia guide the developmental process by determining the fate of cells leading to symmetry-breaking events in the embryo (kim2018).

### Key components

Epithelial function primarily depends on the tissue’s structure and its microenvironment. It can be described in three parts: first, cell structure; second, microenvironment; and lastly, cell-matrix interactions.

#### Cell structure

In general, cell structure helps cells maintain their shape along with providing mechanical support to perform vital functions like division and migration (alberts2015). This structure is known as the cell cytoskeleton. In particular, the Eukaryotic cell cytoskeleton is mainly constructed out of filamentous proteins that hold 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 (mofrad2009). Unlike actin filaments and microtubules, intermediate filaments are constructed from several different subunit proteins. All three types of filaments dynamically alter themselves in reaction to signals from microenvironments and cell networks.



Mechanically, actin filaments are stiffer than microtubules in extension, but they rupture at lower extension. The intermediate filaments exhibit an intermediate extensional stiffness at lower extensions and can sustain much larger extensions than the other two types of filaments while exhibiting a nonlinear stiffening response (wen2011). Differences in strength and stability come from the properties of the individual subunits. The persistence length can vary from 1µm for intermediate filaments to 1 mm for microtubules (fletcher2010). Stiffest of all, actin filaments, have a persistence length of a few microns.

The assembly and disassembly of these filaments are dictated by the dynamics of their macromolecular components and accessory proteins. Actin filaments in conjunction with myosin motors form the actomyosin cortex which is critical for producing intra/intercellular forces. In the case of epithelial layers, the actomyosin cortex and intercellular junctions make cell-cell contacts stronger and provide integrity to the tissue (braga2016). 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 (brugues2014).We will discuss the actomyosin network more in detail in Chapter 3.

Figure: Quilling of the human body and then showing apical-basal polarity of epithelial and monolayer including all the cytoskeleton and cell junctions and their mechanics

Multiple membrane molecules can mediate adhesion between cells. One of these is cadherins, a critical component 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 a key factor in the mechanical regulation of division and tissue rearrangement during development and homeostasis (godard2019, mertz2013). Desmosomes are another type of intercellular junction. They are coupled with intermediate filaments, and the resulting supracellular network confers mechanical resilience on cell layers (hatzfeld2017, latorre2018). Tight junctions perform a barrier function and enable the transport of ions across epithelial layers to be actively regulated (marchiando2010). This plays an important role in controlling fluid pressure in the tissues (chan2020).

#### Microenvironment

The extracellular matrix (ECM) is the cell environment or substrate to which cells adhere; it is also known as the matrix, mesenchyme, 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 also provides an aqueous environment for the diffusion of nutrients, ions, hormones, and metabolites between the cell and the capillary network (alberts2015). 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 (waters2012, walma2020). It is shown that the ECM regulates cell shape, orientation, movement, and overall function in response to biophysical forces (alberts2015).

ECM is a fibrous network of proteins; its three primary structural constituents are collagen, elastin, and proteoglycans. Collagen is one of the most abundant proteins in the body. Elastin is the most elastic and chemically stable protein, and proteoglycans 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 (casares2015).

Moreover, the collagen network can remodel under the influence of cells aiding in migration or mechanical forces (humphrey2014). Like most cytoskeletal proteins, most extracellular components turnover. Some continuously and some very slowly. For example, collagen in the periodontal 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 (humphrey2013). In response to altered physical stimuli, disease, or injury the rates of synthesis and degradation of collagen can increase many folds to have a rapid response.

#### Cell-matrix interaction

Cells and ECM have a symbiotic relationship with each other through various sensors on the cell surface. The biophysical cues are primarily sensed using integrins and focal adhesion complexes in cell-substrate adhesion (kechagia2019). Through these adhesions, cells can sense the stimuli like matrix stiffness, ligand density, or chemotactic gradients (fortunato2022). Recently, it has been shown that they could the cells could even respond to the viscoelasticity of the matrix (elosegui-artola2022). The cells also secrete ECM on their own or remodel the substrate to promote growth/invasion of cancer or reorganize the cytoskeleton altogether (malandrino2018). Due to the deeper ties of focal adhesions to the nucleus and in turn transcriptional factors, cell-matrix adhesions could affect the tissue behavior fundamentally (venturini2020, lomakin2020). Precise control of cell-cell and cell-substrate interactions enables cell sheets to transform themselves into intricate curved forms (schamberger2022).

Figure: ECM and focal adhesion.

### Role in disease and development

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. For example, epithelial cells have one of the fastest turnover rates in the body. The entire gut cell lining turns over in 5–7 days (barker2014). 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 (torras2018, eisenhoffer2013). Not only this, but it could easily disrupt the barrier function, as no gaps should emerge around dying or dividing cells.

If the fluid compartmentalization goes awry, it has profound implications for epithelial and stromal homeostasis, fluid and/or electrolyte balance, and the generation of inflammatory states. Several bacterial toxins are known to target junctions and cause changes in the tight junction protein ZO1, resulting in compromised barrier function and pathologies such as diarrhea and colitis (fasano1991). The compromised ZO1 barrier 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 (mullin2005).

Moreover, epithelia participate in physiological events such as epithelial–mesenchymal transformation (EMT), a developmental process when epithelial cells gradually transform into mesenchymal-like cells by losing their epithelial functionality. EMT plays a vital role in normal biological functions like repair and differentiation; and abnormal pathological activity like organ fibrosis and promoting carcinoma progression (alberts2015). It endows cells with stem cell properties. Thus, the mesenchymal state enables cell migration to distant organs and allows their subsequent differentiation into multiple cell types during development and the initiation of metastasis (thiery2009).

Epithelia undergo drastic changes in shape with deformation and reorganization from the embryonic to the adult stage. Unsurprisingly, any improper function would lead to damage and disorder. Defects in morphogenesis results in congenital malformations, which are one of the leading causes of infant mortality around the world (clarke2021). Moreover, epithelial dysfunction is a precursor of diseases such as chronic obstructive pulmonary disease, asthma, cystic fibrosis, or pulmonary fibrosis (carlier2021).

### Forms of epithelia

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 a free surface on the apical side. 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: Different forms of epithelia and the range of shapes it takes

The classification in the nineteenth century was based on structure and physiological characteristics. In parallel, embryologists expanded the epithelial nomenclature with germ layer theory (maccord2012). During early embryogenesis, three layers: endoderm, mesoderm, and ectoderm emerge and maintain separate identities. Ectoderm creates the epithelia lining the skin, mouth, and nervous system. Parts such as the digestive tract, respiratory system, and liver are from the endoderm. While mesoderm develops the endothelia which covers much of the circulatory and lymphatic systems.

Our focus is simple epithelial monolayers, which can form and organize themselves in an assortment of 3D shapes: ranging from a simple sphere to complex branched tubules. In this thesis, we will explore epithelial morphogenesis in a mechanical context.

## The mechanical basis of Morphogenesis

### The complexity of the morphogenesis

During embryonic development, epithelia form transient structures, such as the neural tube, somites, and precardiac epithelium, that serve as progenitors for the development of more complex organs. Different epithelia acquire diverse morphological forms and perform their specific functions like branched lungs, looped gut, kidney tubules, thyroid follicles, and sinusoids in the livers. Owing to its multifaceted regulation and hierarchical organization, epithelial morphogenesis is a complex phenomenon dependent on coordinated processes at multi-spatial-temporal scales (trepat2018).

Some processes appear to be happening fast at the local level, like a series of cells changing their shape by undergoing apical constrictions to create a global change like the formation of a ventral furrow in a Drosophila embryo (martin2009). However, at the same time, the chemical signaling events activating this process are slow and at a global level. Similar events are observed in *in vitro* systems. A cluster of dissociated stem cells cultured in an appropriate medium assemble itself into an organoid or a gastruloid (collinet2021). Just like the drosophila embryo, this structure also undergoes local changes creating global folds in response to global signals of culture conditions. It gets even more complex when we consider the details of these processes. For example, genes responding to the morphogen gradients, molecular machinery implicated in apical constriction, or mechanical stresses involved tissue scale deformations (schock2002, lecuit2011).

Figure: Multi spatial and temporal scale events in the morphogenesis

Rudolf Virchow’s third tenet of the cell theory stated ‘*omnis cellula e cellula’* meaning ’all cells come from cells’ (virchow1860)[[3]](#footnote-4). All tissues come from cells that contain essentially the same genetic information. Nonetheless, every tissue exhibits a distinct architecture and function. This raises many questions such as: what makes cells different from each other? Is it all because of the genes? or environmental factors? What drives the shape changes in tissue morphogenesis? Since the advent of cell theory two centuries ago, the field of developmental biology has answered a lot of these questions, but it has also raised new issues and left open questions. The field, until last decade, had been focused on the studies tracking and mapping patterns of cell movements to patterns of a gene or protein expression (gorfinkiel2021). These studies, while being greatly influential and important to understand morphogenetic patterns; fall short of explaining how cells and tissues are shaped physically. (veenvliet2021, odell1981). Because physical understanding was only limited to the kinematic description, which is the deformation of the tissue or motion of the cells. As we know, the cell and tissues are actively driving these shape changes and movements through the generation of mechanical forces (lecuit2011). Thus, to have an integrated grasp of morphogenesis, we must consider the role of forces and mechanics.

### On growth and form

Historically, the form for animate and inanimate objects has been tied to function. As per the twentieth-century architecture principle of “Form Follows Function”; where the organization of a structure should be based on its intended function[[4]](#footnote-5). In developmental biology, there are many examples of this principle at work in self-assembling systems like intestinal organoids, cancer spheroids, and gastruloids (gjorevski2016, ishiguro2017, morizane2017). Each emerges out of a set of cells in an appropriate environment changing and adapting itself in a specific form to perform its biological function (vianello2019). However, exactly the opposite design principle is at work in numerous in vitro experiments with a controlled cellular environment; illustrating geometric constraints drive biological function (xi2018). For instance, seeding stem cells in bio-printed three-dimensional geometry of the gastrointestinal tract produced functional tissues with physiological characteristics of the intestine. The formation of a villus-like structure can be controlled with curvature. (brassard2021).

Figure: Form and function examples paralleling design to not design

Advanced microscopy techniques have enabled us to visualize each of the developmental processes with clarity. We can monitor each cell and its motion throughout the morphogenetic process: starting from a spherical embryo to a complete organism (shah2019). Cells undergo shape changes and large-scale flows to accomplish the task of morphogenesis (Reviewed in labernadie2018, trepat2018). Mechanical forces are driving these shape changes alongside the biochemical processes of gene patterning (lecuit2011). Therefore, the dichotomy of form and function is incomplete if we isolate it from the physical laws of mechanics.

Figure: fish embryo development with velocities

More than a hundred years ago, D’Arcy Wentworth Thompson[[5]](#footnote-6) published the classical text “On Growth and Form” (thompson1979), where he tries to unravel the dialectics of morphogenesis by exploring the geometric and physical constraints on living entities during development and across evolution. Thompson compiled examples of how mathematics and biology are related. Such as his theory of transformations, which shows the differences between the forms of related species can be represented geometrically. A famous example of this is transforming one type of fish picture into another by deforming it[[6]](#footnote-7). According to Thompson’s daughter, he used to entertain children by drawing pictures of dogs on rubber sheets and stretching them to make poodles into dachshunds (wolfram2022). This distortion of the shape is supposed to be representative of significant alterations in various forces or rates of growth throughout the developmental processes of different organisms.

Thompson’s work is highly speculative. However, his broad idea was to identify generalized principles behind the various biological forms and patterns. He tried to illustrate this by comparing growth curves of haddock, trees, or tadpoles; or by finding logarithmic spirals in shells, horns, and leaf arrangements[[7]](#footnote-8). Essentially, this book emphasizes two points: first, all material forms of living things—cells, tissues, and organs—must obey the laws of physics, and second, quantitative measurements are necessary to unravel the physical principles of biology.

Figure: Transformation theory

Thompson’s work has inspired many scientists from Alan Turing to Stephan J. Gould; me and my advisers. Right as I began my Ph.D., the centenary of the book's publication was being celebrated in the fields of developmental biology and biophysics (heer2017, nat2017, natphys2017). Even more so by the field of mechanobiology, an interdisciplinary field that studies the role of biophysical forces in cell and tissue functioning.

### Mechanobiology

Figure: Tissues as a control system with different inputs and outputs; mechanotransduction

Each cell in epithelial tissue can be imagined as a mathematical system which integrates several input types to result in an output behavior. Such input cues may be mechanical, such as lung stretching, or chemical, such as morphogen gradients in developing embryos, while outputs can be cell deformation, migration, differentiation, or proliferation (kumar2017). Some outputs could also feed back into the system as an input, as in the case of cells remodeling the matrix (malandrino2018). The sensing of the environment is mediated by mechanochemical switches at the membrane, cell-cell junctions, or cell-matrix adhesions (roca-cusachs2017). This sensing triggers the biochemical cascade leading to a cellular response. This crosstalk between biochemistry and mechanics is called ‘Mechanotransduction’.

During morphogenesis, mechanotransduction occurs across the scales: from a single cell to complex multicellular tissue. To parse out the role of the different variables, experiments at different scales are needed. It is seen that individual cells can sense their environment and respond by altering their behavior through mechanical or biochemical processes. However, the multicellular system can transmit forces and information at a longer length scale (heer2017, lecuit2011). This enables them to exhibit emergent characteristics such as collective migrations, oscillations, rearrangements, and even turbulent flows (trepat2018).

A perfect example of tissue-environment interaction is durotaxis. The epithelial cells can sense the matrix rigidity and migrate from low to high stiffness. In vitro, cells in a monolayer collectively expand and relocate to stiffer regions (sunyer2016). Similarly, durotaxis is observed in vivo *Xenopus laevis* neural crest migration (shellard2021). Not just the matrix affects the cells; the reverse is also true. The durotactic gradient is generated by the migrating neural crest cells themselves. In another example, Drosophila oogenesis, the disorganized matrix is remodeled by cells into a polarized matrix, which aligns with the actin bundles in the follicular epithelium through coordinated rotation of the cells (haigo2011, cetera2014). These polarized fibers can guide the directed motion of cells.

There is an interplay between an individual cell, its neighbors, and exogenous stimuli. It is challenging to decouple various biophysical aspects of the environment, such as forces, pressures, matrix stiffness, spatial confinement, porosity, or viscoelasticity. Direct force measurements in and out of tissues are even more difficult. To address these challenges, scientists from various disciplines have attempted to recreate experimental systems with precise control over the biochemical and mechanical environments of the cells (xi2018). This has been made possible by continuous technological advancement in fluorescent probes, imaging, microfabrication, and force measurements (roca-cusachs2017). In the following section, I highlight some relevant techniques and experiments for mechanobiology.

#### Synthetic substrates

The simplest technique is to culture cells or tissues on 2D synthetic substrates. Commonly, substrates like plastics (PET, PEGDA), hydrogels (Polyacrylamide, collagen gels), and elastomers (soft PDMS) are used to study mechanics because one can control rigidity, topography, and mechanical forces (xi2018).

Polyacrylamide and soft PDMS gels have allowed researchers to investigate mechanical interactions at cell-substrate adhesion. Just seeding cells on different stiffness hydrogels reveals a drastic effect on the actin cytoskeleton, shape, or on cell lineage specification (yeung2005, engler2006). These substrates because of their known elastic response are also used to measure forces through techniques like traction force microscopy (TFM) (harris1980, gomez-gonzalez2020). TFM studies showed that cells and tissues can exert higher forces on the stiffer substrate because of remodeling of the cytoskeleton (elosegui-artola2016). Higher matrix stiffness has been shown to induce Yes-associated protein (YAP) translocation from the cytoplasm to the nucleus, which could be considered as a sensor for mechanotransduction (elosegui-artola2017). However, increasing ECM ligand density alone can induce YAP nuclear translocation too without changing substrate stiffness (stanton2019).

#### Geometric control

In 2D substrate spatial control on cells or tissues could be imposed by using micropatterning adhesion proteins or microfabricated stencils. At a cellular level, cells respond to the confinement by remodeling the actin cytoskeleton and focal adhesion complexes according to the shape (vignaud2012). Confined tissues undergo rearrangement at a larger scale producing fascinating topological defects or oscillations (tlili2018, balasubramaniam2021, guillamat2022). These experiments also uncover the mechanism of force transmission throughout the tissues and cells during collective cell migration, and epithelial growth regulation in two dimensions (nelson2005, vedula2012, deforet2014).

The experiments with 2D embryonic stem cells show that differentiation depends on the shape and size of the confinement. The tissue patterning of a 3D gastruloid can be reproduced in 2D with a circular monolayer of stem cells (warmflash2014). Also, the same cells confined in a triangle could create high tension at the vertices and lead to Wnt signaling (muncie2020). This signaling promotes differentiation to the mesoderm.

New photopatterning technologies aid in precisely controlling for multiple proteins on the same substrate (guyon2021, prahl2022). This enables us to establish a viable co-culture system for increased complexity mimicking in vivo events.

As most of the epithelia in vivo are three-dimensional, the curvature of epithelia is also a key factor to control. Using microfabrication technologies like 3D printing and photolithography, we can create substrates with topographical cues (schamberger2022). Epithelial monolayers can sense curvature and respond by regulating cell migration, orientation, cell/nucleus size, and shape (marin-llaurado2022, schamberger2022). The epithelial monolayer on hemispheres of elastomers would act as a fluid with increasing curvature (tang2022). However, at a smaller scale with cells attached to a corrugated hydrogel, curvature induces variations in lamins, chromatin condensation, and cell proliferation rate (luciano2021). As mentioned before, bio-printing three-dimensional geometry of tissue architecture could create functional tissues (brassard2021, breau2022).



Figure: Substrate and geometric control over the tissues

#### Mechanical control

Living systems do not just have spatial control, they also have mechanical control through physical forces emerging out of growth, deformation, and remodeling of ECM, and fluid pressure in closed geometries*.* In our body, intestinal epithelia are stretched during peristaltic movements in the gut and lung alveoli deform during breathing. Not just tension, compression is known to guide several morphogenetic events involving tissue bending and folding, such as the formation of the optic cup, gut villi, and cortical convolutions in the brain (okuda2018, shyer2013, tallinen2016). To understand tissue behavior under external perturbation, cells and tissues are probed at the molecular and subcellular scales using, atomic force microscopy, magnetic beads, optical tweezers, and micropipettes (bao2003). At a larger scale, various kinds of stretching devices, tissue rheometers and force plates can be used (xi2018).

These tools allow for probing biological materials with controlled force or displacement, which is also useful in characterizing their rheological properties. These experiments reveal that cells have a complex viscoelastic behavior at different regimes of deformation involving different parts of the cytoskeleton (mofrad2009). Whereas on stretching the tissue, the cells within the tissue can reorganize or stretch depending on the timescale of the stretch (guillot2013). Also, with the help of rheological experiments, the role of signaling pathways in terms of transcription factors like YAP in mechanosensing is elucidated (wagh2021).

Figure: Mechanical control over the tissues

#### Microfluidic chips

In the purview of cell mechanics, the microfluidic system—cells on a chip— has been proven as an outstanding tool for mimicking in vivo conditions and controlling biophysical cues; enabling us to study cell behavior (ingber2018). It is a practical way for applying stretch/shear forces or creating a controlled microenvironment. Organ-level cues, like surface tension at an air-liquid interface in the case of the lungs, as well as both fluid flow through the vasculature and cyclic mechanical stretch of the tissue-tissue interface due to breathing motions, can be recreated (huh2010).

In developmental biology, culturing iPSC-derived motoneurons and brain microvascular endothelial cells together on a similar chip produced the neuromuscular unit with in vivo-like maturation of spinal cord neural tissue (sances2018). This opens new avenues to develop the current view of self-organization and embryo functions with controlled physical conditions (samal2019).

Figure: Microfluidics and cell aggregates

#### Matrix independent

s mentioned earlier, the tissue-matrix interaction is playing a critical role in sensing and transmitting forces rapidly (tambe2011, sunyer2016, serra-picamal2012). However, in early embryonic epithelia where little or no ECM is present, stresses generated by actomyosin contraction of the cells in one tissue are transmitted over long ranges via intercellular adhesions to other tissues. Thus, the system of studying just simple free-standing epithelial monolayer is very appealing in terms of characterizing mechanical response to stretch at different time scales. There are only two techniques available for this. One, Harris and colleagues created a suspended monolayer by culturing a cell monolayer on a collagen matrix on two rods; later matrix was removed using enzymatic digestion (harris2012). Second, epithelial domes, where MDCK cells pump ions to form fluid-filled blisters (lever1979). Recently, the control over the curvature, shape, and size of the domes is enhanced even more by my colleagues, Ernest Latorre and Ariadna Marin-Llaurado (latorre2018, marin-llaurado2022, Details on this system in the next chapter). These experiments showed that elasticity measurements of the monolayer were two orders of magnitude larger than that of individual cellular parts, and the monolayer could sustain more than 200% strain before the rupture of cell-cell junctions. The cell cytoskeleton particularly the actomyosin network and cadherin junctions actively remodel during stretching, also the keratin network reinforces the monolayer integrity at higher strains (latorre2018, duque2023). At sustained stretching, the tissue undergoes significant realignment and rearrangement via division (wyatt2015). The experiments on tissue devoid of the matrix also revealed epithelial actions such as superelasticity and buckling (latorre2018, wyatt2020).

#### 3D tissues

Many of these in vitro experiments with 2D or 2.5D systems have improved our understanding of cell mechanics in morphogenesis. We could measure deformations and forces along with controlling the environmental conditions which are inaccessible in vivo systems. However, to elucidate further the mechanics must be probed at systems closer to in vivo systems.

Cell aggregates have become viable in vitro systems where mechanics could be probed. The engineering techniques for synthetic matrix and mechanical measurement tools could be used for this system too. Cell clusters are shown to respond to the matrix as planar tissues but with increased complexity. They could sense matrix stiffness, confinement, and ECM concentration along with undergoing 3D shape transformations. Our lab has shown that cell aggregates perform durotaxis and are actively wet or de-wet dependent on stiffness (perez-gonzalez2019, pallares2022). The cell aggregates in suspension resemble a viscous droplet. This behavior is exploited to measure rheological properties. Aggregates squeezed between plates, probed with AFM or micropipette inform us about its mechanics (xi2018). Even coalescing two aggregates could allow for measuring the viscoelastic properties (oriola2022).



Cell aggregates can be embedded into a hydrogel. The most common types of hydrogels are PEG, polyacrylamide, collagen, or Matrigel. Naturally extracted hydrogels like Matrigel provide a similar architecture to native ECM. Most of the epithelia are polarized, so when embedded into a hydrogel they tend to form a spherical structure with a hollow lumen. However, under the action of hepatocyte growth factor, the branching morphogenesis is induced (bryant2008).

The cell-driven self-assembly in organoids leads to the formation of tissue with features mimicking an organ. However, the reproducibility of shape and composition is often tricky (nelson2008, hofer2021). 3D hydrogel system has proven greatly useful with epithelial organoids. The control over ligand presentation, crosslinking, and degradability of synthetic hydrogels allows for control of cell fate (gjorevski2016, gjorevski2022).

3D gel-based culture systems are also developed with spatiotemporal control on the mechanical properties that correspond to the in vivo-like functional structures (torras2018). Interestingly, some recent publications show tissue transformation of planar tissue to complex organ-resembling tissue without finer control of the environment. Intestinal epithelium mechanically compartmentalizes itself, and 2D stem cells transform into a 3D neural tube (perez-gonzalez2021, karzbrun2021).

#### In vivo systems

In developing embryos, embryonic and extraembryonic fluids generate forces: frictional and tensional stresses when flowing, or hydrostatic pressures when in confined spaces (vianello2019, chan2020). Measuring forces poses a great challenge in this system. Micropipette has been one of the prominent methods to manipulate these tissues.

Micropipette experiments, where the needle is inserted into the embryo to control pressure, revealed that internal hydrostatic pressure determines the embryonic size and directs the allocation of cell fates (chan2019). As a fluid-filled structure, imagine a balloon, the hydrostatic pressure inside relates to tension in the surfaces. Any changes in luminal volumes are sensed by cells through the increased cortical tension, which in turn induces changes in cell shape and cytoskeletal organization (chan2019, choudhury2022). Micropipette aspiration is an effective tool in measuring the surface tension of individual cells or the whole blastomeres (dumortier2019). These experiments explain the role of the actin cortex in governing the contractility of preimplantation embryos (ozguc2022, firmin2022).

Measuring forces in the interior of the embryos could be solved by inserting a deformable probe into the tissue such as hydrogel, oil, or magnetic droplets (dolega2017, campas2014, serwane2017). The shape changes allow for measurement of local forces as well as osmotic pressures inside (mongera2023).

Besides embryos, explant systems are utilized to understand the organogenesis of the brain, gut, or lungs. Lung explant research has been a fantastic way to understand different aspects of shape formation. Lungs start as an evagination of a foregut tube which later buds and bifurcates to form a branched organ. This occurs under different stimuli of pressure and growth factors. The explant system allows direct control of the chemical and mechanical environment right at a specific stage of development. Work with mouse airway epithelium shows pressure and the matrix stiffness affect the number of branches (palmer2021, varner2015, nelson2017).

There are other tools like optical tweezers, laser ablation, or optogenetic excitations used at different levels for probing the mechanics of development (lecuit2011, gomez-gonzalez2020). Yet, independent control over different discerning factors is incredibly hard and the force measurement remains indirect.

To sum up, epithelia are actively responding to various biophysical forces and constantly undergoing remodeling at different lengths and timescales. There are technologies available to manipulate from single cells to embryos with controlled forces and deformation. Epithelial tissue may be thought of as active material based on how it behaves. In the next chapter, we delve into how these tissues are active and what molecular machinery drives them.

## Active tissue mechanics

### Actin cytoskeleton and force generation

Most of the morphogenetic processes involve cells changing shapes to sculpt a specific form. In the nineteenth century, embryologists, observing the mechanical processes of individual cells in a tissue, thought that there must be an external vital force guiding the morphogenesis (thompson1979). Experiments of Wilhelm His and Wilhelm Roux and later ideas of D’Arcy Thompson made it clear that the physical forces guide the shape change of the cells[[8]](#footnote-9) (clarke2021). Now we know, what was unknowable in the 19th century, the machinery for generating the forces is the actin cytoskeleton. In a cell just beneath the plasma membrane, the actomyosin cortex forms a mesh containing actin filaments along with myosin motors (alberts2015). This mesh is organized into various higher-order arrays capable of dynamic remodeling. We can understand the actomyosin cortex step by step: from its basic organization of single actin filament to higher-order supracellular actomyosin cables.

#### Actin filaments

Actin filaments are helical polymers of actin proteins (G-actin). Asymmetrical actin proteins connect to each other in the same direction giving the filament structurally two different ends and polarity. These two ends are referred are barbed or pointed ends because of their appearance in electron micrographs. They are dynamically assembling and disassembling. Because the distinct ends have different kinetics rates, the actin filament grows in the direction of the barbed end. As there is a pool of monomers in the cell and nucleotide hydrolysis process, a filament with both sides exposed will maintain its length but the subunits would be in constant flux. This event is known as treadmilling. Although, if one end is capped the filament will continue to grow and apply pushing force in the outward direction.

#### Actin networks

Actin filaments can also form branched networks. These are assembled by creating nucleation sites on a filament with proteins that contain actin-binding motifs. Actin nucleation can be catalyzed by two factors: the ARP 2/3 complex or the formins. ARP 2/3 complex creates a pointed end in the middle of a filament leading to the formation of a new branch from that site. These branches turn into a tree-like web that can apply pushing forces enough to push a part of the cell membrane. The formins along with profilin enhance the growth of the filaments. Profilin in this process is used as a staging area for the rapid addition of monomers to the filaments. These structures could be dendritic actin networks that enable membrane protrusion at lamellipodia or spike-like projections of the plasma membrane that allow a cell to explore its environment. The pushing forces generated at a molecular level are around 1 piconewton order.

Figure: Actin filament to bundles

#### Actomyosin bundles

Another way actin filament organizes is by forming tight or loose bundles with help of crosslinking proteins. With fimbrins, multiple actin filaments can arrange themselves in parallel. As it is a small monomer, forms closely packed bundles that exclude myosin for connecting to the filaments. On contrary, α-actinin crosslinks actin filaments with opposite polarity into a loose bundle. This allows myosin to bind and create contractile bundles. Myosin II oligomerizes into a bipolar short filament that can connect multiple actin filaments and move across the filaments creating a pulling effect.

The loose bundle forms the gel-like network for the cell cortex. There are a number of other actin crosslinking proteins that can lead to a different structure. Filamin creates a loose and viscous gel needed for migration. Whereas spectrin creates a strong and flexible weblike network of short actin filaments allowing cells to reversibly deform. The actomyosin bundles in the cortex could generate two orders of magnitude more force than a single filament (clarke2021).

#### Actin structures organized at a larger scale

During epithelial morphogenesis, cells change shape individually by altering the contractility or actin turnover to develop the curvature of the tissue. As seen earlier the epithelial cells have apicobasal polarity. This polarity creates a non-homogeneous distribution of actin cytoskeleton, which affects the cell shape and tissue architecture.



Geometrically, the cells of columnar or wedge-like can only be organized in specific ways in a monolayer (gomez-galvez2021). Columnar cells together will make up a flat tissue. Wedged cells with a narrow top will create convex curvature. Another way is concave curvature with a narrow bottom. By monitoring the actin cytoskeleton, we can discern the specific mechanism of the tissue shape. Apical constriction with concentrated actin cortex on the apical surface is implicated in multiple convexly curved tissues like invagination of the intestinal crypt, drosophila mesoderm, and vertebrate lens placode (perez-gonzalez2021, lecuit2011, houssin2020). Opposite curvature is seen after basal constriction in an optic cup and mid-hind brain fold of zebrafish (sidhaye2017, gutzman2018).

However, similar convex curvature in the tissue can be produced through basal expansion as in the case of the drosophila wing disc. Some parts of the wing disc locally relax the basal side without affecting the apical side causing basal expansion (sui2018). Besides apical and basal surfaces, lateral surfaces can also contract or expand with myosin II activity. This could produce tissue folding in the wing and leg disc of drosophila (sui2018, monier2015); or produce cell-cell rearrangements by changing junction lengths during its germ band extension (yu2016, collinet2015).

Not only the coordinated actin reorganization in cells but also tissue-wide supracellular actin structures can emerge. Junctional actomyosin organizes to form bundles connected across multiple cells to perform vital tasks such as wound healing and morphogenesis (brugues2014, clarke2021). These supracellular networks could exert forces at the embryo scale seen in cases such as drosophila’s dorsal closure and parasegment boundary formation, or zebrafishes epiboly (ducuing2016, calzolari2014). These supracellular networks also change the material properties of the specific regions in the embryo making it easier for them to deform to form folds or invaginations. During Drosophila gastrulation, tissue level actin cortex is altered in direction of the anterior-posterior axis giving it more bending strength in that direction. This in turn aids in the internalization of the mesoderm by supporting folding in a perpendicular direction (yevick2019). Interestingly, in even larger systems such as Hydra, vertebrate smooth muscle, and heart highly organized actin bundles are often found (maroudas-sacks2021, palmer2021, cetera2014, helm2005). They assist in generating mechanical force patterns to create global coordinated tissue movement.

#### Controlling cortical tension

However, the magnitude of contractile or extensile forces applied by cells depends greatly on the tissue and its environment. The signaling of crosslinkers and nucleators of actin bundles is regulated by external biochemical and biomechanical stimuli. As the actin filaments, the actomyosin bundles are also dynamic. They constantly undergo contraction, polymerization, and depolymerization, in the normal state, while producing a homeostatic level of cortical tension. As many moving parts are involved in the actin network, cortical tension of cells can be easily controlled with pharmacological treatments targeting specific molecules (cartagena-rivera2016).

The contractility can be reduced with Latrunculin which binds to the actin monomers leading to the depolymerization of the network. Inhibiting myosin activity with Blebbistatin results in a decrease of cortical tension too. Blebbistatin hinders myosin II ATPase activity. While Calyculin-A increases contractility by enhancing the rate of Myosin II phosphorylation. Cortical tension could also be boosted by sequestering ARP 2/3 monomers with CK666. There are other factors like Rho-GTPases or calcium levels further away in the signaling pathway which could affect the stability of the network (valon2017).

Optogenetic tools give finer and more localized control over contractility. The tools based on the regulation of RhoA can locally control cell protrusion, tissue tension, and traction (valon2017). A recently developed tool controlling Shroom3 gives finer control over apical constriction. It can be used to recreate tissue folding (martinez-ara2022).

### Modeling active tissue dynamics

We are increasingly able to understand the tissue dynamics and molecular pathways responsible for morphogenesis. However, it has become very important to interpret biological experiments with a theoretical model. The model and its hypothesis could inspire us to come up with intriguing predictions or launch new trials to validate them.

There are mathematical models describing physics and biology at multiple scales. There are hyperelastic continuum material models at larger tissue scales, for describing the behavior of the cardiovascular system. There are agent-based models for relatively smaller scales explaining epithelial tissue behavior in terms of cell sorting or reorganization. I will introduce the reader to pertinent modeling approaches in a brief manner.

#### Vertex model

D'Arcy Thompson, in the chapter on “The forms of tissues”, makes a super intuitive argument regarding the role of surface tension or capillarity to organize cells together in a tissue. This was visible to him ubiquitously in nature from two connected cells to the organization of cells in the dragonfly wing. These resemble associations of soap bubbles or foams.

Monolayered epithelial tissue has a polygonal cellular pattern on its surface. This enables one to describe/track cell motion and shape change easily in terms of vertices and edges. Vertex models have been valuable to understand the complex interaction between cellular shape, the forces generated inside epithelial cells, and mechanical constraints externally imposed on the tissue (Reviewed in alt2017). Models could be 2D or 3D depending on the system being modeled, but cells are consistently defined as each one has an apical and basal surface and lateral interfaces between neighbors. More complexities have been added to describe specific systems like intercalations in 3D epithelia using geometric shape as Scutoid.

To determine the motion of the vertex, mechanics must be specified. It is often done using the virtual work function (W). There are two components: internal and external.



$$ \delta W = \delta W\_i + \delta W\_e $$

The changes in internal virtual work, $\delta W\_i$, can result from changes in the cell volumes, in the areas of surfaces, or in the lengths of bonds. By defining the cell pressure, the surface tension, and the line tensions, the differential of the internal virtual work for vertex movements can be written.

$$ \delta W\_i =

\Sigma\_{cell \alpha} -P^\alpha \delta V^\alpha

+ \Sigma\_{surface k}T^k \delta A^k

+ \Sigma\_{edge \lambda} \Lambda^\lambda \delta l^\lambda

- \Sigma\_{vertex \nu}f\_i^\nu \delta x^\nu $$

Similarly, the external virtual work, $\delta W\_e$, can be written according to the external forces that come from external mechanical forces applied to the tissue through the matrix, or fluid pressure acting on apical or basal cell surfaces.

The state of the monolayer is estimated by minimizing the virtual work function. Here, molecular complexities are incorporated in the definition of surface tension and line tensions. Particularly for the epithelial layers, the actin cortex plays a huge role in determining tensions along the edges. In 2D models, vertex model simulations show the role of interfacial tensions in guiding cell orientation, collective migrations, and tissue rearrangement through cell divisions.

Whereas the 3D or 2.5D models captured the physics of different morphogenetic processes from appendage formation on drosophila eggshell to mechanical compartmentalization of intestinal epithelia. Also, these simple models provided unique insights about cell packing and the jamming/unjamming transitions. In some cases, there are phase transitions from solid to fluid states emerging due to localized proliferation and oriented divisions. The epithelial tissue behaves as an active material with viscoelastic properties.

#### Active surfaces and Continuum models

Viscoelastic properties of the tissues are captured in the vertex models. However, in the instances where the focus is on larger-scale deformations or flows, we can model them as a continuous material. Here, I would discuss two tactics for thinking about these models. One is related to the material properties of the tissues. It involves probing the tissue behavior in terms of rheological properties. The second is about shape transformations. How a continuous sheet of cells can fold up during development. It can be thought of as an active surface and using this model the physics of single cell to embryo can be captured at once.

The soft tissues respect the basic postulates of mechanics, and basic concepts such as stress, strain, and entropic elasticity. Hence, much of the continuum models focus on the formulation of reliable constitutive relations and then on the solution of initial-boundary-value problems. Constitutive relations describe the response of a material to applied loads, which depends on the internal constitution of the material.

However, the determination of constitutive relations is tricky for materials such as epithelial monolayers. These tissues are much more complicated materials than those simple metals or passive polymers. However, the complex material behavior can be understood by characterizing mechanical response using standard material testing techniques. Typically, it is done by probing the tissue mechanically in a biologically relevant manner. For example, biaxial or uniaxial stretching of the tissues to recapitulate the in vivo tissue behavior. These experiments reveal the viscoelastic nature of the material.

##### Viscoelasticity

Solids like rubber are considered elastic where the material can reversibly deform under the application of force. On contrary, the fluids are viscous. They flow on the application of force. The viscoelastic material behavior is a combination of solid-like and fluid-like properties. The simple models can represent the rheology through a combination of the elastic parts as springs and viscous parts as dashpots. The elastic response is non-dissipative as compared to the viscous one.

$$ \sigma = E\epsilion $$

$$ \sigma = \eta \frac{d\gamma}{dt} $$

The quasi-static stretching or compressing can reveal the stiffness or Poisson’s ratio. However, dynamic properties are understood through frequency sweep, creep, or stress relaxation experiments. Rheological experimentation has been greatly useful in understanding the mechanical response of different biological materials: from reconstituted cytoskeletal proteins to large multicellular aggregates.

The rheological properties often correlate with their physiological state and are crucial for their specific functions (Park, J.-A. et al. 2015; Vedula, S. R. K. et al. 2015, Vedula, S. R. K. et al. 2014). For example, Heart failure is often due to the loss of contractility of heart muscle cells; as it is observed in remodeling under mechanical stretch (Fung YC, 1990). So, one must test rheological properties along with different microenvironments. The mechanical information includes deformation, rates of deformation or velocity fields, traction forces exerted by cells on the substrate, and intercellular mechanical stress. Coupling these parameters with information on cellular architecture, obtained by imaging, can provide a mechanistic understanding of tissue rheology (roca-cusachs2017). These kinds of experiments reveal complex mechanisms of strain stiffening and viscoelastic behavior at different regimes of deformation involving different parts of the cytoskeleton.

##### Hyperelasticity

However, in certain cases like modeling cardiovascular mechanics or the growth of organs, we can rely on hyperelasticity or composite material framework. The basic kinematics assumes a mapping, $ x = \chi (X, t)$, deformation from reference to deformed configuration. The deformation gradient and Green’s strain tensor are defined.

$$ F = \nabla\_X (\chi(X, t))); E = (F^T F – 1)/2 $$

The elastic and growth can be delineated in the deformation gradient through decomposition.

$$ F = F\_e F\_g $$

Here, in the theoretical framework of finite elasticity, one can assume a strain energy function relates to stress. The stress-strain data extracted from the experiment allows for predicting the form of the strain energy function.

$$ S = \frac{\delta W}{\delta E}

In the case of the bladder, heart tissue, skin, and arteries, hyperelastic form has been useful in capturing the material response. This kind of formulation is also flexible in adding extra physical constraints as anisotropy of the tissue microstructure or its incompressibility. Borrowing from composite materials, transversely isotropic material models have been instrumental in understanding mechanisms of myocardium infarction and various aneurysms. Slight modification to these constitutive relations could capture material response, such as explaining strain stiffening, or inhomogeneity in the material like accounting for collagen content and crosslinking in the tissue.

These models are also used in understanding growth and remodeling by using the theory of kinematical growth. It has pointed out the existence of residual stresses in growing tissue to make compatible elastic and inelastic growth-induced deformations, which in turn remodel the tissue properties modifying the material into a spatially inhomogeneous and anisotropic one. This process is crucial in solid tumor growth mechanobiology, the residual stresses directly influencing tumor aggressiveness, nutrients walkway, necrosis, and angiogenesis.

##### Nematic active mechanics

At the cellular scale, we know that the mechanical properties of the tissue are controlled by the biopolymeric cytoskeleton. Filaments and their cross-linkers (molecular motors) continuously transduce energy (ATP to ADP) to contract or extend the network. This system can be considered a physical gel because of its cross-linked actin filament network. But phenomena like treadmilling, active polymerization-depolymerization of filaments, and mobility of molecular motors like myosin makes the tissue system an active gel. Also, note that the cellular system lacks time reversal symmetry because it is constantly transducing energy.

Moreover, these filaments are polar, as constituents can acquire orientational order. Thus, one can model tissues as active gels; are used to model active systems like flocks for birds and schools of fish using hydrodynamics of active matter. Active matter systems are a subclass of continuum models used to describe the dynamics of packed active particles; based on liquid crystal theories of soft condensed matter. Like liquid crystals cells too have orientation and the ability to move past each other. In this framework, one characterizes the orientation of filaments in the cytoskeleton or elongation of cells in the tissue by nematic order parameter matrix.

$$ Q = 3S(nn – I/3)/2 S = [cos2\theta] $$

$$ \sigma\_active = \Zeta Q $$

This formulation aids in defining active forces generated by the network. The stress is divided into two parts: active and passive. Where passive stress will be arising from the mesoscopic viscoelasticity of the material and the bending, splaying, and twisting of the aligned agents. Active stresses are obtained using a combination of zeta, the strength of activity, and the nematic order matrix. Zeta’s sign determines the nature of the force dipole. If negative, the system contracts; if positive, the system expands along the nematic axis. For example, actomyosin systems are contractile. Active stress is very crucial for the motion of the system. Even in low Reynold’s number systems, the motion could get chaotic. In a dense bacterial system of Bacillus subtilis, jet flows and turbulent flow patterns were observed. Also, independent vortices have been observed in the expanding monolayers. Nematic equations have captured physics very well in 2D confined systems or expanding systems.

##### Active surface of the actin cytoskeleton

For 3D, active surfaces are used. The actomyosin cortex near cell membrane or epithelium in the embryo is like a thin sheet of matter, which drives shape changes at the cellular or tissue level by causing deformation due to the generation of internal forces and torques. These three-dimensional structures resemble a curved active two-dimensional surface. The framework developed for active matter can be used by applying mathematical tools from differential geometry. The curved surface is defined in generalized coordinates $X$. The metric tensor $g$ and curvature tensor $C$ are used to describe the kinematics. And forces and torques are defined as, where $dl$ is the length of the line element with tangential unit vector $v$ normal to the line, and $t$ and $m$ are tension and moment respectively. In this framework, the mirror and rotation symmetries of the surface elements are also considered.

Figure: active surfaces from Salbreux and Julicher 2017

Salbreux and Julicher show that flat active membranes with up-down asymmetry have stability dependent on active tension and active tension-curvature coupling term. This tension-curvature dependency can be seen in the pancreas of mice the morphology of epithelial tumors is determined by the interplay of cytoskeletal changes in transformed cells and the existing tubular geometry (messal2019). Consistent with theory predictions: small pancreatic ducts produced exophytic growth, whereas large ducts deformed endophytically. Another example shows that curls of high curvature form spontaneously at the free edge of suspended epithelial monolayers (fouchard2020). It is pointed out that the curling originates from an enrichment of myosin in the basal domain that generates an active spontaneous curvature. It was shown that the extent of curling is controlled by the interplay between internal stresses in the monolayer.

Overall, the molecular level behind the epithelial morphogenesis, actin cytoskeleton, is well understood. There are modeling approaches to elucidate the functioning at different scales from filaments to whole tissues. The phenomenological experiments provide insights into the constitutive relations of cytoskeletal components and tissues in specific conditions. Vertex and continuum models capture the physics of morphogenesis at the tissue scale. The theoretical and experimental framework is lacking to bridge the gap between molecular dynamics and tissue scale deformations. The vertex models and active surface mechanics could be combined to give finer control over the individual cell surface. In the next chapter, we will discuss bottom-up morphogenesis.

## Bottom-up morphogenesis

### Learning by building

It is very clear that the mechanics and biology of the epithelial tissues are complicated; intertwined by mechano-chemical signaling; and multiscale in their behavior. The lens of active material has been very helpful in providing information on the role of molecular elements in performing the biological function. These studies have also led to examining emergent behaviors which would be impossible in vivo. The mechanistic understanding has been enhanced with newer mathematical tools and advanced microscopy; enabling us to measure forces involved in the tissues.

Engineering in biological systems has pushed our limits in understanding physiological response, morphogenesis, and pathologies. However, engineers are not just attracted to this subject for its application in health and disease, but for its potential to inspire the construction of new materials or engineering systems. In many instances, where new materials have been fabricated inspired by biological matter. Also, an improved understanding of biological systems has provided new methods for creating organically optimized systems.

Biomimetics is a field where nature continuously inspires human innovation: from hydrophobic surfaces to supersonic passenger planes! Here, epithelial tissue has displayed incredible capabilities such as self-assembly, self-healing, and self-replicating. This makes it a very interesting material for engineers as its study provides new ideas in the strength, adaptability, flexibility, and functional aspects of the material.

However, experimentation with systems like organoids or embryos can only provide insights into the autonomously formed structures. This information is useful, but the topological transitions these structures are not fully understood. An interesting proposal is to learn by doing morphogenesis. The critical pedagogist, Paolo Freire, had the philosophy of learning through an active practice called ‘praxis/learn by doing.’ We can extend it to learn by building epithelial structures.

A lot of synthetic biology is based on the idea of learning about biology through recreating it from scratch. Also, it gives an enhanced understanding of which components are indispensable for the desired function. Researchers are working on different scales to recreate life: starting from synthetic proteins to a cell. However, I would focus on the mesoscale (~10-104µm) structures of epithelia.

From physics perspective, at this scale of multicellular forms we will be in a perfect position to recreate emergent properties which play a key role in many systems. This reminds me of the example of cars and traffic (good2018 [[9]](#footnote-10)). We can understand the function and behavior of each of the car components and drivers, but this information would not allow us the study the behavior of the traffic jams. One could even think about the traffic flows as a living and breathing organism. Assuming to solve the problem at the scale of traffic, we could learn much more about the transportation issue by just building the network.

What is even more interesting is that while building a structure we could encounter instabilities, emergent phenomena, or see the system self-organize around an alternative path. A perfect example of this is a desired path page on the internet, where people tend to carve energy-efficient paths around the instructed paths.

In this work, we have tried to create a system to engineer an epithelial structure with a controlled microenvironment. At the same time, it is sensitive to undergo self-organization and mechanical instabilities. In the following sections, I will explain how we can make these structures from the scratch, and relevant mechanical instabilities.

### How to build epithelial structures?

#### Elementary shapes

Before starting to build the epithelial structure, one must ask questions related to form and function. Despite of the diversity in forms and functions of epithelia, there are elementary shapes emerge everywhere through coordination of physical force and biochemical signaling. Shapes such as spherical blastocysts, ellipsoidal embryos, or cylindrical vessels.

To construct any of these structures, the well-established cell lines are used. Researchers choose the cells according to the relevant systems. For example, epithelial cells for recapitulating intestinal crypts, endothelial cells for vasculature, or fibroblasts for cancer stroma. However, the building of the synthetic structure is done through different techniques from controlling geometry to engineering localized folding (many of these are discussed earlier in chapter 2 mechanobiology section).

##### Scaffolding

Scaffolding is the most intuitive way to create these structures from engineering point of view. A scaffold is created using 3D printing or various microfabrication techniques. Then cells are seeded to create the desired shape. In this approach the cells are provided well controlled microenvironment in terms of geometry, stiffness, adhesion proteins, and appropriate cell culture media. The structures created through this method could be used to study tissue behavior in response to the forces and curvature.

Dessalles and colleagues prepared a hydrogel with a cylindrical hole, which they used for culturing the endothelial cells to form a micro-vessel (dessalles2021). The hydrogel with cells inside is housed in a microfluidic device which can control the pressure and the flow in the vessel. They were able to uncover the role of poroelastic properties of hydrogel in regulating dynamics of the vessel.

The scaffolds could even dynamically change the shape. As in the case of cell monolayer on a flexible membrane allows to change curvature of the tissue (blonski2021); or the cell-laden hydrogel through combination of stretching and un-stretching creates distinctive patterns and folds (chan2018). In some fascinating studies, researchers used 4D bioprinting, where 3D printed object transforms with time (arif2022). A flat hydrogel sheet with endothelial cells on photo-crosslinking can be transformed into a tube (zhang2020).

Some have used the contractility of fibroblasts and hepatoma cells to fold 2D structures into 3D (he2018). Here, the origami folding pattern is used to create microplates, and on seeding cells apply forces to construct 3D shape. While others have used the ability of cells to self-organize only dictating the geometry and hope that the cells differentiate and localize themselves into specific regions. This externally imposed geometric constraint improves the efficiency of organoid-like systems. In case of intestinal organoids, even controlling the stiffness of the matrix in certain areas lead to growth and differentiation at softer regions to produce a highly reproducible structure.

##### Biochemical control

Another way of constructing epithelial structures is to control biochemical signaling induce shape transformation. It involves using similar natural processes in the embryo morphogenesis like apical constriction in ventral furrow formation or cell jamming in normal elongation of the zebrafish. A localized apical constriction can be controlled using optogenetic Rho signaling tools (izquierdo2018). Using this technique, the furrow can be induced with spatial temporal control.

#### Epithelial domes

### Mechanical instabilities: Buckling

Spontaneous pattern formation and mechanical instabilities. Splashes of fluids analogous to plants. This has been confirmed through quantitative studies. He was speculative but now it has been shown quantitively. Gut, brain, shapes of plants, and non-Euclidean plates.

### What is to be done?

The principles which govern tissue form and function are very important; on two fronts. First, to understand fundamental physical rules of biology, and second for inspiration of new engineering tools and design principles. We want to use state-of-the-art technologies such as bioprinting, microfluidics, and 3D cell cultures to control morphogenetic driving factors individually; allowing us to test tissues from the material science point of view. This specific probing allows us to comprehend the intricate mechanism of the generation of forces, and shape change at the cellular and tissue levels. Using a microfluidic setup, we subject tissues to unravel emergent phenomena at different spatial and temporal scales.

This is a process of deformation or growth of the tissue under the combination of endogenous and exogenous mechanical forces that include contractility of the epithelium itself and the surrounding matrix as well as hydraulic pressure from the lumen. These stresses are applied to different material components of the tissues, such as cells and the extracellular matrix, that display distinct viscoelastic properties and remodeling time scales. Understanding how the complex interplay between tissue stresses and viscoelastic properties gives rise to specific morphogenetic events in vivo poses outstanding technical and conceptual challenges. These include difficulties to disentangle the relative role of the distinct components involved in a system, the lack of tools for quantitative measurements of stresses and mechanical properties, and the inability to impose controlled stresses over a broad range of amplitudes and rates. As a complementary strategy, bottom-up approaches aim at understanding the role of each component of the system and its morphogenetic potential, with the ultimate goal of building complexity through rational engineering of the building blocks that form functional tissue. These approaches have been successful at engineering elementary morphogenetic processes such as epithelial bending or buckling. However, despite the emerging success of bottom-up approaches, we still lack tools to simultaneously measure and control the shape and stress of 3D epithelia. In addition, we lack computational models that integrate cellular and tissue shape with the subcellular determinants of epithelial mechanics such as the contractility, turnover, and viscoelasticity of the actomyosin cortex.

Here we present a microfluidic-based technique to impose a controlled deformation on an epithelial monolayer while continuously monitoring its state of stress. With this technique, we probe the active viscoelasticity of epithelial layers over a range of physiological time scales. We present a 3D model of the epithelium, which shows that the observed phenomenology can be explained by the active viscoelastic properties of the actomyosin cortex. Finally, we show that these viscoelastic properties combined with adhesion micropatterning can be harnessed to engineer epithelial wrinkles of predicted geometry.

## References

‘A ton for Thompson’s tome’ (2017) *Nature Physics*, 13(4), pp. 315–315. Available at: <https://doi.org/10.1038/nphys4096>.

Alberts, B. (2015) *Molecular biology of the cell*. Sixth edition. New York, NY: Garland Science, Taylor and Francis Group.

Alt, S., Ganguly, P. and Salbreux, G. (2017) ‘Vertex models: from cell mechanics to tissue morphogenesis’, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1720), p. 20150520. Available at: <https://doi.org/10.1098/rstb.2015.0520>.

*Are All Fish the Same Shape if You Stretch Them? The Victorian Tale of On Growth and Form* (no date). Available at: <https://writings.stephenwolfram.com/2017/10/are-all-fish-the-same-shape-if-you-stretch-them-the-victorian-tale-of-on-growth-and-form/>.

Balasubramaniam, L. *et al.* (2021) ‘Investigating the nature of active forces in tissues reveals how contractile cells can form extensile monolayers’, *Nature Materials*, 20(8), pp. 1156–1166. Available at: <https://doi.org/10.1038/s41563-021-00919-2>.

Bao, G. and Suresh, S. (2003) ‘Cell and molecular mechanics of biological materials’, *Nature Materials*, 2(11), pp. 715–725. Available at: <https://doi.org/10.1038/nmat1001>.

Barker, N. (2014) ‘Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration’, *Nature Reviews Molecular Cell Biology*, 15(1), pp. 19–33. Available at: <https://doi.org/10.1038/nrm3721>.

Braga, V. (2016) ‘Spatial integration of E-cadherin adhesion, signalling and the epithelial cytoskeleton’, *Current Opinion in Cell Biology*, 42, pp. 138–145. Available at: <https://doi.org/10.1016/j.ceb.2016.07.006>.

Brassard, J.A. *et al.* (2021) ‘Recapitulating macro-scale tissue self-organization through organoid bioprinting’, *Nature Materials*, 20(1), pp. 22–29. Available at: <https://doi.org/10.1038/s41563-020-00803-5>.

Breau, K.A. *et al.* (2022) ‘Efficient transgenesis and homology-directed gene targeting in monolayers of primary human small intestinal and colonic epithelial stem cells’, *Stem Cell Reports*, 17(6), pp. 1493–1506. Available at: <https://doi.org/10.1016/j.stemcr.2022.04.005>.

Brown, T.M. and Fee, E. (2006) ‘Rudolf Carl Virchow’, *American Journal of Public Health*, 96(12), pp. 2104–2105. Available at: <https://doi.org/10.2105/AJPH.2005.078436>.

Brugués, A. *et al.* (2014) ‘Forces driving epithelial wound healing’, *Nature Physics*, 10(9), pp. 683–690. Available at: <https://doi.org/10.1038/nphys3040>.

Bryant, D.M. and Mostov, K.E. (2008) ‘From cells to organs: building polarized tissue’, *Nature Reviews Molecular Cell Biology*, 9(11), pp. 887–901. Available at: <https://doi.org/10.1038/nrm2523>.

Calzolari, S., Terriente, J. and Pujades, C. (2014) ‘Cell segregation in the vertebrate hindbrain relies on actomyosin cables located at the interhombomeric boundaries’, *The EMBO Journal*, 33(7), pp. 686–701. Available at: <https://doi.org/10.1002/embj.201386003>.

Campàs, O. *et al.* (2014) ‘Quantifying cell-generated mechanical forces within living embryonic tissues’, *Nature Methods*, 11(2), pp. 183–189. Available at: <https://doi.org/10.1038/nmeth.2761>.

Carlier, F.M., de Fays, C. and Pilette, C. (2021) ‘Epithelial Barrier Dysfunction in Chronic Respiratory Diseases’, *Frontiers in Physiology*, 12. Available at: <https://www.frontiersin.org/articles/10.3389/fphys.2021.691227> (Accessed: 23 December 2022).

Cartagena-Rivera, A.X. *et al.* (2016) ‘Actomyosin Cortical Mechanical Properties in Nonadherent Cells Determined by Atomic Force Microscopy’, *Biophysical Journal*, 110(11), pp. 2528–2539. Available at: <https://doi.org/10.1016/j.bpj.2016.04.034>.

Casares, L. *et al.* (2015) ‘Hydraulic fracture during epithelial stretching’, *Nature Materials*, 14(3), pp. 343–351. Available at: <https://doi.org/10.1038/nmat4206>.

Cetera, M. *et al.* (2014) ‘Epithelial rotation promotes the global alignment of contractile actin bundles during Drosophila egg chamber elongation’, *Nature Communications*, 5(1), p. 5511. Available at: <https://doi.org/10.1038/ncomms6511>.

Chan, C.J. *et al.* (2019) ‘Hydraulic control of mammalian embryo size and cell fate’, *Nature*, 571(7763), pp. 112–116. Available at: <https://doi.org/10.1038/s41586-019-1309-x>.

Chan, C.J. and Hiiragi, T. (2020) ‘Integration of luminal pressure and signalling in tissue self-organization’, *Development*, 147(5), p. dev181297. Available at: <https://doi.org/10.1242/dev.181297>.

Choudhury, M.I. *et al.* (2022) ‘Kidney epithelial cells are active mechano-biological fluid pumps’, *Nature Communications*, 13(1), p. 2317. Available at: <https://doi.org/10.1038/s41467-022-29988-w>.

Clarke, D.N. and Martin, A.C. (2021) ‘Actin-based force generation and cell adhesion in tissue morphogenesis’, *Current Biology*, 31(10), pp. R667–R680. Available at: <https://doi.org/10.1016/j.cub.2021.03.031>.

Collinet, C. *et al.* (2015) ‘Local and tissue-scale forces drive oriented junction growth during tissue extension’, *Nature Cell Biology*, 17(10), pp. 1247–1258. Available at: <https://doi.org/10.1038/ncb3226>.

Collinet, C. and Lecuit, T. (2021) ‘Programmed and self-organized flow of information during morphogenesis’, *Nature Reviews Molecular Cell Biology*, 22(4), pp. 245–265. Available at: <https://doi.org/10.1038/s41580-020-00318-6>.

Deforet, M. *et al.* (2014) ‘Emergence of collective modes and tri-dimensional structures from epithelial confinement’, *Nature Communications*, 5(1), p. 3747. Available at: <https://doi.org/10.1038/ncomms4747>.

Dolega, M.E. *et al.* (2017) ‘Cell-like pressure sensors reveal increase of mechanical stress towards the core of multicellular spheroids under compression’, *Nature Communications*, 8(1), p. 14056. Available at: <https://doi.org/10.1038/ncomms14056>.

Ducuing, A. and Vincent, S. (2016) ‘The actin cable is dispensable in directing dorsal closure dynamics but neutralizes mechanical stress to prevent scarring in the Drosophila embryo’, *Nature Cell Biology*, 18(11), pp. 1149–1160. Available at: <https://doi.org/10.1038/ncb3421>.

Dumortier, J.G. *et al.* (2019) ‘Hydraulic fracturing and active coarsening position the lumen of the mouse blastocyst’, *Science* [Preprint]. Available at: <https://doi.org/10.1126/science.aaw7709>.

Duque, J. *et al.* (2023) ‘Fracture in Living Cell Monolayers’. bioRxiv, p. 2023.01.05.522736. Available at: <https://doi.org/10.1101/2023.01.05.522736>.

Eisenhoffer, G.T. and Rosenblatt, J. (2013) ‘Bringing balance by force: live cell extrusion controls epithelial cell numbers’, *Trends in Cell Biology*, 23(4), pp. 185–192. Available at: <https://doi.org/10.1016/j.tcb.2012.11.006>.

Elosegui-Artola, A. *et al.* (2016) ‘Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity’, *Nature Cell Biology*, 18(5), pp. 540–548. Available at: <https://doi.org/10.1038/ncb3336>.

Elosegui-Artola, A. *et al.* (2017) ‘Force Triggers YAP Nuclear Entry by Regulating Transport across Nuclear Pores’, *Cell*, 171(6), pp. 1397-1410.e14. Available at: <https://doi.org/10.1016/j.cell.2017.10.008>.

Elosegui-Artola, A. *et al.* (2022) ‘Matrix viscoelasticity controls spatiotemporal tissue organization’, *Nature Materials*, pp. 1–11. Available at: <https://doi.org/10.1038/s41563-022-01400-4>.

Engler, A.J. *et al.* (2006) ‘Matrix Elasticity Directs Stem Cell Lineage Specification’, *Cell*, 126(4), pp. 677–689. Available at: <https://doi.org/10.1016/j.cell.2006.06.044>.

Fasano, A. *et al.* (1991) ‘Vibrio cholerae produces a second enterotoxin, which affects intestinal tight junctions.’, *Proceedings of the National Academy of Sciences*, 88(12), pp. 5242–5246. Available at: <https://doi.org/10.1073/pnas.88.12.5242>.

Firmin, J. *et al.* (2022) ‘Mechanics of human embryo compaction’. bioRxiv, p. 2022.01.09.475429. Available at: <https://doi.org/10.1101/2022.01.09.475429>.

Fletcher, D.A. and Mullins, R.D. (2010) ‘Cell mechanics and the cytoskeleton’, *Nature*, 463(7280), pp. 485–492. Available at: <https://doi.org/10.1038/nature08908>.

Fortunato, I.C. and Sunyer, R. (2022) ‘The Forces behind Directed Cell Migration’, *Biophysica*, 2(4), pp. 548–563. Available at: <https://doi.org/10.3390/biophysica2040046>.

Gjorevski, N. *et al.* (2016) ‘Designer matrices for intestinal stem cell and organoid culture’, *Nature*, 539(7630), pp. 560–564. Available at: <https://doi.org/10.1038/nature20168>.

Gjorevski, N. *et al.* (2022) ‘Tissue geometry drives deterministic organoid patterning’, *Science* [Preprint]. Available at: <https://doi.org/10.1126/science.aaw9021>.

Godard, B.G. and Heisenberg, C.-P. (2019) ‘Cell division and tissue mechanics’, *Current Opinion in Cell Biology*, 60, pp. 114–120. Available at: <https://doi.org/10.1016/j.ceb.2019.05.007>.

Gómez-Gálvez, P. *et al.* (2021) ‘The complex three-dimensional organization of epithelial tissues’, *Development*, 148(1), p. dev195669. Available at: <https://doi.org/10.1242/dev.195669>.

Gómez-González, M. *et al.* (2020) ‘Measuring mechanical stress in living tissues’, *Nature Reviews Physics*, 2(6), pp. 300–317. Available at: <https://doi.org/10.1038/s42254-020-0184-6>.

Gorfinkiel, N. and Martinez Arias, A. (2021) ‘The cell in the age of the genomic revolution: Cell Regulatory Networks’, *Cells & Development*, 168, p. 203720. Available at: <https://doi.org/10.1016/j.cdev.2021.203720>.

Guillamat, P. *et al.* (2022) ‘Integer topological defects organize stresses driving tissue morphogenesis’, *Nature Materials*, 21(5), pp. 588–597. Available at: <https://doi.org/10.1038/s41563-022-01194-5>.

Guillot, C. and Lecuit, T. (2013) ‘Mechanics of Epithelial Tissue Homeostasis and Morphogenesis’, *Science*, 340(6137), pp. 1185–1189. Available at: <https://doi.org/10.1126/science.1235249>.

Gutzman, J.H. *et al.* (2018) ‘Basal constriction during midbrain–hindbrain boundary morphogenesis is mediated by Wnt5b and focal adhesion kinase’, *Biology Open*, 7(11), p. bio034520. Available at: <https://doi.org/10.1242/bio.034520>.

Guyon, J. *et al.* (2021) ‘Co-culture of Glioblastoma Stem-like Cells on Patterned Neurons to Study Migration and Cellular Interactions’, *Journal of Visualized Experiments*, (168), p. 62213. Available at: <https://doi.org/10.3791/62213>.

Haigo, S.L. and Bilder, D. (2011) ‘Global Tissue Revolutions in a Morphogenetic Movement Controlling Elongation’, *Science*, 331(6020), pp. 1071–1074. Available at: <https://doi.org/10.1126/science.1199424>.

Harris, A.K., Wild, P. and Stopak, D. (1980) ‘Silicone Rubber Substrata: A New Wrinkle in the Study of Cell Locomotion’, *Science*, 208(4440), pp. 177–179. Available at: <https://doi.org/10.1126/science.6987736>.

Harris, A.R. *et al.* (2012) ‘Characterizing the mechanics of cultured cell monolayers’, *Proceedings of the National Academy of Sciences*, 109(41), pp. 16449–16454. Available at: <https://doi.org/10.1073/pnas.1213301109>.

Hatzfeld, M., Keil, R. and Magin, T.M. (2017) ‘Desmosomes and Intermediate Filaments: Their Consequences for Tissue Mechanics’, *Cold Spring Harbor Perspectives in Biology*, 9(6), p. a029157. Available at: <https://doi.org/10.1101/cshperspect.a029157>.

Heer, N.C. and Martin, A.C. (2017) ‘Tension, contraction and tissue morphogenesis’, *Development*, 144(23), pp. 4249–4260. Available at: <https://doi.org/10.1242/dev.151282>.

Helm, P. *et al.* (2005) ‘Measuring and Mapping Cardiac Fiber and Laminar Architecture Using Diffusion Tensor MR Imaging’, *Annals of the New York Academy of Sciences*, 1047(1), pp. 296–307. Available at: <https://doi.org/10.1196/annals.1341.026>.

Hofer, M. and Lutolf, M.P. (2021) ‘Engineering organoids’, *Nature Reviews Materials*, 6(5), pp. 402–420. Available at: <https://doi.org/10.1038/s41578-021-00279-y>.

Houssin, N.S. *et al.* (2020) ‘Formation and contraction of multicellular actomyosin cables facilitate lens placode invagination’, *Developmental Biology*, 462(1), pp. 36–49. Available at: <https://doi.org/10.1016/j.ydbio.2020.02.014>.

Huh, D. *et al.* (2010) ‘Reconstituting Organ-Level Lung Functions on a Chip’, *Science*, 328(5986), pp. 1662–1668. Available at: <https://doi.org/10.1126/science.1188302>.

Humphrey, J.D. (2002) *Cardiovascular Solid Mechanics*. New York, NY: Springer New York. Available at: <https://doi.org/10.1007/978-0-387-21576-1>.

Humphrey, J.D., Dufresne, E.R. and Schwartz, M.A. (2014) ‘Mechanotransduction and extracellular matrix homeostasis’, *Nature Reviews Molecular Cell Biology*, 15(12), pp. 802–812. Available at: <https://doi.org/10.1038/nrm3896>.

Ingber, D.E. (2018) ‘From mechanobiology to developmentally inspired engineering’, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1759), p. 20170323. Available at: <https://doi.org/10.1098/rstb.2017.0323>.

Ishiguro, T. *et al.* (2017) ‘Tumor-derived spheroids: Relevance to cancer stem cells and clinical applications’, *Cancer Science*, 108(3), pp. 283–289. Available at: <https://doi.org/10.1111/cas.13155>.

Karzbrun, E. *et al.* (2021) ‘Human neural tube morphogenesis in vitro by geometric constraints’, *Nature*, 599(7884), pp. 268–272. Available at: <https://doi.org/10.1038/s41586-021-04026-9>.

Kechagia, J.Z., Ivaska, J. and Roca-Cusachs, P. (2019) ‘Integrins as biomechanical sensors of the microenvironment’, *Nature Reviews Molecular Cell Biology*, 20(8), pp. 457–473. Available at: <https://doi.org/10.1038/s41580-019-0134-2>.

Kim, E.J.Y., Korotkevich, E. and Hiiragi, T. (2018) ‘Coordination of Cell Polarity, Mechanics and Fate in Tissue Self-organization’, *Trends in Cell Biology*, 28(7), pp. 541–550. Available at: <https://doi.org/10.1016/j.tcb.2018.02.008>.

Kumar, A., Placone, J.K. and Engler, A.J. (2017) ‘Understanding the extracellular forces that determine cell fate and maintenance’, *Development*, 144(23), pp. 4261–4270. Available at: <https://doi.org/10.1242/dev.158469>.

Labernadie, A. and Trepat, X. (2018) ‘Sticking, steering, squeezing and shearing: cell movements driven by heterotypic mechanical forces’, *Current Opinion in Cell Biology*, 54, pp. 57–65. Available at: <https://doi.org/10.1016/j.ceb.2018.04.008>.

Latorre, E. *et al.* (2018) ‘Active superelasticity in three-dimensional epithelia of controlled shape’, *Nature*, 563(7730), pp. 203–208. Available at: <https://doi.org/10.1038/s41586-018-0671-4>.

Lecuit, T., Lenne, P.-F. and Munro, E. (2011) ‘Force Generation, Transmission, and Integration during Cell and Tissue Morphogenesis’, *Annual Review of Cell and Developmental Biology*, 27(1), pp. 157–184. Available at: <https://doi.org/10.1146/annurev-cellbio-100109-104027>.

Lever, J.E. (1979) ‘Regulation of dome formation in differentiated epithelial cell cultures’, *Journal of Supramolecular Structure*, 12(2), pp. 259–272. Available at: <https://doi.org/10.1002/jss.400120210>.

Lomakin, A.J. *et al.* (2020) ‘The nucleus acts as a ruler tailoring cell responses to spatial constraints’, *Science*, 370(6514), p. eaba2894. Available at: <https://doi.org/10.1126/science.aba2894>.

Luciano, M. *et al.* (2021) ‘Cell monolayers sense curvature by exploiting active mechanics and nuclear mechanoadaptation’, *Nature Physics*, 17(12), pp. 1382–1390. Available at: <https://doi.org/10.1038/s41567-021-01374-1>.

MacCord, K. (2012) ‘Epithelium’. Available at: <https://hpsrepository.asu.edu/handle/10776/3946> (Accessed: 19 December 2022).

Malandrino, A. *et al.* (2018) ‘Complex mechanics of the heterogeneous extracellular matrix in cancer’, *Extreme Mechanics Letters*, 21, pp. 25–34. Available at: <https://doi.org/10.1016/j.eml.2018.02.003>.

Marchiando, A.M., Graham, W.V. and Turner, J.R. (2010) ‘Epithelial Barriers in Homeostasis and Disease’, *Annual Review of Pathology: Mechanisms of Disease*, 5(1), pp. 119–144. Available at: <https://doi.org/10.1146/annurev.pathol.4.110807.092135>.

Marín-Llauradó, A. *et al.* (2022) ‘Mapping mechanical stress in curved epithelia of designed size and shape’. bioRxiv, p. 2022.05.03.490382. Available at: <https://doi.org/10.1101/2022.05.03.490382>.

Maroudas-Sacks, Y. *et al.* (2021) ‘Topological defects in the nematic order of actin fibres as organization centres of Hydra morphogenesis’, *Nature Physics*, 17(2), pp. 251–259. Available at: <https://doi.org/10.1038/s41567-020-01083-1>.

Martin, A.C., Kaschube, M. and Wieschaus, E.F. (2009) ‘Pulsed contractions of an actin–myosin network drive apical constriction’, *Nature*, 457(7228), pp. 495–499. Available at: <https://doi.org/10.1038/nature07522>.

Martínez-Ara, G. *et al.* (2022) ‘Optogenetic control of apical constriction induces synthetic morphogenesis in mammalian tissues’, *Nature Communications*, 13(1), p. 5400. Available at: <https://doi.org/10.1038/s41467-022-33115-0>.

Mertz, A.F. *et al.* (2013) ‘Cadherin-based intercellular adhesions organize epithelial cell–matrix traction forces’, *Proceedings of the National Academy of Sciences*, 110(3), pp. 842–847. Available at: <https://doi.org/10.1073/pnas.1217279110>.

Mofrad, M.R.K. (2009) ‘Rheology of the Cytoskeleton’, *Annual Review of Fluid Mechanics*, 41(1), pp. 433–453. Available at: <https://doi.org/10.1146/annurev.fluid.010908.165236>.

Mongera, A. *et al.* (2023) ‘Mechanics of the cellular microenvironment as probed by cells in vivo during zebrafish presomitic mesoderm differentiation’, *Nature Materials*, 22(1), pp. 135–143. Available at: <https://doi.org/10.1038/s41563-022-01433-9>.

Monier, B. *et al.* (2015) ‘Apico-basal forces exerted by apoptotic cells drive epithelium folding’, *Nature*, 518(7538), pp. 245–248. Available at: <https://doi.org/10.1038/nature14152>.

Morizane, R. and Bonventre, J.V. (2017) ‘Generation of nephron progenitor cells and kidney organoids from human pluripotent stem cells’, *Nature Protocols*, 12(1), pp. 195–207. Available at: <https://doi.org/10.1038/nprot.2016.170>.

Mullin, J.M. *et al.* (2005) ‘Keynote review: Epithelial and endothelial barriers in human disease’, *Drug Discovery Today*, 10(6), pp. 395–408. Available at: <https://doi.org/10.1016/S1359-6446(05)03379-9>.

Muncie, J.M. *et al.* (2020) ‘Mechanical Tension Promotes Formation of Gastrulation-like Nodes and Patterns Mesoderm Specification in Human Embryonic Stem Cells’, *Developmental Cell*, 55(6), pp. 679-694.e11. Available at: <https://doi.org/10.1016/j.devcel.2020.10.015>.

Nelson, C.M. *et al.* (2005) ‘Emergent patterns of growth controlled by multicellular form and mechanics’, *Proceedings of the National Academy of Sciences*, 102(33), pp. 11594–11599. Available at: <https://doi.org/10.1073/pnas.0502575102>.

Nelson, C.M. *et al.* (2017) ‘Microfluidic chest cavities reveal that transmural pressure controls the rate of lung development’, *Development*, 144(23), pp. 4328–4335. Available at: <https://doi.org/10.1242/dev.154823>.

Nelson, C.M., Inman, J.L. and Bissell, M.J. (2008) ‘Three-dimensional lithographically defined organotypic tissue arrays for quantitative analysis of morphogenesis and neoplastic progression’, *Nature Protocols*, 3(4), pp. 674–678. Available at: <https://doi.org/10.1038/nprot.2008.35>.

Odell, G.M. *et al.* (1981) ‘The mechanical basis of morphogenesis’, *Developmental Biology*, 85(2), pp. 446–462. Available at: <https://doi.org/10.1016/0012-1606(81)90276-1>.

Okuda, S. *et al.* (2018) ‘Strain-triggered mechanical feedback in self-organizing optic-cup morphogenesis’, *Science Advances*, 4(11), p. eaau1354. Available at: <https://doi.org/10.1126/sciadv.aau1354>.

Oriola, D. *et al.* (2022) ‘Arrested coalescence of multicellular aggregates’, *Soft Matter*, 18(19), pp. 3771–3780. Available at: <https://doi.org/10.1039/D2SM00063F>.

Özgüç, Ö. *et al.* (2022) ‘Cortical softening elicits zygotic contractility during mouse preimplantation development’, *PLOS Biology*, 20(3), p. e3001593. Available at: <https://doi.org/10.1371/journal.pbio.3001593>.

Pallarès, M.E. *et al.* (2022) ‘Stiffness-dependent active wetting enables optimal collective cell durotaxis’, *Nature Physics*, pp. 1–11. Available at: <https://doi.org/10.1038/s41567-022-01835-1>.

Palmer, M.A. *et al.* (2021) ‘Stress ball morphogenesis: How the lizard builds its lung’, *Science Advances*, 7(52), p. eabk0161. Available at: <https://doi.org/10.1126/sciadv.abk0161>.

Pérez-González, C. *et al.* (2019) ‘Active wetting of epithelial tissues’, *Nature Physics*, 15(1), pp. 79–88. Available at: <https://doi.org/10.1038/s41567-018-0279-5>.

Pérez-González, C. *et al.* (2021) ‘Mechanical compartmentalization of the intestinal organoid enables crypt folding and collective cell migration’, *Nature Cell Biology*, 23(7), pp. 745–757. Available at: <https://doi.org/10.1038/s41556-021-00699-6>.

Prahl, L.S. *et al.* (2022) ‘Independent control over cell patterning and adhesion on hydrogel substrates for tissue interface mechanobiology’. bioRxiv, p. 2022.11.16.516785. Available at: <https://doi.org/10.1101/2022.11.16.516785>.

Roca-Cusachs, P., Conte, V. and Trepat, X. (2017) ‘Quantifying forces in cell biology’, *Nature Cell Biology*, 19(7), pp. 742–751. Available at: <https://doi.org/10.1038/ncb3564>.

Samal, P. *et al.* (2019) ‘Grow with the Flow: When Morphogenesis Meets Microfluidics’, *Advanced Materials*, 31(17), p. 1805764. Available at: <https://doi.org/10.1002/adma.201805764>.

Sances, S. *et al.* (2018) ‘Human iPSC-Derived Endothelial Cells and Microengineered Organ-Chip Enhance Neuronal Development’, *Stem Cell Reports*, 10(4), pp. 1222–1236. Available at: <https://doi.org/10.1016/j.stemcr.2018.02.012>.

Schamberger, B. *et al.* (2022) ‘Curvature in biological systems: its quantification, emergence and implications across the scales’, *Advanced Materials*, p. e2206110.

Schöck, F. and Perrimon, N. (2002) ‘Molecular Mechanisms of Epithelial Morphogenesis’, *Annual Review of Cell and Developmental Biology*, 18(1), pp. 463–493. Available at: <https://doi.org/10.1146/annurev.cellbio.18.022602.131838>.

Serra-Picamal, X. *et al.* (2012) ‘Mechanical waves during tissue expansion’, *Nature Physics*, 8(8), pp. 628–634. Available at: <https://doi.org/10.1038/nphys2355>.

Serwane, F. *et al.* (2017) ‘In vivo quantification of spatially varying mechanical properties in developing tissues’, *Nature Methods*, 14(2), pp. 181–186. Available at: <https://doi.org/10.1038/nmeth.4101>.

Shah, G. *et al.* (2019) ‘Multi-scale imaging and analysis identify pan-embryo cell dynamics of germlayer formation in zebrafish’, *Nature Communications*, 10(1), p. 5753. Available at: <https://doi.org/10.1038/s41467-019-13625-0>.

Shellard, A. and Mayor, R. (2021) ‘Collective durotaxis along a self-generated stiffness gradient in vivo’, *Nature*, 600(7890), pp. 690–694. Available at: <https://doi.org/10.1038/s41586-021-04210-x>.

Shyer, A.E. *et al.* (2013) ‘Villification: How the Gut Gets Its Villi’, *Science*, 342(6155), pp. 212–218. Available at: <https://doi.org/10.1126/science.1238842>.

Sidhaye, J. and Norden, C. (2017) ‘Concerted action of neuroepithelial basal shrinkage and active epithelial migration ensures efficient optic cup morphogenesis’, *eLife*. Edited by D.Y. Stainier, 6, p. e22689. Available at: <https://doi.org/10.7554/eLife.22689>.

Stanton, A.E., Tong, X. and Yang, F. (2019) ‘Extracellular matrix type modulates mechanotransduction of stem cells’, *Acta Biomaterialia*, 96, pp. 310–320. Available at: <https://doi.org/10.1016/j.actbio.2019.06.048>.

Sui, L. *et al.* (2018) ‘Differential lateral and basal tension drive folding of Drosophila wing discs through two distinct mechanisms’, *Nature Communications*, 9(1), p. 4620. Available at: <https://doi.org/10.1038/s41467-018-06497-3>.

Sunyer, R. *et al.* (2016) ‘Collective cell durotaxis emerges from long-range intercellular force transmission’, *Science*, 353(6304), pp. 1157–1161. Available at: <https://doi.org/10.1126/science.aaf7119>.

Tallinen, T. *et al.* (2016) ‘On the growth and form of cortical convolutions’, *Nature Physics*, 12(6), pp. 588–593. Available at: <https://doi.org/10.1038/nphys3632>.

Tambe, D.T. *et al.* (2011) ‘Collective cell guidance by cooperative intercellular forces’, *Nature Materials*, 10(6), pp. 469–475. Available at: <https://doi.org/10.1038/nmat3025>.

Tang, W. *et al.* (2022) ‘Collective curvature sensing and fluidity in three-dimensional multicellular systems’, *Nature Physics*, 18(11), pp. 1371–1378. Available at: <https://doi.org/10.1038/s41567-022-01747-0>.

‘The 100-year-old challenge to Darwin that is still making waves in research’ (2017) *Nature*, 544(7649), pp. 138–139. Available at: <https://doi.org/10.1038/544138a>.

Thiery, J.P. *et al.* (2009) ‘Epithelial-Mesenchymal Transitions in Development and Disease’, *Cell*, 139(5), pp. 871–890. Available at: <https://doi.org/10.1016/j.cell.2009.11.007>.

Thompson, D.W. (1979) *On Growth and form*. Repr. Cambridge: Univ. Pr.

Tlili, S. *et al.* (2018) ‘Collective cell migration without proliferation: density determines cell velocity and wave velocity’, *Royal Society Open Science*, 5(5), p. 172421. Available at: <https://doi.org/10.1098/rsos.172421>.

Torras, N. *et al.* (2018) ‘Mimicking Epithelial Tissues in Three-Dimensional Cell Culture Models’, *Frontiers in Bioengineering and Biotechnology*, 6. Available at: <https://www.frontiersin.org/articles/10.3389/fbioe.2018.00197> (Accessed: 10 January 2023).

Trepat, X. and Sahai, E. (2018) ‘Mesoscale physical principles of collective cell organization’, *Nature Physics*, 14(7), pp. 671–682. Available at: <https://doi.org/10.1038/s41567-018-0194-9>.

Valon, L. *et al.* (2017) ‘Optogenetic control of cellular forces and mechanotransduction’, *Nature Communications*, 8(1), p. 14396. Available at: <https://doi.org/10.1038/ncomms14396>.

Varner, V.D. *et al.* (2015) ‘Mechanically patterning the embryonic airway epithelium’, *Proceedings of the National Academy of Sciences*, 112(30), pp. 9230–9235. Available at: <https://doi.org/10.1073/pnas.1504102112>.

Vedula, S.R.K. *et al.* (2012) ‘Emerging modes of collective cell migration induced by geometrical constraints’, *Proceedings of the National Academy of Sciences*, 109(32), pp. 12974–12979. Available at: <https://doi.org/10.1073/pnas.1119313109>.

Veenvliet, J.V. *et al.* (2021) ‘Sculpting with stem cells: how models of embryo development take shape’, *Development*, 148(24), p. dev192914. Available at: <https://doi.org/10.1242/dev.192914>.

Venturini, V. *et al.* (2020) ‘The nucleus measures shape changes for cellular proprioception to control dynamic cell behavior’, *Science*, 370(6514), p. eaba2644. Available at: <https://doi.org/10.1126/science.aba2644>.

Vianello, S. and Lutolf, M.P. (2019) ‘Understanding the Mechanobiology of Early Mammalian Development through Bioengineered Models’, *Developmental Cell*, 48(6), pp. 751–763. Available at: <https://doi.org/10.1016/j.devcel.2019.02.024>.

Vignaud, T., Blanchoin, L. and Théry, M. (2012) ‘Directed cytoskeleton self-organization’, *Trends in Cell Biology*, 22(12), pp. 671–682. Available at: <https://doi.org/10.1016/j.tcb.2012.08.012>.

Virchow, R. *et al.* (1860) *Cellular pathology as based upon physiological and pathological histology; twenty lectures delivered in the Pathological Institute of Berlin during the months of February, March, and April, 1858*. London: John Churchill, pp. 1–546. Available at: <https://doi.org/10.5962/bhl.title.110759>.

Wagh, K. *et al.* (2021) ‘Mechanical Regulation of Transcription: Recent Advances’, *Trends in Cell Biology*, 31(6), pp. 457–472. Available at: <https://doi.org/10.1016/j.tcb.2021.02.008>.

Walma, D.A.C. and Yamada, K.M. (2020) ‘The extracellular matrix in development’, *Development*, 147(10), p. dev175596. Available at: <https://doi.org/10.1242/dev.175596>.

Warmflash, A. *et al.* (2014) ‘A method to recapitulate early embryonic spatial patterning in human embryonic stem cells’, *Nature Methods*, 11(8), pp. 847–854. Available at: <https://doi.org/10.1038/nmeth.3016>.

Waters, C.M., Roan, E. and Navajas, D. (2012) ‘Mechanobiology in Lung Epithelial Cells: Measurements, Perturbations, and Responses’, *Comprehensive Physiology*, 2(1), pp. 1–29. Available at: <https://doi.org/10.1002/cphy.c100090>.

Wen, Q. and Janmey, P.A. (2011) ‘Polymer physics of the cytoskeleton’, *Current Opinion in Solid State and Materials Science*, 15(5), pp. 177–182. Available at: <https://doi.org/10.1016/j.cossms.2011.05.002>.

Wyatt, T.P.J. *et al.* (2015) ‘Emergence of homeostatic epithelial packing and stress dissipation through divisions oriented along the long cell axis’, *Proceedings of the National Academy of Sciences*, 112(18), pp. 5726–5731. Available at: <https://doi.org/10.1073/pnas.1420585112>.

Wyatt, T.P.J. *et al.* (2020) ‘Actomyosin controls planarity and folding of epithelia in response to compression’, *Nature Materials*, 19(1), pp. 109–117. Available at: <https://doi.org/10.1038/s41563-019-0461-x>.

Xi, W. *et al.* (2018) ‘Material approaches to active tissue mechanics’, *Nature Reviews Materials*, 4(1), pp. 23–44. Available at: <https://doi.org/10.1038/s41578-018-0066-z>.

Yeung, T. *et al.* (2005) ‘Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion’, *Cell Motility*, 60(1), pp. 24–34. Available at: <https://doi.org/10.1002/cm.20041>.

Yevick, H.G. *et al.* (2019) ‘Structural Redundancy in Supracellular Actomyosin Networks Enables Robust Tissue Folding’, *Developmental Cell*, 50(5), pp. 586-598.e3. Available at: <https://doi.org/10.1016/j.devcel.2019.06.015>.

Yu, J.C. and Fernandez-Gonzalez, R. (2016) ‘Local mechanical forces promote polarized junctional assembly and axis elongation in Drosophila’, *eLife*. Edited by J.B. Wallingford, 5, p. e10757. Available at: <https://doi.org/10.7554/eLife.10757>.

Zampieri, F., Coen, M. and Gabbiani, G. (2014) ‘The prehistory of the cytoskeleton concept’, *Cytoskeleton*, 71(8), pp. 464–471. Available at: <https://doi.org/10.1002/cm.21177>.

1. Ruysch is referred to as a “Artist of death” because of his famous anatomical collection. He was the first to use arterial embalming, which allowed for visualizing and dissecting smallest arteries. He also was part of the macabre practice of public dissections. (halley2019) [↑](#footnote-ref-2)
2. Finding a fundamental unit of living entities comes from the philosophy of Gottfried W. Leibniz. It was based on the idea of “monad”. Thanks to progress in microscopy and philosophy, naturalists were able to put together ideas for cells, fibres, and even cytoskeleton! (zampieri2014) [↑](#footnote-ref-3)
3. The famous epigram was coined by François-Vincent Raspail. Virchow is regarded as influential biomedical scientist of 19th century, but more interesting part is as a radical who took part in the March revolution of 1848. He was one of the first to advocate for the social origins of illness (wright2012, brown2006). [↑](#footnote-ref-4)
4. Louis Sullivan is credited with this maxim, but Sullivan’s protégé Frank Lloyd Wright, designer of New York City’s Guggenheim Museum said “…that has been misunderstood. Form and function should be one, joined in a spiritual union.” (Guggenheim2022) [↑](#footnote-ref-5)
5. Thompson was a polymath, interested in Greek classics to anatomy. The work has been influenced by Goethe’s structuralism and the functionalism of Bertrand Russell. At that time, the dominant explanation in biology was “Darwinism.” To the extent that the ideas of fitness and natural selection would filter in every discipline. Thompson believed in physics rooted in classical mechanics. [↑](#footnote-ref-6)
6. consider *Argyropelecus olfersii* fish mapped onto a cartesian coordinate system can be transformed into *Sternoptyx diaphana* fish by shear transformation of the grid. This theory of transformation has demonstrated its relevance in a new field of geometric morphometrics (abzhanov2017). [↑](#footnote-ref-7)
7. Funnily, He criticized the zoologists and morphologists of the time of assigning shapes to psychical instinct of the organism or some divine interference for creating the perfect shapes: “He finds a simple geometric construction, for instance in the honeycomb structure, he would fain refer it to psychical instinct or design rather than in the operation of physical forces. ... When he sees in snail, or nautilus, or tiny foraminiferal or radiolarian shell a close approach to sphere or spiral, he is prone of old habit to believe that after all it is something more than a spiral or a sphere, and that in this "something more" there lies what neither mathematics nor physics can explain.”

   He was also strong critic of the teleology, where the form can be explained because of its function. The intestine has large surface area because of its absorption function, or in evolution wolf has sharp teeth because of wolf’s dietary requirements. Even today in it is challenging to abstract oneself from teleology. Thompson has a special affinity to the physical explanation by saying,’...to seek not for ends but for antecedents is the way of the physicists, who finds causes in what he has learned to recognize as fundamental properties, or inseparable concomitants, or unchanging laws, of matter and of energy.” [↑](#footnote-ref-8)
8. While, in the introduction of the D’Arcy Thompson’s Growth and Form we can see that he is not completely able be free of vitalism. Especially when comparing dead with alive humans. [↑](#footnote-ref-9)
9. A fantastic commentary of state of engineered living systems research. Matthew Good talks about biologist perspective of building cells from interacting molecules and the complexity it entails. Xavier Trepat applies the same philosophy for building tissues at mesoscale and gives the analogy for the traffic jams. [↑](#footnote-ref-10)