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REVIEW

Mechanical forces in cell monolayers

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ABSTRACT

In various physiological processes, the cell collective is organized in a monolayer, such as seen in a simple epithelium. The advances in the understanding of mechanical behavior of the monolayer and its underlying cellular and molecular mechanisms will help to elucidate the properties of cell collectives. In this Review, we discuss recent *in vitro* studies on monolayer mechanics and their implications on collective dynamics, regulation of monolayer mechanics by physical confinement and geometrical cues and the effect of tissue mechanics on biological processes, such as cell division and extrusion. In particular, we focus on the active nematic property of cell monolayers and the emerging approach to view biological systems in the light of liquid crystal theory. We also highlight the mechanosensing and mechanotransduction mechanisms at the sub-cellular and molecular level that are mediated by the contractile actomyosin cytoskeleton and cell–cell adhesion proteins, such as E-cadherin and α-catenin. To conclude, we argue that, in order to have a holistic understanding of the cellular response to biophysical environments, interdisciplinary approaches and multiple techniques – from large-scale traction force measurements to molecular force protein sensors – must be employed.

KEY WORDS: Cell–cell junctions, Mechanobiology, Actin cytoskeleton, Collective cell migration, Active matter

Introduction

The ability of metazoan cells to interact, migrate, segregate and coordinate with each other is crucial for animal ontogenesis. As embryogenesis and histogenesis proceed, specific cell adhesion structures are formed between embryonic cells that act as platforms that allow cell communication and contribute, on the one hand, to the mechanical cohesion of tissues and, on the other hand, to the segregation of cell ensembles (Lecuit et al., 2011; Takeichi, 1988). In adult tissues, dysfunction of cell adhesion and mechanics frequently leads to loss of tissue homeostasis, with serious physio-pathological consequences, such as tumor development and metastasis (Scarpa et al., 2015; Thiery et al., 2009). Most of the morphogenetic processes during embryogenesis, tissue repair and cancer invasion represent collective cell dynamics that are associated with mechanical rearrangements and, in the latter case, the disorganization of tissues. Such arrangements are directly controlled through cell adhesion, actomyosin-mediated contractions and properties of the environment (Barriga et al., 2018; Friedl and Gilmour, 2009; Friedl and Mayor, 2017; Lecuit and Lenne, 2007; Szabó et al., 2016; Takeichi, 2014).

This large-scale reorganization of epithelial sheets or monolayers not only require, but are also regulated by, mechanical forces. These forces can be exerted actively, such as traction forces on underlying extracellular matrix (ECM) (Cetera et al., 2014), forces occurring during cell constrictions (Köppen et al., 2006) and at cellular protrusions (Reffay et al., 2014), as well as stresses at cell–cell contacts (Borghi et al., 2012). In addition, passive forces can be imposed by the physical properties of their environment (Barriga et al., 2018; Haeger et al., 2014; Vedula et al., 2014). It is thus important to understand how cells regulate contacts with the ECM and their neighbors, and apply forces on them. Even though recent techniques have been developed to measure forces (Bambardekar et al., 2015; Campàs et al., 2014) and stiffness *in vivo* (Barriga et al., 2018), the direct measurement of mechanical forces remains challenging. In this context, *in vitro* methods are expedient approaches to achieve multi-scale analysis from molecular to multicellular levels; at the frontier between physics and cell biology, they have been developed to understand the mechanoregulation of collective cell behaviors (Ashby and Zijlstra, 2012; Ladoux and Mège, 2017; Trepat and Sahai, 2018).

In this Review, we explore the role of mechanics-related processes in collective cell behaviors that are unraveled in *in vitro* systems of epithelial monolayers. We will discuss large-scale dynamics and mechanical properties of cellular monolayers, the effects of external physical constraints and internal long-range coordinated tension with a special focus on cell extrusion and tissue nematics. We also highlight the underlying cellular structures and molecular mechanosensing mechanisms that are involved in the regulation of monolayer dynamics. Last, we outline the experimental techniques for studying monolayer mechanics and the need for interdisciplinary approaches in future research.

Large-scale mechanical properties of cellular monolayers

Cell movements in a monolayer

The collective dynamics of cellular monolayers are regulated by mechanisms that take place not only at the front of leading cells but also in the inner cells of the tissue. The migration of cellular monolayers leads to the formation of large-scale coordinated movements that can extend over 10–15 cells, depending on the cell type and their geometrical confinement within the tissue (Poujade et al., 2007; Tambe et al., 2011; Vedula et al., 2014, 2012) (Fig. 1A). The extent of collectivity of cell movements has been well characterized by measuring the velocity correlation length within monolayers (Petitjean et al., 2010; Poujade et al., 2007) that determines, on average, to what extend pairs of velocity vectors are still correlated to each other. The coordinated movement of cells in the monolayer depends on the strength of cell–cell junctions, since fibroblast-like cells or cells with altered adherens junctions exhibit short-range interactions and, consequently, cell movements that are more like those of individual cells (Petitjean et al., 2010; Vedula et al., 2012).

Cell movements within confined patterns

The emergence of coordinated movements of monolayers has been further investigated using small circular micro-patterns on which endothelial and epithelial cells break the angular symmetry and move in a specific direction in a rotational fashion (Deforet et al.,

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Glossary

Angular motion and vorticity: In a small circular micropattern, cells rotate together around their center of mass in so-called angular motion. The vorticity field in tissue measures the local rotational movement in any part of a material, and is defined as $\omega = (\partial v_y/\partial x) - (\partial v_x/\partial y)$, where v_x and v_y are the *x*- and *y*-components of the velocity field.

Catch bond: In contrast to a slip bond (see below), a catch bond is a non-covalent bond, whose lifetime increases with increasing tensile force.

Inertia: Tendency of any physical body to resist change in states of motion and, preferentially, to move with the same velocity. The degree of inertia in a body is measured by its momentum, $P=mv$, where m is mass and v is velocity; more forces have to be exerted on the body to incur changes in its momentum according to Newton's second law.

Kinematics: The study of motion of objects without referring to the forces that generate the motion.

Mechanical wave: Oscillation of mechanical parameters within tissue – such as the tissue strain rate and mechanical stress – in space and time, which can travel from one point to another, like ripples in water.

(Monolayer) mechanical stress: When cells exert traction on the substrate, the reaction forces from the substrate can be transmitted within the tissue to other cells. In cellular systems, since the local mass is very small and negligible, the total substrate reaction force under any local portion has to be balanced out by local mechanical stress within the tissue according to Newton's second law. This is an important concept used in techniques, such as monolayer stress microscopy (MSM) and Bayesian inversion stress microscopy (BISM) to infer mechanical stress in the tissue.

Nematic liquid crystal: A liquid crystal (LC) are made up of anisotropic molecules that flow like particles in a liquid but can orient in a crystal-like way (long-range orientation order). There are different types of LC, the simplest of which is the nematic LC – with a single orientation axis at each local point. Nematic LCs have enormous technological impact as they are used in LC displays and the average orientation can be easily switched using electric fields.

Reynolds number: Used to characterize a fluidic system and defined as $Re=\rho vL/\eta$, where ρ is mass density, v is velocity, L is system length scale and η is fluid viscosity. At a high Re, the system has huge inertia and can produce turbulent flows owing to the tendency of each of its local parts to maintain constant movement. At a low Re, viscosity effects dominate and dampen momentum; flows in passive liquids should be laminar and can only be driven to move by the constant exertion of external forces on the system. An example of a high-Re system is seawater, whereas honey has a low-Re system. In active, low-Re systems, the components produce their own forces that maintain its motion.

Slip bond: A typical non-covalent chemical bond, whose dissociation lifetime decreases with increasing tensile force, i.e. it is easier to pull apart two interacting molecules when force is increased.

Tissue strain rate: Spatial gradient of velocity fields (see entry below) within the tissue. For example, the change of the x component of the velocity in the x-direction in space is written as $\dot{\varepsilon}_{xx} = \partial v_x/\partial x$. Thus, taking into account all possible velocity components in all possible directions within 2D, the full information on strain rate can be incorporated in a 2×2 matrix called the strain rate tensor. This quantity describes the rate of deformation at each local point in the tissue.

Traction forces: Through actin stress fibers and the connection to the underlying substrate through focal adhesion, cells can exert traction forces on the substrate that depend on the contractility of the cell, usually conveyed by non-muscle myosin II.

Velocity correlation length: This length determines the collectivity of cell movements in the tissue, and is determined from the correlation function, $C_u(\rightarrow\mathrm{r}) = \langle(\langle u^*(\rightarrow\mathrm{r}'+\rightarrow\mathrm{r},t)\times u^*(\rightarrow\mathrm{r}',t)\rangle\rightarrow\mathrm{r}')/(\left[\langle u^*(\rightarrow\mathrm{r}'+\rightarrow\mathrm{r},t)^2\rangle\langle u^*(\rightarrow\mathrm{r}',t)^2\rangle\right]^{1/2})\rangle t$, where u^* is the deviation of the velocity from the mean velocity, $\rightarrow$r is the vector of the coordinates t is time, and $\langle\ \rangle$ is the average over the respective parameters. The correlation length can be calculated as the distance when the correlation function (which starts at value=1 at distance=0) approaches zero.

Velocity field in tissue: A grid of velocity values is assigned within the tissue at each space and time point, not related to any particular single cell, determined by techniques such as particle image velocimetry (PIV) (Deforet et al., 2012) or optic flow methods (Vig et al., 2016). This determines the local kinematics of the tissue.

2012; Doxzen et al., 2013; Huang et al., 2005). This is reminiscent of the movements of clusters of several breast epithelial cells grown in 3D collagen gels, and is termed coherent angular motion (see Glossary) (Tanner et al., 2012). This synchronized rotation within the circular pattern (Fig. 1A) depends on several parameters that include cell density, cell–cell adhesion and the size of the confinement (Deforet et al., 2012; Doxzen et al., 2013; Huang et al., 2005). As density increases through cell proliferation, cellular velocity within the monolayer decreases, possibly as a result of higher intercellular friction (Deforet et al., 2012; Garcia et al., 2015). This phenomenon has also been interpreted in the framework of 'jammed' systems in analogy to colloidal particles in physics (Angelini et al., 2011; Atia et al., 2018; Park et al., 2015). In such systems, particle movement slows down and the size of particle clusters increases with particle density (Hakim and Silberzan, 2017). However, such an analogy with jammed particles cannot fully explain the observed behaviors of living cells, where systems with similar densities can either remain fluid or jammed (Mongera et al., 2018; Park et al., 2015). It appears that other parameters, such as cell contractility, cell–cell contacts and cell–substrate adhesions also have to be taken into account (Bi et al., 2016; Garcia et al., 2015). Finally, the size of the confined micro-patterns can trigger various collective dynamics. When confined to patterns that are larger than the velocity correlation length of the tissues, cell clusters do not show a fully coordinated rotational movement but, rather, display transient vortices or swirls that are smaller than the tissue (Doxzen et al., 2013; Vedula et al., 2012) (Fig. 1A).

Mechanical coordination of cell monolayers

The expansion of migrating cell monolayers leads to particular force patterns, in which the largest traction forces (see Glossary) exerted by cells on their substrate are localized at the leading front (du Roure et al., 2005; Trepat et al., 2009). From force balance between cell–cell and cell-substrate interactions (Trepat et al., 2009), intercellular stress exerted through cell–cell junctions within the monolayer can then be calculated as the sum of the traction forces. As a result, this mechanical stress (see Glossary) is increasing from the edges of the monolayer towards the bulk of the tissue, exhibiting a tensile state for the entire monolayer. The built up tension from the free edges has been further confirmed by measuring the E-cadherin-mediated tension using Förster resonance energy transfer (FRET) biosensors (Gayrard et al., 2018). It confirmed a tension gradient that is low in front cells and high in the back of the tissue. In addition, the dynamic mapping of forces and velocities within expanding monolayers has revealed the emergence of large-scale propagating mechanical waves of tissue strain rate (see Glossary) and stress from the front towards the center of the monolayer (Serra-Picamal et al., 2012) that covers distances of up to several hundreds of microns. These kinematics (see Glossary) and force patterns lead to the interpretation that large-scale mechanical signals propagate within cohesive cell monolayers through cell–cell junctions (Serra-Picamal et al., 2012). Since the largest traction forces are observed at the location of leader cells at the edge (Reffay et al., 2014; Trepat et al., 2009), together with higher intercellular stresses towards the bulk, the propagation of physical waves and the associated collective motion may be

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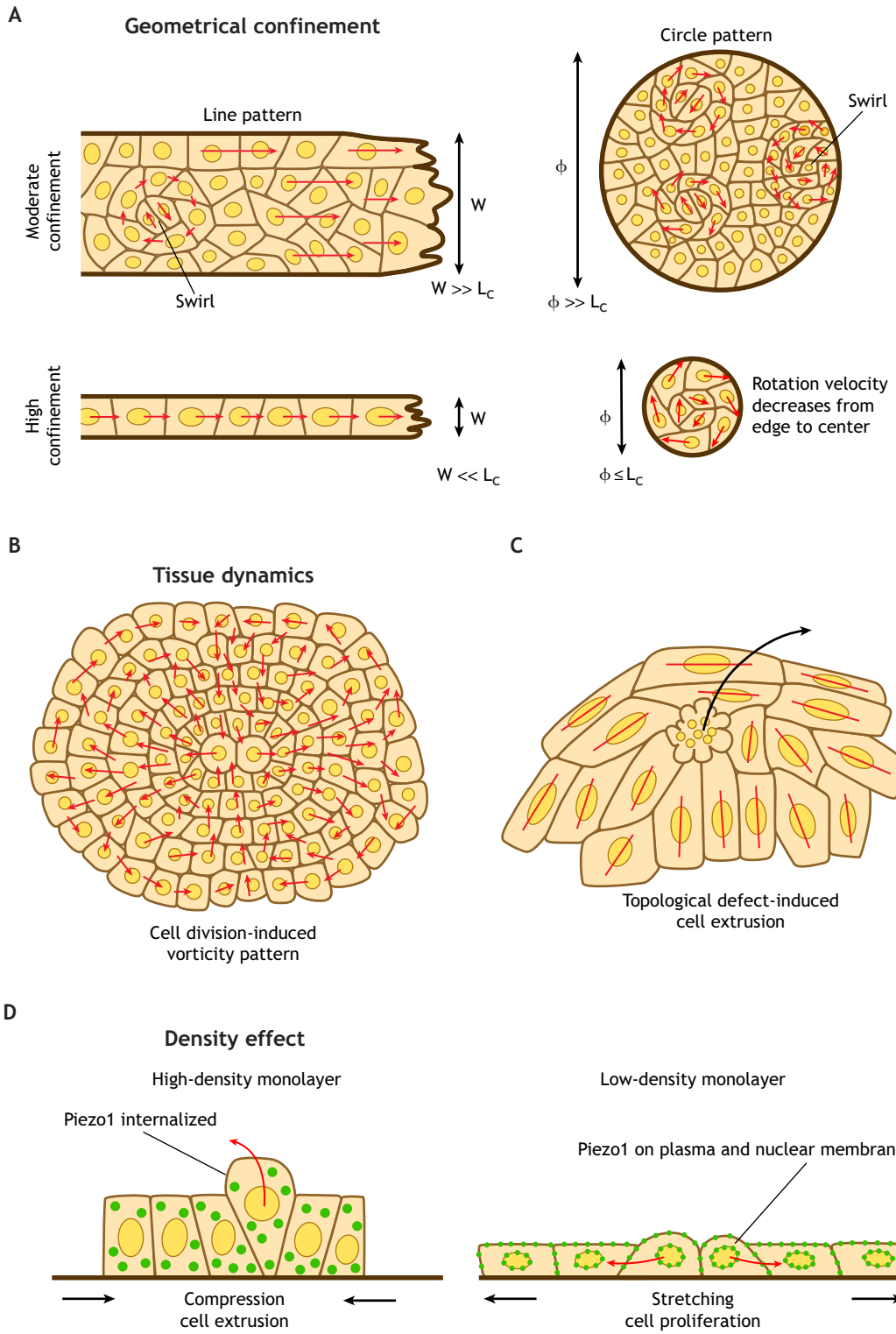

Fig. 1. Monolayer mechanics at tissue scale. (A) Tissue motion depends on geometrical confinement. Regarding line patterns, swirls can form inside the monolayer when the width (W) of the line pattern is larger than the correlation length (L_C), e.g. 400 µm versus ∼150 µm for a MDCK monolayer; $W>>L_c$ (see 'velocity correlation length' in Glossary), but cells can engage in unidirectional motion on highly confined patterns, where $W\ll L_C$, for example, when the width is 20 µm. Similarly, regarding circle patterns (right side), swirls form inside the monolayer when the diameter Φ (e.g. 500 µm) of the pattern is much larger than L_C ($\Phi>>L_c$). However, the tissue is able to rotate consistently when Φ is smaller or comparable to L_C, for example when diameter is 100 µm ($\Phi\leq L_c$). Red arrows signify the local velocity vectors. (B,C) The dynamics of the monolayer can be affected by physiological events, such as cell division and extrusion. Cell division can induce vortexes (red arrows) in neighboring cells (B). In turn, cell extrusion (C) is also affected by large-scale tissue geometries, such as topological defects (see Box 1). Red lines signify the principle orientation of each cell. Black arrow initiates within the cell that is to be extruded. (D) High-cell density in monolayers can induce mechanical compression and low density can lead to stretching forces, which can induce extrusion or proliferation, respectively. This could be regulated by the location and activity of the mechano-sensitive ion channel Piezo1 (green dots), which localizes to the plasma membrane and peri-nuclear region in cell-sparse monolayers but forms cytosolic aggregates in cell-dense monolayers.

interpreted from a biochemical perspective. For instance, a high intercellular stress in a follower cell has been shown to promote the relocalization of merlin, a tumor suppressor protein, from the cell–cell junctions to the cytoplasm (Das et al., 2015). This eventually leads to the activation of the small GTPase Rac, which in turn promotes lamellipodial protrusion. Such mechanism may, thus, favor a newly polarized state of the follower cell that could protrude cryptic lamellipodia underneath the cell in front of it (Farooqui and Fenteany, 2005). From this perspective, merlin appears to be an interesting candidate acting as 'mechanochemical transducer' at the cell–cell junction, which may translate mechanical information sensed by the cell into biochemical signals and, hence, promote large-scale tissue polarization.

Role of cell division and extrusion in tissue mechanics

The aforementioned velocity waves during tissue expansion can even extend further when cell division is blocked (Tlili et al., 2018). This suggests that cell division can perturb monolayer flows by modifying the local dynamics. In accordance, it has been shown that long-range dynamics were induced by cell divisions in endothelial cell monolayers (Doostmohammadi et al., 2015; Rossen et al., 2014) (Fig. 1B). Rossen et al. reported the emergence of well-ordered vortex patterns around cell divisions that could extend to several cell diameters away from the division event (Rossen et al., 2014). From a mechanical point of view, this might be explained by a local pressure increase within the tissue, which leads to particular flow patterns reminiscent of turbulent flows characterized by chaotic changes in pressure and flow velocity. Interestingly, turbulent flows are usually associated with hydrodynamic systems of a high Reynolds number (*Re*), i.e. high inertia (see Glossary) dominating over viscous effects that dampen mechanical motion. Yet, cellular systems that have a very low Reynolds number (Purcell, 1977) still produce turbulent migratory movements and this conundrum turns out to be attributed to the active forces that cellular systems can exert. Apart from actomyosin forces, cell division events can also inject energy into the monolayer (Doostmohammadi et al., 2015).

Monolayer dynamics is not only perturbed by cell division but also by cell extrusion events, apoptotic extrusion (Kocgozlu et al., 2016; Rosenblatt et al., 2001) (Fig. 1C) and live cell delamination (Eisenhoffer et al., 2012; Gudipaty et al., 2017) can also affect monolayer dynamics and are regulated by monolayer mechanics. Here, the extrusion process does not only involve contraction of the extruding cell and its immediate neighbors (Kuipers et al., 2014; Rosenblatt et al., 2001) but is also accompanied by long-range inward migration of several layers of surrounding cells (Kocgozlu et al., 2016). This, together with division events, can fluidize the tissue (Ranft et al., 2010), meaning that the cells within the tissue can flow more easily – like particles in a liquid, rather than being 'caged' within a particular location – like particles in a solid. Indeed, it was shown that local density and mechanics trigger either cell division or extrusion in epithelial monolayers (Eisenhoffer et al., 2012; Gudipaty et al., 2017; Marinari et al., 2012). Stretch reduces cell density, thereby inducing cell division; whereas cell crowding increases cell density that leads to cell extrusion (Fig. 1D). Both mechanisms appear to depend on the activation of the mechanosensitive ion channel Piezo1, but through different pathways. At low cell density, Piezo1 localizes to the plasma membrane, possibly facilitating the transduction of tissue mechanical tension into biochemical signals to induce cell division, whereas at high density, Piezo1 aggregates in the cytoplasm (Gudipaty et al., 2017) (Fig. 1D). However, the mechanisms for this spatial redistribution of Piezo1 on the basis of cell density, and the mechanism of its aggregation in the cytoplasm at high cell density and how it triggers cell extrusion are unclear.

Tissue as active nematics

In addition to the above, cell shape and actomyosin activity of cell monolayers can lead to cell-packing arrangements that play an important role in explaining collective cell migration and cell extrusion (Saw et al., 2017). Specifically, epithelial cells within the monolayer have slightly elongated shapes, and this anisotropic shape prompts them to spontaneously align along preferential directions in a dense tissue environment (Fig. 2A) that is similar to elongated rod-like shaped particles in a nematic liquid crystal (Glossary). As the name suggests, the anisotropic particles in a liquid crystal have a long-range orientation order as in crystals, i.e. they order on a length-scale that is much larger than the size of a particle but, at the same time, can move as easily as particles in a liquid (de Gennes and Prost, 1995).

The spontaneous movement of cells that arises from ATP hydrolysis and actomyosin activity leads to the generation of active mechanical stresses (Marchetti et al., 2013; Prost et al., 2015), the defining feature that sets living systems, such as epithelial tissues apart from passive nematic systems. Such internal activity can induce cellular bend and splay configurations (Fig. 2B), and even local spots of misaligned cells that present different patterns of organization (Box 1). The misalignment points are locations with undefined local cell orientation axes that, in the framework of liquid crystals, are referred to as topological defects (de Gennes and Prost, 1995). It was found that cellular monolayers present misalignments that are similar to the ones observed in nematic liquid crystals. Predominantly, they form comet-like or triangular shapes that are called +1/2 and −1/2 defects, respectively (Duclos et al., 2016; Saw et al., 2017) (Box 1).

In the context of active systems, the formation of defects can be understood through the forces that cells can exert on each other. In theory, there are two different types of force pattern that cells can exhibit towards their neighbors: outward or inward forces along the long axis of the cell body, which causes them to extend or contract in that direction, respectively (Fig. 2C). When cells are in a well-aligned region, the local cellular forces are balanced and the arrangement is stable (Fig. 2D). However, when the alignment is distorted – as is the case in defect positions – a spatial gradient of active forces emerges, which can drive large-scale motion in defects that have well-defined head and tail directions, such as the +1/2 defect (Fig. 2E). By contrast, a −1/2 defect, which has a three-fold symmetry, should not exhibit dynamics (Box 1). The direction of motion of a +1/2 defect can be used to distinguish whether the cells are extensile or contractile. Specifically, extensile (contractile) nematic particles have a force imbalance at the +1/2 defect core that drives the defect in the comet tail-to-head direction (head-to-tail) (Fig. 2E). Interestingly, these emergent mechanical properties of cell monolayers appear to depend on the cell type, since fibroblasts are contractile (Duclos et al., 2016), whereas epithelia and neural progenitor cells are extensile (Kawaguchi et al., 2017; Saw et al., 2017). Owing to these local activities, defects are continuously generated and annihilated, which drives a chaotic motion in living nematic systems and can be understood as a source of collective epithelial dynamics.

Apart from driving tissue dynamics, the appearance of comet-shaped topological defects in epithelial monolayers provides a mechanism that explains cell extrusion; i.e. the misalignment of cells causes significant bending of cells, leading to high compressive stresses in these regions (Saw et al., 2017). These

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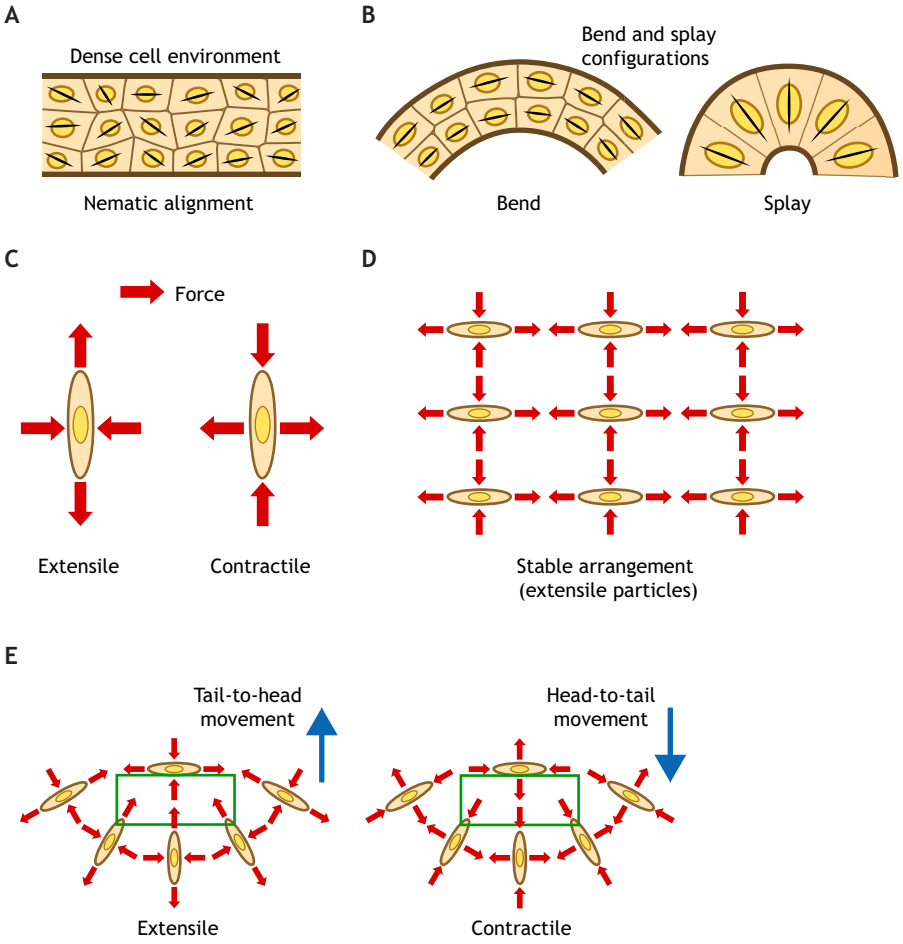

Fig. 2. Tissue as an active nematic. (A) Cells have elongated bodies and, when contained in a dense environment, tend to orient along a common axis with their local neighbors, while still being able to 'flow' around like particles in a liquid (black lines show local orientation directions). These properties fit the material descriptions of a nematic liquid crystal (see Glossary), where rod-shaped particles have long-range orientation order, yet fluidic motion. (B) When cell alignment becomes distorted, bend (left) and splay (right) configurations appear in the epithelial monolayer. These configurations are also found in other nematic physical systems. (C) The prominent feature of active cellular systems is that they can exert forces on other cells. As cells exhibit dipolar forces, in theory, there are two different types of force pattern that they can generate on their neighbors, i.e. outward or inward forces (red arrows) along the long axis of their bodies, causing them to, respectively, extend or contract in that direction. (D) When cells are in a well-aligned region, local cellular forces are balanced and the arrangement is stable. (E) When alignment is distorted, as in a defect position, the force balance is broken (green box indicates force imbalance at a +1/2 comet-shaped defect; see also Box 1). The direction of motion of a +1/2 defect is dictated by its dipole force distributions and can be used to distinguish whether the cells are extensile or contractile. Specifically, extensile nematic particles have a force imbalance that drives the +1/2 defect to move from tail to the head of the comet whereas contractile ones move from head to tail (blue arrows show movement direction).

stresses are then sufficient to trigger apoptosis and extrusion of a nearby cell. Overall, the active contractility of the cells that are coupled through their cell–cell junctions, and the division and extrusion events in the monolayer give rise to complex tissue dynamics.

In summary, the tissue-level kinematics and mechanics have a complex reciprocal relationship with underlying cell–cell adhesion, actomyosin cell activity, and cell division and extrusion events. These local events are, in turn, controlled by mechanisms of mechanosensing and mechanotransduction at cellular and molecular levels. In the following sections, we discuss these mechanisms with regard to the actomyosin cytoskeleton and molecular organization, as well as the regulation at the cell–cell junctions.

The regulation of collective migration of monolayers

The collective movement of cell monolayers requires the coordination of cells within the cohort and the transmission of physical forces between neighboring cells, which relies on force-bearing structures, such as adherens junctions and the dynamic regulation by the actin cytoskeleton. At the sub-cellular level, actin and the cadherin complex can form cell-type specific structures (Takeichi, 2014; Yonemura et al., 1995), such as the zonula adherens in mature epithelial cells. Equipped with parallel contractile actomyosin belts that are located close to the plasma membrane at the apical surface of the cell, these structures are important for the establishment of cell polarity and morphogenetic processes, such as apical constriction (Lecuit and Lenne, 2007; Mason et al., 2013) (Fig. 3A). The forces that are exerted on cell–cell junctions by the contraction of the actomyosin network contribute to cell intercalation in *Drosophila* germ-band extension (Rauzi et al., 2010). Pulses of actomyosin contraction towards dorso-ventral junctions create a polarized flow that leads to shrinkage of these junctions and their planar polarized remodeling. The actomyosin network undergoes polarized flow owing to the asymmetric coupling to E-cadherin clusters at junctions (Levayer and Lecuit, 2013; Rauzi et al., 2010).

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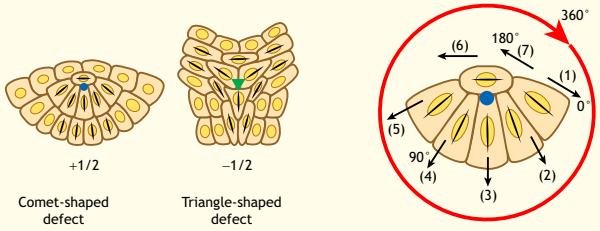
Box 1. Topological defects in an active system



The points of cell misalignment are analogous to singularities in an orientation field that are called topological defects. The most commonly occurring defect patterns are the comet-shaped (+1/2) and triangle-shaped (−1/2) defects (centers of which are marked with a blue dot and a green triangle, respectively). The – +1/2 versus −1/2 – nomenclature for these defects stems from the fact that the direction of local orientation (black arrows) either changes by turning clockwise (follow the black arrows according to the numerical order 1 to 7) (for comets) or counterclockwise 180° (for triangles) when we trace one full circle, i.e. 360° in the clockwise direction (red arrow) around a defect core.

Organization of free edges of cell monolayers

In an expanding monolayer, a leader cell with enhanced motility and large lamellipodium is often observed at the tip of finger-like multicellular structures (Poujade et al., 2007) (Fig. 3B). The leader cell exerts large traction forces on the substrate but, at the same time, also exerts a pulling force on the follower cells (Reffay et al., 2014). Strikingly, in doing so, they together behave as a single migrating entity with coordinated directionality and polarity (Reffay et al., 2011). The adhesion between leader and followers can be homotypic – as in epithelial monolayers, or heterotypic – as in between cancer cells and cancer-associated fibroblasts. The formation of leader cells itself is also regulated by mechanical forces (Riahi et al., 2015). Multicellular contractile actin cables that are observed along the sides of the finger-like structures (Fig. 3B,C) can be involved in suppressing the formation of new leader cells (Reffay et al., 2014; Vishwakarma et al., 2018).

During epithelial wound healing and *Drosophila* dorsal closure, a supracellular structure that is composed of thick actomyosin cables and connected by adherens junctions and/or tight junctions between neighboring cells forms around the edge of the wound/gap, and contracts in a purse string-like manner to close it (Brock et al., 1996; Danjo and Gipson, 1998; Tamada et al., 2007). A recent study has challenged the essential role of the actin cable during *Drosophila* dorsal closure (Ducuing and Vincent, 2016); however, it remains unclear whether it applies also to other cases and whether the actin cable serves other functions, such as coordination of the monolayer migration. *In vitro* force measurements of wound closure over circular non-adhesive gaps in cell sheets of keratinocytes revealed that cells first exert traction forces on the substrate that point away from the gap and, once they have extended over the gap – the contractile purse string-cables have now formed across the leading edge cells – the radial component of the force reverts direction and points towards the gap (Vedula et al., 2015). The 'tug-of-war' mechanism that was identified in that study, as well as studies showing that cells are capable to form multi-cellular bridges (Sharma et al., 2017; Vedula et al., 2014), provide a clear demonstration of how a group of cells exert directional forces in order to facilitate epithelial gap closure with supracellular actin cables, while still maintaining adhesion to the matrix. Interestingly, actin structures are also regulated by the geometrical information that is sensed by the cell (Elliott et al., 2015; Parker et al., 2002; Thery et al., 2006). During epithelial wound healing, multi-cellular actin cables are promoted at concave edges (negative curvature) and, in cooperation with lamellipodium protrusions at convex (positive curvature) edges, their contraction pulls the monolayer forwards (Klarlund, 2012; Ravasio et al., 2015) (Fig. 3C).

The collective behavior of the monolayer is, therefore, a result of the interactions between neighboring cells. The aforementioned studies on sub-cellular structures, such as the adherens junctions and associated actin cytoskeleton help to elucidate how cells within the monolayer can adopt different roles, e.g. leader and follower (Khalil and Friedl, 2010), establish local polarity with differential distribution of proteins and asymmetric organization of the actomyosin contractility (Barlan et al., 2017; Das et al., 2015; Hayer et al., 2016), as well as transmit intercellular forces with adhesion proteins and actin cables (Reffay et al., 2014; Vedula et al., 2014). Together, these insights help us to understand the large-scale dynamics and mechanics of the monolayer.

Molecular mechanosensing of adherens junctions

At the molecular level, the mechanical interconnection between cells is mediated by the interaction between transmembrane proteins, such as cadherins (Takeichi, 2014; Yonemura et al., 1995). Although other cadherin proteins, such as N-cadherin, VE-cadherin and P-cadherin (Bazellières et al., 2015), as well as other junctional structures, such as tight junctions and desmosomes also play a role in the regulation of intercellular mechanics depending on the context and cell type (Ladoux and Mège, 2017), E-cadherin present at adherens junctions is the most-important and best-studied adhesion protein in epithelial cells (Harris and Tepass, 2010; Mège and Ishiyama, 2017). E-cadherin-mediated adhesion is mediated by trans-interactions between the extracellular domains of E-cadherin from two neighboring cells, reinforced by cis-interactions between E-cadherin molecules within the same cell (Harrison et al., 2011; Strale et al., 2015; Truong Quang et al., 2013; Wu et al., 2015). The intracellular domain of E-cadherin recruits accessory proteins, like, α-catenin and β-catenin, which physically link the adhesion complex to the actin cytoskeleton (Mège and Ishiyama, 2017) (Fig. 3D).

Molecular clustering stabilizes E-cadherin adhesions

Transmembrane proteins, such as E-cadherin, undergo fast 2D diffusion on the plasma membrane but can also form clusters through protein-protein interactions (Bihr et al., 2012; Cavey et al., 2008; Mancini et al., 2017), which help to stabilize them. Spontaneous clustering of E-cadherin molecules in the plasma membrane can induce the formation of micron-sized punctae (Adams et al., 1998; Engl et al., 2014; Lambert et al., 2007) or oligomers (Strale et al., 2015; Wu et al., 2013) during adhesion formation and resist the perturbation of thermal fluctuation and external load (Fenz et al., 2017). Interestingly, the clustering of cadherin is also influenced by the interaction of the cytoplasmic domain of cadherin with actin and by myosin contractility (Hong et al., 2013; Lambert et al., 2007). The ability of E-cadherin to cluster and form force-bearing mechanotransducing structures provides a biophysical basis for the mechanical integrity of cell monolayers. However, the capability of cells to modify E-cadherin clustering through actomyosin-generated forces could allow cell monolayers to change their mechanical properties at a molecular level, but remains to be further investigated.

α-catenin as a molecular mechanosensor

Adherens junctions are connected to contractile actomyosin cytoskeleton and under active force (Lecuit and Yap, 2015). *In vivo*

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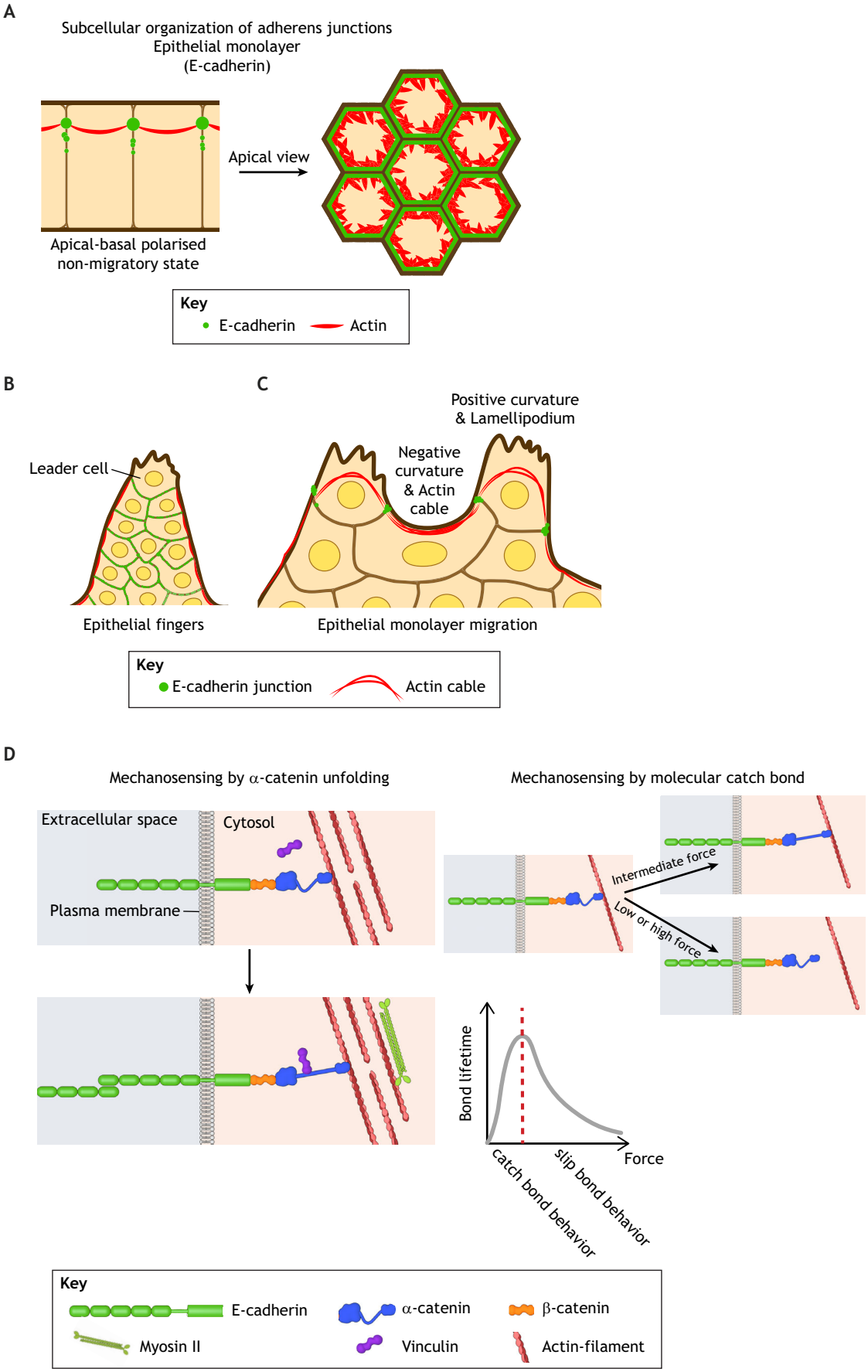

Fig. 3. See next page for legend.

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Fig. 3. Cell adhesion structures and molecular mechanisms of mechanosensing. (A) The subcellular organization of adhesion structures in a monolayer varies depending on the context. In apical–basal polarized epithelial monolayers, apical actin belts (red) connect with E-cadherin (green) to form the zonula adherens (adherens junctions). (B) Multicellular finger-like structures form at the leading front of an expanding epithelial monolayer, with a leader cell at the tip and supracellular, i.e above-cell-level, actin cables at the two sides of the finger-like protrusion. (C) The leading front of a migrating monolayer shows lamellipodia formation at regions of positive curvature but forms supracellular actomyosin cables at regions of negative curvature. (D) The molecular mechanism of force-sensing at the adherens junction can be explained by the force-dependent unfolding of α-catenin (blue) and the subsequent binding of vinculin (purple). The binding between the E-cadherin–β-catenin–α-catenin complex (green/orange/blue) and F-actin (red) exhibits a catch bond behavior where the bond lifetime initially increases with force, then reaches a maximum (graph, red dashed line) and then decreases with force (slip bond; see Glossary).

measurements of the molecular forces exerted on E-cadherin have been performed with FRET tension sensors inserted into its cytoplasmic domain, and revealed a load of 1–2 pN force per molecule on average at stable junctions between cell doublets (Borghi et al., 2012). α-catenin and vinculin have emerged as the main force sensors at adherens junctions (Ladoux et al., 2015). The recruitment of vinculin to E-cadherin complexes depends on myosin II-generated cellular forces (le Duc et al., 2010). Seminal work from S. Yonemura's laboratory revealed that α-catenin can undergo force-induced unfolding and expose a vinculin-binding site (Yonemura et al., 2010) (Fig. 3D). In addition, single-molecule force spectroscopy experiments revealed that this vinculin-binding site on α-catenin becomes exposed under a stretching force of 5 pN (Maki et al., 2016; Yao et al., 2014), which facilitates binding between α-catenin and the vinculin head domain. Importantly, the force-dependent binding between vinculin and α-catenin shows biphasic behavior, i.e. at high forces of >30 pN, the vinculin-binding domain further unfolds, which causes loss of affinity of α-catenin towards vinculin (Maki et al., 2016; Yao et al., 2014). These data suggest that the junction is stable within a certain force range and is disrupted once high stress is exerted by the actomyosin cytoskeleton. Furthermore, binding between α-catenin and the actin cytoskeleton is also sensitive to force. Indeed, by using optical tweezers to probe the interaction between a trapped actin filament and a tertiary complex of the cytoplasmic domains of α-, β-catenin and E-cadherin, Buckley et al. revealed that the interaction between α-catenin and F-actin can be characterized as a catch bond (Glossary), with an optimum binding force at ∼8 pN (Buckley et al., 2014) (Fig. 3D).

Molecular mechanosensing mechanisms contribute to large-scale monolayer dynamics

One central goal of the multi-scale studies of monolayer mechanics is to understand how small-scale molecular and cellular mechanisms can explain large-scale physiological functions of the cell collective, such as migration. The mechanosensing ability of the molecular force transducers is essential for regulation of the large-scale mechanics of the monolayer. Not only do different cadherins regulate the magnitude and dynamics of the intercellular tension within the monolayer (Bazellières et al., 2015), but the protein–protein interaction-induced clustering of these adhesion proteins on the membrane also plays an essential role in regulating the stability of cell–cell junctions, as well as their collective migration (Strale et al., 2015). The molecular mechanosensor of adherens junction α-catenin and its interaction with vinculin are also involved in the regulation of intercellular tension within monolayers and the collective migration dynamics both *in vitro* (Seddiki et al., 2018) and *in vivo* (Han et al., 2016). Suppression of α-catenin protein expression – or its force-dependent interaction with vinculin by decreasing the strength of cell–cell adhesions at the molecular scale – decreases collective cell migration correlation length *in vitro* and increases cell migration and the exchange of neighboring cells (Seddiki et al., 2018). Furthermore, during embryonic development in zebrafish, the mechanosensing function of α-catenin is not required for epithelial barrier formation; it is, however, required for directional cell migration during convergent extension (Han et al., 2016).

In relation to topological defects and cell extrusion in the monolayer, α-catenin knockdown increases defect density and extrusion rate (Saw et al., 2017). However, the size of the defects decreases after α-catenin knockdown, possibly owing to a decrease in orientational elasticity of the cells in the monolayer (Saw et al., 2017). Along these lines, the nematic description of cell monolayers, such as fibroblasts or epithelial cells, does not display the same behavior, being contractile or extensile, respectively. Since fibroblastic cells develop weaker cell–cell adhesions than epithelial cells (Duclos et al., 2016; Kawaguchi et al., 2017), these differences suggest that the mechanical strength developed at cell–cell junctions triggers various dynamical responses of cellular monolayers. The development of novel techniques that can probe monolayer mechanics at various scales is also essential for future studies to bridge the gap between molecule and tissue scales.

Techniques and challenges in studying monolayer mechanics

Recent technical advances have enabled researchers to study the mechanical properties of the cell monolayer in more depth, especially *in vitro* (Polacheck and Chen, 2016). Here, the physical environment can be controlled and multiple physical parameters, such as velocity, strain, force, rigidity of the substrate are easily probed. Moreover, various experimental conditions, such as substrate composition, geometrical confinement and pharmaceutical perturbations are possible to implement (Vedula et al., 2014). However, current methods still face considerable difficulties for measuring certain physical parameters, particularly when high spatial-temporal resolution is desired and 3D information is required to make sense of biological processes. Also, it remains challenging to probe the mechanical properties of tissues *in vivo*, even though recent advances have been made in this direction (Bambardekar et al., 2015; Campàs et al., 2014). To measure forces that cells apply on the substrate *in vitro*, traction force microscopy (Trepat et al., 2009) or micro-pillar arrays (du Roure et al., 2005; Tan et al., 2003) can be used. Both methods allow researchers to measure traction forces at the multicellular scale in 2D together with the option to modify the rigidity of the substrate or to create rigidity gradients. Traction force microscopy functions on the basis of single-particle tracking and has been shown to achieve high-resolution information below the scale of a single focal adhesion (Plotnikov et al., 2012). By embedding cells in a 3D gel, researchers have also developed methods to measure traction force in three dimensions (Legant et al., 2010). The challenge in studying monolayer mechanics, however, lies in measuring inter-cellular tension. Although intercellular forces have been measured by using the dual-pipette assay (Chu et al., 2004) and also for detached cell collectives, it is still impossible to directly measure tension within an adhesive and migrating monolayer. Computational inferences from traction forces can provide us with the calculation of intercellular stresses within cellular monolayers (Nier et al., 2016, 2018; Tambe et al., 2011). As mentioned above, FRET-based tension sensors have been developed to measure molecular forces exerted on cell adhesion proteins (Borghi et al., 2012). This has proven to be

useful in studying forces at the pico-Newton scale but was also applied to studying monolayer mechanics at larger scale with access to local forces at resolution below that of a single cell (Kim et al., 2015) and *in vivo* (Cai et al., 2014). However, caution should be exerted when interpreting the results, as the readout is an average of the dynamic forces of a group of molecular sensors. Furthermore, the rheology of the cell and the cytoskeleton can be probed by atomic force microscopy (AFM) (Alcaraz et al., 2003) and magnetic tweezer beads or optical tweezer-controlled beads that are attached to the cell membrane (Muhamed et al., 2016). AFM has also been successfully used to measure tissue rigidity *in vivo* (Barriga et al., 2018; Koser et al., 2016). Perturbation of cell contractility or controlled disruption of force-bearing cellular structures by laser ablation can also be used to qualitatively or semi-quantitatively infer the forces within the monolayer or cytoskeleton (Hara et al., 2016; Kumar et al., 2006).

In summary, culturing cellular monolayers on engineered substrates that mimic the composition and mechanical properties of their physiological microenvironment provides a means to precisely test and measure cellular responses and forces. The various techniques to shape the substrate surface provide ample possibilities to test the effect of physical confinement, substrate rigidity and geometry on monolayer dynamics (Doxzen et al., 2013; Nikolić et al., 2006; Poujade et al., 2007; Vedula et al., 2012). For instance, the interplay between actomyosin cable formation and cell crawling mechanisms during epithelial closure can be better dissected when geometrical constrains and traction forces exerted by cells on their underlying substrate are controlled (Brock et al., 2003; Parker et al., 2002; Ravasio et al., 2015). Along the same line, new challenges have been recently addressed by mimicking *in vivo* environments through the use of curved surfaces (Hu et al., 2014; Yevick et al., 2015), such as villus-like structures (Salomon et al., 2017; Viswanathan et al., 2016) or microtubules (Xi et al., 2017).

Clearly, a collaborative effort that involves physical and biological disciplines is needed to advance our technical capabilities in order to effectively quantify biophysical parameters when investigating monolayers. Future technical development that enables us to measure physical forces in biological systems at higher spatial-temporal resolution and in 3D, as well as non-invasive techniques to measure forces *in vivo*, will significantly advance our understanding of monolayer mechanics.

Conclusion and perspectives

The mechanical *in vitro* studies discussed here have provided important insights into how physical parameters can affect cell–cell contacts and may lead to various changes in tissue reorganization and dynamics at the multicellular scale. Understanding collective cell mechanics of monolayers, thus, requires integrated approaches from molecular to multicellular scales and the combination of engineering, cell biology and soft matter physics. Concepts on the basis of physical principles that have been developed for non-living systems – such as liquid crystals – exemplify that it may help to define a biophysical framework in order to understand cellular organization, cellular dynamics and tissue homeostasis (Duclos et al., 2018; Kawaguchi et al., 2017; Saw et al., 2017).

Future directions of these integrated research approaches on the mechanobiology of cellular assemblies are the application of well-controlled external signals, the use of pressure-controlled systems, electric fields (Cohen et al., 2014) or mechanical forces, but also the development of active microenvironments that can mimic interaction with other cell types and adapt to tissue mechanical properties that play important roles in the regulation of collective cell behaviors *in vivo*.

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Competing interests

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