Mechanics of epithelial layers subjected to controlled pressure and tension

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Contents

0.1 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, we developed a microfluidic chip and a computational framework to engineer 3D epithelial tissues with controlled shape and pressure. In the setup, an epithelial monolayer is grown on a porous surface with circular low adhesion zones. On applying hydrostatic pressure, the monolayer delaminates into a spherical cap from the circular zone. This simple shape allows us to calculate epithelial tension using Laplace's law. Through this approach, we subject the monolayer 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 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 allow us to pattern epithelial folds by rationally directed buckling. Our study establishes a new approach for engineering epithelial morphogenetic events.

0.2 Preface

0.3 Aims of the thesis

0.3.1 General aim of the thesis

Study the mechanics of epithelial layers subjected to controlled pressure and tension.

0.3.2 Specific aims of the thesis

General aims are divided into specific goals:

1. Review the literature on engineering epithelia, and effect of physical forces such as pressure and tension on morphogenesis.
2. Develop a new technology to control shape, size, and forces in three dimensional epithelial monolayer.

a. Invent a microfluidic system for forming domes, blisters like epithelial structures, with aid of controlled lumen pressure and protein patterning.
b. Verify the functioning of the device.
c. Optimize an imaging technique to capture fast dynamics of large epithelial structures enabling its rheological characterization.

3. Study the rheology of pressurized epithelial structure.

a. Obtain the material response of the tissue by subjecting different regimes of cyclic pressure.
b. Analyse the stress and strain relation and understand it in context of computational framework developed in close collaboration by Adam Ouzeri.

4. Investigate the buckling phenomena

1 Introduction

1.1 Epithelial tissues

1.1.1 What is an epithelial tissue? And what does it do?

Epithelial tissues are cell sheets with strong intercellular bonds that form physical barriers for major organs (lung, skin, intestine, etc.) It protects said organs from external physical, chemical, and microbial onslaughts. Besides protection, the main functions of epithelial cells include secretion, selective absorption, transcellular transport, and detection of sensation (Powell, D.W., 1981). It also plays a key role in developmental stages of by acting as growth support and driving critical shape changes. (Figure 1: Epithelial Caco2 cell monolayer labelled for a tight junction protein in blue and nuclei in red. CellBiol_MRCLMB)

1.1.2 Physiological function in the body and polarity of the tissue

Epithelial cells are polarized, i.e., their apical side, facing the lumen of the organ, differs in shape and composition from the basolateral side. Its polar organization is reflected in the vectoral functions like transporting epithelia such as those of the renal tubule, absorptive epithelia such as those of the intestine, and secretory epithelial cells such as the hepatocytes are typical examples of epithelia that create and maintain concentration gradients between the separated compartments (Simons, K. and Fuller, S.D., 1985). In addition to this, polarized epithelia guide cell fate specification in development (Kim, E.J.Y., Korotkevich, E. and Hiiragi, T., 2018). (Figure 2: Polarized epithelia adapted from Chen, Jia, et al. 2018)

1.1.3 Introduction to different components

Epithelial function primarily depends on tissue's structure and microenvironment. It can be described completely in three parts: first cell structure; second cell connection; and lastly microenvironment.

1.1.3.1 Cell structure In general, cell structure helps cells maintain the shape along with providing mechanical support to perform vital functions like division and migration. This structure is known as cell cytoskeleton. It includes different components, playing various roles together. For eukaryotic cells, it is constructed out of filamentous proteins to support the cell and its cytoplasmic constituents. There are three major filaments, which differ in size and protein. Microtubules are the largest type of filament of protein tubulin, with a diameter of about 25 nanometers (nm). Actin filaments are the smallest type, with a diameter of only about 6 nm. Finally, Intermediate filaments are mid-sized, with a diameter of about 10 nm. Unlike actin filaments and microtubules, intermediate filaments are constructed from several different subunit proteins. These filaments dynamically alter themselves in reaction to signals from microenvironments and cell networks (Alberts, B., et al., 2013; Fletcher, D.A. and Mullins, R.D., 2010). 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 nonlinearly stiffening response (reference to the figure) (Janmey et al., 1991; Mofrad, M.R., 2009). In case of epithelial layers, actin cytoskeleton in conjunction with intercellular junctions makes stronger cell-cell contacts and creates a tissue level integrity (Braga V. 2016). The perfect example of these tissue level structures can be seen in wound healing assays; where a wound is created in epithelial monolayer, and 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 case of circular islands of epithelial cells showed radial patterns in the actin organization (Jalal S., et al., 2019).

1.1.3.2 Description of cell-cell junctions

A 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 wit h intermediate filaments and the resulting supracellular network confers mechanical resilience on cell layers (Hatzfeld, M., Keil, R. & Magin, T. M. 2017; Latorre, E., et al., 2018). Tight junctions (TJ) perform a barrier function and enable the transport of ions across epithelial layers to be actively regulated. This plays an important role in the control of fluid pressures in tissues. Together, adherens junctions, desmosomes and tight junctions are the major mediators of epithelial cell–cell adhesion and their regulation enable emergent behaviors in cell sheets that are not observed in single-cell systems (Figure reference here) (Trepat, X. and Sahai, E., 2018; Ladoux, B. and Mège, R.M., 2017).

1.1.3.3 Extracellular matrix

The cell surroundings or a substrate on which cells are adhered on is called extracellular matrix (ECM); This substrate is interchangeably referred to as matrix/cellular microenvironment. ECM serves many functions: it endows a tissue with strength and resilience 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). In many respects, therefore, it is the ECM that regulates cell shape, orientation, movement and overall function. Cells and ECM have a symbiotic relationship between each other from signalling cues to various sensors on the cell surface. These cues are primarily sensed using integrins and focal adhesion complex in cell-substrate adhesion (Kechagia, J.Z., Ivaska, J. and Roca-Cusachs, P., 2019). (Figure reference) Thus, triggering complex molecular processes which are required to maintain homeostasis and strongly affect processes in development or tumorigenesis (DuFort, C. C., Paszek, M. J. & Weaver, V. M. 2011; Northey, J. J., Przybyla, L. & Weaver, V. M. 2017). ECM is a fibrous network of proteins, from mechanical perspective, the three primary structural constituents of the ECM are typically collagen (the most abundant protein in the body), elastin (the most elastic and chemically stable protein) and the proteoglycans (which often sequester significant water as well as growth factors, proteases, etc.). Due to its water content, deformation of ECM can produce cracks in epithelial layers. ECM acts as a poroelastic material soaking water upon stretch (like sponge) and releasing it under compression causing hydraulic fracture effect (Casares, L., et al, 2015). Moreover, collagen remodels itself under influence of cells aiding in migration or under stress (Shields, M. A., et al., 2012; Humphrey J D, 2003). Like most of the cytoskeletal proteins, most extracellular components turnover continuously, albeit some very slowly. For example, collagen in the peridontal ligament appears to have a half-life of the order of days, whereas that in the vasculature may have a normal half-life of 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).

1.1.4 Epithelial Homeostasis

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 turning over rates in the body. The entire gut cell lining turns over in 3-4 days. This turn over implies constant cell division and death. The excessive rate of division than the death may give rise to tumors. It is known that 90% of cancers emerge in simple epithelia (Torras, N. et al. 2018; Eisenhoffer, G.T. and Rosenblatt, J., 2013). Not only this but it could easily disrupt the barrier function as no gaps should emerge around dying or dividing cells. There is a spectrum of distinct disease states that have in common their effect of breaking down epithelial and/or endothelial barrier function.

1.1.4.1 Disease and Cancer from leaky barriers If the fluid compartmentalization goes awry, it has profound implications for epithelial and stromal homeostasis, fluid and/or electrolyte balance, generation of inflammatory states, and even tumor microenvironment. There are number of bacterial toxins known to target junctions and cause alteration in TJ protein ZO1 which leads to compromised barrier function and causing pathologies like diarrhea and colitis (Fasano, A. et al. 1991). Cancer cells have tendency to spread and disperse metastatically, by using their very high rate of cell motility and diminished sense of cell adhesion. This elimination and/or reduction of TJ barriers in cancer are essential to allow metastatic cells to break in and out of blood vessels. Leaky barrier also allows a growing epithelial tumor an additional source of nutrients from luminal fluids (Mullin, J.M. et al., 2005).

1.1.4.2 Developmental disorders Epithelia undergoes changes in shape with deformation and reorganization from embryo to adult stage. It is unsurprising that any improper function would lead to damage and disorder. Like in case of Epithelial–mesenchymal transition (EMT), a developmental process when epithelial cells gradually transform into mesenchymal-like cells by losing their epithelial functionality, which is involved in the pathogenesis of numerous lung diseases ranging from developmental disorders, fibrotic tissue remodeling to lung cancer. Another example, Bronchopulmonary dysplasia (BPD) is 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 on a very immature lung (Bartis, D. et al., 2014).

1.2 Epithelial mechanics

1.2.1 Cell as a mathematical integrator

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)

1.2.2 Complex emergent behaviour and matrix interaction

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)

1.2.3 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).

1.2.4 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).

1.2.5 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).

1.2.6 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).

1.3 Morphogenesis

1.3.1 Embryonic development and functional organs

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 (Put some references for each structure). Different epithelia acquire diverse morphological forms appropriate for their specific functions, such as the thyroid follicles, the kidney tubules, the interconnected bile canaliculi and sinusoids in the liver, and the complex branching structures found in the lung and salivary glands (Gumbiner, B.M., 1992) (Figure reference here). Owing to its multifaceted regulation and hierarchical organisation, epithelial morphogenesis is a complex phenomenon dependent on factors at multi spatial-temporal scales.

1.3.2 Local and global changes

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

1.3.3 Introduce form, and function

At the end of the day, all cells come from cells ('omnis cellula e cellula') (Virchow RLK 1858), all tissues come from cells that contain essentially the same genetic information. Nonetheless, every tissue exhibits a distinct architecture and function (Figure reference). One could ask many questions from here, as how form-function work in synchrony? How organisation is triggered physically? Reductionists would ask whether function follows form, or it is other way around. As per the twentieth century architecture principle of "Form Follows Function"; where the organisation 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 compartmentalisation and an open and 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 reference). One should easily reach a conclusion that there are more things involved in understanding dialectics of form and function (Figure reference). This was a subject of D'Arcy Wentworth Thompson's classical text "On Growth and Form" (Thompson, 1917). Thompson tries to explore biological forms during development and across evolution with considering geometric and physical constraints. After more than 100 years of its publishing we can answer more specific questions about shape and function using advances in bioengineering and microscopy.

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

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

1.4 Engineering and Measurement

1.4.1 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.

1.4.2 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.

1.4.3 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.

1.4.3.1 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.

1.4.3.2 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 the 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).

1.4.4 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). For 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).

1.4.5 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.

1.4.6 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).

1.4.7 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-beta (TGF beta) 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

1.4.8 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.

1.4.9 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.

2 References