Trends in Cell Biology

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Review

Leveraging computational modeling to explore epithelial and endothelial cell monolayer mechanobiology

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Endothelial cells (ENCs) and epithelial cells (EPCs) form monolayers whose barrier function is critical for the maintenance of physiological processes and extremely sensitive to mechanical cues. Computational models have emerged as powerful tools to elucidate how mechanical cues impact the behavior of these monolayers in health and disease. Herein, the importance of mechanics in regulating ENC and EPC monolayer behavior is established, highlighting similarities and differences in various biological contexts. Concurrently, computational approaches and their importance in accelerating mechanobiology studies are discussed, emphasizing their limitations and suggesting future directions. The aim is to inspire further synergies between cell biologists and modelers, which are crucial for accelerating cell mechanobiology research.

Highlights

Computational models enable quantitative predictions of the cell monolayer's behavior that can be experimentally tested.

Models can help infer biomechanical parameters of monolayers that can hardly be measured *in vitro*.

The accuracy of computational models depends on the quality of data, highlighting the need to improve *in vitro* and *in vivo* techniques for measuring monolayer biomechanics.

Models can help explain the biophysical processes governing endothelial cell (ENC) and epithelial cell (EPC) monolayer behavior in health and disease.

Models can expose differences in EPC and ENC mechanobiological responses likely stemming from the unique functions of their respective tissues.

Computational modeling can facilitate ongoing ENC and EPC monolayer mechanobiology studies

The endothelium is composed of a single layer of ENCs lining the inner lumen of blood vessels, thereby separating the bloodstream from underlying tissues. By contrast, the epithelium consists of one or more layers of EPCs lining organ surfaces and cavities, thus separating these organs from their environment. Despite these differences, the behavior of both cell linings is sensitive to mechanical cues. For example, ENCs are constantly exposed to shear stresses due to blood flow, and bladder EPCs are constantly exposed to hydrostatic pressure. These extracellular forces critically impact cell monolayer functions and tissue integrity by directly influencing cell biomechanics and the forces that cells exert on the extracellular matrix (ECM) and on each other [1–3] (Figure 1A, Key figure). Despite the numerous studies focused on understanding how mechanics regulate the integrity of the cell monolayers [1–3], relatively less is known regarding the crosstalk with the underlying biochemistry under conditions that mimic human (patho)physiology *in vitro*. This is due to experimental limitations in replicating such environments while concurrently measuring cell-generated forces [4]. To address some of these challenges, mechanics-based mathematical models have emerged as invaluable tools, because they can estimate cell-generated forces and predict biological responses, bridging gaps and limitations of *in vitro* and *in vivo* studies [5].

This review focuses on advances highlighting the similarities and differences in ENC and EPC monolayer biomechanics (i.e., focus is on single-layer cell linings), with a special emphasis on computational models that allow predicting their biomechanical behavior. Specifically, first the cell and its cytoskeleton are examined regarding how cell-generated forces regulate monolayer integrity and barrier function. The discussion then focuses on how cell monolayer biomechanics are modulated by the mechanical properties of the ECM, and finally the importance of integration

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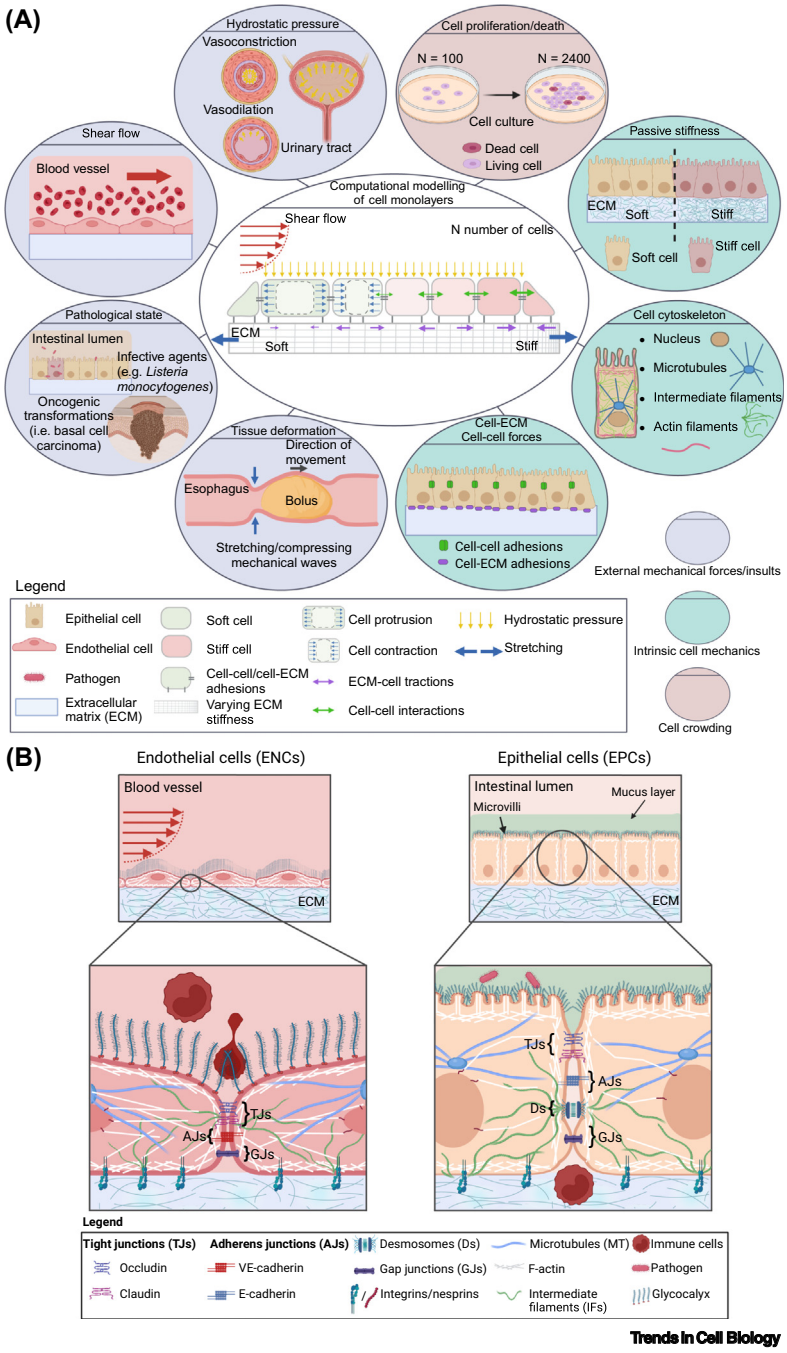
Key figure

Endothelial cells/epithelial cells (ENCs/EPCs) in monolayers are exposed to extracellular physical forces that they sense and transduce into biomechanical responses, which can be modeled computationally



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of additional extracellular physical cues to build a comprehensive picture of monolayer mechanobiology is examined (e.g., tissue stretching, shear flow) (Figure 1A). Relevant modeling efforts, their underlying philosophies, contributions, and limitations are highlighted throughout, suggesting potential future directions. For a detailed discussion on individual topics, readers are referred to reviews on modeling [5] and cell biomechanics [6]. This review aims to inspire further synergies between cell biologists and modelers, accelerating the understanding of ENC and EPC monolayer mechanobiology and **mechanotransduction** (see Glossary) as it pertains to human health and disease.

Passive cell stiffness arising from the cytoskeleton and active cytoskeletal forces transmitted through cell–cell and cell–ECM adhesions regulate the behavior of the monolayer

From a mechanical perspective, the cytoskeleton of adherent cells like ENCs and EPCs can be seen as an **active material**, which generates and transmits forces to and from the microenvironment through cell–cell and cell–ECM adhesions (Box 1).

The cytoskeleton and passive mechanical properties of ENCs and EPCs

The cytoskeleton is generally composed of distinct polymers, namely, F-actin, microtubules, and intermediate filaments (IFs; e.g., vimentin, cytokeratins). The higher apicobasal polarity of EPCs in monolayers as compared with the flatter ENCs can be attributed in part to differing cytoskeletal composition and arrangement [6,7] (Figure 1B and Box 1). For example, EPCs do not express vimentin unless they undergo epithelial-to-mesenchymal transition, while ENCs typically do not express cytokeratins [8,9]. While all components of the cytoskeleton contribute to the mechanical stability of the cell and its overall shape, they differ in **persistence length** (ℓ_p, directly proportional to the bending stiffness of the polymer), resulting in distinct structural and mechanical properties [10]. Actin filaments (ℓ_p ~10–20 μm) provide **tensile strength**, enabling cells to resist pulling forces, while microtubules (ℓ_p ~1–10 mm) can bear compressive load [10]. IFs, the most flexible cytoskeletal components, allow cells to withstand significant deformation without breaking and have an ℓ_p ranging from ~0.3–1 μm for keratins in EPCs to ~1–3 μm for vimentin in ENCs [11] (Box 1). Techniques to measure ℓ_p include direct mechanical testing (e.g., optical/magnetic tweezers), a combination of atomic force microscopy (AFM) and curvature distribution analysis, and fluorescence microscopy paired with computational simulations [10,11]. Mechanics-based computational models further elucidate the role of ℓ_p in defining the overall mechanical properties of the cytoskeleton [10].

The varying cytoskeletal composition and organization of ENCs versus EPCs can explain the different mechanical properties of these cells and the resulting cell monolayers. For example, EPCs

Figure 1. (A) Purple peripheral sketches illustrate different extracellular physical forces (hydrostatic pressure, shear flow, stretching/compressive forces) or insults (infection) cells are subject to [4,9,65,72]. Green blobs show how cells actively alter their mechanics, such as in response to extracellular matrix (ECM) stiffness variations [22,41]. Brown blob shows that cell crowding alters cell mechanics. The center of part (A) illustrates how these parameters can be integrated, allowing a computational model to be built that predicts the biomechanical behavior of a cell monolayer. Depicted is a side view of an EPC monolayer on a matrix exhibiting a stiffness gradient, thus generating a gradient of traction (purple arrows) and monolayer (green arrows) stresses across the monolayer. (B) Schematic comparing ENCs and EPCs in monolayers. ENCs (left) experience blood shear stresses, have squamous morphology, a thick glycocalyx, and reside on an ECM. EPCs (right) are columnar, exhibit extensive cell–cell contacts, and are topped with microvilli and mucus. Zoomed-in views show differences in cytoskeletal structures – F-actin (white), microtubules (blue), and intermediate filaments (green) – and cell–cell adhesion complexes – adherens junctions, tight junctions, gap junctions, and desmosomes (exclusive to ENCs). Both cell types anchor to the ECM via integrin-based focal adhesions, with immune cells and bacteria depicted on their respective apical or basal sides. Simplified representations focus on key adhesion players. Inspired by [6,7,41]. This figure was created using BioRender (https://biorender.com/).

Glossary

Active material: a material that uses energy to modify its mechanics, including in response to external forces applied to it. A cell is considered an active material. By contrast, an inert material (e.g., steel) does not exhibit active responses to external forces.
Anisotropic: refers to whether a material (e.g., cells, ECM) has uniform mechanical properties in all directions. For example, an isotropic material exhibits the same stress/strain response, regardless of the direction in which the load is applied. By contrast, in an anisotropic material, the stress/strain response depends on the direction of the applied load.
Cell contractility: the ability of a cell to actively generate forces and change its shape through contraction, important for driving many cellular processes such as cell motility and division.
Cell viscosity: measure of the internal resistance of the cells cytoplasm to flow.
ECM topography: the set of micro- and nanoscale geometrical features that define the ECM (e.g., pore size, alignment of ECM fibrillar proteins, curvature).
Inter- and intracellular stresses: the stresses experienced within the cell (intra), arising from the cell cytoskeleton and action of motor proteins, and the stresses experienced between cells (inter), which are altogether crucial for the maintenance of tissue integrity and monolayer barrier function.
Kinematics and dynamics: kinematics is the study of the motion of objects without consideration of the causes that generate this movement. By contrast, dynamics is the study of motion and the forces that produce this motion.
Mechanotransduction: a process by which cells convert the mechanical stimuli they sense from the environment into chemical or mechanical cellular responses.
Persistence length: denoted as ℓ_p, is a measure of the length scale over which a polymer (e.g., cytoskeletal filament) remains roughly straight and is proportional to its bending stiffness.
Tensile strength: the maximum stress (force per unit area) a material can withstand before breaking when tested in a uniaxial tensile test.
Traction forces: the contractile forces generated by a cell and exerted on its ECM.

Box 1. How do ENCs/EPCs sense and generate forces?

From a mechanical perspective, the cell nucleus, cytoskeleton, and cell adhesion complexes form a structural network enabling adherent cells (e.g., ENCs, EPCs) to sense/transmit forces from/to their environment. Numerous cellular processes, including cell motility and ECM anchorage, are dictated by the cytoskeleton, a biopolymer network composed of F-actin filaments, microtubules, and intermediate filaments (IFs) and their associated crosslinking proteins, spanning the entire cell, thereby providing cell shape and mechanical strength. These cytoskeletal elements interact physically through linker proteins, such as spectraplakins, which associate with all three elements of the cytoskeleton (F-actin, microtubules, IFs), allowing integration of these distinct networks; consequently, disruption of one network can influence the organization or function of the others [79]. The cytoskeleton can autonomously and actively reorganize in response to changes in the extracellular environment (e.g., ECM stiffness). This adaptability is driven by a series of biomechanical processes through which cells sense the geometry and physical forces of their environment via different cell surface receptors, a process known as 'mechanosensing.' These mechanosensing receptors transduce mechanical signals into intracellular biochemical signaling. For example, fluid shear stresses exerted onto the apex of ENCs are detected through mechanosensitive receptors and the glycocalyx (sugar-rich coating of ENCs that forms a highly hydrated fibrous meshwork), leading to a cascade of signaling events that reorganize the cytoskeleton and focal adhesions, altering the forces cells exert on their matrix and each other. This is partially driven by changes in the activity of molecular motors such as myosin II, which generate contractility by sliding along actin filaments using ATP hydrolysis-derived fuel, while actin undergoes polar polymerization. But F-actin polymerization alone can also generate protrusive forces (ratchet model) [80,81]. The cytoskeleton is connected to the ECM through focal adhesions, consisting of many proteins. Among them, transmembrane heterodimeric integrins allow attachment of cells to ECM proteins and thus exertion of traction forces. Focal adhesions, such as the cytoskeleton, are dynamic, which explains why ENC and EPC traction forces vary spatiotemporally. Cells in monolayers also transmit forces to each other through their cell–cell interfaces due to protein complexes such as adherens junctions and tight junctions. Those include multiple proteins that allow binding to the underlying cytoskeleton and transmission of forces between cells. Understanding this adaptable machinery and its responses to extracellularly applied and cell-generated forces is crucial for understanding ENC and EPC function, monolayer collective behavior, and overall tissue function.

Viscoelastic materials: material that exhibits a time-dependent mechanical behavior. The deformation of the material is dependent on the external loads, the velocity of application of these forces, and the time these forces are acting on the material.

Young's modulus: measure of stiffness or resistance of a material to deformation/strain in response to an applied force.

in monolayers are in general softer than ENCs, with their stiffness (**Young's modulus**) and viscosity depending greatly on the chemical and mechanical environment and on the cell type examined [12,13]. Accurately characterizing the stiffness and viscosity of EPCs and ENCs is crucial, since alterations of these variables are directly linked to pathologies [14] (Box 2). In various EPC types, an increase in **cell viscosity** directly correlates with invasive potential during cancer metastasis [14], whereas ENCs typically exhibit an increase in their stiffness with increasing ECM stiffness *in vitro* and similarly *in vivo* in pathologies where fibrosis occurs (ECM stiffening), such as atherosclerosis [15–17]. Moreover, in ENCs, ECM stiffening can increase transcriptional

Box 2. Techniques for (in)direct measurement of cellular forces *in vitro*

To understand the biomechanics of *in vivo* systems, *in vitro* experiments are often performed on cell monolayers, as mechanical variables are more accessible to measure. Advances have allowed some measurements of cell-generated forces also *in vivo* [82]. Data from such experiments serve as inputs for *in silico* models that recapitulate cell monolayer biomechanics.

Various experimental techniques allow measuring physical cell parameters *in vitro*, such as the mechanical properties of the cytoskeleton and cell–cell and cell–ECM junctions (Figure I) [83]. Micropipette aspiration measures mechanical properties of individual cells (e.g., their elastic modulus or surface tension) by observing cell deformation given a pressure suction, but these techniques perturb cells, albeit minimally (Figure IA). Likewise, atomic force microscopy (AFM) enables the measurement of cell stiffness through a flexible cantilever that interacts with the cell and measures the forces between the probe and the cell through the displacement of it (Figure IB). Techniques such as optical/magnetic tweezers measure tension at cell–cell junctions by manipulating particles/beads injected into cells using light/magnetic fields (Figure IC). To quantify active forces in confluent monolayers, traction force microscopy (TFM) is often used to measure traction stresses (forces per unit area) exerted by cells on an elastic matrix embedded with tracer beads [84] (Figure ID). This technique measures the deformations of the ECM by following the displacements of embedded beads in the ECM using image correlation techniques such as particle image velocimetry (PIV). Using those displacements, one can analytically or numerically calculate cellular traction stresses [83]. These tractions serve as input for conducting MSM and indirectly infer monolayer stresses (i.e., inter- and intracellular stresses), which can be thought of as a proxy of barrier integrity (Figure IE). Another approach to retrieve monolayer stresses is through force inference, based on the analysis of cell shape changes, such as through Bayesian inference [85]. More invasive but direct methods for assessing tension built within a monolayer also exist, such as laser wounding/ablation of a part of a monolayer, which allows measuring tension via the recoil velocity cells display upon wounding [86] (Figure IF). Moreover, Förster resonance energy transfer (FRET)-based molecular force sensors allow measuring molecular forces on cell–cell and cell–ECM adhesion complexes, but they are more suited for measuring forces on the subcellular scale [39] (Figure IG).

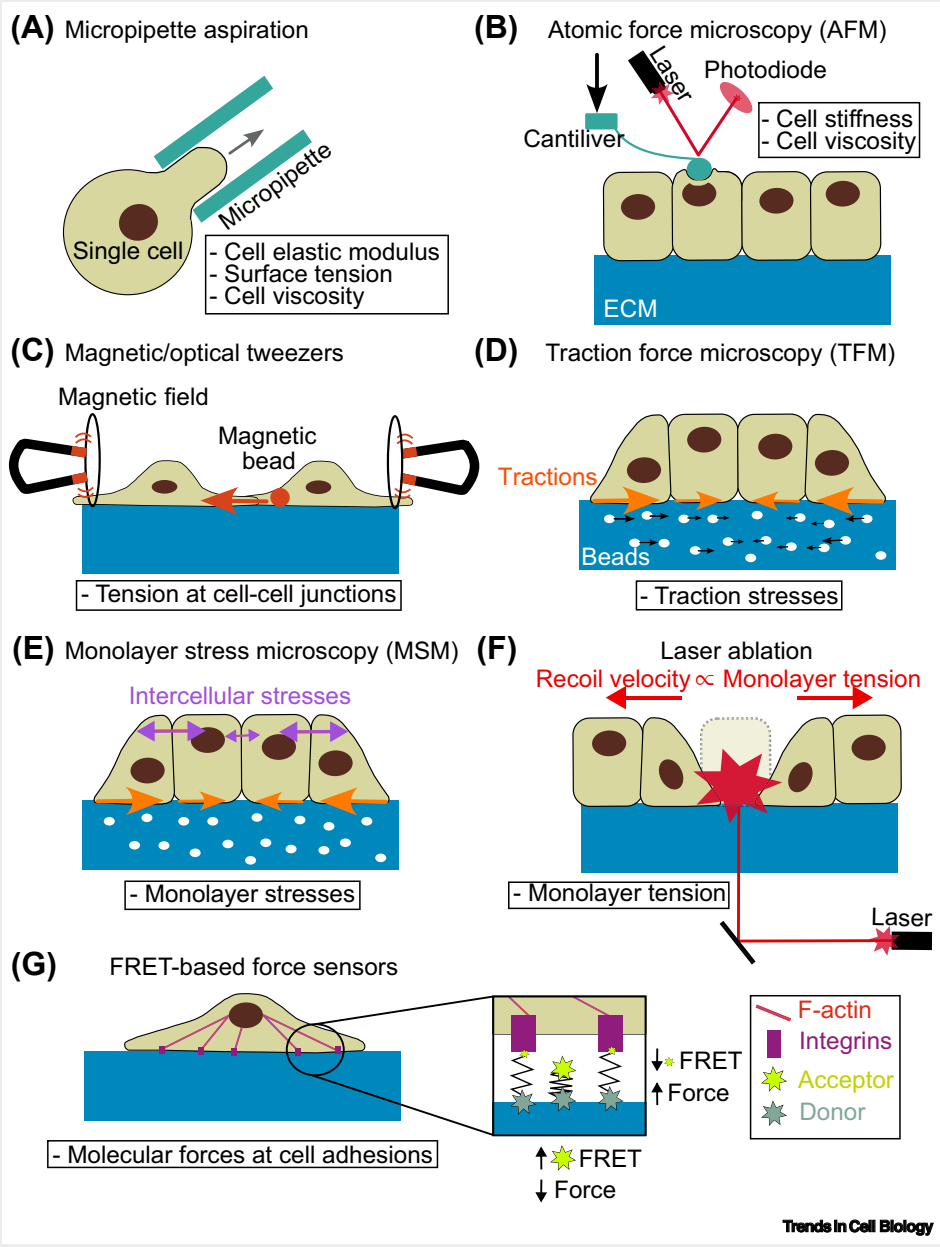

Figure I. Visual illustration of techniques to measure *in vitro* cell monolayer biomechanics. Abbreviation: FRET, Förster resonance energy transfer.

heterogeneity of cells, as evidenced by single-cell RNA sequencing, but how that impacts cell biomechanics remains obscure, although *in situ* RNA sequencing could be performed side by side with mechanical characterization of cells [18]. Computational models can help clarify how individual cytoskeletal components contribute to the overall *in vitro* mechanical properties of cells in a monolayer. Different computational approaches can be used, depending on the length and time scales of interest (Box 3). For instance, molecular dynamics simulations capture the detailed interactions of molecular motors, thermal fluctuations, and steric effects within individual cell

Box 3. Techniques/approaches for modeling cell monolayer biomechanics *in silico*

Various computational approaches can simulate ENC/EPC monolayer biomechanics, each providing distinct outputs (Figure I). The simplest mechanical models for cell collectives are discrete agent-based models (ABMs; Figure IA). Here, cells are represented as individual particles interacting on the basis of predefined rules, allowing the study of cell–cell interactions, proliferation, and differentiation. However, ABMs cannot capture changes in cell shape. To study cell shapes within monolayers, the vertex model (VM; Figure IB) is more suitable. Cells are represented as polygons with vertices and shared edges forming a network. Cell shape changes are governed by equations of motion or energy functions, accounting for adhesion, cortical tension, and contraction forces. However, this method does not account for the dynamics of intracellular structures like the cytoskeleton. If changes in individual cell shapes, along with the shape of the monolayer, need to be studied in detail, the cellular Potts model (CPM; Figure IC) framework can be employed. This approach uses a rectangular lattice where each cell spans multiple lattice sites. Each cell occupies a subset of the lattice with a unique identifier and associated properties. The dynamics of the CPM are governed by minimizing an energy function (typically a Hamiltonian), accounting for cell volume resistance and adhesion, which influences cell distributions within the domain. Alternatively, if the research question lies in understanding the average behavior of cells and monolayers, continuum modeling frameworks can be used. One popular approach is the finite element method (FEM; Figure ID), where the domain is divided into smaller regions called 'elements.' The differential equations describing cell mechanics are numerically solved. FEM can be highly detailed regarding mechanical and chemical properties and can simulate the geometry of cells, including their deformations and stresses. However, significant changes of cell shape or monolayer topology over time may be challenging for FEM to model. To overcome this drawback, another technique that can be used is the phase field model (PFM; Figure IE), which consists of different phases (e.g., inside or outside the cell) within the monolayer with smooth transitions between them. The mechanical equilibrium is calculated through the minimization of an energy function that includes terms related to surface tension, cell adhesion, or elasticity.

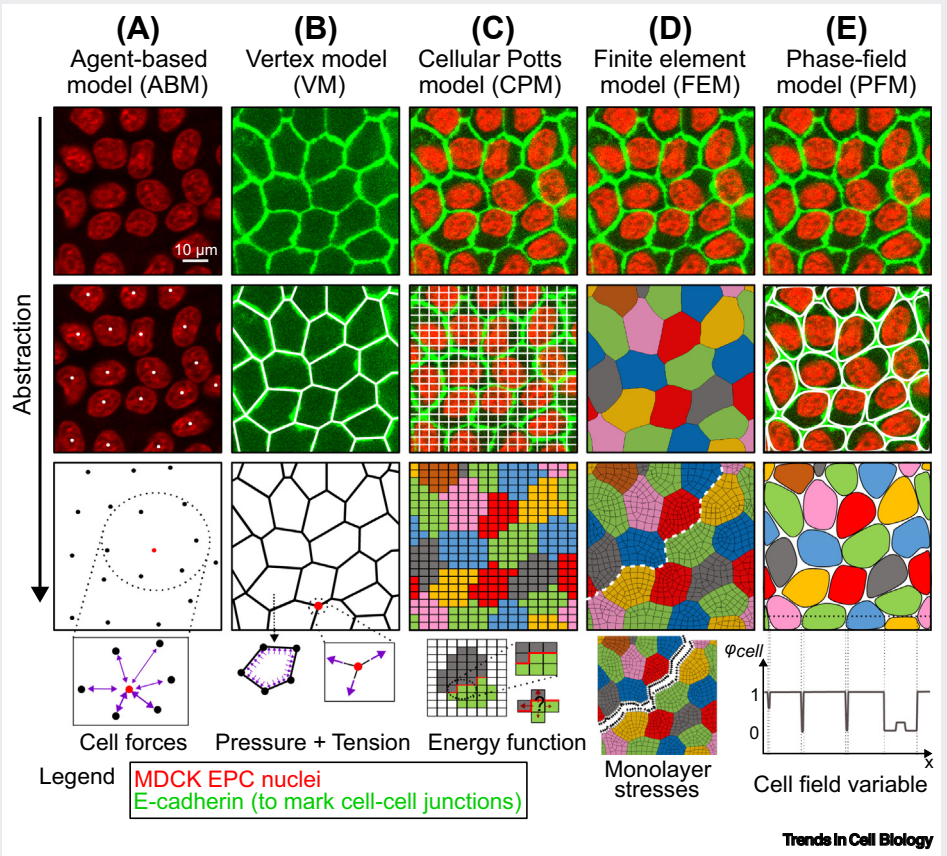

Figure I. Schematic of techniques/approaches for modeling cell monolayer biomechanics. Abbreviations: EPC, epithelial cell; MDCK, Madin–Darby canine kidney.

cytoskeletal components and reveal how microscopic dynamics of individual filaments influence the overall cytoskeletal organization and cell mechanics [19]. By contrast, continuum mechanics simulations model the averaged behavior of the entire cytoskeleton, overcoming the computational challenges of simulating every individual component. Using such a continuum approach, a study explored the role of cell stiffness in influencing competition between two different EPC populations, as this is crucial in developmental biology and cancer research. Simulating EPC dynamics in a crowded environment, the model predicted that stiffer cells tend to die off faster in competition, allowing softer cells to dominate, emphasizing that mechanical properties are key factors in determining cell survival and tissue dynamics [20]. Using a multiscale model, which integrates processes occurring at different spatial and temporal scales, and experiments, another study revealed that mechanical cell competitions are driven by how cells respond to crowding, with loser cells being more sensitive to increased cell density, which ultimately leads to their elimination [21]. In contrast to the former study, this work showed that 'loser' cells are typically softer and can thus more easily be compressed by surrounding 'winner' cells, leading to increased local cell density and subsequent apoptosis [21] (Figure 2A). These results are in good agreement with another study on mechanical cell competition, where softer infected cells get eliminated by uninfected bystander cells [9]. It should be noted that the outcome of cell competition is influenced by not just mechanical but also biochemical factors, which may explain discrepancies between studies.

Cell–ECM adhesions and traction forces

Like any adherent cell, ENCs and EPCs are able to generate active forces and transmit them to the ECM by exerting **traction forces** through focal adhesions, which link the ECM to the underlying F-actin cytoskeleton (Boxes 1 and 2, and Figure 1B) [22]. Alterations in focal adhesion organization and traction forces can have a causal role in the onset or progression of different diseases, such as the development of hypertension and atherosclerosis or the spread of a bacterial infection through cell monolayers [9,23,24].

To computationally model the forces that ENC or EPC monolayers exert on their environment, different techniques are available (Box 3). The choice of the most appropriate model depends on the questions asked and the available experimental input parameters (Table 1). Modeling techniques include discrete modeling methods, such as the agent-based model, the vertex model (VM), and the cellular Potts model (CPM) and variations thereof, as well as continuum modeling methods, such as the finite element model (FEM) and the phase-field model (PFM) (Box 3). Discrete models treat cells as individual entities connected by *ad hoc* force relations [25,26] or energy constraints [27,28], while continuum models represent the average behavior of cell populations using predefined constitutive laws [29–31]. Hybrid models that combine the strengths of both discrete and continuum approaches have also gained prominence [32,33]. Regardless of the method employed, the accuracy of the results depends largely on the quality of the experimental data input into the model.

For example, a study combining *in vitro* experiments and modeling focused on investigating intestinal EPC mechanical competition during late-stage infection with intracellular bacterial pathogen *Listeria monocytogenes* [9]. The study revealed dramatic changes in the **kinematics** (i.e., migration speed, directionality) and **dynamics** (i.e., traction and monolayer stresses) of host EPCs during infection, which are not observed in healthy quiescent epithelia. Here, bystander cells move directionally with high speeds toward infected cells, squeezing them, and driving their collective extrusion and cell death, suggesting that this mechanism acts in favor of the host in limiting infection spread. This movement is driven by stiffer bystander cells exerting higher traction forces on the ECM, as confirmed using an FEM. The model predicted that increasing bystander traction or stiffness would lead to greater infected cell extrusion (Figure 2B) and that loss of intercellular adhesion would prevent this, which was then confirmed experimentally [9,34].

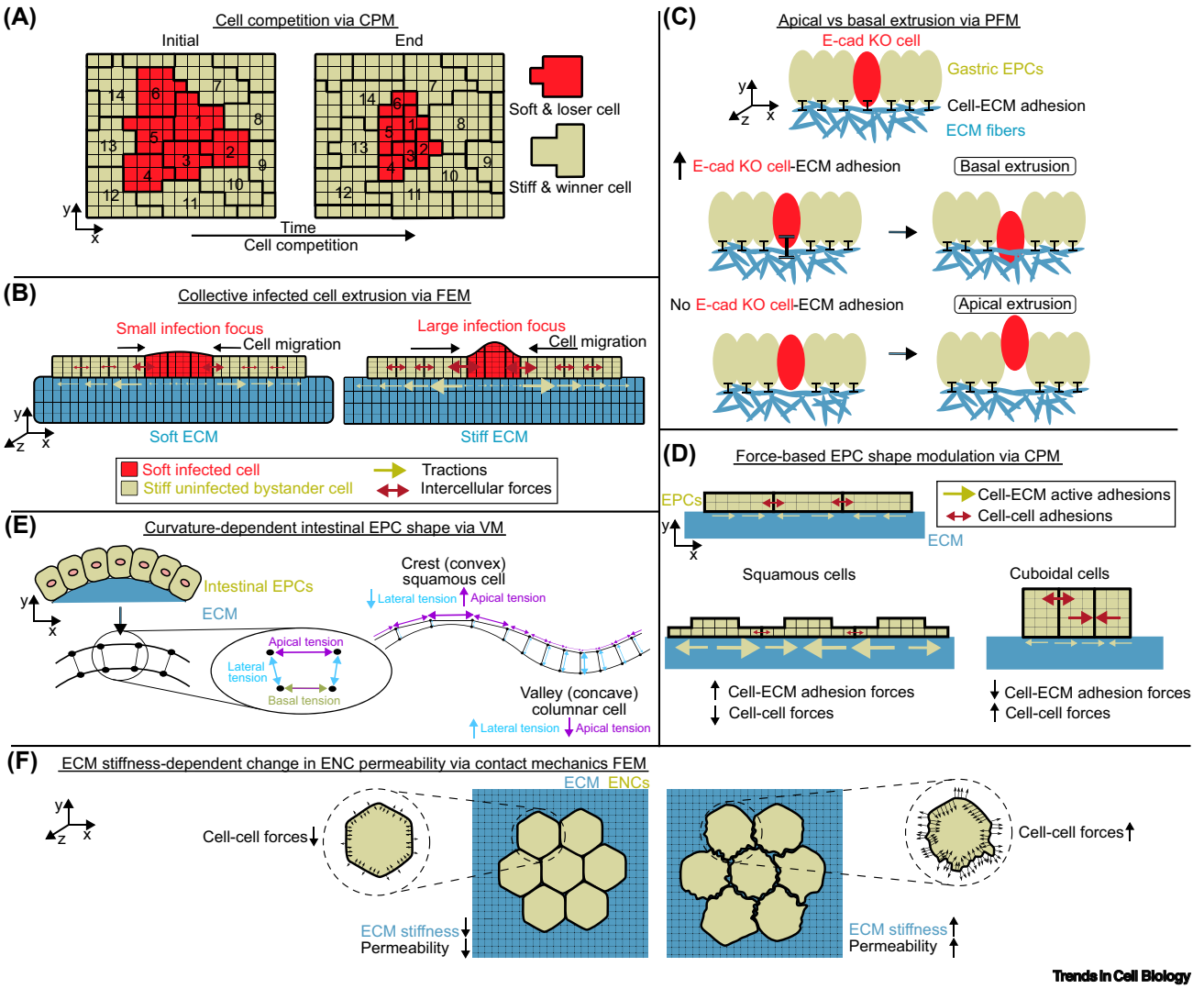

Figure 2. Examples of studies combining modeling with experimentation to uncover the mechanobiology of cells in monolayer. (A) Schematic showing how a cellular Potts model (CPM) revealed that, during cell competition, the stiffer cells (beige) will consistently outcompete softer cells (red) [21]. (B) Finite element modeling (FEM), paired with *in vitro* measurements of epithelial cell biomechanics during bacterial infection, showed that uninfected 'surrounder' cells on a stiff matrix migrate more readily toward infected cell clusters, enabling their elimination via extrusion [9,34]. (C) Sketch of a study using a phase-field model (PFM) to explain how cell–extracellular matrix (ECM) adhesion forces dictate whether E-cadherin-deficient cells in an epithelial monolayer extrude apically or basally [38]. (D) Schematic of a study employing CPM which demonstrated that increased cell–ECM forces, paired with decreased intercellular tension, promote squamous cell morphology in epithelial monolayers, while the opposite conditions favor a cuboidal shape [48]. (E) Illustration of how combination of *in vitro* experiments and a vertex model (VM) revealed how curvature in the intestinal epithelium influences cell-generated tension, leading to distinct cell shapes in crypts versus villi and affecting nuclear morphology [7]. (F) An FEM for endothelial cells (ENCs) showed that intercellular forces in response to ECM stiffness crucially impact the ENC monolayer permeability, with increased ECM stiffness leading to augmentation of intercellular forces, thus increasing monolayer permeability [29]. Abbreviations: EPC, epithelial cell; KO, knockout.

Whether a similar behavior occurs in ENC monolayers to limit intracellular pathogen spread remains to be determined.

Single-cell extrusion has been studied in EPC monolayers and, to a lesser degree, in ENC monolayers and is a potent mechanosensitive mechanism for maintaining tissue homeostasis [35–37]. A study investigated the contribution of cell–ECM adhesions to the extrusion behavior of E-cadherin-defective cells in gastric EPC monolayers [38]. Using both a PFM and a VM of EPCs in

Table 1. Studies that used computational modeling to investigate ENC or EPC mechanobiology

Model	Topic studied	Extracellular forces?	ECM mechanics?	*In vitro* data?	Refs
2D VM	Impact of shear stress-driven mechanical cell deformations on EPC monolayers	Yes	No	No	[77]
1D FEM	Role of ECM stiffness gradients on collective EPC migration (durotaxis)	No	Yes	No	[47]
2D (CPM + FEM)	How intercellular tension guides EPC spatial patterning	No	Yes	Yes	[87]
2D ABM+VM	Role of ECM stiffness on elastic force propagation through EPCs	Yes	Yes	Yes	[64]
3D PFM/VM	Role of cell–ECM adhesion in guiding basal versus apical EPC extrusion	No	Yes	Yes	[38]
2D CPM	How mechanics drives transition from mono- to multilayer EPCs	No	Yes	No	[48]
2D VM	Mechanical interactions of multiciliated cells with EPCs	Yes	No	Yes	[88]
2D PFM	Relative contribution of cell–cell and cell–ECM forces to the regulation of EPC behavior	No	No	Yes	[80]
2D CPM	Examined factors that regulate mechanical EPC competition	Yes	Yes	Yes	[21]
3D FEM	How mechanics dictate EPC competition that arises during infection with bacteria	No	Yes	Yes	[9,30,34]
3D VM	Effect of curvature on EPC monolayers	No	Yes	Yes	[52]
3D FEM	Role of shear stress, contractility, ECM stiffness, and curvature in modulating ENC monolayer permeability	Yes	Yes	No	[29]
2D FEM	Role of mechanics in determining the orientation of EPCs during division	Yes	No	Yes	[78]
2D VM	How EPC shape is controlled by rigidity and active cell stresses	No	Yes	Yes	[89]
3D FEM	Studied the influence of ENC monolayer curvature on vessel permeability	Yes	No	No	[31]
2D Multiscale hybrid	How ENC dynamics and their heterogeneous responses impact angiogenesis	Yes	Yes	No	[90]
2D Molecular dynamics + FEM	Methods to simulate ENC monolayers under different ECM stretching conditions	Yes	Yes	No	[32]

monolayers, it was predicted that defective cells with increased cell–ECM adhesion are more likely to undergo basal extrusion, leading to ECM invasion, while absence of cell–ECM adhesion forces favors apical extrusion and apoptosis, supporting the supposed role of cell–ECM adhesions in determining the extrusion fate of EPCs (Figure 2C). Moreover, the model identified that the cylindrical structure of gastric glands strongly promotes basal extrusion of cells with defective E-cadherin, a feature overlooked in 2D *in vitro* experiments. Since extrusion is influenced by the balance between intercellular and cell–ECM adhesions, the larger cell–cell contact area in EPCs compared with ENCs may explain why apical extrusion is more prevalent in EPCs.

Cell–cell adhesions and transmission of intercellular forces

Cells in monolayer are also able to transmit forces to each other through various dynamic adhesion complexes (Figure 1B and Box 1). Förster resonance energy transfer (FRET)-based molecular force sensors allow measuring these forces on the molecular scale [39], but on the supracellular scale, mostly indirect methods are used to infer intercellular forces (Box 2). For

example, monolayer stress microscopy (MSM) allows **inter- and intracellular stresses** (referred also as monolayer stresses) to be inferred [40]. Although the composition and area of adhesion complexes may differ between ENCs and EPCs (i.e., EPCs columnar and ENCs squamous) [41], their mechanical functions are conserved. That is, intercellular pulling and pushing forces are crucial to maintain tissue function and preserve barrier integrity, thereby preventing disease onset. Increased tension at cell–cell adhesions typically leads to enhanced barrier integrity in both ENC and EPC monolayers up to a certain point above which integrity is lost [42]. However, the organization and increased turnover of vascular E-cadherins (VE-cadherins) allow ENCs to more rapidly alter barrier integrity and permeability in response to different mechanical and chemical signals as compared with EPCs [41,43]. Using a mechanical testing device to compare the tensile properties of VE-cadherins and E-cadherins, both were found to behave as **viscoelastic materials** [44] but to exhibit different tensile strengths with varying loading rates [45].

Discrete [46] and continuum [29,47] modeling techniques have been applied to simulate either individual cell–cell junctions and/or cell collective behavior, showing how the force they bear impacts cell morphology, mechanics, and ultimately cell monolayer behavior. For example, continuing the work on the battle between infected versus bystander EPCs [9], the FEM predicted that the monolayer stresses built by uninfected bystander cells are key in eliciting squeezing of adjacent infected cells. On the basis of these predictions, MSM experiments were conducted that confirmed that spatiotemporal changes in EPC monolayer barrier integrity determine the outcome of infection processes [34]. In another example, a CPM was used to model monolayer-to-multilayer transitions that can occur during tumorigenesis [48]. This model analyzed the role of cell mechanics in regulating cell–cell and cell–ECM adhesions and how alterations in **cell contractility** impact a monolayer's structural morphology. The model predicted that squamous cells are more likely to appear when cell–ECM adhesion forces are stronger than cell–cell adhesion forces (Figure 2D). Moreover, when cell–ECM forces are increased and cortical contractility is reduced, extrusion of cells and thus monolayer-to-multilayer transition are promoted [48]. Finally, another study based on a continuum modeling approach assessed the critical crosstalk of cell–ECM and cell–cell mechanics with biochemical signaling in ENC monolayers [49]. Simulations showed that while cell tension can stabilize junctions, excessive RhoA signaling disrupts them and high Rac1 levels weaken them, with actin polymerization assisting in ENC gap closure. The model also predicted the impact of pharmacological treatments on junction stability and ENC barrier integrity, which were then validated experimentally, highlighting the power of such models.

ECM geometry and mechanics modulate ENC and EPC monolayer mechanobiology

Effect of ECM geometry on EPC and ENC monolayer biomechanics

ECM geometrical properties, such as matrix porosity, curvature alterations (e.g., as they occur in areas of vessel bifurcations), confinements arising from pathologies (e.g., atherosclerotic plaques in vascular endothelium), and **anisotropic** topography, critically modulate the behavior of cells in monolayers [50,51]. For example, *in vitro*, anisotropic **ECM topography** leads to deformation and alignment of single ENCs through contact guidance, whereas in confluent ENC monolayers, this effect is somewhat attenuated [50], an aspect that is important to consider when building patterned endovascular devices for tissue engineering. Likewise, EPCs can collectively sense ECM curvature, which in turn regulates collective migration, with higher or lower ECM curvature leading to more fluid- or solid-like cell movements, respectively, as revealed by a 2D dynamic VM adapted to study EPC monolayers embedded on 3D spherical surfaces [52]. This model also predicted that the more solid-like cell behavior in low curvature ECM results in more regular hexagonal cell shapes, as also verified experimentally. Since EPCs tend to adopt more regular hexagonal shapes than ENCs, while the latter tend to align better with each other [53], it would be of interest

to address ENC curvature sensing through modeling. A biomechanical model for ENCs has shown how intercellular forces change in response to curvature [29]. To adapt such a model to varying ECM curvature, one would need to incorporate curvature-sensing proteins such as septins, which localize specifically to curved regions and regulate cytoskeletal forces, influencing both their direction and their magnitude [54]. Additionally, careful consideration of the problem's dimensionality is essential, particularly when extending 2D models to 3D.

In another seminal study, a VM was used to investigate how physical parameters such as apical, lateral, and basal tensions, which are not directly measurable through *in vitro* experiments, impact the shape of EPCs and the mechanical interactions between the nucleus and cell surface (Figure 2E). Along with *in vitro* experiments, this analysis revealed that convex zones (crests) lead to compression of the in-plane nuclei (squamous-like cell shape), while thicker concave zones (valleys) compress the nuclei along the apicobasal axis (columnar-like cell shape) [55]. Mechanical cytoskeletal forces transmitted to the nucleus via the LINC (linker of nucleoskeleton and cytoskeleton) complex, which includes nesprins, and the resulting nuclear deformation can significantly influence chromatin condensation and gene expression, thereby altering essential EPC/ENC functions – an area of ongoing research [56–58] (Figure 1B).

The impact of ECM topology on the behavior of ENC monolayers has remained broadly unexplored in the modeling field, and so is the crosstalk between ECM geometry and cell/nuclear mechanics, which would require the development of multiscale mathematical models.

Effect of ECM mechanics on EPC and ENC monolayer dynamics

Beyond geometry, the mechanical properties of the ECM, such as its Young's modulus, and viscosity also significantly impact cellular functions and are altered in various (patho)physiological processes, such as aging, fibrosis, and cancer [59]. However, most *in vitro* studies have neglected such factors and have considered only matrices that are purely elastic, or viscoelastic, often with constant material properties [60]. These studies mostly examined how ECM stiffness and/or composition impacts important cell functions such as proliferation, motility, apoptosis, lineage differentiation, and tissue organization and function [51,61,62]. Although both EPCs and ENCs are responsive to ECM stiffness, ENCs appear to be more sensitive than EPCs, possibly because of their very mechanically dynamic environment. For example, in response to a threefold increase in matrix stiffness, both EPC and ENC monolayers increase the **traction forces** on their matrix, but ENCs to a larger extent than EPCs, with consequences on ENC permeability [23,29]. A 3D continuum model predicted that ENC monolayer permeability increases with ECM stiffening because of elevation in ENC cytoskeletal contractile and cell–cell forces [29] (Figure 2F), in line with experimental observations [63].

A study employing both experimental and computational approaches showed that the distance over which elastic forces spread within an EPC monolayer over short timescales depends on ECM stiffness, with EPCs on stiffer ECM (i.e., more fibrotic) exhibiting lower displacements and force propagation through the tissue than softer ECM [64]. This suggests that on stiffer ECM, cells in monolayers tend to mechanically isolate more (i.e., a given cell is less able to sense and respond to mechanical signals generated by neighboring cells) because increased ECM stiffness reduces the extent of deformation and signal propagation through the ECM, critical for long-range intercellular communication. Nonetheless, through a 2D VM, it was revealed that ECM stiffening increases cell–cell and cell–ECM forces [34]. This is consistent with another study, where an FEM calibrated with *in vitro* data of EPC monolayers residing on varying stiffness ECM predicted an increase in traction and monolayer stresses with increasing ECM stiffness, but no significant changes in cell motility [34].

Extracellular mechanical forces impact the functions and integrity of ENC and EPC monolayers

To better understand the functioning of ENCs and EPCs in monolayers in a physiological context, it is crucial to also consider the extracellular physical forces at play. While ENC and EPC monolayers experience similar extracellular mechanical forces, differences in the magnitude of these forces, along with differences in cell and ECM properties, may lead to distinct biomechanical responses (Figure 1A). For example, apically imposed fluid shear stress, on the one hand, helps maintain barrier integrity in ENCs by regulating junctional permeability [65]; on the other hand, it enhances differentiation and mucus production in intestinal EPCs, which is critical for the maintenance of intestinal homeostasis [66]. Stretching forces are exerted on ENCs when pressure waves propagate through the aorta after a heartbeat, while in EPCs, these forces arise from peristalsis, which is essential for moving a food bolus through the esophagus. In response to cyclic mechanical stretching, both ENCs and EPCs polarize and reorient themselves to maintain barrier integrity despite the load applied to them [6,67]. However, an excessive (nonphysiological) magnitude of cyclic stretching can increase ENC permeability and eventually lead to calcification [68]. Likewise, in bronchial EPCs during allergen-induced bronchoconstriction, acute unregulated stretching can contribute to damage and associated pathologies [4]. ENCs generally exhibit a greater capacity to withstand strain before failing than bronchial EPCs, which could be a result of their natural mechanical environment, structural composition, and/or physiological roles [69,70]. However, the specific response to large strain might also vary depending on the cell type (macro- versus microvascular ENCs) and other characteristics of the applied strain (e.g., direction, frequency). Hydrostatic pressure is another example of extracellular physical force that is experienced by ENCs in the blood vasculature and EPCs in areas such as the urinary tract. If unregulated, this can impair, for example, ENC barrier functions [71], while when properly regulated, it is key in driving cell deformations important for tissue morphogenesis and remodeling in the urinary tract [72].

While numerous studies have explored the effects of individual extracellular mechanical stimuli on the behavior of EPC or ENC monolayers using specialized devices to replicate human (patho)physiology, there is still limited understanding of how cells integrate and respond to all these stimuli simultaneously. Concurrent application of shear stress and cyclic stretch was shown to alter ENC alignment/organization and increase nitric oxide production, which are crucial for maintaining vascular tone and reducing inflammation [73] and can also improve the mechanical strength of ENC-based vascular grafts that can be successfully implanted to patients [4]. Further insights could be achieved by developing *in silico* models that disentangle the effects of shear stress, cyclic stretch, or other combined mechanical stimuli. For example, models of mechanosensing pathways, such as those involving Rho GTPases or YAP/TAZ [74–76], could help clarify how cells integrate multiple mechanical signals. Additionally, integrating multiscale information – from molecular force sensors to cellular mechanical responses – could reveal how molecular and cellular scales are interconnected and bidirectionally influence ECM force sensing.

To that end, a study used a VM to examine how shear stresses can drive fluid-like to solid-like transitions in EPC monolayers, a process that is important in driving processes ranging from wound healing, over embryonic development to cancer metastasis [77]. This model demonstrated that shear-driven rigidity occurs when the tissue reaches a critical strain, resembling behaviors observed in other soft matter systems (e.g., colloids or polymers). It also showed that accounting solely for the linear mechanical response of EPC monolayers to shear-imposed strains cannot fully explain their behavioral responses and that accounting for nonlinearities is crucial to mimic the monolayer's mechanical response. Mechanical stretch imposed on EPC

monolayers can also dictate whether cells will divide in-plane, as they should to maintain barrier integrity, or out-of-plane, which is problematic and can lead to cancer metastasis, for example [78]. A study combining FEM with experiments investigated how tension at the intercellular junctions affects the orientation of cell division in EPC monolayers, revealing that reduced tension increases the frequency of out-of-plane divisions [78]. The model facilitated the identification of both the level of stress experienced by cells under mitosis and the correlation between intercellular stresses and cell contractility, which is challenging to measure *in vitro*.

Using Brownian dynamics-based simulations and experiments, another study addressed the question of how extreme mechanical stretch, as it occurs during development, impacts the mechanical behavior of entangled IF-based filament networks in EPCs [8]. The IF cytoskeleton was found to switch from a wavy configuration (at low cell stretching) to a taut configuration (at high cell stretching), crucial for providing structural integrity in highly stretched cells. Continuous experimental and computational investigations on the response of additional cytoskeletal elements to stretch will allow unraveling how they regulate cell mechanics, tissue function, and barrier integrity under extreme mechanical conditions or other commonly encountered insults.

Concluding remarks

EPCs and ENCs exhibit key similarities and differences in their mechanochemical properties, including shear flow sensing, ECM sensing and mechanotransduction, barrier function, cytoskeletal structures, and dynamics. Integrating mechanobiology with computational modeling offers the opportunity to predict the impact of mechanical properties, such as cell and matrix stiffness, on physiological processes such as cell extrusion and barrier function. However, models simultaneously accounting for the multiple mechanical and chemical cues that EPCs and ENCs are exposed to have yet to be developed. Such models, calibrated with data from EPCs or ENCs, could help predict how molecular features influence monolayer mechanics, barrier function, and tissue behavior. They could also act as 'virtual laboratories' and support hypothesis generation, tissue engineering, and therapeutic strategies to improve, for instance, barrier integrity and the mechanical strength of tissues. A major challenge in the development of multimodal models is the integration of very different data types, such as multiomics and mechanical data, to deepen our understanding of the interplay between molecular and mechanical properties of ENCs and EPCs (see Outstanding questions). Another challenge is to bridge different scales, from molecular motors to multicellular architectures. Addressing these challenges requires stronger collaboration between experimentalists and modelers. While these advancements promise transformative potential, outstanding questions warrant further investigation.

Acknowledgments

We thank all our colleagues whose studies could not be cited in this review due to space limitations and journal policy, which restricts citations to primary research published within the past 3–5 years. We also thank Dr Libera Lo Presti for continuous discussions and for revising the manuscript and materials. Figures 1 and 2 were created with BioRender.com. This initiative was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2124 – 390838134 (E.E.B.), by the DFG grant - project number 490839690 (E.E.B.), by the BBSRC grant BB/V002708/1 (F.S. and P.K.), by the UKRI Future Leaders Fellowship (MR/T043571/1) (F.S.), by the MCIN/AEI/10.13039/501100011033 (R.A.-Y. and M.J.G.-B.) and ERDF A way of making Europe under the grant agreement no. PID2021-124271OB-I00 (R.A.-Y. and M.J.G.-B.), and by the European Research Council (ERC) Advance grant agreement ICoMICS no. 101018587 (R.A.-Y. and M.J.G.-B.). R.A.-Y. acknowledges support from the Spanish Ministry of Universities (grant no. FPU 20/05274).

Declaration of interests

The authors have no interests to declare.

Outstanding questions

The adoption of *in silico* methods for understanding ENC and EPC biology is not yet widespread among cell biologists. Could developing more user-friendly platforms or improved communication between modelers and biologists make these approaches more attractive?

A computational model is only as good as the data it is based on. Techniques to probe mechanical forces in 3D monolayers *in vitro* remain limited, and doing so *in vivo* is even more challenging. Could we better understand ENC and EPC mechanotransduction by developing advanced *in vitro* techniques that measure forces in 2D and 3D matrices? If achieved, could this significantly improve existing models?

To what extent can computational models predict the complex interactions between cytoskeletal dynamics, cell adhesions, and resulting cell shapes in ENC and EPC monolayers? Could these models eventually integrate biochemical signaling dynamics? As live-cell imaging advances, could it provide more data on how cell mechanics and biochemistry interact, leading to more accurate models? Moreover, could these models be valuable beyond cell biology, such as in tissue engineering, where building stronger monolayers for implantation is crucial?

What are the limitations of current *in silico* models in capturing the heterogeneity within ENC and EPC populations that is observed *in vitro*, and how could future models account for that? Along this line, how can patient-specific computational models (e.g., digital twins) be developed to predict individual responses to therapeutic interventions targeting mechanobiological pathways in ENCs or EPCs? Could multiscale models be built that function in a patient-specific manner?

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT 4 with the intent to improve the clarity and readability of the text. After using this tool/service, the authors reviewed and edited the content as needed, taking full responsibility for the content of the publication.

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