Engineering tools for quantifying and manipulating forces in epithelia

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The integrity of epithelia is maintained within dynamic mechanical environments during tissue development and homeostasis. Understanding how epithelial cells mechanosignal and respond collectively or individually is critical to providing insight into developmental and (patho)physiological processes. Yet, inferring or mimicking mechanical forces and downstream mechanical signaling as they occur in epithelia presents unique challenges. A variety of *in vitro* approaches have been used to dissect the role of mechanics in regulating epithelia organization. Here, we review approaches and results from research into how epithelial cells communicate through mechanical cues to maintain tissue organization and integrity. We summarize the unique advantages and disadvantages of various reduced-order model systems to guide researchers in choosing appropriate experimental systems. These model systems include: 3-D, 2-D, and 1-D micromanipulation methods, single cell studies, and non-invasive force inference and measurement techniques. We also highlight a number of *in silico* biophysical models that are informed by *in vitro* and *in vivo* observations. Together, a combination of theoretical and experimental models will aid future experiment designs and provide predictive insights into mechanically driven behaviors of epithelial dynamics.

I. INTRODUCTION

This paper reviews approaches for inferring and applying forces while observing epithelial dynamics. Our ultimate goal is to help researchers close the loop between *in silico* and experimental (*in vivo* and *in vitro*) biophysical models for both understanding and predicting collective epithelial cell behaviors. Section I motivates the need to understand cellular migration, the role of mechanosignaling in its regulation, and the need for mapping *in silico* biophysical models onto *in vivo* and *in vitro* experiments. Section II introduces a range of *in vitro* reduced order models from 3-D tissues to single cells in controlled environments and how we can obtain new insights at these different scales (Fig. 1). Section III reviews the three most common classes of *in silico* biophysical models and their benefits and limitations. Finally, Section IV discusses how these experimental and *in silico* biophysical models can be integrated and inform each other.

I.A. Properties of epithelial dynamics

The collective properties of epithelia define their abilities to perform tasks integral to life including tissue growth and development[1–10], wound repair[11–14], nutrient absorption[15], and preventing pathogenic invasion[16]. As an active material, the epithelium and its collective properties are defined by changes at the cellular level. These collective properties are a combination of cellular movements within the tissue, cell renewal, cell death, and inter/intracellular tensions[17–20]. For example, epithelial migration patterns facilitate changes in cell division and cell death necessary for tissue morphogenesis[14,21,22]. In the healthy intestinal epithelium, migration from the intestinal crypt drives cellular extrusion at the top of the villus[22]. In zebrafish injury, significant cell migration, proliferation, and extrusion are observed to occur simultaneously during wound repair[14]. For many processes that dictate epithelial dynamics, collective cell migration is a reoccurring trait that defines the physical state of the epithelium.

Development. The controlled collective movements of epithelial cells are necessary for the growth and formation of tissues during development[1–10]. Embryonic processes including gastrulation, epiboly, oogenesis, and convergent extension that require epithelial migration are seen ubiquitously across different model organisms. During gastrulation, the migration of cells drives furrow formation and creation of germ layers[2,3]. During epiboly, epithelial cells spread in a coordinated manner to cover the entire embryo[5]. During convergent extension as portions of the embryo change shape or elongate, cells must actively migrate and rearrange to mitigate tissue deformation and maintain tissue integrity[6–10]. Even after development, epithelial migration continues to be essential for the homeostasis of mature tissues across all organisms.

Homeostasis. Adult tissue is often thought of as an immobile epithelium that provides functions like filtration of waste products or nutrients while also acting as a barrier to infectious agents. However, continuous cell renewal and collective epithelial movements are critical for regulating many aspects of adult tissue. The repair of epithelial damage (i.e., wounds) occurs both internally (e.g., lungs, gut) and externally (e.g., skin) and requires the coordinated movement of epithelial cells[11–14]. Without proper epithelial homeostasis and wound repair, our organs are at risk of pathogenic invasion, poor nutrient absorption, and ultimately organ failure.

Dynamic Signaling. Collective cell migration can be prompted by various cues: directional fluid flow[23], chemical (chemotaxis)[24], physical (plithotaxis)[25,26], bio-interface (haptotaxis)[27], substrate stiffness (durotaxis)[28], and even electrical (galvanotaxis)[29]. These migratory regulators, or taxis processes, have been extensively reviewed[27,28,30–33].

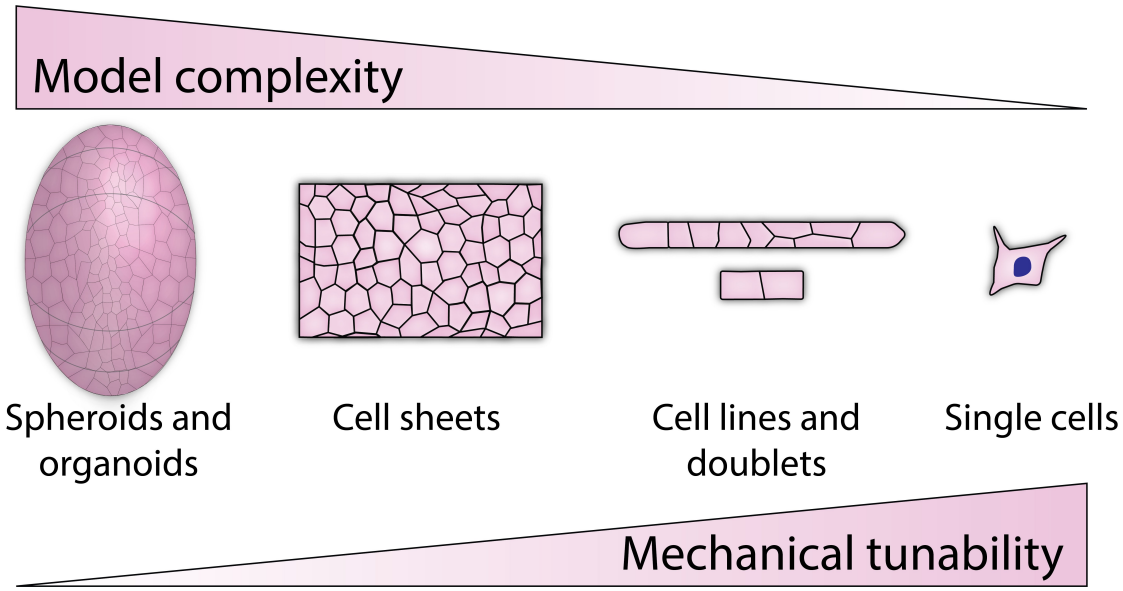


Figure 1. *In vitro* experimental models of varying complexity used to study collective epithelial mechanics. (left) Spheroids and organoids are the most complex *in vitro* models. Cell sheets and cell lines are amenable to engineering interventions that allow researchers to investigate the impact of external forces on the epithelium. (right) The simplest model, a single cell, can also be useful to infer properties of collective cell behavior.

However, existing studies on the relationships between mechanosignaling pathways and epithelial migration at the tissue level often neglect localized, intercellular mechanical forces and long range dynamic changes that can influence migration[34–36]. Whether in a developing tissue or adult tissue, the epithelial environment is not static. It is incredibly dynamic. Many mechanical forces occur within these environments in a localized or regionalized context via local tissue deformation. Developing tissues continuously bend, fold, constrict, or stretch, imposing local forces on groups of cells[8,22,37–45]. An open question is to what extent mechanical perturbations regulate cell migration, and if these resulting cellular movement patterns can be predicted *in silico*. An increased understanding of epithelial cell migration will be critical to predicting and controlling deviations from normal development and adult epithelial tissue homeostasis. Testing and validating these models requires new controlled techniques for applying mechanical perturbations *in vitro*.

I.B. Mechanosignaling in the dynamical epithelium

At the cellular level, epithelial cells exert forces on neighboring cells as well as on the extracellular matrix (ECM). Neighboring cells are "stitched" together by cell-cell adhesions, where homotypic binding of E-cadherins is thought to initiate binding of like cells. E-cadherins then indirectly link to junction-supporting F-actin bundles via intracellular intermediate proteins such as β-catenin and α-catenin[46], making up adherens junctions[46] (Figure 2a). Cadherin complexes are further stabilized by other proteins such as vinculin and p-120 catenin[47,48]. Mechanical cues can either release the catenin proteins from the adherens junction or recruit them, which have implications on the stability of cell-cell adhesion and cell behavior[49–52]. Cell-cell contacts are then stabilized by other junctions, e.g., tight junctions and desmosomes, which further reinforce or regulate cell-cell adhesion[53,54]. Mechanically linked cells also bind to the ECM via focal adhesion complexes and hemidesmosomes[55]. These complexes anchor to F-actin and other cytoskeletal filaments[56,57]. During migration, branching F-actin pushes the cell membrane forward within protrusions known as lamellipodia. The adhesion sites between cells and their substrate that guide lamellipodia formation are known to experience mechanical cross-talk. For example, the number of adhesion sites, types of integrin mediated adhesions (via different ECM proteins), or viscoelastic relaxation can all influence the persistence and speed of cell migration[27,28,58]. Many of these mechanisms operate through mechanotransduction pathways to indirectly modulate intracellular signaling. For example, branching F-actin can be regulated by rhoGTPases, including cdc42 and Rac1[59,60]. Lamellipodia at the front of the cell work in unison with actomyosin driven contractions at the rear to propel the cell forward with a lateral front-rear polarity (Figure 2b). While lamellipodia are most commonly observed on leader cells at the edge of an expanding epithelium, cryptic lamellipodia are produced by follower cells to chase leader cells[61,62]. As leader cells migrate, they can induce tension on follower cells to guide follower cell polarization via E-cadherin and Merlin[4,34]. However, cells behind leader cells can help determine leader cell migration[63,64]. Therefore, there exists an intricate network of mechanical regulation among cell-cell and cell-ECM interactions to guide cell migration.

Many of these mechanical complexes (e.g., intercellular tension, cell contractility and cell traction), along with shape descriptors (e.g., cell area and perimeter) can be used as in-

puts within *in silico* biophysical models. For example, factors that affect cell-cell and cell-ECM adhesion (e.g., cadherins, integrins, desmosomes, and hemidesmosomes) can be modeled as frictional forces[65,66]; cells must overcome these adhesions to migrate along the substrate. By manipulating these different variables experimentally, experiments and theoretical models can help inform one another to help researchers both understand and predict intercellular signaling and force propagation.

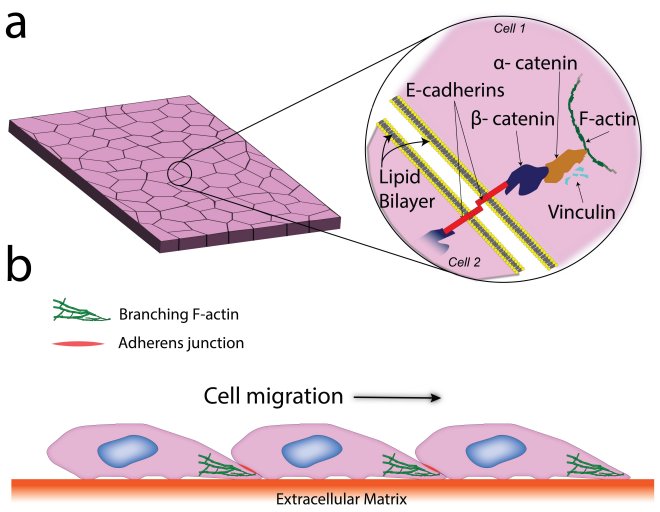


Figure 2. Mechanosignaling at the adherens junction aids in collective cell migration (a) The force-sensitive adherens junction has been shown to regulate mechanical forces between cells and dissipate them via intercellular signaling. (b) Cross-section of collectively migrating epithelial cells, which rely on adherens junction to mechanically link cells together and transduce signals.

I.C. Choosing an experimental model system: Setting the stage for *in vitro* studies

In vivo epithelial models have illustrated the necessary role for collective migration in tissue repair and development. These models most commonly include zebrafish (e.g., epiboly, gastrulation) and *Drosophila* (e.g., germ band extension, gastrulation, border cell migration, wing development) though other systems exist[67]. To isolate mechanistic elements associated with cell migration, observational studies often rely on genetic knockouts and pharmacological approaches combined with imaging techniques. Light microscopy has been particularly valuable. For example, fluorescent labels have helped researchers understand protein localization during specific developmental events, including the distribution of myosin during cellular movements in *Drosophila*[68]. More recently, several engineered interventions have helped identify the role of mechanical forces in *in vivo* epithelia[69]. For example, Förster Resonance Energy Transfer (FRET) sensors encoded into *Drosophila* revealed that mechanical tension through cell-cell contacts is critical in directing cell movement[4,70,71]. Oil and actuated ferrofluid droplets have been used to infer tissue forces in zebrafish using known mechanical properties of the droplets[72,73]. Recent advances in cell segmentation (i.e., reducing cell-cell borders to a polygonal packing structure) have offered a non-invasive method to infer forces between cells within *Drosophila* and quail[39,74–77]. Laser ablation, a more invasive technique, has helped validate these force inferences[77] (Figure 3b). Laser ablation can also be used to induce wounds to study collective cell dynamics during wound healing of the *Drosophila* pupae[78]. More recently, "synthetic mechanosignaling" tools (i.e., optogenetics) have been expanded to inhibit or facilitate protein function in a spatiotemporal manner. For example, optogenetically controlled RhoA has been used to manipulate mechanical forces during *Drosophila* tissue folding and morphogenesis[79,80] (Figure 3a).

Despite the physiological relevance of *in vivo* models, they are complex. Numerous interacting variables in the *in vivo* environment make it difficult to isolate the effect of mechanical forces on epithelial growth and migration. Specifically, it is difficult to directly connect cell-cell mechanics and local mechanical tissue deformations to tissue rheology (i.e., migration). Furthermore, *in vivo* biophysical studies are often expensive with experimental setups that may be difficult or elaborate. *In vitro* studies can supplement those done *in vivo* by providing information under more controlled conditions where single variables (e.g., traction force) are manipulated. Biophysical *in silico* models can then help by providing continuous feedback between theory and experiments.

In this review, we describe micromanipulation tools available to probe collective epithelial dynamics (with a focus on migration) in four scales of *in vitro* models: spheroids/organoids, tissue sheets, cell lines/doublets, and single cells. For each of these cellular structures, we review the following themes: i) methods to infer forces, ii) methods to apply forces (including controlling the biophysical environment and local vs. global mechanical manipulation), and iii) limitations/advantages of the model system. Finally, we discuss biophysical *in silico* models that can inform and guide our understanding of the experiments.

II. *IN VITRO* MODELS AND MECHANICAL MANIPULATION TECHNIQUES FOR THE STUDY OF COLLECTIVE EPITHELIAL MIGRATION

In vitro experiments allow control of boundary conditions and tissue geometry while quantitatively describing the relationship between spatiotemporal mechanical inputs and epithelial migration. A number of devices that allow the application of mechanical perturbations to an epithelium *in vitro* have been proposed and will be reviewed below.

II.A. 3-D spheroids and organoids

Spheroids and organoids are powerful 3-D *in vitro* models that offer the possibility of carrying out high-throughput assays in a controlled microenvironment. Many 3-D *in vitro* models are valuable for answering questions related to dis-

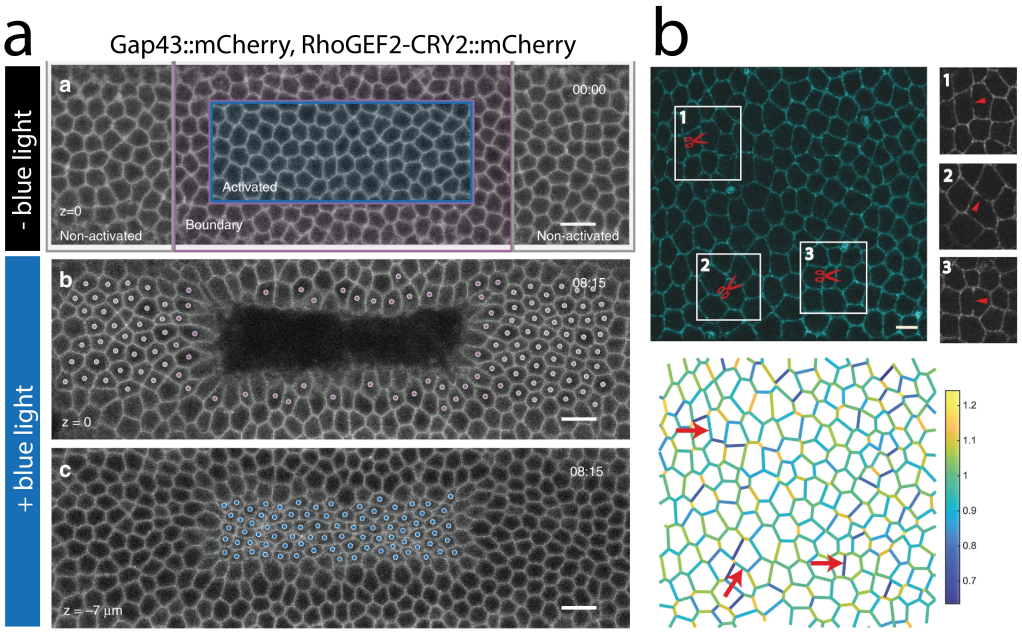


Figure 3. *In vivo* experimental models. (a) Optogenetic RhoGEF2 can be used to trigger apical constriction of specific cells in the *Drosophila* embryo. (b-top) Laser ablation of cell-cell junctions in the *Drosophila* notum has validated (b-bottom) segmentation inferred intercellular tension levels. Figures adapted from (a) Izquierdo, Quinkler, and De Renzis, Nature Communications. **9**, 1-13 (2018)[79] and (b) Kong *et al.*, Scientific Reports. **9**, 1-12 (2019)[77] under Creative Commons Attribution 4.0 International (CC BY 4.0). Scale bars: a= 10 μm, b= 5 μm.

ease or understanding tumor invasion (i.e., metastasis), phenomena extensively reviewed elsewhere[81–83]. Our interest is in how organoids have been used to study epithelial collective dynamics (i.e., migration and mechanosignaling)[84].

In vitro organoids are most commonly generated from stem cells that differentiate into clusters representative of the cell types found in organs, typically on glass or gel substrates (sometimes referred to as "bottom-up" assembly)[85–87]. Alternatively, spheroids are manufactured "top-down" either by mixing multiple cell types in a mold or by allowing a sheet of a single cell type to fold or aggregate into a 3-D structure[88–90]. Spheroid and organoid models have both been deployed to mimic healthy and pathological tissue microenvironments, reviewed here[91]. For example, stem cells can differentiate in 3-D to form an intestinal epithelium for disease modeling[85] or be directed to form an intestinal lumen through biofabrication methods[92,93].

II.A.1. Methods to infer force from 3-D spheroids and organoids

Inferring forces exerted or transmitted by epithelia help researchers connect mechanical cues to epithelia behavior. When considering how engineering tools can be implemented for 3-D *in vitro* studies to measure migration and mechanosignaling, we do not need to look much further than methods used *in vivo*. These shared methods for *in vitro* applications have answered a variety of questions on epithelial cell behavior. Additionally, they have utilized human derived cells. For example, cell segmentation via image analysis has been used to discover an increase in fluidity of human carcinoma epithelial cells during tumor progression[88] (Figure 4a). Other methods referenced earlier, including FRET molecular tension sensors[94] and oil droplets embedded in the tissue[90] (Figure 4b) are amenable to *in vitro* 3-D systems as well.

While both *in vivo* and 3-D *in vitro* epithelia consist of large multi-cellular tissues, *in vitro* environments offer several additional approaches for inferring cellular forces. Since organoids or spheroids can exist on a substrate or within an extracellular medium[95], traction force microscopy (TFM) or monolayer stress microscopy can be used to infer local cellular stresses to help researchers understand the forces that drive migration[96]. To perform TFM, gels such as polyacrylamide (PA) or polydimethylsiloxane (PDMS) are fabricated with embedded fluorescent microbeads[96,97]. Cells exert traction on the gels, resulting in measurable displacements of the beads, from which one can infer the forces or traction stresses that generate the corresponding displacements[98]. TFM is a particularly powerful force inference technique as it can be used to dissect the temporal and spatial variations of tractions associated with collective cell movements in different environments. For example, studies of ovarian tumor spheroids have shown that cancer cell clusters generate force to gain access to the submesothelial environment. By blocking force transmitting proteins (i.e., $\alpha 5\beta 1$ integrin, talin I and myosin II), researchers were able to block metastatic escape of ovarian cancer cells[99]. Furthermore, TFM can be combined with protein dysregulation methods to determine how specific proteins regulate epithelia traction and migration. For example, suppres-

sion of E-cadherin and induction of basement membrane expression influenced branching morphogenesis (i.e., migration) of stratified epithelia in a 3-D spheroid model[100]. An alternative form of TFM involves individual cells or whole spheroids grown on fabricated elastic micropillars[101,102], where pillar deflection provides a direct measurement of traction force. Additional methods include elastically compressed PA "force sensors" embedded *within* the tissue itself, which can connect local mechanical forces to changes in cell behavior. This method has shown that increased pressure near the core of a carcinoma spheroid is accompanied by cell shape anisotropy and minimal cell proliferation[103]. Custom tools have also been built, such as microtweezers, to measure the stiffness of organoids[102,104]. Methods that can quantify and measure mechanical stresses within 3-D epithelial models can help researchers understand the complicated relationship between intercellular forces and migration. Direct manipulation of the microenvironment can also provide information on causal relationships, as discussed in the next section.

II.A.2. Methods to apply force to 3-D spheroids and organoids

a. Engineering the biophysical microenvironment. There are several methods to engineer the microenvironment of a spheroid or organoid, including altering substrate stiffness, ECM composition, or geometric confinement. Each method may be used to mimic specific conditions (e.g., disease states) and connect these inputs to changes in collective epithelia behavior.

Tailoring substrate stiffness is one method that enables researchers to measure changes in tissue fluidity under specific pathological conditions. For example, altering the substrate rigidity of murine sarcoma spheroids resulted in different forms of motile behavior, ranging from a “running spheroid” to a flattened spheroid moving as a film[105] (Figure 4d). In other studies, tunable substrate stiffness can regulate collective migration via changes in tissue folding[106].

Changing the ECM is another minimally invasive way to alter the collective dynamics of a spheroid. Some studies have shown that the concentration of ECM can change the degree of migration of tumor spheroids[107]. In collagen for example, human mammary gland organoids show increased branching and migration[108].

Spheroids or organoids can be subjected to varying degrees of geometric confinement as well. By varying the spatial constraints, researchers can recapitulate a diseased or developmental microenvironment. For example, patterned substrates of different geometries have been used to regulate the tube size and folding architecture in the development of neural tube organoids[43](Figure 4c). Tailoring geometric confinement is commonly done using pre-determined geometric patterns of ECM for cell growth and migration. These shapes can be sized and designed for diverse studies ranging from single cells[109–111] to entire monolayers and tissues[25,112]. Several methods can be used to fabricate specific shapes or patterns of ECM. Popular methods include microcontact printing[113], co-polymerization via hydrogel “lift-off”[109], and light-induced photomolecular adsorption of proteins[114–116].

Beyond changing the substrate stiffness or the geometry of the microenvironment, cellular biomechanics can also be tuned by introducing biochemical reagents, pharmacological approaches, or gene editing[94,95]. For example, genetic mutation of force sensitive α-catenin revealed that tension at the adherens junction is critical to the formation of multi-cellular structures during epithelial spheroid development[117].

Organoid and spheroid models have largely focused on engineering the microenvironment of 3-D *in vitro* systems and measuring force generation, deformations, or fluidity. Thus far, there are limited engineered devices (e.g., cell stretchers) applied to loading or deforming epithelial spheroid or organoids[118,119].

b. Local force application. In vitro spheroids and organoids are also advantageous in that they allow local force gradients to occur naturally as they would *in vivo*. Relating local mechanical deformations to global cell migration or tissue rheology remains, however, challenging. The methods used to apply local forces to 3-D cellular structures are largely the same *in vivo* and *in vitro*. Optogenetic "synthetic mechanosignaling" has for instance been used to demonstrate the role of the WNT pathway in embryonic dynamics[120] and that of the Notch1 pathway in breast cancer cells[121]. Laser ablation has been used to relax tension on ECM fibers and reduce tumor invasion[89].

II.A.3. Advantages and limitations of 3-D spheroids and organoids

3-D *in vitro* multi-cellular structures provide important models for the study of *in vivo* mechanosignaling. They are cost-effective, relatively high-throughput, and offer control over cell types and the microenvironment. They retain, however, substantial complexity. Multiple cell types or self-organizing structures can make it difficult to introduce and isolate mechanical variables. Their large size can also hinder imaging accessibility and dissection at the molecular level. Overall, spheroid and organoid models are largely advantageous for modeling disease or cancer invasiveness, but don’t often re-capitulate collective migration seen in developing embryos or wound repair.

II.B. 2-D cell sheets

The reduced dimensionality of 2-D cell sheets allows researchers to introduce mechanical perturbations that control, limit, or enhance cellular movements (Figure 5). Within an *in vitro* 2-D environment, cells explore the XY plane typically on an ECM coated substrate made from a hydrogel, plastic, or glass. 2-D epithelial sheets also exist *in vivo* (e.g., lungs, kidneys, intestines), though may have different regions of folding and diverse topologies depending on the organ.

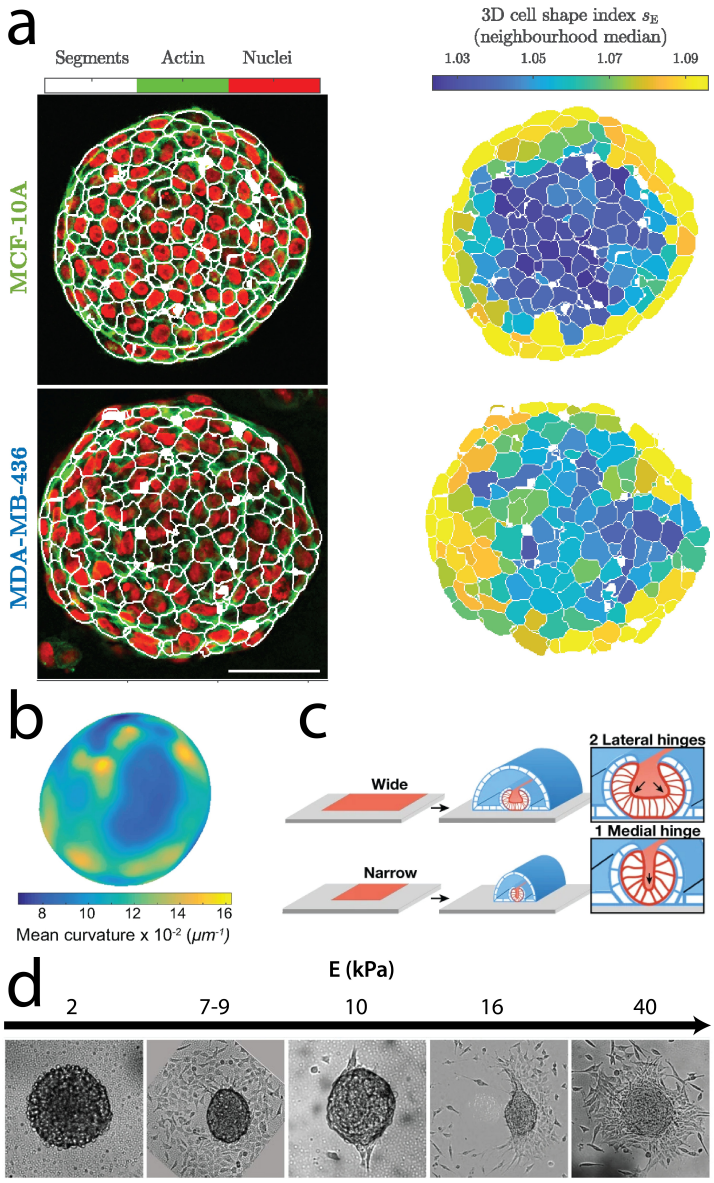


Figure 4. *In vitro* 3-D experimental models (a) Cell segmentation has been used to quantify cell shape and subsequent increase in tissue fluidity for cancerous (MDA-MB-436) compared to noncancerous (MCF-10A) breast epithelial spheroids[88]. (b) 3-D fluorocarbon oil droplets embedded in mesenchymal cell aggregates can map out the internal forces exerted by cells[90]. (c) To control organoid development, the underlying geometry of the ECM can be controlled[43] or (d) the stiffness to regulate tissue organization and cell migration[105]. Figures adapted from (a) *Grosser et al*., Phys. Rev. X. **11**, (2021)[88] and Lucio *et al*., Scientific Reports **7**, 1-11 (2017)[90] under Creative Commons Attribution 4.0 International (CC BY 4.0). Figure (c) adapted with permission from Karzbrun *et al*., Nature **599**, 268-272 (2021)[43]. Copyright 2021 Springer Nature. Figure (d) adapted from Beaune *et al*., Proc. Natl. Acad. Sci. U.S.A **115**, 12926-12931 (2018)[105]. Copyright 2021 National Academy of Sciences of the United States of America. Scale bars: a=100 μm

II.B.1. Methods to infer force from 2-D cell sheets

Just as in other experimental models, extensive information about cellular behavior can be extrapolated through time-lapse or video microscopy. In a 2-D system, however, researchers are able to answer questions about collective cell behavior in a more controlled environment with specific mechanical inputs. For example, wound healing assays are a common method to better understand cell migration. Open gaps or "wounds" in an otherwise confined system are most commonly created using physical barriers[34,122,123] or scratch assays[124,125]. Cellular migration in these 2-D systems can be imaged by wide-field fluorescence and bright-field imaging modalities (Figure 6a)[63]. Fluorescence microscopy combined with wound healing assays and genetic knockdown experiments has matched transcription factor (TF) regulation or activation of pathways to migration of cells. For example, mechanical tension between cell-cell contacts causes the release of junctional merlin

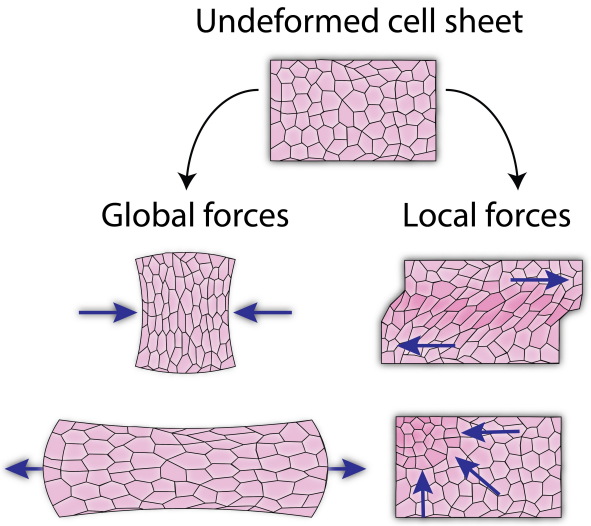


Figure 5. Global and local deformations of epithelia. Epithelial tissues experience a range of mechanical forces, some of which are (left) global and (right) local. Most engineered devices, including cell stretchers, have mimicked global forces (e.g., compression and tension) by applying a more evenly distributed strain field throughout the tissue using elastomeric substrates. Few studies have focused on local forces and their impact on epithelial collective behavior. These forces can originate from local regions of deformation (e.g., shear forces, tissue bending, or apical constriction).

to the cytoplasm, which activates Rac1 and subsequent lamellipodium formation for cell migration[34].

Time-lapse image stacks can be used to quantify cell migration and can also be combined with multiple force inference techniques that are more difficult in 3-D systems. Particle image velocimetry (PIV) is a widely used tool to quantify cellular movements by capturing the migratory "flow" of cells across the XY plane[35,126,127]. Images are broken down into an array of windows, each with a distinct arrangement of pixels that can be tracked between frames. PIV is especially useful in quantifying migration directionality and velocity. Combining migration tracking with TFM in expanding cell monolayers has revealed a highly heterogeneous spatiotemporal distribution of traction forces that extend throughout an epithelial sheet[64]. TFM combined with fluorescent live-cell imaging can further reveal how cytoskeletal filaments influence cell migration[128] or change structure as a function of mechanical tension at cell-cell contacts[129–131].

Beyond video microscopy used for TFM or PIV, segmentation tools are increasingly deployed to relate cellular structure to epithelia mechanics[76,132,133]. For example, cell morphology obtained from segmentation of tissue imaging has helped predict changes in cell tractions or cell movements during cell migration[63]. Shape descriptors derived from epithelial cells (e.g., area, perimeter, mean cell shape) can then be used as useful inputs for *in silico* biophysical models. Since segmentation can also be used to infer intercellular tension[134], combining tools such as segmentation and PIV provides powerful opportunities to connect the mechanical state of the cell-edge network and epithelial migratory behavior.

With imaging methods on an even smaller spatial scale, researchers can start to determine the proteins responsible for transmitting mechanical load during traction and migration. For example, E-cadherin FRET probes have shown E-cadherin is under tension as cell-cell contacts are actively stretched[135]. Interestingly, this tension seems to relax over time[129]. These same sensors have also shown E-cadherin tension is highest inside expanding monolayers compared to the leading edge, corroborating TFM data[64,128].

A more invasive method to infer force properties of cell-cell contacts is laser ablation. Laser ablation can also be used to induce wound closure for single cells (e.g., apoptotic induced extrusion), which can be used to assess the mechanics behind localized wound closure[21,136]. However, as mentioned in the previous section, laser ablation is invasive, requires specific equipment, and single cuts can affect the tension states of other regions of the tissue.

II.B.2. Methods to apply force to 2-D cell sheets

a. Engineering the biophysical microenvironment. Many methods exist to manipulate the microenvironment of epithelial sheets for the purpose of connecting mechanical inputs to collective epithelia behavior: geometric confinement, substrate stiffness, substrate topography, and ECM composition.

Geometric confinement within a 2-D environment provides a passive way to constrain a tissue and thereby manipulate interfacial tension on specific regions of cells. Geometric confinement via protein micropatterning indirectly applies edge tensions and intercellular tensions to cells which can influence their collective dynamics. For example, protein shapes designed for higher amounts of interfacial tension (e.g., star vs. circle) have been shown to control locations of cellular extrusion events within the tissue via changes in nematic order[21]. Furthermore, protein patterns have regulated migratory oscillations and proliferation patterns as a function of geometric confinement[112,137,138].

Beyond regulating the geometric confinement to control the collective motion of cellular sheets, the stiffness of the underlying substrate can also be tuned by creating hydrogels of varying polymerization parameters[38,139]. Different stiffnesses can be used to mimic specific disease states and correlate these conditions to changes in the expansion of an epithelium. Physiological stiffnesses of epithelia are generally reported in the range of 1-500 kPa (depending on the tissue or if malignant)[140,141] accompanied by changes in collective cell dynamics (e.g., stiffness and migration[142]). Stiffer substrate conditions can also regulate the rate of wound closure by increasing actomyosin dynamics, YAP activation, and subsequent migration rates[143–145].

One can additionally engineer substrates with nanofabricated topography to investigate substrate based mechanotransduction (e.g., microgrooves[146]) of expanding or confluent epithelial sheets. Substrate topography has been shown to increase the persistence of migration of MCF10A epithelial cells[147], which is likely influenced by their ability to re-orient

and elongate along the direction of the groove[148]. On a larger scale, the topography of the ECM has been altered to have curvature (i.e., hills and valleys) on the order of microns[149], which has implications for tissue thickness and nuclear morphology.

Studies have also manipulated collective cell migration by tuning the underlying ECM. Different ECM proteins (e.g., laminin, fibronectin, collagen, and their various sub-types) have different binding domains that connect to various integrin α and β subunits of cells. These integrin sub-units subsequently regulate cell migration differently, as quantified in an expanding 2-D epithelium. For example, fibronectin has been shown to enhance migration and alter epithelial organization in both developing and adult tissues[150–152]. In another study, fibronectin *gradients* have been attributed to directional cell migration, with implications for cellular collectives[153]. ECM concentration has also been shown to affect migration speed of epithelial keratocytes, where cell collectives had a lower velocity on denser collagen networks[154].

The way in which researchers modify the biophysical environment, whether it be via ECM modification or altering substrate stiffness, will largely depend on the questions at hand. 2-D epithelial models enable a large combination of force inference techniques such as traction force microscopy or migration analysis within a confined or expanding sheet.

b. Global force application. To directly study the effect of cell-cell mechanics on collective cell migration, engineers have designed and utilized novel systems such as elastomeric cell stretchers. These polymer stretching devices are made from commonly used elastomers (i.e., PDMS or silicone rubber) and when stretched are assumed to apply a homogeneous, global strain field across the *entire* tissue. Cell stretchers can provide a useful approach for tackling a number of open questions in epithelia mechanics. Future studies may for instance examine temporal variations, downstream effects on cell migration, or employ different substrates, such as ones based on viscoelastic materials.

Cell stretchers can vary in several ways, starting with their actuation methods. Stretch actuation can be induced pneumatically via vacuum[155–157], mechanically[158–160], or through a dielectric elastomer actuator[161,162] (Figure 6b and 6c). Furthermore, cell stretchers can induce either uniaxial or biaxial strain on a tissue in a static or cyclic dependent manner. Some have been 3-D printed[159,163] or have been easily fabricated[133,158] to improve accessibility. They have even been commercialized (*Flexcell International Corporation, Red Dog Research, Strex Cell*). Several reviews have been written solely on cell stretching devices[164,165]. Here we will focus on studies that have examined the role of stretch in regulating epithelia organization and intercellular signaling within a 2-D sheet.

Uniaxial tension: The simplest uniaxial stretchers apply a static stretch to the substrate and enable the observation of the tissue response. These methods may be useful to gain fundamental insight into how epithelial cells handle mechanical load. For example, uniaxial tension has connected force sensitive proteins at cell-cell contacts to mitotic activity and division along the axis of stretch[155]. It has also shown that mechanical strain on E-cadherin drives the initial stages of the cell cycle via β-catenin and YAP activation[156,166]. In addition to demonstrating the importance of direct cell-cell linkages, such experiments have established that stretch activated ion channels such as Piezo1 influence cell division, causing phosphorylation of the ERK pathway and driving cells into mitosis[167]. Intercellular tension can also orient the mitotic spindle during cell division[155,168]. Taken together, preferential division along the long axis of stretched cells[155] may be an adaptive mechanism for epithelial cells to combat external mechanical stresses[169] and facilitate tissue elongation and renewal. Beyond the role of static stretch in epithelial cell division, static stretch has been shown to limit epithelial cell migration[133]. However, this migratory response can depend on the cell type, as stretch increased directed migration of fibroblasts[170]. Additional experiments will help elucidate the mechanisms of static stretch in collective epithelial behavior.

Devices with more complex actuation methods (e.g., vacuum controllers[171] or innovative systems built from LEGOs^{TM}[158]) have been engineered to study the role of cyclic stretch on epithelial behavior. Studies have shown that during cyclic uniaxial stretch, cell orientation changes periodically in intestinal epithelial cells[172] and endothelial cells[173,174]. This phenomenon has been observed in fibroblasts as well[175,176]. On a molecular level, changes in cellular reorientation have been correlated with restructuring of the cytoskeleton[173,177] or to the growth of focal adhesions in osteosarcoma epithelial cells[178]. Interestingly, cyclic stretch has shown minimal effect on nuclei positioning or nuclei orientation[158]. Stretch-induced reinforcement mechanisms, including cell division and cytoskeletal restructuring, are, however, limited in their capacity to maintain epithelia integrity, as the tissue eventually ruptures when subjected to high stretch amplitudes and frequencies[179].

Biaxial tension: Biaxial cell stretchers can apply uniform strains resulting in uniform tissue growth and may be more physiologically relevant for certain tissue types (e.g., lung). Most studies employing biaxial stretch have focused on cyclic protocols. It was found that, while both cyclic uniaxial and biaxial stretch induce gap formation[179,180], biaxial cyclic stretch can also increase the ability of epithelial monolayers to withstand chemical disruption, as seen with human pulmonary artery endothelial cells[180]. Cyclic stretch under physiologically relevant stretch also induced a quicker recovery of gaps/wounds compared to higher pathological stretch, potentially due to increased activation of Rac[181]. The variations in the ability of epithelial wounds to form or repair may also be associated with the magnitude of applied stretch or the density of the epithelium itself[179].

Cell stretchers continue to be optimized for higher imaging resolution[160] and fabrication accessibility[133,158,159,183]. In addition to cell stretchers that exert tension, devices that apply both uniaxial and biaxial compression to manipulate cell packing and extrusion have also been developed[158,159,184]. Devices have been custom engineered to probe the mechanics of suspended epithelia[185] in either compression[186] or tension[185]. Unlike traditional cell stretching studies, suspended monolayers are devoid of cell-ECM interactions and

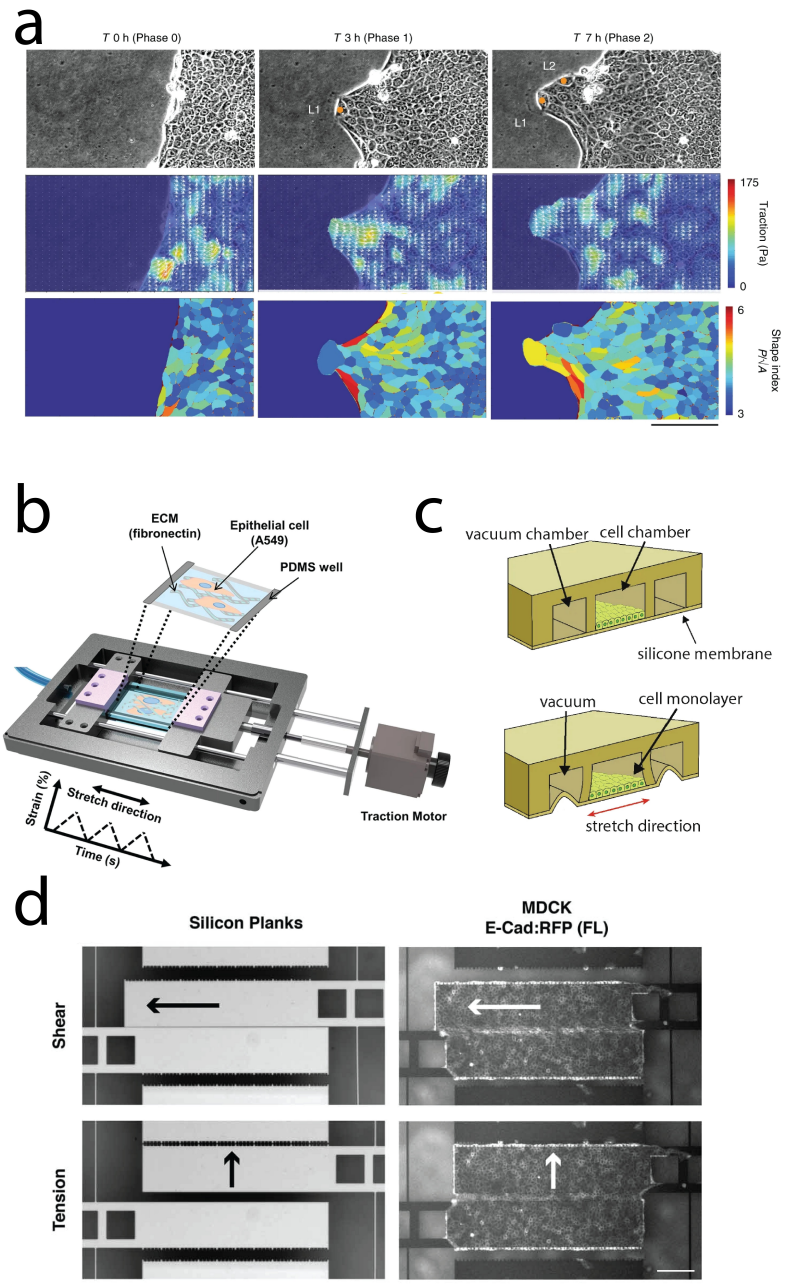


Figure 6. *In vitro* 2-D experimental models. Several techniques have been used in conjunction with light microscopy to quantify migration and force exertion by migrating epithelial sheets. (a) An expanding MDCK epithelium in brightfield (top) can be combined with TFM (middle) and segmented (bottom) to correlate changes in physical changes in cell shape with cell migration[63]. When applying external forces to epithelia, cell stretcher can be actuated (b) mechanically via motors[174] or pneumatically[133]. (d) More limited studies of *local* actuation include an MDCK epithelia grown on a microfabricated silicon platform, where the center of the epithelium can be subjected to either a mid-plane tension or shear strain to induce collective migration[35]. Figures adapted from (a) Vishwakarma *et al*., Nature Communications **9**, 1-12 (2018)[63], (b) Roshanzadeh et al., Scientific Reports **10**, 1-14 (2020)[174], and (c) Hart *et al*., Cell. Mol. Bioeng. **14**, 569-581 (2021)[133] under Creative Commons Attribution 4.0 International (CC BY 4.0). Figure (d) adapted with permission from Garcia *et al*., J. Micromech. Microeng. **30**, (2020)[182]. Scale bar: a= 100 μm, d= 200 μm

can be used to isolate the effect of cell-cell interactions on epithelia behavior. Mechanical stretch of a suspended epithelium has validated that stretch causes division along the long axis rather than along the monolayer stress axis[187]. When subjected to compression, a suspended epithelium buckles in a manner reminiscent of an elastic material, but recuperates tension in an actomyosin dependent manner[186]. Of course, the limitation of studying suspended epithelia is its lack of cell migration. An open question moving forward is how observed changes at cell-cell contacts correlate to changes in collective cell migration throughout the tissue.

Devices that apply global forces to epithelia have been instrumental in unlocking how cell-cell contacts respond to mechanical load, but more local approaches are needed to dissect how these mechanical loads are propagated through an epithelium.

c. Local force application. Engineering a method to apply local forces to an epithelium can be difficult, though necessary to recapitulate local forces *in vivo* that regulate epithelia behavior. Existing strategies to apply local forces to an epithelium have utilized tunable substrate stiffness, protein micropatterning, high precision micromanipulation tools, and more recently "synthetic mechanosignaling" approaches. These strategies have been used, and continue to be innovated, to answer questions around how local mechanics influence epithelial force transmission and collective migration.

In a transition from global to local mechanical inputs, gradients of substrate stiffness can be applied to cell collectives using methods such as light-induced gel polymerization[188–190]. Such stiffness gradients exist *in vivo*, for example in tumor development[191]. By mimicking these mechanical gradients *in vitro*, researchers can determine how regional cues transmit signals to influence intercellular signaling. For example, controlled *in vitro* studies have revealed that cell migration can occur in a stiffness dependent fashion[192], the basis for durotaxis. Cells migrate towards regions of higher stiffness but can also influence long-range intercellular force transmission[193]. There are several helpful reviews of migration on stiffness gradients[28,30,33,194].

Protein micropatterns with defined gaps may be used[195–197] to answer questions surrounding protrusion dynamics of lamellipodia or to understand epithelial gap closure (i.e., wound healing). Though, there are limited studies using this method to explore local stretch on regulating collective migration. Other local stretch methods have been attempted for epithelial sheets using elastomeric substrates with “trenches”[63] on an expanding (i.e., nonconfluent) epithelium.

Micromanipulation tools, including microfabricated platforms, have also been engineered to directly apply local boundary forces to an epithelium. By adjusting the mechanical inputs (e.g., strain) and subsequently observing epithelial behavior (e.g., signaling or migration), researchers can understand how mechanical forces are transmitted between cells. Many of these tools are exclusively compatible with 2-D epithelial sheets. For example, microfabricated silicon devices have been used to exert a local shear or tension on an epithelium as observed in tissue morphogenesis[35,182](Figure 6d). Following local shear strain, cell collectives actively migrate towards the shear zone in an oscillatory manner for several hours after the mechanical perturbation. To understand which protein complexes facilitated the collective migratory response, the authors combined their micromanipulation methods with an E-cadherin mutant cell line and pharmacological approaches. Unlike the global mechanical strain induced by cell stretchers that have caused epithelial cells to re-orient or divide, local boundary mechanical strain has found that the epithelium may dissipate mechanical stresses via collective and directed cell migration. Other micromanipulation methods have investigated the importance of local mechanics in more specific applications (e.g., disease). For example, pneumatically actuated PDMS chambers were used to apply a local mechanical stress on regions of a retinal pigment epithelium[198] to assess changes in the progression of choroidal neovascularization. Various other micromanipulation methods exist that are capable of applying local forces to epithelial sheets, (e.g., micropipette aspiration, magnetic twisting cytometry, and atomic force microscopy) though are largely confined to single-cell force applications and rarely used to investigate downstream collective epithelial migration or other dynamics. Furthermore, many of these techniques have been exclusively used to characterize mechanical properties of the *static* epithelium and not as a means to study the *dynamic* epithelium.

The last form of manipulating mechanical inputs on an epithelial sheet involves local "synthetic mechanosignaling" via optogenetics. Optogenetics advances in the last decade allow both spatial and temporal activation of force-sensitive proteins within epithelial sheets[199], which are more amenable to quantifying migration. These experiments allow researchers to cross new mechanical inputs between experimental and *in silico* models and determine their effects on collective epithelial behavior. For example, local control of RhoA has been shown to remodel cell-cell junctions[80] and alter cell shape[200]. Light-activated RhoA can also control cell traction and subsequent activation of transcriptional regulator YAP[199], a key player in both cell proliferation and migration. Other proteins that induce apical constriction (e.g., Shroom) can induce folding of an epithelial sheet[201]. Aside from contractility based optogenetic tools used in epithelial sheets, optogenetic tools have also been developed for the migration regulating proteins cdc42 and Rac1[202–204]. While still in its infancy, future studies in optogenetics have the potential to exert unique, tunable control over cell migration and tissue behavior by regulating force propagation from specific cellular regions. By utilizing optogenetics in epithelial sheets, local synthetic signaling can be combined with highly quantifiable cell migration methods.

II.B.3. Advantages and limitations of 2-D cell sheets

2-D epithelial sheets importantly preserve the relevance of cell-cell contacts, a key component of collective migration. Unlike complex *in vitro* 3-D systems, 2-D epithelial sheets are easily accessible for cell migration experiments and analysis. Furthermore, they are tunable with respect to ECM modification, substrate stiffness, and amenable to culture on microfab-

ricated devices[35,158,171]. Since the 2-D epithelium is a more general model, it also allows researchers to control the uncontrollable endogenous factors that are unique to specific *in vivo* or organoid environments (e.g., the effect of cell packing density or cell confluency on collective cell migration). Such biophysical parameters can then be translated more easily into *in silico* models. However, mechanical forces applied to 2-D *in vitro* systems can be outside the context of the forces that occur *in vivo*. Therefore, force application methods within these simplified systems should be considered carefully.

II.C. 1-D cell lines and cell doublets

Even more spatially reduced models than cell sheets are linear epithelial models (1-D). Within 1-D models, both force transmission and migration is confined along the direction of a single axis. These "cell train" models are not just limited to 1-D cell lines or cell rings (Figure 7a), but can include cell doublets which are 1-D by nature. Cell lines are commonly used to study migration, which can be created through either spatial confinement or protein micropatterning. Cell doublets can also be spatially confined but are more commonly used to isolate force transmission at single cell-cell contacts.

II.C.1. Methods to infer force from 1-D cell lines and cell doublets

As in other models we've discussed, TFM is a powerful inference tool for cellular traction stresses. Between cell lines and cell doublets, TFM has been more widely used for cell doublets to assess how cell-ECM tension influences stability and strength of a cell-cell interaction[129,205,206]. TFM studies on epithelial cell pairs may be advantageous for dissecting the relationship between geometric confinement and cell contractility, or manipulating mechanical regulation at a single cell-cell contact. The mechanical regulation of single cell-cell contacts can also be combined with FRET to return semi-quantitative feedback on intercellular molecular mechanics. For example, changing tension at cell-cell contacts of micropatterned cell doublets showed no change in E-cadherin tension[129], suggesting that tension over long time-scales may be supported by junction reinforcement from the cytoskeleton.

Image analysis, including video microscopy, is again a useful tool for force inference and quantifying cellular migration. While PIV is commonly used for 2-D epithelial sheets, analysis can be simplified when cells are confined to moving in a single direction. PIV has pointed to increased speed and persistence when confining either breast or kidney epithelial cells to a 1-D style of movement[25,147], which can be explained by a simple random walk diffusion anisotropy model. Cells have less room to migrate in the y direction and therefore redistribute that migration to the x direction. When these migration studies were distilled to a cell doublet experimental model, the mode of contact dissociation influenced how cells migrated away from one another via change in the protrusion angle[207]. Taken together, these results from 1-D model systems has brought new insight regarding how the orientation of force at cell-cell contacts influences migration direction.

At the subcellular level, immunohistochemistry (IHC) can answer questions surrounding protein localization and concentrations that may be more difficult to observe in larger tissues. For example, IHC has been used to determine protein localization at cell-cell contacts during single direction movement, including how cryptic lamellipodia form under epithelial kidney cell-cell contacts. Further examination of Rac1 activity has helped determine the relationship between adherens junctions and downstream signaling to influence cell polarity[61]. For cell doublets, IHC has shown that E-cadherin and F-actin bundle together at cell-cell contacts[208], where tension is localized to edges of the cell-cell contact[205]. These studies also show that increasing tension at cell-cell contacts does not change E-cadherin localization or intensity[129]. Even though IHC is just a snapshot of cellular and protein structure, positioning of the centrosome and other organelles can be used as an indicator of cell polarity during dynamic processes (i.e., migration direction)[209].

II.C.2. Methods to apply force to 1-D cell lines and cell doublets

a. Engineering the biophysical microenvironment. Cell lines are commonly created through either spatial confinement or protein micropatterning. Alternative methods have also been utilized, such as migration across a cylindrical wire[210]. In spatial confinement, cells are trapped in microfabricated glass or polymer channels anywhere from 5-30 microns wide[211,212]. Protein micropatterns can be fabricated on similar dimensions, but rely on biopassivation techniques to restrict the spreading of cells[25,61,114,213,214]. These same techniques can also be used for cell doublets[129,206,215,216].

Several modifications to the protein microenvironment for 1-D cell models can be implemented to answer new questions regarding how cells collectively migrate. For example, researchers have made new conclusions regarding how colliding cells interact to overcome contact inhibition[61,217] (Figure 7a and c). Protein micropatterns can also guide directional migration of melanoma cells using asymmetric "ratcheted" micropatterns[218]. There is also evidence that increasing cell-ECM tension via substrate stiffness proportionally increases cell-cell tension[205]. Furthermore, varying the collagen gradient can impact the migratory behavior[161] in corroboration with haptotaxis.

Protein micropatterning has been used to regulate cell-cell junctions for 1-D cell doublets in various ways. For example, rectangles of increasing aspect ratios can be used to alter the forces at cell-cell contacts[129]. Specific shapes for cell doublets, including I-shapes or bowties, can alter the location or stabilization of the cell-cell contact[206,216]. Other geometric shapes (e.g., circle, triangle, or square) of cell doublets have shown changes in junction maturation which has implications for cell-cell signaling and properties of collective migration[219] (Figure 7b).

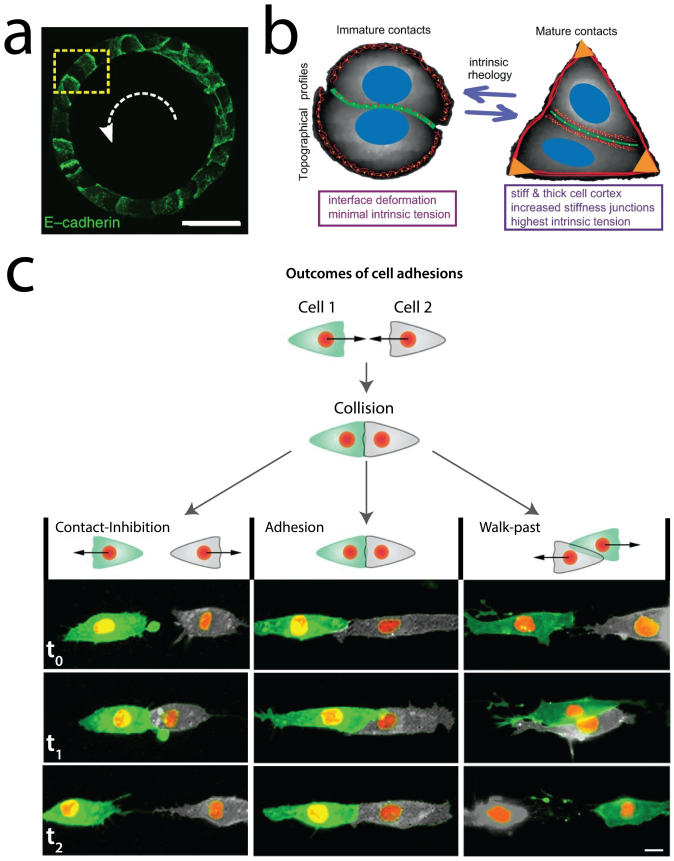


Figure 7. *In vitro* 1-D experimental models. (a) MDCK epithelial cells have been grown on micropatterned ECM rings to direct their single-file motion in a circle[61]. (b) Alternatively, linear tracks have also been used to force cell-cell collisions which can have different implications for cell migration depending on how they interact[217]. Figure (a) adapted with permission from Jain *et al*., Nature Physics **16**, 802-809 (2020)[61]. Copyright 2020 Springer Nature. Figure (b) adapted from Sri-Ranjan *et al*., Nature Communications **13**, 1-20 (2022)[219] under under Creative Commons Attribution 4.0 International (CC BY 4.0). Figure (c) adapted from Scarpa **et al**., Biol. Open **2**, 901-906 (2013)[217] under Creative Commons Attribution 3.0 Unported (CC BY 3.0). Scale bars: a= 50 μm c= 10 μm

b. Global force application. Several studies have globally stretched cell doublets[36,216] to determine how single cell-cell contacts respond to different mechanical inputs. For example, stretch of a mesendoderm cell doublet on a uniaxial stretcher showed lamellipodia extension away from the cell-cell contact[36]. This experimental finding supports a biophysical model in which intercellular tension directs migration away from the direction of mechanical pull. Beyond elastomeric cell stretchers for cell doublets, a recently printed two-photon polymerization device showed strain rate dependent rupture of cell-cell contacts[216]. Even more recently, a method using a DNA E-cadherin hybrid linker was engineered to tune the cell-cell adhesion strength in cell doublets[220]. Tuning the adhesion properties of cell-cell contacts may have significant applications for migrating cell collectives.

c. Local force application. In a cell train, a local force would imply mechanically perturbing one region of the train. For a cell doublet, a local force would imply perturbing only one of the cells. Optogenetics has been useful so far in locally perturbing 1-D epithelial models. For example, an Opto-Rac1 tool was used to locally perturb migration[61] within a cell line. Less common methods have been used to dissect cell doublets, though in one study a mechanical probe was used to pull at the cell-cell contact and induce E-cadherin tension[135].

II.C.3. Advantages and limitations of 1-D cell lines and cell doublets

The 1-D model simplifies the mechanical forces acting on cell-cell contacts by reducing the number of cell-cell contacts per cell. Force exchanges can be directly in line with cell migration and can make the relationship between cell-cell signaling and migration easier to understand[132] and analyze. Cell doublets allow researchers to more easily calculate forces acting at single cell-cell contacts with more certainty compared to larger clusters or collectives[132]. Both lines and doublets also offer high control and tunability, and are useful with high resolution microscopy.

The largest limitation of these reduced 1-D models is their lack of physiological relevance compared to *in vivo* systems or more complex *in vitro* systems.

II.D. Single cell micromanipulation systems

The most reduced cellular system for studying cell migration and force transmission is the single cell. However, fewer single cell epithelial studies exist since cell-cell contacts are so critical to epithelial behavior. Most studies with single epithelial cells primarily focus on either single cell mechanics (e.g., stiffness) or single cell migration. Some cleverly designed studies use single cell epithelial models to connect intercellular forces to cell migration.

II.D.1. Methods to infer force from single cell systems

For single cells, TFM is a common method to infer force of a moving cell as a way to understand how its microenvironment influences migration[221–223]. Other methods for single cell force inference are not necessarily unique from other models, which include tools such as FRET[224], atomic force microscopy[222], IHC[223], and live cell imaging[36]. Since the focus of this review is on collective cell mechanics, we will not go in depth into many single cell force inference studies.

II.D.2. Methods to apply force to single cell systems

a. Engineering the biophysical microenvironment. As covered previously in this review, a basic component of the microenvironment for epithelial cells is substrate composition and mechanics (e.g., the ECM and stiffness), which is easily tuned for single cell environments *in vitro*. With innovative experimental design, many conclusions regarding collective cell mechanics have been extrapolated from these findings. For example, cell culture substrates functionalized with cell contact mimicking E-cadherin has shown stiffness dependent adhesion regulation[221].

More complex methods to tailor the microenvironment for single cells involve protein micropatterning, which offer controlled physical pathways for single cell migration. Many of these studies involve 1-D tracks or channels where only single cells are allowed to move in the tracks[211,213]. For example, microfabricated tapered channels helped determine how cancerous vs. noncancerous breast epithelial cells navigate something akin to a tumor microenvironment during metastasis[211].

b. Global/local force application. In contrast to using protein micropatterns to create migration tracks for single cells, numerous studies have used protein micropatterns to regulate various aspects of cytoskeletal dynamics (e.g., actin orientation, interfacial tension, chirality, and overall traction force[110,111,225,226]). More active approaches have utilized cyclic cell stretchers, where reorientation of NRK epithelial cells occurred through microtubule extension during the relaxation phase[177]. Interestingly, many single cell epithelial studies using cell stretchers have mainly focused on changes in focal adhesion and cytoskeletal dynamics, rather than changes in migratory activity[160,177,178,224].

There are few single cell studies that combine an applied force and the subsequent effect on cellular migration. Limited studies exist in part because there are few tools (besides TFM) that can apply force and measure migration. Techniques like atomic force microscopy, magnetic or optical tweezers, and micropipette aspiration apply and quantify forces on cells, but often do not look at actively migrating cells or how such forces have differing impacts on cell migration. However, one study utilized magnetic tweezers to connect force to migration, where E-cadherin coated magnetic beads were pulled against single mesendoderm cells to measure cell polarization (i.e., migration)[36].

II.D.3. Advantages and limitations of single cell systems

Unlike *in vitro* models that utilize cell collectives, observational studies of the fundamental single cell can reveal more information about cell morphology. For example, the lamellipodia can be easily visualized, unlike the cryptic lamellipodia of moving cells in a sheet.

However, while many single cell epithelial studies attempt to extrapolate collective behavior, epithelial cell-cell contacts are integral to both migration and tissue integrity. The lack of this additional complexity should be considered in single cell studies aiming to make conclusions about collective cell properties. Furthermore, many tools that study single cell mechanics largely investigate non-moving cells outside the dynamic nature of epithelia.

III. BIOPHYSICAL *IN SILICO* MODELS OF EPITHELIAL DYNAMICS

The physical state of epithelia has important consequences for how cells communicate and how biological tissues respond to force and deformation. Confluent epithelia can exist in a solid-like (jammed) state or a liquid-like (unjammed) state. A jammed state is characterized by compact, roughly isotropic cells with reduced motility. Cell-cell adhesions are highly mature with few to no neighbor exchanges. The unjammed state is characterized by a disordered configuration of anisotropic cells with frequent neighbour exchanges. Neighbour exchanges lead to tissue level rearrangements which allow the tissue to "flow" and remodel, thus affecting the tissue's response to forces and deformations[17,227,228]. A physical property that distinguishes between the two states is the mean cell shape, as quantified for instance by $q = \langle P_i/\sqrt{A_i}\rangle$, where P_i and A_i are the perimeter and area of the i-th cell and the brackets denote an average over all cells in the tissue[229]. Theoretical work[229] supported by experiments[230] has indicated that a thereshold value $q^* \simeq 3.81$ separates jammed (q, q^*) from unjammed $(q > q^*)$ tissue.

Confluent epithelia can undergo jamming-unjamming transition by tuning cell-edge tension, cell motility, and the rate of cell division[17]. The tissue jamming transition has been predicted theoretically[231,232] and has been observed *in vivo* in the body axis elongation of Zebrafish embryos[18] where unjamming drives morphogenetic flows[18–20]. Unjamming is also thought to play a major role in cancer cell invasion[107,233].

The iterative back and forth between *in silico*, *in vitro* and *in vivo* studies has brought forth new understanding of collective cell motion in epithelia. *In silico* models are useful to quantify the role of distinct mechanisms that cannot be disentangled experimentally. For example, they have provided quantitative understanding of a number of processes that affect the onset of cell jamming/unjamming, such as cell motility[107,231], cell-cell adhesion and cortex contractility[233,234], the interaction of cells with their environment[107,233], and the rate of cell division[235,236].

We can classify existing models of epithelia in three groups: Cell Edge Network models, continuum models, and agent based models[237,238].

III.A. Cell Edge Network models

Under the heading of Cell Edge Network models we group a number of 2-D models that describe cells in confluent tissues as irregular polygons covering the plane, with no gaps between cells and shared edges representing the network of adherens junctions that connect neighboring cells[240–242]. This 2-D representation models a cross section (e.g., apical or basal surface) of the epithelial tissue layer[243,244].

The most popular models of this type are vertex models that describe the tissue as a network of N polygonal cells labelled by $i = 1, 2, \cdots, N$, each with area A_i and perimeter P_i. The mechanical and structural properties of the tissue are described by an energy:

$$\mathscr{E}_{\mathscr{V}\mathscr{M}} = \sum_i \frac{K_i}{2}\left(A_i - A_i^0\right)^2 + \sum_{<\mu,\nu>} \Lambda_{\mu\nu} l_{\mu\nu} + \sum_i \frac{\Gamma_i}{2} P_i^2 \,. \quad (1)$$

The first term in Eq. (1) quantifies the energy cost for deviation of the i-th cell area from its target value A_i^0 and capture cell area elasticity, with associated stiffness K_i. Although tissues are generally practically incompressible, this term accounts for the fact that cells can change their 2-D cross-sectional area by changing their height. The second term describes line tension $\Lambda_{\mu\nu}$ along cell edges connecting vertices μ and ν, with $l_{\mu\nu}$ the corresponding cell-edge length, and is summed over all neighbouring cell edges ($<\mu,\nu>$). The line tension is positive when cortical tension dominates intercellular adhesion and negative otherwise. The last term describes contractility of the cell perimeter of strength Γ_i, which is set by various processes in the cell, such as acto-myosin ring contraction[241].

Vertex models have been widely successful in studies of morphogenetic tissue remodelling and deformations *in vitro* in 2-D[20,244–246] and in 3-D epithelial layers and organoids [106,247–250]. They have been modified to include a variety of active processes, such as cell motilty, as in Active Vertex Models[251], cell-edge tensions fluctuations[252], cell proliferation and death[169,235], and even to allow for local tearing of the tissue[253].

A related class of models consists of Voronoi models, where the cell energy is written entirely in terms of cell area and perimeter as:

$$\mathscr{E}_{\mathscr{V}_l} = \frac{1}{2}\sum_i \left[K_i\left(A_i - A_i^0\right)^2 + K_i^P\left(P_i - P_i^0\right)^2\right] \,. \quad (2)$$

The Voronoi energy can be obtained from the vertex model energy by assuming uniform cell edge tensions $\Lambda_{\mu\nu} = \Lambda$. An important distinction between the two models is that in vertex models the degrees of freedom are the cell vertices, while in the Voronoi model the degrees of freedom are the centroids of the polygonal cells. This makes Voronoi models more directly suitable for including cell motility, as implemented in the Self Propelled Voronoi (SPV) model [231,251]. In the SPV the cell centers have a noisy self-propulsion velocity and are acted upon by forces derived from the energy given in Eq. (2). This model has been used to study the effect of cell motility on the tissue jamming-unjamming transition[231]. The further addition of alignment interactions has revealed the possibility of flocking liquid and solid states, with possible relevance to collective cell migration[254,255].

A related model is the Active Tension Model (ATN) that neglects area elasticity, but includes the mechanical feedback between cell-edge tension and myosin activation[256]. This model can explain the variable apical areas of ventral cells in early stages of gastrulation in the fruit fly embryo. Another modified vertex model study links contraction pulses acting like mechanical ratchets to tissue-scale deformations during morphogenesis[80].

The idea of representing tissues as networks has been shown to be useful even in the analysis of *in vivo* experimental data. Using networks of cell centers, called cell connectivity networks, a recent study linked changes in tissue rigidity and tissue viscosity to variation in cell packing fraction[234].

Cell Edge Network models are generally appropriate for confluent tissue. They have been adapted to allow for the creation of extra-cellular spaces in vertex models[257,258]. Like vertex models, the "active foam" model[258] predicts that edge tension fluctuations control stress relaxation and tissue fluidization, which is corroborated by *in vivo* studies[258]. In spite of these adaptations, Cell Edge Network models may not be ideal for systems that can develop inter-cellular gaps, as for instance near the transition from epithelial to mesenchymal behavior. In such cases agent based models, such as active particles or multi-phase field models may be more appropriate (see section III.C.).

III.B. Continuum models

Continuum models describe epithelial tissues on scales much larger than the size of individual cells[259,260]. In a continuum model the tissue is described in terms of a few coarse-grained fields that vary in space and time, such as the mean cell density, velocity, and polarity. The tissue dynamics is then controlled by conservation laws and constitutive equations that capture the system's symmetries and mechanical state. Epithelial monolayers are often modeled as active viscoelastic[261] fluids or viscoplastic elastic sheets, with

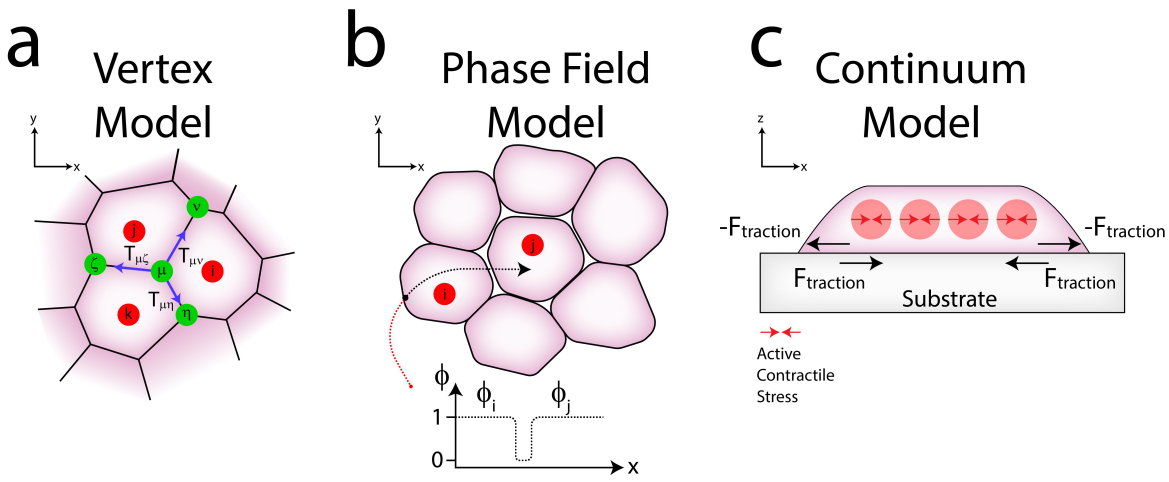


Figure 8. *In silico* models. (a) Vertex model of cells $i, j,$ and k sharing a vertex μ under tension from three edges. (b) Multi-phase field model of deformable cells. The bottom graph shows how the total phase field $\Phi = \sum_i \phi_i(\mathbf{x},t)$ varies in the x direction as we move along the arrow. (c) An example of a continuum picture, where an epithelial monolayer sits atop a substrate with the edges free to move. Contractile elements generate active contractile stress and cells move by generating traction with the substrate. Figure (c) adapted from Banerjee et al., Phys. Rev. Lett. 114, 228101[239]. Copyright 2015 American Physical Society.

couplings to internal degrees of freedom that account for active processes, such as contractility and cellular polarization. Continuum models have been shown to account for the heterogeneous spatial distribution of cellular stresses inferred from TFM in both expanding[64,130] and confined[262] monolayers, and even at the level of cell clusters[132] and individual cells[205]. They have also captured collective waves in migrating monolayers[239] as observed in *in vitro* experiments[263]. Continuum models have also been used in conjunction with *in vitro* studies to explain cell extrusion and apoptosis[21], intercellular force transmission during migration[63], long range force transmission leading to durotaxis[193], and curvature dependent mechanics of purse-string contraction during gap-closure[197].

They have also been used to predict the causal relation between stress patterns induced by myosin contractility and cell flow in *in vivo* morphogenesis in *Drosophila* gastrulation[68] and ventral furrow formation[264], as well as viscosity and cell ingression rates in Zebrafish body axis elongation[19] and surface displacements in early starfish oocyte development[265].

Continuum approaches do not attempt to faithfully incorporate intracellular processes, but rather aim at characterizing quantitatively the modes of organization and the materials properties of cell collectives in terms of a few macroscopic parameters, such as cell density and shape, cell-cell adhesiveness, contractility, polarization and division/death rates. Each of these quantities may describe the combined effect of a number of molecular processes and signaling pathways. An important open challenge is relating the coefficients of continuum models to both the parameters of mesoscopic (e.g., Vertex) models and to quantities controlled in experiments.

Another key limitation of the most current continuum modeling approaches lies in the assumption of fixed materials properties of tissues, which is encoded in the choice of a particular constitutive law. Tissues are able to adapt their mechanical response to perturbations (both external and internal) and are characterized by multiple relaxation times. This demands a rheological model capable of capturing both active solid-like and fluid-like behavior in different regimes of stress response and to dynamically transition between the two. In other words one needs to incorporate the feedbacks between cellular mechanics, polarized motility, and the regulatory biochemistry of actomyosin contractility[259,266]. These couplings play an essential role in the transmission of spatial information in large cell monolayers, mediated by waves, pulses, and a tug of war between cell-cell and cell-substrate forces.

III.C. Agent based models

Agent based models describe each individual cell as a discrete agent with its own biophysical properties and interaction forces with other cells and with the surrounding environment[267]. Agent based models can be defined on a lattice with cells constrained to occupy spaces on a discrete grid, as for instance in Cellular Potts models[268–270] or Cellular Automaton type models[123,233]. They can also be defined off-lattice as multi-phase field models[271,272], deformable particle models[179,273], various Spring link models[274,275] or 1-D train models [61,276,277]). Recently a Cellular Potts model was used to study the role of ECM confinement on collective and single-cell dynamics[233]. Phase field models were originally developed to study multi-component micro-structure materials[278] and have been adapted to represent epithelial cells as deformable active particles[271,272,279–284]. Each cell (i) is represented by a phase field ($\phi_i(\mathbf{x},t)$) defined to have value 1 in the region occupied by the cell and 0 otherwise (refer to Fig. 8). Cell-cell interactions and cell shape are controlled by a Cahn-Hillard phase field free energy, whose minimum determines the ground state configuration of the tissue[238]. Phase

field models can then be augmented to include cell-individual properties like polarisation, contractility, self-propulsion, as well as cell-cell interactions and active stresses[281,283]. Phase field models additionally allow for variations in both cell density and cell shape and can resolve arbitrary cell shapes. These models have been used to explore many behaviors of biological tissue, such as liquid-solid transitions[282], spontaneous emergence of collective cell migration[272], collective sustained oscillations[112], emergent nematic behavior and defects on mesoscopic scales[283], differential elasticity of cancerous versus healthy cells[271,285], and how contractile cells can yield tissue scale extensile behavior[283].

Many more modeling approaches exist[238] beyond the ones described above, such as hybrid multiscale models linking continuum description to agent based description[270,286]. *In silico* modeling is a powerful tool for filling in gaps in both *in vivo* as well as *in vitro* experiments, and can motivate new directions of experimental inquiry.

IV. DISCUSSION

IV.A. Summary of local vs. global mechanical perturbation

There is a broad spectrum of devices that can be used to exert forces on epithelia for the purpose of understanding collective cell behavior. These devices can be soft or rigid, transparent or opaque, low-throughput or high-throughput, etc. However, an important distinction we want to highlight is the ability for a device to apply a local force (i.e., region of cells within the tissue) or global force (i.e., the entire tissue) (Figure 9). Epithelial tissues *in vivo* experience a range of mechanical deformations that are not always uniform across the tissue. Tissue forces and strains are often regionalized with high variability across different parts of the tissue depending on the microenvironment or biochemical gradients. So far, most devices in the literature apply mechanical deformation across all cells (e.g., cell stretchers) or on a single cell (e.g., atomic force microscopy, optical tweezers, micropipette aspiration). Future studies focused on applying regional or local strains to epithelia will help elucidate how mechanical cues propagate through the epithelium.

Our limited understanding of how forces propagate through epithelia subsequently hinders our understanding of how local forces influence migration. Currently in the literature, there are conflicting models with respect to how intercellular forces direct migration. Many biological models suggest that cells follow local force[4,34], corroborating the theory that leader cells pull on follower cells to direct them forward. However, some biophysical models suggest that cells move *away* from increasing tension[36,64,130]. These models are reminiscent of contact inhibition, where collision of cell-cell contacts cause cells to polarize in the opposite direction and turnaround[217,276,287]. Perhaps these different models can be explained by differences in cell-cell contact regulation for leader-follower cells vs. neighboring cells moving within a confluent epithelium.

To elucidate the discrepancies between these migration models, a new class of *in vitro* studies would be beneficial. Many studies regarding force-induced repolarization have been modeled through simulations and theory[276,287], but few have experimentally taken into account the complex variables of the cell-cell contact. Even experimental studies that have made conclusions about intercellular forces are often based on TFM without external mechanical manipulation[26,129].

In vitro models will be a useful platform to dissect these complex variables that exist in biology, but require micromanipulation techniques to directly probe and manipulate intercellular cues.

IV.B. Additional variables to consider when mechanically loading cell-cell contacts

Beyond devices and tools that mechanically manipulate *regions* of tissue, there are also limited studies on the impact of different *kinds* of mechanical strain (e.g., strain rate, magnitude, frequency, or directionality) of mechanical force and subsequent effects on collective dynamics. Many different forces exist at cell-cell contacts and these changes are likely to influence force propagation and subsequent changes in collective cell behavior.

For example, several studies have shown the physiological significance of oscillatory strains as they are needed to drive tissue deformations or junction remodeling[80]. However, many studies apply either step or single strains to the epithelium. Beyond step vs. cyclic strain application, the rate of strain is another important variable that can be implemented in future studies[288].

Mechanical forces can also vary with respect to their orientation on cell-cell contacts (e.g., tension and shear). Tugging at cell-cell contacts of different orientations may have implications for junction adhesion and downstream signaling[289]. Tensile tugging forces, where force is exerted perpendicular to the cell-cell contact, occur regularly in developmental events such as invagination, neural tube development, and routinely in lung epithelial stretch or in intestinal crypts[39,41,45,51,290–292]. Shear forces, where cell membranes are pulled laterally past one another, also occur regularly in development (e.g., gastrulation and *Drosophila* genitalia development)[39,289,293]. Beyond potential downstream signaling for cellular function, the orientation of cell-cell contacts under mechanical load may influence their molecular interactions and ability to collectively migrate. This spectrum of molecular interactions at cell-cell contacts could help explain how different stages of embryo development utilize different modes of cell migration with regard to retaining cell neighbors. In zebrafish eyelid closure, cell monolayers utilize collective cell movements while retaining neighbors[291] via cell-cell adhesion. In germband extension, cells actively migrate past one another by breaking and reforming transmembrane binding proteins in a process known as intercalation[9]. Alternatively, cell-cell contacts under tension may promote adhesion reinforcement.

Whether cell junctions experience predominantly shear or tension depends on the dynamics of the local microenvironment (e.g., bending, folding, or constriction events)[41,45,294].

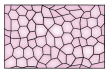
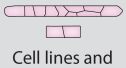
	Local mechanical manipulation methods to study collective cell behavior	*Global* mechanical manipulation methods to study collective cell behavior	Methods to *infer* force and collective cell behavior
 Spheroids and organoids	• Optogenetics • Actuatable droplets	• Biochemical reagents, pharmacological approaches, or gene editing • Substrate modifications • Geometric confinement	• Traction Force Microscopy (via beads and micropillars) • Segmentation and shape analysis • Molecular tension sensors • Laser ablation • Incompressible droplets • Time-lapse microscopy (including particle image velocimetry) • Immunohistochemistry and fluorescence microscopy • Micropipette aspiration
 Cell sheets	• Optogenetics • MEMS platforms and custom micromanipulation setups • ECM or stiffness gradients • ECM shapes • Atomic Force Microscopy	• Biochemical reagents, pharmacological approaches, or gene editing • Substrate modifications • Geometric confinement • Cell stretchers (static or cyclic) • Suspended epithelia • Shear flow	• Traction Force Microscopy (via beads and micropillars) • Segmentation and shape analysis • Molecular tension sensors • Laser ablation • Time-lapse microscopy (including particle image velocimetry) • Wound healing assays • Immunohistochemistry and fluorescence microscopy • Atomic Force Microscopy
 Cell lines and doublets	• Optogenetics • MEMS platforms and custom micromanipulation setups • ECM or stiffness gradients • ECM shapes • Atomic Force Microscopy	• Biochemical reagents, pharmacological approaches, or gene editing • Substrate modifications • Geometric confinement • Cell stretchers (static or cyclic)	• Traction Force Microscopy (via beads and micropillars) • Molecular tension sensors • Time-lapse microscopy (including particle image velocimetry) • Immunohistochemistry and fluorescence microscopy • Atomic Force Microscopy
 Single cells	• Optogenetics • Atomic Force Microscopy	• Biochemical reagents, pharmacological approaches, or gene editing • Substrate modifications • Geometric confinement • Micropipette aspiration • Magnetic bead trap • Cell stretchers (static or cyclic)	• Traction Force Microscopy (via beads and micropillars) • Molecular tension sensors • Time-lapse microscopy (including particle image velocimetry) • Immunohistochemistry and fluorescence microscopy • Magnetic Twisting Cytometry • Tweezers • Atomic Force Microscopy • Micropipette aspiration

Figure 9. Engineered approaches used to probe and infer mechanics of epithelia. Differentiating local vs global force application techniques can increase our understanding of how forces are transmitted between cells to influence collective behavior. These methods are discussed in more detail throughout Section II with appropriate references that provide more information on their applications.

Other cell-cell mechanics can include compression, which has been shown to lead to extrusion and and subsequent gap closure[295–297].

IV.C. Applications for synthetic biology and biomimetics

Epithelia are biological tissues that create and maintain life. At the core of these studies researchers stand to gain fundamental understanding of how tissues develop, regenerate, and provide protection for our organs. However, epithelia are also nature's best examples of active matter. Understanding the collective and regenerative dynamics of epithelia will enable the advancement of synthetic materials with far reaching applications in society. For example, synthetic membranes could be engineered to alter filtration specificity as a function of environmental inputs (e.g., mechanical stress). Other materials could self-repair when "wounded" from a rip or tear.

Several other reviews focus on additional aspects of collective cell migration and engineered platforms that have been used for *in vitro* cell studies[84,298–301]. Several methods in mechanobiology have also been commercialized in recent years beyond cell stretchers mentioned earlier (*Forcyte Biotechnologies, CYTOO, Alvéole, 4D-Cell*). These commercialized platforms will help to accelerate mechanobiology studies in both academia and industry.

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AUTHOR DECLARATIONS

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Liam P. Dow: Conceptualization (lead); Visualization (lead); Writing - original draft (lead); Writing - review and editing. Toshi Parmar: Conceptualization (supporting); Visualization (supporting); Writing - original draft (supporting); Writing - review and editing. M. Cristina Marchetti: Conceptualization (supporting); Funding acquisition; Writing - review and editing. Beth L. Pruitt: Conceptualization (supporting); Funding acquisition; Writing - original draft (supporting); Writing - review and editing. Liam P. Dow and Beth

L. Pruitt led discussion on biophysical experimental models, while Toshi Parmar and M. Cristina Marchetti led discussion on *in silico* biophysical models.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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