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Modeling tissue polarity in context

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Abstract

Polarity is critical for development and tissue-specific function. However, the acquisition and maintenance of tissue polarity is context dependent. Thus, cell and tissue polarity depend upon cell adhesion which is regulated by the cytoskeleton and influenced by the biochemical composition of the extracellular microenvironment and modified by biomechanical cues within the tissue. These biomechanical cues include fluid flow induced shear stresses, cell-density and confinement-mediated compression, and cellular actomyosin tension intrinsic to the tissue or induced in response to morphogens or extracellular matrix stiffness. Here, we discuss how extracellular matrix stiffness and fluid flow influence cell-cell and cell-extracellular matrix adhesion and alter cytoskeletal organization to modulate cell and tissue polarity. We describe model systems that when combined with state of the art molecular screens and high resolution imaging can be used to investigate how force modulates cell and tissue polarity.

Introduction

Polarity, which is the asymmetric organization of cellular proteins, membranes, organelles and the cytoskeleton, is a key regulator of cell fate and is important for tissue development and homeostasis. The establishment of apical-basal tissue polarity, which first emerges when a polarized sheet of epithelial cells forms the trophectoderm, is arguably one of the most critical events in early embryonic development. As development progresses apical-basal polarity continues to play a major role by directing the organization and function of cell clusters that create the distinct interfacial tissue layers that comprise the endoderm, ecotoderm and mesoderm [1]. When more complicated tissue-level structures develop, planar polarity emerges to modulate tissue orientation, as has been documented during wing morphogenesis and hair follicle formation [2,3]. Planar polarity, which establishes cell and

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tissue orientation, is also critical for cell and tissue function in the adult organism. Back to front orientation is key for directed migration and facilitates neutrophil infiltration into injured tissues and orients the directed collective migration of keratinocytes during wound healing [4]. Not surprisingly, during development and in the adult organism, both apical-basal and planar polarity are important for the organization and maintenance of the structure-function of cells and tissues. Indeed, polarity enables the afferent and efferent biochemical information flow in neurons, facilitates directed migration during gastrulation, and permits efficient nutrient exchange and polarized secretion in differentiated epithelial and endothelial sheets [5,6].

Cell and tissue polarity are regulated by the asymmetric targeting of proteins and membranes mediated by directed vesicle trafficking and cytoskeletal reorganization in response to soluble cues such as growth factors and morphogens [3,7–9]. The establishment and maintenance of cell and tissue polarity are tightly regulated by cell-extracellular matrix (ECM) and cell-cell adhesion that are in turn influenced by biomechanical cues within the tissue microenvironment [10–12]. For instance, the acquisition and maintenance of apical-basal and planar tissue polarity both depend upon adhesion to the ECM through specialized matrix adhesion receptors such as integrins (cell-ECM adhesions), and to other cells via adherens, tight, and scribble junctional complexes (cell-cell adhesions) [13,14]. Cell-ECM and cell-cell adhesions and soluble factors such as growth factors and morphogens synergize to direct cellular and tissue polarity by modulating the activity of GTPases including Rac, Cdc42, and Rho, which are molecular switches that regulate actin cytoskeletal dynamics and organization [14,15]. Apical-basal polarity in an epithelium requires the continuous apical and basolateral sorting of proteins through the trans-golgi network (TGN), and this protein trafficking is influenced by the actin cytoskeleton that is modulated by the activity of GTPases [16]. Planar polarity is also regulated by GTPases that modulate protein trafficking and reorganize the actin cytoskeleton [15,17,18].

Cell-ECM and cell-cell adhesion assembly and strength as well as Rac and Rho GTPase activity are enhanced in response to biomechanical forces such as exposure to a shear force or ECM stiffening [19]. Shear flow for instance stimulates Rac activity and modulates actin reorganization and integrin adhesion dynamics to modulate endothelial tissue integrity and orientation [20]. Furthermore, a stiff ECM promotes integrin engagement and signaling and activates GEFs that stimulate Rho to induce mDia-dependent actin remodeling and ROCK-induced type-II myosin contractility that then reinforce integrin adhesion assembly [21]. The elevated RhoGTPase-dependent stress fiber formation and ROCK-induced actomyosin contractility also perturb the polarized sorting of proteins through the transgolgi network (TGN) and destabilize tight junction and adherens junction integrity that compromise apical-basal polarity. In tumors chronically elevated cellular actomyosin tension induced by oncogenes such as mutant Ras or by enhanced integrin focal adhesion signaling in response to a stiffened fibrotic ECM, disrupt apical-basal polarity [22–26]. Similarly, amplification of erbB2 (Her2) receptors hyperstimulate Ras to enhance ROCK-dependent cellular tension that disrupts PAR/scribble cell-cell complexes and redistributes scribble to ECM adhesions to promote mammary epithelial cell invasion[22,27–30]. Importantly however, biomechanical forces are also important for normal tissue development and for maintaining tissue homeostasis. For instance, flow can enhance planar polarity in cells and tissues by activating

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RhoGTPases that reorient the cytoskeleton and stimulate actomyosin tension to strengthen cell-cell versus cell-ECM adhesion [13,31–34]. Thus, a stiff ECM that enhances integrin adhesion assembly and signaling also stimulates the relocalization of Scribble from apical-lateral adhesions, where the protein resides in a complex with Crumbs and PARs, to the basal plasma membrane, where it assembles with Rac1 via Rac1GEF βPix [35–37] , PTEN [30], and MCC [38] to direct polarized cell migration.

The molecular mechanisms by which cell-cell and cell-ECM adhesions regulate apical-basal and planar polarity have been clarified by studies that have employed two and three dimensional organotypic culture models and natural and synthetic biomaterials with defined biochemical and biophysical properties [39–47]. Recent innovations in cell culture models using architecturally defined tissues with microfluidics that recapitulate flow dynamics in the vasculature and lymphatic systems are now being used to clarify how fluid flow and shear stress regulate cell and tissue polarity. In this review, we discuss cell and tissue polarity in the context of mechanical signals derived from cell contractility, ECM elasticity, and fluid flow. We outline tractable model systems that include mechanically-tuned biomimetic cell culture devices and fluid flow devices that are available to study how these biomechanical cues regulate cell and tissue polarity.

Cell-extracellular matrix adhesion: the physical foundation of cell polarity

Cell adhesion to the extracellular matrix (ECM) or to other cells via cell-cell adhesions establishes the physical context in which a cell orients its functional structures and intracellular proteins and chemical gradients. This is especially auspicious in the context of endothelial cells lining vascular and lymphatic networks and epithelial cells lining secretory ductal trees, as these cell types adhere to a basement membranes and orient their endo/exocytic machinery towards their fluid filled lumens [48,49]. Epithelial and endothelial barriers orient their polarity within these anisotropic physical conditions by adhering to a basement membrane through a plethora of transmembrane ECM receptors including syndecans, discoidin receptors, and integrins. Of these ECM adhesion receptors, integrins are the best studied, and their role in cell and tissue polarity has been well-established. Integrins are a family of transmembrane adhesion receptors comprised of 24 αβ heterodimeric members that bind specific regions of large macromolecular ECM proteins. Upon binding to the ECM, activated integrins cluster to form focal complexes that associate with adhesion plaque proteins such as talin that in response to either an externally-applied force or intrinsic actomysosin tension unfold to recruit vinculin and assorted cytoskeletal binding and signaling molecules to drive the assembly of integrins into mature focal adhesions [50–52].

Cells engage outside-in and inside-out integrin signaling to develop apical-basal polarity in multi-cellular tissues. For example, when epithelial cells such as MDCK (Madin-Darby Canine Kidney Epithelial Cells) are cultured in suspension they depolarize, however, once they aggregate to form cystic structures they repolarize to form multi-cellular structures with the apical domain localized to the outside surface of the cyst [53]. This inversed cell polarity is reverted towards the tissue lumen when the MDCK cysts are embedded within an isotropically soft collagen gel in a beta 1 integrin-Rac1 GTPase dependent manner [53–55]. Perturbations in alpha 2 beta 1 integrin-collagen interactions compromise MDCK cyst

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polarization, emphasizing the key role of cell-ECM adhesion in apical-basal polarity regulation, possibly by regulating polarized protein trafficking [56,57].

Integrin adhesion assembly and signaling are exquisitely modulated by rigidity sensing of the viscoelasticity of the ECM and by intracellular actomyosin tension [22,58–60]. Physical force alters the conformation and localization of integrins and their adhesion plaque proteins including talin and vinculin and to foster the assembly of mature focal adhesions. A stiff ECM can influence tissue polarity by modulating cell-ECM adhesions which can destabilize cell-cell adhesions and compromise tissue organization. Under extreme conditions a chronically stiffened ECM will collaborate with increased growth factor receptor signaling to promote cell invasion and may foster the malignant transformation of an epithelial tissue [61]. Moreover, the speed and persistence of cell migration is also tuned by ECM substrate compliance suggesting a stiff ECM could foster the migration of transformed cells into the interstitial stroma [62,63]. Indeed, a stiffened, fibrotic ECM also permits a TGFb-dependent epithelial to mesenchymal transition that has been implicated in tumor metastasis (EMT) [64]. Nevertheless and importantly, ECM stiffening is also critical for normal tissue development and homeostasis. For example, a stiffened ECM permits the directed, coordinated, collective migration of keratinocytes and instructs neutrophil infiltration and monocyte differentiation into macrophages to facilitate proper wound healing. The wound-activated macrophages secrete MMPs that induce ECM remodeling and TGFβ that stimulate the expression of ECM proteins and the transdifferentiation of fibroblasts into contractile myofibroblasts that stiffen the wound stroma [65,66]. The stiffened ECM in collaboration with macrophage and fibroblast secreted chemokines and the TGFb induce a normal physiological EMT in the keratinocytes and then foster their directed migration into the wound to repopulate and heal the injured tissue site [67–69].

In vitro studies using substrates with defined elasticity, cell adhesive peptides, and MMP-degradable materials revealed that the formation of polarized tissue structures is tuned by ECM stiffness and depends upon ECM remodeling [62,63,70–72]. This phenomenon has also been observed during development where gradients of a stiffened ECM modulate integrin-vinculin-talin mediated mechanotransduction to direct the collective sheet migration critical for neural crest development [73]. In fact directed migration of cells towards increasingly rigid adhesion substrates has been experimentally demonstrated using materials that are resistant to ECM degradation and has been termed “durotaxis” [42,74,75]. Durotaxis is consistent with the findings that efficient cell migration is a dynamic balance between adhesive and protrusive forces supported by a spatiotemporally variable program which integrates type-II myosin activity, focal adhesion assembly/disassembly, and remodeling of actin cytoskeleton [76].

ECM stiffness can also modulate tissue polarity indirectly by altering the synthesis and secretion of soluble factors that regulate polarity through auto-, juxta-, and paracrine signaling. This paradigm was illustrated by Przybyla et al. who employed protein-functionalized polyacrylamide gels with tuned elasticity to demonstrate that substrate stiffness modulates human embryonic stem cell polarity and differentiation by regulating the expression and secretion of key wnts and their inhibitors [77]. Secreted wnt gradients function as directional cues which alter cell polarity [78], which implicates secreted signals as another

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possible mode of polarity disruption caused by aberrantly stiff ECM. Indeed, endo and exocytosis are regulated by membrane tension, which is modulated by intrinsic and extrinsic physical force [79]. These mechanically altered secretory responses may lead to the loss of polarity by disrupting the maintenance of the basement membrane. Substrate rigidity also alters the production and secretion matrix metalloproteinases (MMPs), which remodel the basement membrane [80] and are exocytosed in a polarized fashion to support Planar Cell Polarity (PCP) [81]. These secreted or membrane tethered enzymes foster cell migration, ECM remodeling [11], and the cleavage of cell surface receptors and signaling molecules [82]. Apical-basolateral polarity depends upon polarized localized secretion of MT-MMP (MMP14) [83]. Not surprisingly, the synthesis and secretion of MMPs are responsive to ECM elasticity, such that ECM mechanics modulate MMP levels and activity, and control apical-basal tissue polarity by catalyzing ECM remodeling and releasing soluble factors that stimulate cell migration [71].

Given strong links between cell-integrin ECM adhesions and tissue polarity, it is not unreasonable to suggest that defining how force modulates integrin structure/function to alter tissue polarity could provide critical insight into the role of force in tissue development and homeostasis. Moreover, delineating links between force and polarity should clarify the molecular basis of various pathologies that compromise epi/endothelial barrier function or induce diseases linked to loss of tissue polarity including atherosclerosis and cancer [84]. This objective would be well served through the use of defined cellular model systems embedded within materials that accurately mimic the composition and physical properties of the native tissue and that permit high resolution imaging of live cultures. These approaches have become increasingly prevalent as 3D embedded culture conditions, often referred to as “organoids”, have demonstrated improved phenotypic recapitulation of their *in vivo* tissue counterparts than standard *in vitro* monolayer culture formats [85]. These cell/tissue-specific 3D-culture conditions have become increasingly sophisticated and can be well-defined in terms of chemical composition, soluble factor addition, and physical manipulations required to generate *in vitro* models that mimic healthy and diseased human-like organs using primary and immortalized human and murine cells [86–88]. These advanced and defined cellular materials can be complemented with a toolbox of increasingly elaborate physical microenvironments that include tuneable hydrogels with defined ECM ligands, morphogens, and mechanical properties. These defined biomaterials permit high resolution imaging of live cells and are able to facilitate the systematic assessment of the contributions-of and synergy-between biochemical and biophysical cues in adhesion-regulated cell and tissue polarity [89–91]. To fully recreate the “tissue-like” or “bio-mimetic” microenvironments of normal and diseased tissues researchers can also incorporate biomimetic microfluidic devices as well as incorporate compression and stretch setups that have been successfully adapted to conform to organotypic geometries and function [39]. These reconstituted 3D organotypic models permit the systematic tuning of fluid shear force, compression, stretch and ECM elasticity and composition such that it is now possible to delineate the molecular mechanisms whereby cell ECM adhesion regulates tissue polarity [92]. Complementing these sophisticated models are newly developed “mechanically active organ-on-a-chip” microdevices that permit rapid molecular and drug screening to examine mechanisms regulating the polarized uptake of molecules in an epithelium or endothelium [93].

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Interplay between physical force and cell-cell adhesion

The collective morphogenesis of sheets of cells within a developing tissue depend upon PCP and is modulated by mechanical force. During tissue development polarized epithelial sheets integrate directional cues over extended distances to establish aligned tissue patterns through physical forces and chemical cues mediated predominantly by cell-cell adhesion interactions [2,94–96]. For example, planar polarity in the murine keratinocyte epithelium is dictated by anisotropic physical force that is generated and transmitted through cell-cell junctions that function to align Celsr1, Cadherin EGF LAG Seven-Pass G-Type Receptor 1 [97]. Similarly, during fly embryogenesis, Celsr1, Vangl2, and Fz6 (core PCP proteins) become asymmetrically distributed to the anterior/posterior cell borders in the basal cells to define axial-vectorial asymmetry in response to cell-cell generated tension [25,26]. Thereafter, the force-directed PCP core proteins redistribute into specific plasma membrane domains to form instructive “puncta” at cell-cell adherens junctions.

Adherens junctions (AJ) are composed of cadherin receptors that bridge adjacent plasma membranes of cells through homophilic interactions that are critical for the development of apical-basal polarity [13,98,99]. Cadherins coordinate with cytoplasmic catenins to integrate adhesions to actin filaments and microtubule networks to mechanically couple the contractile cortices of the cell thereby distributing physical stresses across a cellular sheet. In vertebrate polarized epithelia, AJs are part of the tripartite junctional complex comprised of tight junction (zonula occludens), AJ (zonula adherens), and desmosome (macula adherens) that are localized to the juxtaluminal region [100]. A major function of AJs is to maintain the physical association between cells, and disruption of these contacts releases cell–cell tension and compromises tissue organization. The transmission of tension to the cytoskeleton through cadherin-mediated adhesions is thus critical for sculpting the epithelium [13,101–104] and its dysregulation disrupts tissue integrity and can foster disease pathologies including malignancy [22,61,105]. Interestingly, although the application of an external force on E-cadherin can induce cytoskeletal stiffening [99], how E-cadherin transduces tension to the actin cytoskeleton remains unclear. Surely force transmission across the AJ must support the engagement and recruitment of the actin binding proteins that assemble and maintain the AJ [106], favoring some form of dynamic collective mechanical stabilization to generate and maintain PCP.

RhoA plays a critical role in PCP by supporting cell-cell adhesions through actin remodeling and by triggering myosin-induced tension to generate the requisite forces required to reorient the cells in an α-catenin-dependent manner [107,108]. This adherens-localized actomyosin tension appears to be absolutely critical for the establishment of PCP in an epithelium and for the maintenance of tissue integrity [109]. In this regard, tissue integrity depends upon sustaining an optimal range of force across the junction as was illustrated by a series of elegant optical trap studies by Buckley et al. [110] which demonstrated that an optimal range physical force was required for cytoskeletal association with AJs. Physical forces exceeding the optimal range of tension led to AJ deterioration and resulted in a loss of tissue integrity [22,111,112]. Yet, the assembly of branched actin networks is also force-dependent [113] and the restructuring of branched actin networks to catenin/cadherin associated bundles is also critical for AJ stability and is likely mediated through catenin

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actin ARP2/3 competitive binding [114]. Thus, force-dependent destabilization of AJs could be mediated either by changes in actin remolding or by direct destabilization of the AJ protein complex. Indeed, several pathogens that compromise intestinal barrier function also hijack actin cytoskeletal dynamics [115] raising the possibility that they might destabilize AJ integrity through actin remodeling that dysregulates force distribution at cell-cell junctions [116].

When good forces go bad

Normal tissue development and homeostasis and the acquisition of apical-basal and planar tissue polarity depend upon a tightly regulated balance of exogenous and intrinsic cell tension. Not surprisingly, chronically elevated external physical stresses or intracellular actomyosin tension exerted at the sites of adhesion alter the distribution, composition, and subcellular signaling systems within integrin adhesions and at cell-cell adhesion. Chronically modified integrin signaling and cell-cell adhesion integrity/composition ultimately compromise tissue polarity to perturb tissue integrity and tensional homeostasis that may promote disease. For instance, tumor progression in solid tissues is frequently accompanied by fibrosis that progressively stiffen and reorganize the stromal ECM. The stiffened stromal ECM in turn enhances the assembly of focal adhesions that potentiate growth factor receptor signaling through PI3 kinase and destabilize cell-cell adhesions to promote cell invasion and malignant transformation and eventually foster metastasis [22,61,117]. Similarly, oncogenes such as ErbB2 and Ras enhance tumor cell actomyosin tension that promote focal adhesion assembly and induce ECM remodeling and stiffening that also then also destabilize cell-cell adhesions and promotes cell invasion and malignant transformation by enhancing pro-growth, pro-invasion and pro-survival signaling such as elevated β-catenin, Myc and STAT3 activity [117–119]. Consistently, inhibiting FAK activity or reducing RhoA or ROCK activity can phenotypically revert the phenotype of malignant mammary tumors in culture and will impede the malignant transformation of multiple tumor types including squamous cell carcinoma, mammary carcinomas, and pancreatic carcinomas *in vivo* [22,61,118–126]. Importantly, repression of the malignant phenotype in all of these instances associates with either maintenance-of or restoration of cell-cell adhesions and apical-basal tissue polarity. Given that many molecules that modulate tissue polarity such as scribble and discs large are putative tumor suppressor these findings imply that these tissue polarity regulators repress malignancy by maintaining tissue architecture [29,36,127,128]. Consistent with this prediction, cells engage basement membrane proteins via specific integrin heterodimers to establish and sustain cell polarity [129] and integrin-mediated adhesion to laminin [130], directs the localization of polarity mediators, such as Par3, to facilitate the assembly of differentiated acinar structures with a polarized lumen [131,132]. Malignancy ensues when the integrity of this “differentiated and apical-basally polarized tissue” is compromised, as occurs in response to the increasingly fibrotic and stiffened ECM surrounding transformed tissues or following increased expression or activity of oncogenes that elevate actomyosin tension [61,105,117–119,133,134]. Indeed, the levels and subcellular localization of Par3 are not only critical for the development of polarized tissues, but are necessary for the prevention of malignancy [29].

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How do we model the tissue microenvironment to understand polarity regulation?

The physical context of a tissue including the type and organization of the cellular constituents, the composition and architecture and mechanical properties of the ECM together with chemical gradients and tissue level forces including flow, compression and tension cooperate to generate cell and tissue behavior [19,135,136]. The challenge has been to clarify how these various environmental cues independently and collectively influence tissue level behaviors such as polarity. Arguably genetically engineered mouse models in which specific ECM components can be specifically knocked out or mutated and their posttranslational modification manipulated in a tissue specific manner and using inducible constructs has greatly facilitated studies to explore the impact of the microenvironment on tissue development, homeostasis and disease. Nevertheless, despite their elegance, these live model systems present a unique challenge when trying to identify direct causal relationships between ECM composition and organization and delineating the impact of specific stromal cellular components or physical forces and chemical gradients on cell and tissue behavior. To address such issues increasing effort has been exerted to develop tractable culture systems that can accurately deconvolve the impact of ECM composition, stiffness, architecture and even dimensionality on tissue phenotype. These newly developed systems have also been perfected to study the impact of compressive, stretch or tensile forces on cells embedded within collagen or hyaluronidase or synthetic hydrogels [39]. Both synthetic and natural polymer scaffolds are also readily amenable to modulation of matrix compliance and ECM ligand bioavailability and have been used to study the impact of two and three dimensional ECMs on tissue behavior [137].

Hydrogels which are aqueous polymer networks that behave as viscoelastic solids, are a standard for biomimetic 3D encapsulated *in vitro* and *in vivo* material manipulation models. Hydrogels can be generated using naturally-derived biopolymers such as collagen, hyaluronic acid, fibrin, agarose, alginate, and cellulose. Synthetic polymers such as polyethylene glycol (PEG), poly(vinylalcohol) (PVA), poly-lactic-glycolic-acid (PLGA) are also effective 3D cell culture hydrogel models. Natural and synthetic polymers each have their respective benefits and limitations. Historically, collagen I gels and basement membrane-enriched hydrogels have proven to be instrumental for the study of tissue-specific differentiation and have critically illustrated the differences between normal and malignant or diseased tissues [45,126,138–150]. Naturally derived materials, especially ECMs which exist in abundance in the tissue/structure or around the cell-type of interest, provide a biointerfacial cell scaffold that engages integrin or CD44 and RHAMM receptors within collagen and hyaluronic acid gels and that directly support the growth, viability, and tissue-like behavior of cells and tissue. These “natural” hydrogels have been used extensively to study tissue specific differentiation such as in mammary epithelial differentiation [151,152], kidney function [153,154] or endothelial network behavior [155–157] and when appropriately “tuned” to specific elasticities and biodegradability can generate important insight into tissue-specific behaviors including defining what factors control branching morphogenesis [70,158–160] and conceivably promote the malignant behavior (invasiveness) of a tissue [39,133]. Nevertheless, these natural hydrogels are notoriously variable and do not always lend

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themselves to consistent modification [91,161]. By contrast, synthetic polymers are amenable to precise modifications including controlled crosslinking arrangements and density and can be tuned to include a specific biochemical and chemical composition. However, synthetic polymers do not always recapitulate the architecture of native ECMs and are not easily remodeled. An optimal strategy for design and implementation of 3D-cell culture systems likely lies with a combinatorial approach where synthetic of naturally derived polymers are either be modified to accommodate more efficient crosslinking reactions or to optimize and define the presentation of adhesion ligands derived from biopolymers such as fibronectin, collagen, or laminin [91,137,162–164]. These combinatorial materials are readily available from vendors or can be engineered and modified to present specific ECM-derived or ECM-mimetic ligands and are amenable to facile approaches to dynamically stiffen or soften the material [165]. For example, RGD and laminin-111 conjugated PEG-based hydrogels with degradable peptides have been judiciously applied to understand gut development using a combination of purified intestinal organoids and mechanically-tuned ECMs and surprisingly have illustrated differential effects of laminin-derived peptides and full length laminin-11 on intestinal lumen formation [89].

While amorphous 3D-hydrogels provide an exciting platform to study cell polarity, 2D-surfaces and structurally defined 3D-surfaces arguably provide a more readily available and hence appropriate model system for the study of planar and apical basolateral polarity. The most easily adapted model system that can be used to study the impact of substrate elasticity on tissue polarity is the polyacrylamide gel (PA) surface [166]. PA gels can be generated across a wide spectrum of elasticity and can be adapted to present a wide assortment of purified ECMs or modified ECMs or even synthetic adhesion ligands and are amenable to fluorescence-based imaging and protein and RNA harvesting [22]. Although PA gels are not biodegradable and at least for short term culture are not easily fouled, the different concentration of bis-acrylamide crosslinkers used to vary the elasticity does modify the gel pore size and this can influence ligand binding and presentation to inappropriately modify cell behavior [167,168]. Furthermore, traditional PA gels do not lend themselves to super-resolution imaging approaches such as Total Internal Reflection Fluorescence (TIRF) or Scanning Angle Interference Microscopy (SAIM). To this end, silicone gel coatings with suitable refractive indexes that permit high resolution imaging and whose elasticity can be modified across a wide range have been developed and are now readily available for general experimental applications [169].

Soft lithography, a technique borrowed from the microfabrication of electrical circuits, has enabled major advances to generate 3D patterned cell culture hydrogels. Rather than culturing cells within a stochastic assemblage of cells, polymers, solutes, and fluids, soft lithography can generate geometrically defined networks of channels, void spaces, elastic and selectively permeable membranes as well as ports or sensors to allow for real-time monitoring of metabolite production/consumption or addition of pharmacologic compounds. Soft lithography is executed either by fabricating or purchasing a mold that is inversely replicated by an elastic material such as polydimethylsiloxane (PDMS) to form embossed microstructures [170,171]. The PDMS cast around the mold can then be readily bonded to glass to form, what is now typically described as, a microfluidic device. The experimental format afforded by soft lithography not only allows for the culture of biologically relevant cellular

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geometric organizations but the viscoelasticity of the PDMS hydrogel material can also be tuned across a range of stiffness to model different normal and diseased ECMs [172,173]. Experiments to explore the impact of ECM compliance and topology on sheets of epithelial cells in two dimensions and on organized ductal tissues in a three dimensional matrix using ultrasoft lithography (0.1–100 kPa) have illustrated how ECM stiffness gradients are able to induce a durotactic migratory response towards tissue-like structures within a more physiologically-relevant context [172,173].

Integrating Flow

Cells and tissues are constantly experiencing a variable range of shear stresses generated by fluid flow and these shear stresses regulate development and when corrupted may also induce tissue pathologies. Shear stresses play critical role in the maintenance and development of cell polarity primarily through the dynamic regulation of Rac1 and RhoA [174]. For instance, during embryogenesis, heart development is exquisitely regulated by directional fluid flow dynamics which critically induce the maturation of the vasculature through shear stress activation of RhoGTPases that promote tissue polarity and endothelial junction integrity [175–177]. In the adult organism the mechanical forces generated by the dynamics of fluid flow within the lymphatic and vasculature (~0.1–50 dynes/cm^2) [178–181] are absolutely critical for the assembly and maintenance of adherens junctions and tight junctions and control the development of vascular and lymphatic valves which mitigate retrograde fluid flow [182–185]. Indeed, endothelial valve forming cells sense shear stresses associated with fluid flow and adjust their polarity [186] through adhesion dynamics that depend upon ROCK activity [183]. Not surprisingly, compromised fluid flow, as occurs at blood vessel bifurcations can perturb tissue homeostasis to induce cardiovascular disease through the disruption of chemical gradients, altered mechanical signaling, and the regional accumulation of aggregates of insoluble material or cells that can stimulate inflammation and lead to lesion formation [187].

The study of the molecular mechanisms whereby fluid flow regulates cell polarity and tissue microstructure homeostasis has been greatly enabled by the use of microfluidic devices. Such devices have been adapted to support epi/endothelial cells assembled into tube-like columns with a central lumen that is capable of supporting dynamic fluid flows modulated by a microfluidic pump. Using these microfluidic devices and altering the rate of flow through the lumen to proportionally modulate shear stress flow was shown to regulate PCP by altering microtubule stability and activating GSK-3β. These studies further revealed that GSK-3β inhibition not only reversed endothelial PCP but also compromised the ability of the vasculature to elongate [174,188]. These types of microfluidic device models may also be used to analyze whether or not the valve structures within the cardiac, venous, or lymphatic systems degenerate in response to shear stresses above or below a critical threshold [189,190] or if valve degeneration occurs in response to inflammatory cytokines. Combining these microfluidic devices with biochemically defined and elastically-tuned materials has permitted an analysis of the impact of physiological ranges of fluid shear stress and defined the role biochemical and morphological gradients on tissue polarity in a three dimensional tissue-like context [191]. Indeed, the use of microfluidic device models that faithfully mimic the architectural geometry and mechanical forces tissues typically experience *in vivo* have

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strong potential to clarify factors that regulate tissue polarity including morphogen gradients, ECM stiffness gradients (durotaxis) and fluid shear stress [192,193]. For instance, a collagen lined, soft lithography generated (1.5 kPa), 3D-endothelial lumen model revealed the importance of frictional force on the durotaxis-dependent migration and orientation of lymphatic and venous networks [72]. Similar devices have been used to address the impact of the torturous and leaky vasculature on the tumor epithelium and reported that the reduced flow rates found in these vessels fosters high tumor cell proliferation whereas high shear stress (12 dynes/cm^2) promotes G_2/M cell cycle arrest [194–197] and the results of these studies have been used to imply flow dynamics could modulate tumor phenotype [198]. Microfluidic devices have also been used to demonstrate that the oscillatory mechanical stresses produced by breathing motions as occurs in the lungs, play a critical role in regulating the growth of human non-small-cell lung cancer cells [199] and to assess barrier function of the endo/epithelium [200–202]. Furthermore, a 3D microfluidics *in vitro* model of intestinal crypts illustrated the impact of the human specific pathogen norovirus on epithelial barrier function [203] and could constitute a tractable model system to assess the impact of the Listeria monocytogene or Shigella flexneri, pathogens on cytoskeletal organization and adherens junctions integrity [115,204,205]. Clearly, impressive advancements in biomaterials, ultrasoft lithography and microfluidics combined with tissue organ cultures are now available and afford the research community with an unprecedented opportunity to use culture models to study how tissue polarity is molecularly regulated not only by morphogens but also by force.

Conclusion

Mechanical force generated or applied to cell and tissue structures can either enforce or compromise apical-basolateral and planer cell polarity and thereby plays a critical role in development, tissue homeostasis, and disease. Actomyosin tension exerted at cell-cell junctions reinforces these adhesions to promote apical-basal polarity while a stiffened ECM destabilizes cell-cell adhesions by enhancing cell-ECM adhesion and stimulating actin reorganization and receptor tyrosine kinase or G-protein coupled receptor signaling. Gradients of ECM stiffness and directed shear forces influence cell-cell or cell-ECM interactions which orient cytoskeletal organization and membrane receptor signaling that engage the planar polarity machinery to induce durotactic migration that is required for normal homeostatic processes or when corrupted can foster tumor cell migration towards the vasculature to promote metastatic dissemination of tumor cells. Deciphering how these forces operate to differentially modulate normal development and tissue behavior versus disease require sophisticated models that faithfully recapitulate the biochemical and the dynamic and three-dimensional biophysical microenvironment of tissues *in vitro*. Clearly, concerted effort to use these newly available model systems to study how force modulates cell and tissue polarity in context should help to clarify the molecular basis of tissue development, homeostasis and disease.

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Box/figure defining basic concepts

- Mechanical forces are defined as physical forces which deform or accelerate matter in an opposing axis to the origin of the force. These physical forces are measured in Newtons (N) (SI system) and dynes (CGS system). When physical forces are defined across an area of measure, Pascals (Pa) are the unit of measure, which is defined newton per square meter (N/m2).

Mechanical forces pertinent to biology: tensile, compression, and shear.

- When tensile and compressive force is applied to an object the resulting deformation will either increase or decrease the parallel or perpendicular axis of the object. The amount of deformation is defined by the physical characteristics of the object acted upon by force.
- Shear stress occurs when forces act tangentially across a resisting object.

Viscoelasticity is the multifaceted properties of a materials or biological structures exhibiting viscous and elastic mechanical properties.

- Elasticity (stiffness) is the ability of an object to resist deformation in response to a given force (~ solid phase).
- Viscosity is measure of internal friction within a physical system (~ liquid phase).

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Highlights of this review

- Cell and tissue polarity depend upon cell adhesion
- Polarity instructing adhesions are affected by mechanical forces
- Mechanical cues within the microenvironment can disrupt polarity6
- Descriptions of model systems to study mechanical forces and polarity

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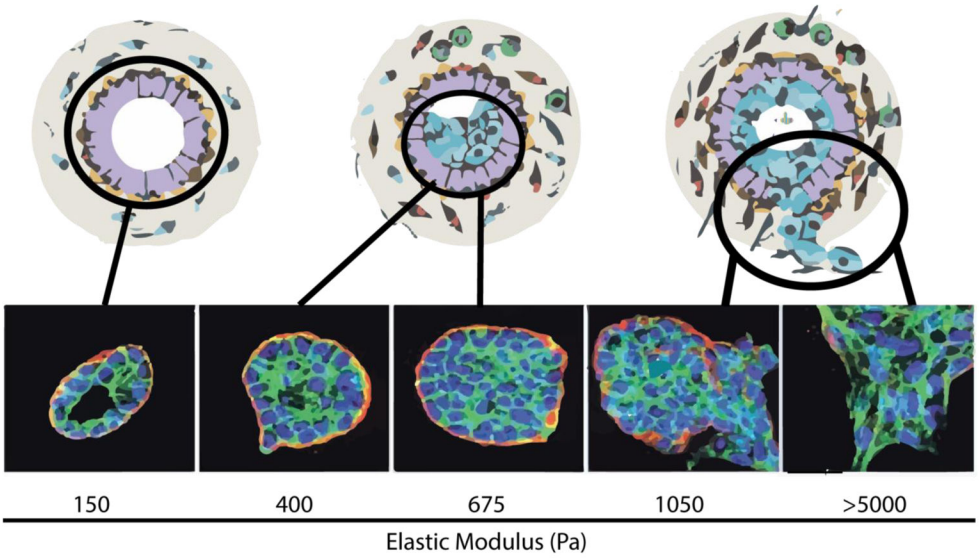

Figure 1. Polarity depends on a delicate balance of physical forces

Increasing ECM stiffness causes the loss of apical basolateral polarity. Schematic depicting the effects of increasing mechanical stress on mammary epithelial cells. Chronic exposure to physical forces compromises the ductal structure and is accompanied by the loss of epithelial polarity. These effects are clearly observable with non-malignant MCF10A cell colonies cultured on a reconstituted basement membrane functionalized polyacrylamide gel surfaces of increasing stiffness (150–5,000 Pa). MCF10A cells cultured on surfaces with a biomimetic ECM stiffness similar to that measured in the normal murine mammary gland (150 Pa) form polarized acini organoids which model the terminal ductal lobular units of a differentiated breast. MCF10A organoids synthesize and localize an endogenous laminin 5 basement membrane (red) to the basolateral surface of the acini. These elastically tuned epithelial acini organoid models demonstrate that stiffening of the basement membrane causes a degeneration of polarity, breakdown of luminal structures, stable cell-cell junctions, and loss of the endogenous laminin 5 basement membrane. Nuclei (blue), F-Actin (green), and laminin 5 (red).

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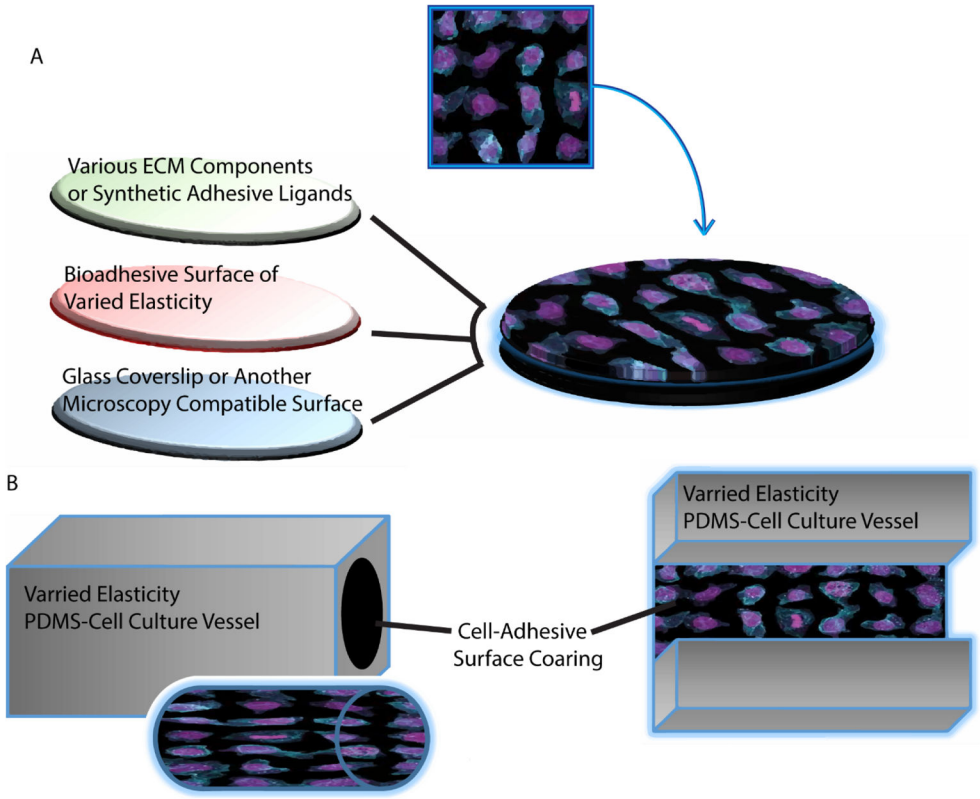

Figure 2. Methods to incorporate control of ECM stiffness into culture models of polarity

A. Typical assembly of materials to generate a mechanically tuned 2D surface for cell culture models. Commonly, polyacrylamide gels are mechanically tuned via alterations in polymer and crosslinking density and bonded to γ-aminopropyltriethoxysilane (APTES) functionalized glass coverslips. The resulting gel surface can be functionalized with ECM proteins or bioadhesive ligands via carbodiimide-mediated crosslinking, N-hydroxysuccinimidyl acrylate (NHS-acrylate), N-succinimidyl ester of acrylaminohexanoic acid (N6), Hydrazine, or polydopamine films functioning as an adhesive interface between the polyacrylamide and the desired surface coating [206,207]. B. Schematic representation of basic cell culture structures which can cast with elastically defined PDMS and are amenable to incorporate fluid flow induced shear stresses. Lumen mimetic tubes or channels may be then be associated with fluid pumps to control the volume and rate of fluid passing through the tube or channel to generate defined fluid flow across cell monolayers or through 3D-cell-lined-lumens.

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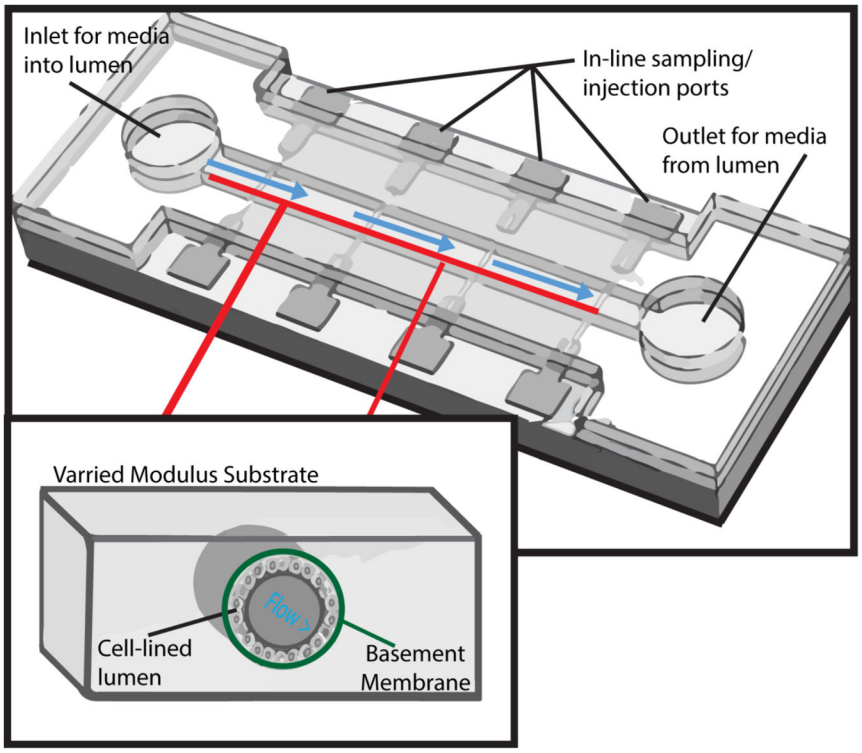

Figure 3. The utility of microfluidic devices

Presented here is a microfluidic device cast of PDMS of varied stiffness and bonded to a glass surface to create a sealed chamber. The glass surface allows for the device to be monitored with microscopy techniques and the in-line ports allow for additions or removal of substances from the outer surface of the lumen. This device has media inlets and outlets at the terminal ends of the channel so that controlled fluid flow of culture media may be added and modulated to vary fluid flow shear stresses. The inner channel would be lined with a cell monolayer mediated via laminin or collagen coating to foster appropriate polarization. Importantly, these devices may be cast in multiple pieces or multiple devices could be interconnected via flexible hoses [208].