Mechanics of inflated epithelia

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# 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 amazing epithelial tissue monolayer. From the perspective of a mechanical engineer, these monolayers are endlessly fascinating. They are shape-changing, self-healing, and continuously deformming or jamming depending on the requirement. They are the most basic system in terms of physical understanding of the larger world of biological morphogenesis. Epithelia are everywhere: covering all our body surfaces and lining body cavities and organs. Epithelial monolayers adopt a range of shapes from simple spherical blastocyst to highly branched and folded lungs. These shapes are developed and maintained through constant adaptation of epithelia. 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. Then a summary of morphogenesis and how we can think of epithelia as an active material, as well as the historical ways of modeling it. Finally, I will conclude with the emerging field of “bottom-up” morphogenesis, where researchers are reconstructing the biological systems from scratch.

# Epithelial Tissues

## Epithelia

A Dutch botanist, Frederick Ruysch, coined the term ‘epithelia’ in 1703 book *Thesaurus Anatomicus*. He created the term to describe the tissue found while dissecting the lips of a cadaver. Combining Greek roots ‘Epi’ for the top and ‘thele’ for a nipple. A few decades later, Swiss scientist Albrecht von Haller started to use the term epithelium/epithelia to describe fiber(s) of the body. The idea of fibers was an old renaissance theory that the body is made of fibers, which a fundamental building block of living things. The eighteenth century understanding was that these fibers/tissues arranged in different arrays gave rise to biological structures (maccord2012, zampieri2014). They were not that far off. In the twenty-first century science, epithelial tissues are understood as cell sheets with strong intercellular bonds that form physical barriers for compartmentalization of the organs. It plays a key role in developmental stages by supporting growth and driving shape changes of the organs and organism. It protects the organs from external physical, chemical, and microbial onslaughts.

Figure: Artist of death. Frederick Ruysch who coined the term epithelia

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 vectoral functions like creating and maintaining concentration gradients between the separated compartment. 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 (Simons, K. and Fuller, S.D., 1985). In addition, polarized epithelia guide the developmental process by determining the fate of cells leading to symmetry breaking events in the embryo. (kim2018).

Epithelial cells have different shapes and may be arranged in single or multiple layers. They are usually classified according to two features: the number of cell layers and the shape of the cells. Simple epithelia are single-cell layers where all the cells contact the underlying basal lamina and have an apical free surface. The shape of the cells can be flat (wider than high), cuboidal (as wide as high), or columnar (higher than wide). However, stratified epithelium contains two or more layers of cells. Our focus is simple monolayers.

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

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

## Cell structure

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

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

Figure: all the cytoskeleton and cell junctions and their mechanics

Multiple membrane molecules can mediate adhesion between cells. One of these are cadherins, critical for epithelial cell cohesion through the formation of adherens junctions. In these junctions, cadherins are coupled to the cell cytoskeleton enabling force transmission between cells. It is finely regulated by both internal and external mechanisms. Desmosomes are another type of intercellular junction. They are coupled with intermediate filaments, and the resulting supracellular network confers mechanical resilience on cell layers (Hatzfeld, M., Keil, R., & Magin, T. M. 2017; Latorre, E., et al., 2018). Tight junctions perform a barrier function and enable the transport of ions across epithelial layers to be actively regulated. This plays an important role in the control of fluid pressure in tissues. Regulation of adherens junctions, desmosomes, and tight junctions together enables emergent behaviors in cell sheets that are not observed in single-cell systems (Trepat, X., and Sahai, E., 2018; Ladoux, B., and Mège, R.M., 2017).

What emergent behaviors? name some

## Microenvironment

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

ECM is a fibrous network of proteins; from a mechanical perspective, the three primary structural constituents of the ECM are typically collagen, elastin, and proteoglycans. Collagen is one of the most abundant protein 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 (Casares, L., et al., 2015). Moreover, collagen remodels itself under the influence of cells aiding in migration or under stress (Shields, M. A., et al., 2012; Humphrey, J. D., 2003). Like most cytoskeletal proteins, most extracellular components turnover. Some continously and some very slowly. For example, collagen in the peridontal ligament appears to have a half-life of a few days, whereas that in the vasculature may have a normal half-life of several months. In response to altered loads, disease, or injury, however, the rates of synthesis and degradation of collagen can increase many folds to have a rapid response (Humphrey J. D., 2003).

## Cell-substrate adhesion

Cells and ECM have a symbiotic relationship with each other through various sensors on the cell surface. These cues are primarily sensed using integrins and focal adhesion complexes in cell-substrate adhesion (kechagia2019). Through these adhesions cells can sense the external mechanical and chemical signals like matrix stiffness, ligand density, or chemotactic gradients (citetation needed). Recently it has been shown that they could the cells could even respond to viscoelasticity of the matrix (alberto havard paper). In response to the signals the cells have shown to secrete ECM on their own or remodel the substrate to promote growth/invasion of a cancer, or reorganize the cytoskeletona all together. Due to recently discovered deeper ties of focal adhesions to the nucleus and in turn transcriptional factors, cell-matrix adhesions could affect the tissue behavior fundamentaly. (DuFort, C. C., Paszek, M. J., & Weaver, V. M. 2011; Northey, J. J., Przybyla, L., & Weaver, V. M. 2017).

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 (Macara, I.G., et al., 2014). For example, epithelial cells have one of the fastest turnover rates in the body. The entire gut cell lining turns over in 3–4 days. This turnover implies constant cell division and death. The excessive rate of division and death may give rise to tumors. It is known that 90% of cancers emerge in simple epithelia (Torras, N. et al. 2018; Eisenhoffer, G.T., and Rosenblatt, J., 2013). Not only this, but it could easily disrupt the barrier function, as no gaps should emerge around dying or dividing cells. 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 (Fasano, A. et al., 1991). Cancer cells tend to spread and disperse metastatically by using their very high rate of cell motility and a diminished sense of cell adhesion. This elimination and/or reduction of ZO1 barriers in cancer is essential to allowing metastatic cells to break into and out of blood vessels. The leaky barrier also allows a growing epithelial tumor to access luminal fluids as an additional source of nutrients (Mullin, J.M., et al., 2005).

Epithelia undergo 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. Like in the case of epithelial–mesenchymal transition (EMT), a developmental process when epithelial cells gradually transform into mesenchymal-like cells by losing their epithelial functionality. EMT is involved in the pathogenesis of numerous lung diseases ranging from developmental disorders to fibrotic tissue remodeling to lung cancer. For example is bronchopulmonary dysplasia (BPD), a chronic lung disease that occurs in very premature infants and is characterized by impaired alveologenesis and vascular development. BPD develops because of injury or infection in a very immature lung (Bartis, D. et al., 2014).

# Mechanics of Morphogenesis

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

To appreciate the complexity of these morphogenetic processes, I find it useful by grouping them in terms of local-global and slow-fast. Some processes appear to be happening fast at local level like series of cells changing their shape by undergoing apical constrictions to create a global change in embryo, the ventral furrow in Drosophila gastrulation. However, at the same time you will find that the chemical signaling events activating this process are slow and at global level. Similar events are happening in vitro systems such as organoids. For example, a cluster of dissociated mouse embryonic stem cells (ESCs) cultured in an appropriate medium assemble itself in a optic cup. It exhibits all the layers of neural retina (Eiraku et al., 2011; Bedzhov, I. & Zernicka-Goetz, M. 2014). Just like the drosophila embryo, this structure also undergoes local changes creating a global invagination in response to global signals of culture conditions. It gets even more complex, when we consider the details of these processes. For example molecular machinery involved in the apical constriction, genes responding the morphogen gradients, or mechanical stresses at the cells or tissue levels causing flows.

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

Rudolf Virchow’s third tenet of cell theory stated ‘omnis cellula e cellula’ meaning all cells come from cells (Virchow R.L.K., 1858). All tissues come from cells that contain essentially the same genetic information. Nonetheless, every tissue exhibits a distinct architecture and function. This raises many questions such as what makes cells different from each other? Is it all because of the environmental factor? 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 alongside has created new debates and unanswered questions #NotInTheGenes. However, we are more interested in the mechanics of morphogenesis: in emergence of the form.

Historically, form for animate and inanimate objects been tied to the function. As per the twentieth century architecture principle of “Form Follows Function”; where the organization of a structure should be based upon its intended function. In developmental biology there are many examples of indicating that the same principle is at work in self-assembling systems like intestinal organoids, cancerous spheroids, and functional kidney tissues (Gjorevski, N, et al. 2016; Ishiguro, T, et al. 2017; Morizane, R. and Bonventre, J.V., 2017). Each emerging out of a set of cells in appropriate environment changing and adapting itself to perform the biological function. However, exactly the opposite design principle is at work in numerous in vitro experiments with controlled cellular environment; illustrating geometric constraints drives biological function. For instance, in a micropatterned collagen scaffold (with structures of intestine) a human small intestinal epithelium was generated that replicates key features of the in vivo small intestine: a crypt-villus architecture with appropriate cell-lineage compartmentalization and an accessible luminal surface (Wang, Y et al., 2017). Or cell reprogramming like in case of fibroblasts turning into induced neurons when supported by specific substrate topography (Kulangara et al. 2014).

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

Knowing the field of developmental biology, it is easy to reach the conclusion that there are more things involved in understanding dialectics of form and function. This was a subject of D’Arcy Wentworth Thompson’s classical text “On Growth and Form” (Thompson, 1917). Thompson tried to explore biological forms during development and across evolution with considering geometric and physical constraints. (*Here talk more about specific examples from the book*) It is quite astounding that Thompson was thinking about these questions when the scientist were still grappling with ideas of vitalism and teleology. Form and function debate still lands up in explaining the form because of its function. Intenstine has large surface area because its function is absorption. There has been a great focus on genetic basis for developmental processes. But it is more clearer now that genes are not the complete answer. There is a specter is haunting this subject—the specter of mechanics. In last couple of decades, there has been a resurgence of interest in physical forces as regulator of development, homeostasis, and disease (Ingber, D. 2005; Barnes, J.M. et al., 2017). This has led researchers across the disciplines to examine the physical mechanisms of tissue formation and its regulation. Unravelling mechanism of Thompson’s mysteriously generated ‘Diagram of the forces’ which governs biological processes (Thompson, 1917; Heer, N.C. and Martin, A.C., 2017). After more than 100 years of its publishing the specific questions about shape and function can be answer using advances in bioengineering and microscopy.

## Mechanics

In simplification, one can imagine each cell in epithelial tissue as a mathematical system which integrates several input types to result in an output behaviour. Such inputs cues can be chemical like soluble chemicals as in morphogen gradients in embryos, and cell-cell/cell-surface signalling molecules; or be mechanical – involving the generation-modification of intra- or intercellular forces as in apical constriction of ventral furrow formation; or external forces of tension/shear like breathing lungs (ref ) and flowing blood through vasculature (Fenech, M., et al., 2019). While outputs could be cells shape change, migration, differentiation, or apoptosis. (Kumar, A. et al., 2017) (Figure reference )

For understanding the epithelial mechanics, we need to have epithelia with controlled biological and mechanical conditions along side the ability to measure forces and deformations. It is not easy to have these conditions met. Here I will give the overview of the system in decreasing order of complexity. Starting from in vivo organisms to simple 2D monolayers.

## Embryos

## Explants

## 3D tissues

## 2D monolayers

Embryos are one system where we see self organization work. apical basal polarity and lumen formation. Also see that the pressure regulates the size (Chi joe chan paper read ari thesis). Other example is zernicka goetz paper. segregation on adhesion properties. Explants of lungs have been useful tool to However, the embryo system lacks better imaging or control over size shape and measurement of the forces

Simple processes like division and deformation in individual cells contribute to complex behaviours which can only be seen at larger scale in collective context like emergence. It has been shown in recent years how guided by biophysical cues in microenvironment give rise to cellular collective migration (Ladoux, B. & Mège, R.-M. 2017), oscillation (Balaji, R. et al. 2017; Serra-Picamal, X. et al. 2012), turbulent motion (Vedula, S. R. K. et al. 2012), or active cell rearrangement (Krajnc, M., et al., 2018). (Figure reference)

Description of actin cytoskeleton and internal force generation Any response to biophysical cues is mainly manifested as physical change in the cell which in turn shows up in tissue. The change in shape is related to mechanical properties of the cell, which are largely defined by the actin cytoskeleton. Actin cytoskeleton is a highly polymorphic and multifunctional cellular system that consists of actin filaments (F-actin) organised into various higher-order arrays capable of dynamic remodelling (Svitkina, T. 2018). Its most important function is to generate force. For pushing, actin filaments typically assemble into branched networks assembled by the Arp2/3 complex (Svitkina, T.M. and Borisy, G.G. 1999), while, for contraction, they form composite networks or bundles with bipolar filaments of myosin II (Verkhovsky, A.B. et al. 1995). These generated forces can also propagate through the tissue through cell–cell junctions which form dynamic cellular contacts and the substrate (Halbleib, J. M. & Nelson, W. J. 2006; Sunyer, R., et al, 2016). Cell–matrix and cell–cell junctions are under constant mechanical stress (Maruthamuthu, V., et al., 2011), which causes remodelling of the junctions (Mège, R. M. & Ishiyama, N. 2017) and triggers cell signalling events within the tissue (Ladoux, B. & Mège, R.-M. 2017). To summarise, any ‘input cues’ start a cascade of biochemical reactions from ‘mechanosensors’ to cytoskeleton resulting in ‘output response’ through contraction/extension. This process is called as ‘mechanotransduction’ (Harris, A.R., et al., 2018). Introduction to models for epithelial mechanics Advances in experimental technologies and better-resolved spatial-temporal measurements provide more detail view of tissue function and its complex physical and molecular underpinnings. Similarly, advances in engineering and mathematical models allows for exploration of hidden physical phenomenon; along with aiding experimentalists to perform physically motivated experiments. For example, the looped pattern of vertebrate gut tube is reproduced by a simple physical system of differentially strained rubber tube with soft latex sheet composite (Savin, T., et al., 2011). This mathematical theory, depended only on geometry and elasticity, could predict quantitatively with observations of intestinal loops at different stages of development in chick embryo. (Figure reference ) As mentioned before epithelial tissues are excellent examples of complexity spanning the molecular, cellular, and tissue scales. The strong interdependence between mechanical and molecular signals makes it difficult to interpret the relative contribution of different cues to the shaping of epithelial tissues. Mathematical models can help to disentangle this complex relationship. Models are perfect tools for discovery and data interpretation. For instance, they have been useful inferring the mechano-chemical properties in different experimental manipulation like laser ablation, force microscopy, and photobleaching assays (ref ; Fradin, C., 2017). History of modelling There is a history to mathematical modelling of epithelia. Almost 70 years ago, Alan Turing put forth his theory of reaction-diffusion of two chemicals. It showed that interaction of two chemicals can generate intriguing spatial patterns in his paper “The chemical basis of morphogenesis” (Turing, A. 1952). These patterns, Turing patterns, can be seen in leopard’s spots, butterfly’s wings, or fish’s scales (Figure reference ). Well now, with much more information of physical forces involved in biological process one can start thinking about mechanochemical basis (Howard, J., Grill, S.W. and Bois, J.S., 2011). Introduction to different models There are mathematical models describing physics and biology at multiple scale. There are hyperelastic continuum material models at larger tissue scales, for example, describing behaviour of cardiovascular system (ref ). There are active fluid models at relatively smaller scales explaining epithelial tissue behaviour in systems such as active dewetting or drosophila wing development (ref ). Active surfaces Fortunately, as anticipated by Descartes and other mechanists, soft tissues respect the basic postulates of mechanics (e.g. conservation of mass, momentum, and energy), and basic concepts such as stress, strain and entropic elasticity apply as well. Hence, much of continuum models focuses 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. Because of the incredible complexity of both the ultrastructure and microstructure of these materials researchers continue to rely primarily on phenomenological descriptors of the behaviours of interest, descriptors that are often motivated by the knowledge of the underlying structure. The uniaxial/biaxial tension and compression testing has been able to provide insights into the constitutive relation of the tissues. In these experiments, one measures deformation in terms of strain tensors, like Green strain tensor (E) E=(F^T.F-I)/2 Where, F is a deformation gradient tensor. Here, in the theoretical framework of finite elasticity, one can assume a strain energy function (W) relates to stress (S). S= ∂W/∂B The stress-strain data extracted from the experiment allows to predict the form of strain energy function. In case of bladder, heart tissue, skin, and arteries, hyperelastic form has been useful in capturing the material response (Fung YC, 1990; Humphrey JD, 2002). 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 mechanism of myocardium infarction and various aneurysms (add references from Humphrey, Holzapfel and Ogden). 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 (Holzapfel, G.A. and Ogden, R.W., 2020). These models are also used in understanding growth and remodelling with using the theory of kinematical growth. It has pointed out 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 tumour growth mechanobiology, the residual stresses directly influencing tumour aggressiveness, nutrients walkway, necrosis, and angiogenesis (Carotenuto, A.R., et al., 2019). The nonlinear continuum models have also been used in mechanical modelling of the brain tissue. As an ultra-soft and heterogeneous material, researchers can model its behaviour in high strain rate scale of brain trauma to slow growth in developmental stages (Budday, S., et al., 2019). F=∇\_x φ F=Fe.Fg  
At cellular scale, we know that the mechanical properties of the tissue are controlled by the biopolymeric system called 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 as a physical gel because of its cross-linked actin filament network. But, phenomenon like treadmilling, active polymerization-depolymerization of filaments, and mobility of molecular motors like myosin makes the tissue system an active gel. Also, to note that 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 (Toner. J. et al., 2005). Active matter systems are sub class of continuum models used to describe dynamics of packed active particles; based on liquid crystal theories of soft condensed matter. Like liquid crystals cells too have orientation and ability to move past each other. In this framework, one characterises the orientation of filaments in cytoskeleton or elongation of cells in the tissue by nematic order parameter matrix (Q). Q=3S(nn-I/3)/2 Where n is a unit vector indicating local average orientation axis, S is the scalar order parameter and theta is the angle of each element with the n vector. S= 〈cos2θ^((m)) 〉 This formulation aids in defining active forces generated by the network. The stress is divided in two parts: active and passive. Where passive stress will be arising from mesoscopic viscoelasticity of the material and from the bending, splaying and twisting of the aligned agents. Active stresses are obtained using combination of zeta, strength of activity, and nematic order matrix. Zeta’s sign determines the nature of the force dipole. If negative, system contracts; if positive, 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, motion could get chaotic. In a dense bacterial system of Bacillus subtilis, jet flows and turbulent flow patterns were observed (Wensink, H.H., et al., 2012 ). Also, independent vortices have been observed in the expanding monolayers (Saw, T.B., et al., 2015). Nematic equations have capture physics very well in 2D confined systems or expanding systems. 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 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 generalised 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 length of the line element with tangential unit vector v normal to the line, and t and m are tension and moment respectively. Total torque is,

The force balance expression becomes

These equations can be used with constitutive equations for specific cases. For example, Salbreux and Julicher show that flat active Helfrich membrane with up-down asymmetry have its stability dependent on active tension and active tension-curvature coupling term. They find that the active flat surface undergoes shape instabilities in two cases. First, classical buckling instability occurring when active stresses are compressive and establish a negative surface tension in the membrane. Second, active buckling instability occurs when tension is dependent on curvature, Because of this dependency, a perturbation of the surface shape results in regions of low and high surface tension. These surface tension differences lead to flows towards region of higher tension. These flows generate further in-plane torques causing further deformation of the surface (Salbreux, G. and Jülicher, F., 2017). This tension-curvature dependency can be seen in the pancreas of mice that the morphology of epithelial tumours is determined by the interplay of cytoskeletal changes in transformed cells and the existing tubular geometry (Messal, H.A., et al., 2019). 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. It is pointed 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 (Fouchard, J., et al., 2020 ). Vertex models Another prominent modelling system is of vertex models. Monolayered epithelial tissue shows a polygonal cellular pattern on its surface. This enables to describe/track cell motion and shape change easily in terms of vertices and edges (Figure reference). Vertex models have been developed to understand the complex interplay between cellular shape, the forces generated inside epithelial cells and mechanical constraints externally imposed on the tissue. Models could be 2D or 3D depending on the system being modelled, but cells are consistently defined as each one has apical and basal surface, and lateral interfaces between neighbours. This enables one to model for cell polarities in either directions. More complexities have been added to describe specific systems like intercalations in 3D epithelia using geometric shape as Scutoid (Gómez-Gálvez, P., et al., 2018) (Figure reference). The molecular and mechanical details are added in formulation of mechanical description. In mechanics to determine the motion of vertex, mechanical forces must be specified. It is done using virtual work function (W); and forces can be obtained by differentiating with vertex position. There are two components to W: internal and external. The Changes in internal virtual work (dWi) 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.

Similarly, the external virtual work can be written external forces that arise from external compression or tension applied to the tissue, fluid pressure acting on apical or basal cell surfaces, or attachment of cells to the underlying basement membrane.

The state of the monolayer is estimated by minimising the virtual work function (Alt, S., Ganguly, P. and Salbreux, G., 2017). Here, molecular details are incorporated in the changes of the surface tension and line tensions (Figure reference). In epithelial layers, the actin cortex plays a huge role in determining tensions along the edges (ref). This has been very useful in capturing physics in systems starting from cell shape distribution and cell packing to appendage formation and growth of epithelial vesicles (Staple DB, et al., 2010; Osterfield M, et al., 2013; Okuda S., et al., 2015). Introduce controlled environment and engineering Due to progress in engineering and material science, new avenues have been explored to study environment sensitive response of the epithelial tissues. Microengineering techniques can be combined to create controllable environments to study epithelial movement and mechanics. By modulating the cell–material interface and by applying principles of active matter, key aspects of epithelial dynamics and mechanosensing mechanisms can be investigated (Xi, W. et al., 2019). The lens of epithelial tissues as active materials with rheological properties has provided new insights from the molecular level to the tissue level. Also, the effects of architecture and stiffness of the microenvironment combined with external mechanical stimuli enables us to precisely study the complicated compromise between competing stimuli (Figure reference). Due to complex nature of material, we need to develop experimental approaches to probe tissues at multiple length scales and timescales through mechano-chemical control (Good, M. and Trepat, X., 2018).

Engineering and Measurement Microenvironment in terms of forces/pressures/matrix As we know apart from genes and biochemical signalling cascades, the mechanical properties of both the tissue and its microenvironment substantially impact epithelial behaviours. The cells engage in continuous feedback loop with its surroundings causing alteration in its cytoskeleton and matrix. Therefore, the rheological behaviour of biological materials, from cortical actin networks to large cell assemblies, is a complicated compromise between competing forces, cellular events and external stimuli. External stimuli through matrix At tissue scale, the epithelia sense its environment through cell-matrix adhesion, where it shows response to stiffness changes—durotaxis; like in neural crest migration in Xenopus laevis (Alkobtawi, M., et al., 2018). It also perceives cell crowding; and cells delaminate from those sites to ease higher intercellular tensions. It can be observed during homeostasis in the colon epithelia (Eisenhoffer, G. T. et al. 2012). Not just matrix affects cells, but reverse is also true. In case of Drosophila oogenesis, disorganised matrix is remodelled into a polarised, restrictive matrix, which aligns with the actin bundles in the follicular epithelium through coordinated rotation of the cells (Cetera, M. et al. 2014; Haigo, S. L. & Bilder, D. 2011). These polarised fibres can in turn guide the directed motion of cells (Cetera, M. et al. 2014; Nam, K.-H. et al. 2016). Similar case is found in tumour mass embedded in collagen-rich matrix (Shields, M. A., et al., 2012), where invasive cancer cells can also remodel the tumour stroma to promote invasion. Examples of engineered environments Due to all these factors, it is difficult to decouple the various biophysical aspects of matrix, such as material stiffness, spatial confinement, porosity, viscoelasticity, material degradability and binding affinity. But efforts have been made to replicate and study tissue dynamics and mechanics in vitro using cell biology, microengineering, materials and modelling approaches.  
Using such a techniques, collective cell migration under confinement (Vedula, S. R. K. et al., 2012; Xi, W., et al., 2017; Yevick, H. G., et al., 2015), collective durotaxis (Sunyer, R. et al., 2016), geometrical and stretching-controlled epithelial extrusion (Eisenhoffer, G. T. et al., 2012; Saw, T. B. et al. 2017) and epithelial growth regulation in two dimensions (Nelson, C. M. et al., 2005) and three dimensions (Salomon, J. et al., 2017) is studied, which led to a better understanding of cell-ECM interactions (Ladoux, B. & Mège, R.-M. 2017) and of tissue as active matter (Prost, J., Jülicher, F. & Joanny, J. F. 2015 ). In vitro studies have elucidated the effects of multiple mechanical cues of the microenvironment on cellular behaviour, and insights into tissue mechanics have promoted the development of new materials and design methods for bioengineering, in vitro modelling, immunotherapy and gene therapy. Substrate Patterning Commonly, synthetic 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. They can be fabricated using basic chemistry and new microfabrication technologies like lithography . For example, 2D substrates are patterned with adhesive or non-adhesive ECM to control tissue/cell shape. This has revealed complex behaviour in arrangement of epithelia in confinement (ref) and mechanism of force transmission throughout the tissue (ref). 2D Surface ECM patterning and topography also have helped in engineering 3D epithelial. Moreover, the integration of different techniques and materials further allows even higher levels of complexity in both two and three dimensions (Xia, Y. N. & Whitesides, G. M. 1998; Théry, M. 2010; Alom Ruiz, S. & Chen, C. S. 2007). For example, multiple proteins can be patterned on flat as well as 3D surfaces using UV patterning and stencilling (Ruprecht, V. et al., 2017) to form adhesive patterns with spatially controlled adhesive strengths. Mechanical forces/pressures Living systems do not just have spatial control, they also have mechanical control through physical forces emerging out of growth, deformation and remodelling of ECM, and flow or arrest of fluids in closed geometries (ref). In our body, intestinal epithelia are stretched during peristaltic movements in the gut and lung alveoli deform during breathing (Ethier CR, Simmons CA, 2007). Not just tension, compression is known to guide several morphogenetic events involving tissue bending and folding, such as the formation of the optic cup (Sidhaye, J. & Norden, C. 2017), gut villi (Shyer, A. E. et al., 2013), and cortical convolutions in the brain (Tallinen, T. et al. 2016) In developing embryo, embryonic and extraembryonic fluids generate forces: frictional and tensional stresses when flowing (Freund et al., 2012), or hydrostatic pressures when in confined spaces (Navis and Bagnat, 2015). The mouse embryo indeed initially floats within intraluminal fluid (Chen et al., 2013), forms numerous fluid-filled cavities upon implantation (Christodoulou et al., 2018), and establishes flows of distinct regimes throughout later development (Freund et al.,2012). These fluids pass on critical information through soluble chemicals (Zhanget al., 2018). However, not much is known about the developmental relevance of the mechanical stresses these fluids also exert (ref). At the blastocyst stage, when the embryo is little more than a fluid filled sphere, internal hydrostatic pressure not only determines embryonic size but also directs allocation of fates by affecting allocation to internal or external compartments (Chan et al., 2019). As a fluid filled structure, imagine a balloon, the hydrostatic pressure inside relates to tension in t he surface. 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 (Chan et al., 2019; Latorre et al., 2018). Later in development, the pressure exerted by the fluid within the amniotic cavity has been postulated to direct notochord convergent extension by exerting anisotropic forces on the underlying axial mesoderm (Imuta et al., 2014). Clearly, the fluid dimension of the embryo plays key developmental functions, strengthening calls for an increased recognition of its role along-side the better-appreciated cues presented by the solid environment of cells and tissues (Kaul and Ventikos, 2015). Rheological measurements Epithelial tissues are much complicated materials than that of simple metals or polymers. However, the complex biological behaviour can be connected to mechanical response using basic material testing techniques (ref). Typically, it is done by probing the material mechanically and then recording its deformation, or by deforming the material and recording force response. In mechanical terms, one records material behaviour with stress (force) strain (deformation) relation, often called as constitutive relation and can be quantified in term of passive and active rheological properties (ref). This depends on the type of deformation as in shear, tension, or compression and is also multiaxial like in composite materials (ref). 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). Fo r example, Heart failure is often due to the loss of contractility of heart muscle cells; as it is observed in remodelling under mechanical stretch (Fung YC, 1990). So, one must test rheological properties along with different microenvironment. The mechanical information include 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-Cusachs, P., Conte, V. & Trepat, X. 2017). This has been done at the molecular and subcellular scales using, atomic force microscopy (AFM) (Emad, A. et al. 1998), magnetic beads (Broders-Bondon, F., et al, 2018), optical tweezers (Bambardekar, K., et al., 2015), and micropipettes (Evans, E. & Yeung, A. 1989); and at larger scale using stretching devices, tissue rheometers and force plates (Huh, D. et al 2010; Gudipaty, S. A. et al. 2017). This reveals complex mechanism of strain stiffening and viscoelastic behaviour at different regimes of deformation involving different parts of cytoskeleton. Also, elucidated the signalling pathways in terms of transcription factors like YAP involved in mechanosensing (ref). Suspended monolayers As mentioned earlier, the tissue-matrix interaction is playing a critical role in sensing and transmitting forces rapidly (Tambe, Dhananjay T., et al. 2011; Sunyer, R., et al., 2016; Serra-Picamal, X., et al., 2012). 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, intrinsic tension generated in some embryonic tissues acts as an extrinsic stress on other tissues. For example, in the Drosophila wing disk, contraction of the wing hinge applies tension on the wing blade (Etournay, R., et al., 2015). During dorsal extension, stresses exerted by the invaginating dorsal mid gut play a role in orienting junction elongation after intercalation (Collinet, C., et al., 2015). During epiboly in zebrafish, an actin belt in the yolk cell applies tension on the enveloping layer (Behrndt, M., et al., 2012). The system of studying just simple free-standing epithelial monolayer is very appealing in terms of characterising mechanical response to stretch at different time scales. This also allows to look at cellular scale phenomenon in terms of actin network remodelling and dynamics of cell-cell adhesion. In experimental setup developed in Harris AR et al., 2012, they were able to create suspended monolayer between two rods. This was done by culturing cell monolayer on collagen matrix on top of two rods; later matrix was removed using enzymatic digestion. The system of two rod allowed them to conduct mechanical tensile testing on the suspended monolayer with force measurement in the tissue. These experiments showed that elasticity measurements of monolayer were two orders of magnitude larger than that of individual cellular parts, and monolayer could sustain more than 200% strain before rupture of cell-cell junction. Moreover, imaging of cell cytoskeleton showed that the acto-myosin network and cadherin junctions actively remodel during stretch, also keratin network reinforces the monolayer integrity at higher strains. A significant rearrangement and realignment of cells is observed. Further, rheological response probed through stress relaxation experiments show stress are dissipated on a minute timescale with increase in length of the tissue. In stress relaxation experiments, suspended monolayer is stretched for some time and then unstretched to measure mechanical response. This increase in length points to the active remodelling of the cell cytoskeleton at molecular scale. However, authors in these experiments did not observe any role played by cell-cell junction and intermediate filaments other than maintaining integrity of the monolayer (Khalilgharibi, N, et al. 2019). As mentioned, earlier the cells have shown complex response to compression in form of apoptosis, differentiation, and extrusion (ref). In Wyatt T., et al., 2020 , the same setup was also used to study monolayer response under fast compression. Just like the slender elastic structures buckles after application of critical compressive stress, this free-standing monolayer buckles after application -35% strain at high strain rates. Intriguingly, the tissue buckling is followed by tissue flattening up to the threshold on -35% strain. The tissue buckling is rapidly eased by actomyosin generated tension within tens of seconds. However, after the threshold strain and higher strain rates stable 3D folds are formed in the epithelia. This revealed mechanisms with which tissues buffer the mechanical stress in response to fast external forces. The similar system has been explored with completely different mechanism in Latorre et al., 2018, where the pressurised epithelia display behaviour equivalent to active superelasticity. They used ion pumping of MDCK cell (kidney epithelial cells) with micropatterned substrate with circular non-adhesive ECM patches to create dome like structures. Due to apical to basal ion pumping, cell monolayer delaminates to form engineered 3D epithelia—dome. This system uses soft PDMS gel with beads embedded into it for later measurement of pressure inside the dome. These domes fit very closely to a spherical cap; making structure analogous to a bubble. This fact allowed use of Laplace’s law relating pressure and surface tension in the bubble/dome. The spherical geometry implies uniform tension as the pressure build up underneath is hydrostatic in nature. Here, they were able to stretch individual cells at extreme strains of 500-800%. Interestingly, the cells under uniform tension showed drastically different cellular strains reminding authors of superelastic materials. The superelasticity describes material response when material can undergo large reversible deformations at constant stress by alteration/phase change in microstructural elements like in Nickel-Titanium alloys. The stress-strain response of cells showed initial increase and then long plateau with subsequent increase. Authors could explain it with high resolution imaging; that the cortical actin depletes as cells are stretched and this induces the plateau where cells are rescued by intermediate filament network at the end. These studies have discussed generic principles of cellular mechanisms . Just like superelasticity could have general application in extra-embryonic tissues or blastocysts systems (Hilbrant, M., et al 2016; Hildebrand, S. et al. 2017; Deglincerti, A. et al. 2016). Just as superelasticity or buckling another interesting physical phenomenon lurking around the corner. Besides providing a framework to understand epithelial mechanics and morphogenesis in vivo, the physics established in these experiments pave the way for a rational manipulation of cell monolayers in organoids and organ-on-a-chip technologies.

On the chip Microfluidics and their applications: Interesting setups to apply forces and creating controlled microenvironment. Cells have been being cultured on synthetic substrates in ex vivo for more than a century now, but the understanding was limited to 2D and passive environment (Carrel and Burrows, 1911). For studying development and immunity in multicellular tissue/organ context, historically researchers have depended on animal studies (Schmeichel and Bissell, 2003). Through last twenty years, incredible progress has been made in controlling matter at small scale. This gave rise to the currently a huge field of microfluidics and microfabrication. As described earlier, control of microenvironment has been very helpful in understanding the maladies from malaria to cancer (Whitesides, G. M. 2006). In purview of cell mechanics, microfluidic system—cells on chip— has been proven as an outstanding tool for mimicking in vivo conditions and controlling biophysical cues; enabling us to study cell behaviour (Vanapalli SA., et al, 2009). Not just in 2D but recent advances in the culture of cells within 3D contexts has enabled us to begin to model more complex behaviours and tissue functions (Eyckmans, J. and Chen, C.S., 2017). Emerging field of organ-on-a-chip or body-on-the-chip: from developmental angle and physics angle. With development of more sophisticated 3D cell cultures, the effort is being made to engineer patient specific drug treatments and eliminate animal use in drug testing (Ingber D., 2018). Leading to development of Human ‘organs on chips’, which are microfluidic devices with separate parenchymal and vascular compartments lined by living human cells that mimic the multicellular architecture and relevant functional units of living organs, while providing dynamic vascular perfusion in vitro. This multi-channel design technology was successfully used to create human lung small airway, skin, kidney, intestine, placenta, blood-retinal barrier, blood-brain barrier, neurovascular unit and neuromuscular unit, among others (Kim et al., 2012; Achyuta et al., 2013; Abaci et al., 2015; Benam et al., 2016a; Musah et al., 2017; Yeste et al., 2017; Workman et al., 2018; Kasendra et al., 2018; Sances et al., 2018; reviewed by Bhatia and Ingber, 2014). Also, these chips showed their application in drug testing and patient specific treatment (more reference). Moreover, these chips have potential for giving insights in developmental and disease mechanics. As our understanding goes that mechanical forces govern cell and tissue development, it is crucial to recreate organ level physical cues, like in the case of the lung involved surface tension at an air-liquid interface, as well as both fluid flow through the vasculature and cyclic mechanical stretch of the tissue-tissue interface due to breathing motions (Huh D., et al., 2010). These chips could also be used in the modelling cancer by imaging with high-resolution the complex interactions between multiple cancer-associated cell types and ECM molecules that are found in the local tissue microenvironment. For example, in an another version of this chip that uses a heterotypic co-culture approach including up to three different cell types (breast cancer cells, stromal cells and monocytes) in combination with gene expression analysis uncovered mechanism of cell types that interacts through paracrine signalling through production of transforming growth factor-β (TGFβ) by breast cancer cells and expression of corresponding receptors by stromal cells (Regier, M. C. et al. 2016). In developmental biology, it has been observed that culturing iPSC-derived motoneurons and brain microvascular endothelial cells together on a chip model the neuromuscular unit with significantly enhanced function and in vivo-like maturation of spinal cord neural tissue (Sances et al., 2018). This opens new opportunities to in developing current view of self-organization and embryo functions. What Is to Be Done? Burning Questions of Our Movement It is very clear that the mechanics and biology of the epithelial tissues is complicated; intertwined by mechano-chemical signalling; and multiscale in its behaviour. The lens of active material has been very helpful in providing information on role of molecular elements in performing biological function. These studies have also led to observe emergent behaviours which would be impossible to see in vivo. The mechanistic understanding has been enhanced with newer mathematical tools and advanced microscopy; enabling us to measure forces involved in the tissues Biomimetics? What remains? Engineering in biological systems have 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 constructions of new material or engineering systems. At many instances, where new materials have been fabricated inspired by biological matter. Also, improved understanding of biological systems has provided new methods for creating organically optimised systems. Biomimetics is a field where nature continuously inspires human innovation: from hydrophobic surfaces to supersonic passenger plane! 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 strength, adaptability, flexibility, and functionality aspects of the material. What we want to do? The principles which governs tissue form and function are very important; on two fronts. First, to understand fundamental physical rules of the biology, and secondly 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; giving us opportunity to test tissues from material science point of view. This specific probing allows us to comprehend intricate mechanism of generation of forces, and shape change at cellular and tissue level. Using microfluidic setup, we subject tissues to unravel emergent phenomenon at different spatial and temporal scales.

# Methods

# Results

# Paper

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 of the surrounding matrix as well as hydraulic pressure from the lumen.

These stresses are applied on 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 a 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.