Role of viscoelasticity in the buckling-to-folding transition of epithelial monolayers under uni-axial compression

Ivana Pajic-Lijakovic [1], Milan Milivojevic [1], and Peter V. E. McClintock[2]

[1] University of Belgrade, Faculty of Technology and Metallurgy, Department of Chemical Engineering, Belgrade, Serbia

[2] Department of Physics, Lancaster University, Lancaster LA1 4YB, UK

Correspondence to: Ivana Pajic-Lijakovic, iva@tmf.bg.ac.rs

Peter V.E. McClintock, p.v.e.mcclintock@lancaster.ac.uk

Abstract

Epithelial tissues often experience, and respond to, in-plane compression. This occurs during embryonic development and continues throughout adult life, driven by both internal and external forces. Gaining insight into such processes is essential for understanding the mechanisms of tissue morphogenesis, and therefore carries significant implications for developmental biology and regenerative medicine. Although the biological mechanisms associated with epithelial folding have been extensively researched, the physical mechanisms are only beginning to be clarified. One of the primary factors contributing to the relaxation of epithelial monolayers, following externally induced buckling and folding, is the viscoelasticity related to energy storage and dissipation resulting from their compression. Physical mechanisms involve the interplay between physical parameters such as: the epithelial surface tension, viscoelastic Poisson's ratio, bending modulus, internally generated strain and corresponding mechanical stress. The main focus of this review is to point out how interconnected relaxation processes influence epithelial buckling and folding as an integral part of the viscoelasticity, and how cells can regulate the extent of the folding depending on the magnitude of the externally applied compressive stress. This complex phenomenon is elaborated on substrate-devoid epithelial monolayers, considered as a simple model system under *in vitro* conditions.

Key words: epithelial surface tension, viscoelastic Poisson's ratio, stress-strain constitutive model, cell contractility, strength of cell-cell adhesion contacts

1. Introduction

Compressive forces, whether applied intrinsically or extrinsically, are fundamental to the intricate shaping of tissues during the process of developmental morphogenesis [1]. Epithelial tissues often experience in-plane compression during both embryonic development and adult life, due to a combination of intrinsic and extrinsic forces. Gaining insight into these mechanisms is vital for understanding tissue morphogenesis and carries significant implications for the fields of developmental biology and regenerative medicine [2-4).

Such compressive forces occur on various time scales, ranging from minutes to hours. Compression may be caused by any or all of (i) actomyosin-involved apical or basal constriction, which arises on a time scale of minutes [5,6], (ii) the migration of epithelial collectives, or rearrangement of the surrounding tissue via migration, occurring on a time scale of hours [4,7], and (iii) cell growth-induced constraints developing on a time scale of days [1,8]. The initial mechanical instability, that happens when in-plane compression exceeds a critical threshold, is buckling. It results in the generation of a small out-of-plane strain, for which the corresponding cell rearrangement can be treated as a linear phenomenon [9]. Compression-induced epithelial buckling is an integral part of numerous morphogenetic processes such as: formation of intestinal villi, lumen formation in early morphogenesis, and the rearrangement of epithelial monolayers on soft substrates [4]. Buckling occurs through a series of interdependent events in which biochemical signals trigger cell shape changes, leading to spatial phenotypic patterning [1,4]. In biological tissues, buckling can give rise to non-linear phenomena such as: folds and large undulations in monolayers [4]. Various types of folding such as invagination (i.e., inward folding) and evagination (i.e., outward folding) take place during morphogenesis [10-12]. The integral formation of an invagination region near the evagination regions via collective cell migration is known as *vertical telescoping*. In this case, central cells move downward, and peripheral cells move upward [4,13]. Successive invagination and evagination represent mechanically unstable structures that need more surface energy, bending energy, and strain energy [14]. Gradients of the epithelial surface tension, the accumulated mechanical stress, and the bending moment can provide the driving forces that cause relaxation to a lower energy state against the internal energy of cells which drives curvature formation [7,15]. The internal cell energy is induced by molecular mechanisms related to cell signalling and gene expression [4]. Cell signalling influences the distributions of the strengths of cell-cell and cell-matrix adhesion contacts and cell contractility via mechano-transduction pathways, while gene expression has a feedback impact on the remodelling of adhesion contacts and cell cytoskeletons [16,17]. Epithelial folding activates mechanosensitive Piezo 1 channels and triggers waves on a timescale of minutes and gene expression on a timescale ranging from ten minutes up to hours [18]. Whereas buckling appears due to the compression of epithelial monolayers or stratified epithelium, wrinkling is caused by a local surface instability [19]. In what follows, we will be interested in the *in vitro*-induced buckling of epithelial monolayers under uniaxial compression.

While biological mechanisms related to epithelial folding have been widely studied, the underlying physical mechanisms are only starting to be elucidated. Viscoelasticity related to energy storage and dissipation (see **Glossary of Terms)** caused by the compression of epithelial monolayers is one of the main factors responsible for the relaxation of epithelial monolayers under various loading conditions [20]. Lecuit and Lenne [21] discussed epithelial folding as a multi time process characteristic of viscoelasticity. Wang et al. [22] revealed that the rate-dependent buckling mode is quantitatively influenced by the viscoelastic and geometrical properties of epithelia. The influence of viscoelasticity on the physical parameters that regulate epithelial rearrangement, including mechanical stress, the

associated strain, Poisson's ratio, bending modulus, and epithelial surface tension, is multi-time and complex. Storgel et al. [23] and Lecuit and Lenne [21] pointed out that the tissue surface tension is a key physical regulator of epithelial morphogenesis, including folding. However, surface tension was discussed as an equilibrium property of the monolayer, while the dilation viscoelasticity, which is related to changes with time of the surface tension, was not considered. A more profound understanding of the viscoelastic characteristics of epithelial rearrangement is essential for comprehending the multi-scale aspects of epithelial folding.

Inhomogeneous energy storage is the main origin of the mechanical instability, which induces epithelial buckling [14,24]. The energy storage depends on the strength of E-cadherin-mediated cell-cell adhesion contacts and cell contractility [25]. It is well known that a prerequisite of epithelial folding is inhomogeneous distributions of actomyosin [15,26] and of the strength of the cell-cell adhesion contacts when under the influence of mechanical stress. The complex impact of viscoelasticity on epithelial folding can be discussed in terms of a set of physical parameters such as: epithelial surface tension, bending stiffness, internal strain, viscoelastic Poisson's ratio, and generated stress, all of which are space-time-dependent [7,27].

The main focus of this theoretical consideration is to point out the inter-relationships of these physical parameters in the physical mechanisms of monolayer relaxation under instantaneous uni-axial compression. The phenomenon is discussed in relation to a simplified model system: a suspended (substrate devoid) epithelial monolayer.

2. Phenomenological description of modelling systems

Two types of model system have been examined in relation to the buckling behaviour of epithelial monolayers under uni-axial compression. One consists of suspended (substrate-devoid) epithelial monolayers [15], while the other is epithelial monolayers on substrate matrices [19,28]. In vitro folding of (substrate devoid) epithelial monolayers under uni-axial compression has been observed in various biological systems such as: eye morphogenesis [29], mouse embryo epiblast folding during implantation [30], and chick midbrain neural tube closure [31].

The epithelial wrinkling-to-folding transition was observed for epithelial Caco-2 monolayers on collagen I substrate matrices under an externally applied uni-axial compressive strain of -0.5 [19]. In this case, the initial wrinkling is a consequence of surface structural changes of the matrix and depends on the strengths of the epithelial matrix focal adhesions (FAs) [28]. When suspended (substrate-devoid) epithelial Madin-Darby Canine Kidney (MDCK) and keratinocyte (HaCaT) monolayers [15] are compressed to a strain higher than -0.35, they undergo the buckling-to-folding transition. The establishment of FAs induces damping effects of initial mechanical instability and protect the monolayers against buckling. In this case, the surface instability of the collagen I matrix and weakening of FAs under compression result in wrinkling [28]. In the case of epithelial monolayers on collagen I matrices, folding occurs at higher compressive strain compared to the substrate-devoid monolayers. When collagen I networks undergo a 20% uniaxial extension aligned with the fibers, the equilibrium Poisson's ratio is estimated to be around $\nu \sim 5$, which is linked to the compression experienced by the network [32]. Consequently, strain stiffening and anisotropy significantly influence the viscoelasticity of a collagen I matrix [33].

Some epithelial cell types are more prone to folding than others, even under the same uni-axial compression. Some (substrate-devoid) epithelial monolayers, such as MDCK cell monolayers, are able to relax the curvature caused by a compressive strain of -0.35, while others such as HaCaT cell monolayers retain the out-of-plane displacement under the same conditions [15]. These two scenarios are shown schematically in **Figure 1**:

Figure 1.

These scenarios under the same external compression are characterized as:

Scenario 1: A lower energetic state occurs after epithelial buckling at $t = \Delta t$, where Δt is the time necessary for epithelial buckling caused by the uni-axial compression applied at $t = 0$. Total relaxation of the out-of-plane displacement has taken place at $t \to \infty$, i.e., $w \to 0$.

Scenario 2: A higher energetic state occurs after the epithelial buckling. Only partial relaxation of the out-of-plane displacement has taken place at $t \to \infty$, i.e., $w \to w_{eq}$, where w_{eq} is the equilibrium out-of-plane displacement.

The folding wavelength varies for different cell types under the same external compressive strain condition, depending on the strength of the adherens junctions (AJs) and the cell contractility. MDCK cells form strong AJs and demonstrate contractility characterized by a higher Young's modulus of the monolayers, whereas HaCaT cells display moderate AJs and inconsistent contractility, as indicated by a lower elastic modulus of the monolayers [34,35]. Consequently, the stress relaxation time of MDCK cells, accompanied by the relaxation time of the out-of-plane displacement, is shorter than that of HaCaT cells. The ability of the monolayers to fold depends on several factors, including: (i) the structural organization of the monolayers, (ii) their thickness, (iii) their viscoelasticity, which accounts for stress and strain relaxation and change in the Poisson's ratio, (iv) their bending modulus, and (v) the epithelial surface tension. The arrangement of the cytoskeleton, cell-cell adhesion complexes, and even small density differences across cells, all affect the mechanical response. Under steadily increasing compression, a more disordered or heterogeneous monolayer might fold earlier and more than a highly ordered one [36]. The monolayers made by bigger cells (i.e., a higher h) have higher bending rigidity; thinner monolayers fold more easily. Viscoelastic relaxation influences epithelial folding. If a monolayer can relax stress quickly, it might not store as much compressive stress, leading to less folding. Higher active contractility can promote folding at lower strains. A monolayer with lower bending modulus will fold more easily — folds will appear with larger amplitude and/or shorter wavelength. The bending modulus depends on cell-cell adhesion strength, cortical tension, and cytoskeletal stiffness. Epithelial surface tension, as a measure of the monolayer cohesiveness, resists bending.

Viscoelastic coupling between epithelial monolayers and substrate matrices contributes to the epithelial buckling while, in the second case, the viscoelasticity of the epithelial monolayer is the only factor responsible for cell response. Viscoelasticity in epithelial monolayers is a complex phenomenon, governed by a set of space-time-dependent physical parameters such as: cell stress-strain, epithelial surface tension, Poisson's ratio, and bending modulus. When epithelial monolayers are attached to substrate matrices, additional physical parameters are needed such as: the matrix surface tension, a stress-strain constitutive model for the matrix, and the epithelial-matrix interfacial tension. The total mechanical stress within the lamellar epithelial-matrix system is equal to the sum of epithelial and matrix stresses, while the strains within both systems are the same.

To gain a more profound understanding of epithelial folding, it is essential to examine the interplay between the physical parameters that facilitate and hinder this process. These parameters are influenced by the strength of the cell-cell adhesion contacts and the contractility of the cells, which enable the cells to modulate folding.

3. Response of epithelial monolayers under uni-axial compression

Compressive force is applied to the right-hand edge of the suspended (substrate-devoid) epithelial monolayer along the *x*-direction. This generates in-plane compressive stress along the *x*-direction equal to ${\sigma_{xx}}^{external} = \frac{F}{A}$ (where F is the applied force and A is the cross-sectional area per unit width). Initially, the compressive stress is uniformly distributed within the epithelial monolayer, but then increases in the middle of the monolayer due to the force action in the opposite direction. When the material experiences in-plane compression for the critical stress of -7 Pa [15], the edges are pushed inward, while non-uniform distribution of mechanical stress leads to the monolayer bending. The out-of-plane displacement (bending) becomes more pronounced over time, as the material fails to resist further compression. This induced epithelial buckling results in the formation of a curvature and, as the compressive force rises, the curvature will increase. The initiated buckling occurs after a few seconds of uni-axial compression applied by the rate of $500\ \%s^{-1}$ [15]. Internally accumulated mechanical stress resists further deformation of the monolayer. The epithelial response to uni-axial compression is considered within three regimes: (i) a non-buckling (lower strain) regime for $\varepsilon_{xx} < \varepsilon_b$ (where ε_b is the buckling threshold strain), (ii) a folding (larger strain) for $\varepsilon_{xx} > \varepsilon_b$, and (iii) a relaxation of the monolayer resulting in a decrease in the strain and corresponding mechanical stress. The epithelial strain $\tilde{\boldsymbol{\varepsilon}}$ is equal to: $\tilde{\boldsymbol{\varepsilon}}\{x, y, z, t\} = \tilde{\boldsymbol{\varepsilon}}^{in-plane}(x, y, t) + \tilde{\boldsymbol{\varepsilon}}^{out-of-plane}(x, y, z, t)$ (where $\tilde{\boldsymbol{\varepsilon}}^{in-plane}(x, y, t)$ is the in-plane strain contribution, i.e., $\tilde{\boldsymbol{\varepsilon}}^{in-plane}(\varepsilon_{xx}, \varepsilon_{yy}, \varepsilon_{xy})$ and $\tilde{\boldsymbol{\varepsilon}}^{out-of-plane}(x, y, z, t)$ is the out-of-plane strain contribution, i.e., $\tilde{\boldsymbol{\varepsilon}}^{out-of-plane}(\varepsilon_{zz})$). The in-plane strain components are $\varepsilon_{ij}^{in-plane} = \frac{1}{2}\left(\frac{\partial}{\partial i}u_j + \frac{\partial}{\partial j}u_i + \frac{\partial}{\partial i}w\frac{\partial}{\partial j}w\right)$ (where $i, j \equiv x, y$, $u_i \equiv u_x$ and $u_j \equiv u_y$ are the components of the displacement field). The non-linear term of the in-plane strain should be included for the case of epithelial folding. The out-of-plane strain component is $\varepsilon_{zz} = -z\left(\frac{\partial^2}{\partial i^2}w + \frac{\partial^2}{\partial j^2}w\right) + \frac{1}{2}\left[\left(\frac{\partial}{\partial i}w\right)^2 + \left(\frac{\partial}{\partial j}w\right)^2\right]$ [37]. The shear strain components ε_{xz} and ε_{yz} are much lower than the normal strain component ε_{zz} and can be neglected [37]. The non-linear contribution to out-of-plane strain should be included for the case of epithelial folding. While the in-plane displacement $\vec{\boldsymbol{u}}(u_x, u_y)$ is a vector, the out-of-plane displacement w has been treated as a scalar [37]. It is in accordance with the fact that the out-of-plane displacement always points in the same direction (normal to the un-deformed monolayer). Consequently, w represents the magnitude of the displacement along the out-of-plane axis. These strain components cause the internal generation of mechanical stress: $\tilde{\boldsymbol{\sigma}}(\tilde{\boldsymbol{\varepsilon}}) = \tilde{\boldsymbol{\sigma}}^{\boldsymbol{in-plane}}(\sigma_{xx}, \sigma_{yy}, \sigma_{xy}) + \tilde{\boldsymbol{\sigma}}^{\boldsymbol{out-of-plane}}(\sigma_{zz})$ (where $\tilde{\boldsymbol{\sigma}}^{\boldsymbol{in-plane}}$ is the in-plane stress and $\tilde{\boldsymbol{\sigma}}^{\boldsymbol{out-of-plane}}$ is the out-of-plane stress, while the transverse shear stress components σ_{xz} and σ_{yz} are often assumed negligible in comparison with normal strain component σ_{zz} [37]). Uni-axial strain of the monolayer causes accumulation of: (i) strain energy equal to $e_s = \Delta V \sum_{ij} \sigma_{ij}\varepsilon_{ij} + \sigma_{zz}\varepsilon_{zz}$ (where $i, j \equiv x, y$ such that the ij components of stress and strain are: xx, yy, and xy components and ΔV is the volume of the monolayer part), (ii) the Helfrich types of bending energy which can be

expressed as: $e_b = \frac{1}{2}E_b\left(\frac{\partial^2}{\partial x^2}w + \frac{\partial^2}{\partial y^2}w - 2C_0\right)^2 + E_b^G\left(\frac{\partial^2}{\partial x^2}w\frac{\partial^2}{\partial y^2}w - \left(\frac{\partial^2}{\partial x \partial y}w\right)^2\right)dA$ [37], and (iii) surface energy $e_A = \gamma_e dA$ (where $\gamma_e(x,y,t)$ is the epithelial surface tension i.e., dynamic surface tension, E_b is the bending modulus, which is space-time dependent for viscoelastic monolayers and expressed as: $E_b(x,y,t) = \frac{h^3 E}{12(1-\nu^2)}$, $\nu(x,y,t)$ is the space-time-dependent Poisson's ratio characteristic of viscoelastic systems, $E(x,y,t)$ is the relaxation modulus, which can be expressed in terms of a suitable stress-strain constitutive model, E_b^G is the Gaussian bending modulus, C_0 is the spontaneous curvature, and $A(x,y,t)$ is the surface area of the monolayer) [37]. Consequently, the viscoelasticity of epithelial monolayers is included through the interplay between physical parameters such as: epithelial surface tension, bending modulus, Poisson's ratio, and internally generated mechanical stress. The viscoelasticity of epithelial monolayers, as well as the level of out-of-plane strain, can be controlled by the cells themselves.

4. Ability of epithelial cells to control viscoelasticity

Viscoelasticity of epithelial monolayers depends on an interplay between cell contractility and the strength of E-cadherin-mediated cell-cell adhesion contacts. Storage of contractile energy within cells and strong cell-cell adhesion contacts ensure transport and storage of elastic energy under internally or externally applied strain. In contrast, the weakening of cell-cell adhesion contacts increases energy dissipation during cell rearrangement. The level of epithelial contractility depends on the concentration and distribution of actomyosin components. Inhomogeneous distribution of actomyosin has been discussed as one of the main factors responsible for the buckling of monolayers [15]. The contractility of epithelial cells enhances the strength of cell-cell adhesion contacts [38]. Local extension of the monolayer also increases the strength of cell-cell adhesion contacts [39], while compression has the opposite effect [40,41].

Cells are capable of self-regulating their cohesiveness, alongside their management of energy storage and dissipation during epithelial response under uni-axial compression, which is essential for their adaptation to micro-environmental conditions. This regulatory mechanism encompasses biological processes such as cell signaling and gene expression [42]. The cellular response to mechanical extension or compression is governed primarily by the interaction between E-cadherin levels and the distribution of actomyosin, which affects: (1) the number of adherens junctions (AJs) formed between adjacent cells, (2) the number of bonds established within individual AJs and their respective strengths, and (3) the cortical tension [40,43].

Sumi et al. [43] found that the radius of an individual AJ ranges from 1 µm to 2 µm, suggesting the presence of one or more AJs between adjacent epithelial cells. The turnover of E-cadherin occurs over a time frame of minutes [7], while the conformational alterations of cadherin take place within milliseconds to seconds. The stretching of AJs facilitates the delocalization of actomyosin from the interior of the cell towards the junctions, thereby preserving junctional integrity [43]. The intracellular pulling force is estimated to be between 20 and 100 nN, which escalates with the expansion of epithelial and endothelial multicellular structures [41=44].

The rupture of E-cadherin bonds necessitates a force of 200 nN [44]. An increase in the pulling force leads to an expansion of the AJ's surface area, ranging from 20 µm² to 100 µm², thereby maintaining a constant stress at the AJ through homeostatic mechanisms [44]. The adhesion stress is influenced

by the type of cell and the surrounding microenvironment; for instance, the adhesion stress in endothelial cells is approximately 1 kPa [44], whereas in Drosophila tissue, it is around 100 Pa [45]. The interplay between the strengths of AJs and the contractility of the cortex is antagonistic in AJs. This is consistent with the observation that cadherins interact with actomyosin, indicating that the cytoskeleton also plays a role in generating tension on the cell-cell adhesion contacts [45].

The complex effect of viscoelasticity on the epithelial response to uni-axial compression can be examined through a series of physical parameters, highlighting their interconnections.

4.1 Physical parameters that control epithelial response under uni-axial compression

We now discuss the impact of viscoelasticity on the generation of out-of-plane strain under uni-axial compression of (substrate-devoid) epithelial monolayers, doing so in relation to physical parameters such as: the epithelial surface tension, Poisson's ratio, and the in-plane and out-of- plane stress components. Our primary objective is to highlight the inter-relationships between these parameters.

4.1.1 Epithelial surface tension

The surface tension within a cellular system denotes an energy per unit area associated with a multicellular interface interacting with a liquid environment [7,38,46]. This physical parameter is space-time dependent, and is thus referred to as *dynamic tissue surface tension* (see **Glossary of Terms)**. The surface energy of a cellular system is contingent upon the intensity of homotypic cell-cell adhesion interactions and the contractile properties of the cells, as articulated by Alt et al. [47] and Koride et al. [48]:

$$e_A = \sum_i \frac{K}{2}\left(A_{ceffi} - A_0\right)^2 + \sum_{i,j} \Lambda l_{ij} + \sum_i \frac{T_{con\,i}}{2} {L_i}^2 \qquad (1)$$

where A_{ceffi} is the effective surface area of the *i*-th cell, K is the effective modulus of the cell around its preferred surface area A_0, l_{ij} is the interface length between the i-th and j-th cells, Λ is the line tension per unit interface length between two cells, $T_{con\,i}$ is the contractility coefficient, and L_i is the perimeter of the i-th cell. As a result, the surface tension, which indicates the variation of surface energy in relation to the surface area of the monolayer, is defined as:

$$\gamma_e = \frac{\partial e_A}{\partial A} \qquad (2)$$

The epithelial surface tension relies on an interplay between: the strength of E-cadherin-mediated cell-cell adhesion contacts, cell contractility, and local extension or compression of the monolayer [7,38,39]. Extension enhances the strength of cell-cell adhesion contacts resulting in an increase in the surface tension [39]. The application of micropipette aspiration forces ranging from 0.5 µN to 1.5 µN at the surface of murine sarcoma (S180) aggregates increases the surface tension from $\sim 7\ \frac{mN}{m}$ to $\sim 22\ \frac{mN}{m}$ [39]. However, compression has the opposite effect. It stimulates cell-cell interactions causing a weakening of cell-cell adhesion contacts [7]. Variations in the contractility of epithelial cells, stemming from the inhomogeneous distribution of actomyosin components, lead to an inhomogeneous distribution of surface tension throughout the monolayer. The tissue surface tension varies significantly between different cellular systems, from several $\frac{mN}{m}$ up to a few tens of $\frac{mN}{m}$ [46,49,50]. The average equilibrium value of the tissue surface tension has been measured for

various multicellular surfaces, rather than the spatiotemporal change of the surface tension resulting from cell rearrangements induced by either external or internal forces.

Epithelial buckling/folding stimulates the contractility of epithelial cells [4] leading to an increase in the epithelial surface tension expressed as [51,52]:

$$\gamma_e(x,y,t) = \gamma_e^{in-plane} + k\nabla^2 w \qquad (3)$$

where $\gamma_e^{in-plane}$ is the in-plane epithelial surface tension, k is a surface tension-curvature coupling coefficient, which depends on the bending modulus, i.e., $k(x,y,t) \sim \frac{E_b}{h}$, h is the thickness of the monolayer, and E_b is the bending modulus. The bending modulus of the tissue is in the range of $3x10^{-13} - 5x10^{-13}$ J [23,53]. For a curvature radius corresponding to a few cell sizes and $h \sim 10$ μm, the second term on the right-hand side of eq. 3 is equal to $\sim 1 \frac{\text{mN}}{\text{m}}$. An increase in the curvature radius causes an increase in the epithelial surface tension, which reduces bending. Changes in the out-of-plane displacement and the in-plane compression influence the surface area of the monolayer expressed as: $A(w) = \int_\Omega \sqrt{1 + |\vec{\nabla} w|} dxdy$. Change of the epithelial surface tension, and its rate of its change, cause a decrease in the surface area of the monolayer; change of the surface area, and its rate of change, have a feedback impact on the epithelial surface tension itself. Nonetheless, surface tension was addressed as an equilibrium characteristic of the monolayer, whereas dilation viscoelasticity [21,23]. This cause-consequence cycle, characteristic of viscoelastic surfaces such as epithelial monolayers, can be expressed in the form of a dilational constitutive model [7].

$$\gamma_e(x,y,t) + \tau_{R\gamma} \frac{d}{dt}(\gamma_e) = E_S \frac{\Delta A(w,t)}{A_0} + \eta_S \frac{d}{dt}\left(\frac{\Delta A}{A_0}\right) \qquad (4)$$

where $\tau_{R\gamma}$ is the relaxation time for epithelial surface tension, E_S is the surface elastic modulus and η_S is the surface viscosity. An increase in cell packing density results in an increase in the relaxation time $\tau_{R\gamma}$.

The gradient of the surface tension represents a driving force for the out-of-plane displacement of the monolayer and the monolayer rearrangement [54]. The phenomenon is known as the Marangoni effect [55]. The displacement occurs from the region of lower surface tension toward the region of larger surface tension. The surface tension for viscoelastic systems such as epithelial monolayers is space-time dependent. The other driving force for epithelial buckling is connected to the generation of in-plane mechanical stress, which will be discussed below.

4.1.2 Viscoelastic Poisson's ratio

For viscoelastic systems such as epithelial monolayers, the Poisson's ratio is time-dependent [27]. If the monolayer thickness h is approximately constant, the uni-axial strain causes the monolayer volume to: (i) increase for $\nu < 0.5$, (ii) stay constant for $\nu = 0.5$, or (iii) decrease for $\nu > 0.5$. Epithelial monolayers are recognized for their anisotropic behaviour (see **Glossary of Terms**), which arises from cell polarization driven by acto-myosin activity, i.e., $\nu \neq 0.5$. Moisdon et al. [56] measured the Poisson's ratio of MDCK and HeLa epithelial monolayers on a flat substrate in frequency-mode for the frequency range $10^{-2} - 10^2$ Hz and pointed out that the Poisson's ratio is frequency independent and equal to $\nu \sim 0.77$, indicating extension of the monolayer in the *y*-direction. Wang et al. [22] supposed a constant value of Poisson's ratio equal to 0.3. Consequently,

the normal strain component in the *y*-direction ε_{yy} is extensional ($\varepsilon_{yy} > 0$), while the normal strain component in the *x*-direction ε_{xx} is compressive $\varepsilon_{xx} < 0$. The strain ε_{yy} relaxes on the strain ε_{xx}. This relaxation can be expressed as [27]:

$$\varepsilon_{yy}(x,y,z,t) = -\nu_0 \varepsilon_{xx}(x,y,z,t) + \int_0^t \nu(x,y,z,t-t') \frac{d\varepsilon_{xx}(x,y,z,t')}{dt'} dt' \quad (5)$$

where ν_0 is the initial Poisson's ratio, and $\nu(x,y,z,t)$ is the viscoelastic Poisson's ratio, while the strain component ε_{xx} relaxes to the externally applied strain ε_d.

The shear strain component ε_{xy} is also generated during the epithelial rearrangement under uni-axial compression, and it can be pronounced during monolayer buckling.

4.1.3 Relaxation of the strain component ε_{xx}

Buckling and folding induce changes in the strain distribution, while the internal strain $\tilde{\boldsymbol{\varepsilon}}(x,y,z,t)$, caused by the externally applied compressive strain ε_d, relaxes exponentially toward the residual strain $\varepsilon_{xx\,r}$, which is $\varepsilon_{xx\,r} \geq \varepsilon_d$. Consequently, we can provide a model for relaxation of the normal internal strain component ε_{xx} under constant external compressive strain applied in the *x*-direction in the form:

$$\varepsilon_{xx} + \tau_\varepsilon \dot{\varepsilon}_{xx} = \varepsilon_{xx\,r} \quad (6)$$

where $\tau_\varepsilon(x,y,z)$ is the strain relaxation time, and $\dot{\varepsilon}_{xx}$ is the strain rate $\dot{\varepsilon}_{xx} = \frac{d\varepsilon_{xx}}{dt}$. The strain relaxation time is the product of the in-plane and out-of-plane force balances, which will be discussed in **Section 4.2**. This parameter can vary along the monolayer. For the initial conditions $t = 0$ the strain is $\varepsilon_{xx} = 0$. The internal strain relaxation can be expressed as [15]:

$$\varepsilon_{xx}(x,y,z,t) = \varepsilon_{xx\,r}\left(1 - e^{-\frac{t}{\tau_\varepsilon}}\right). \quad (7)$$

If ε_d is a compressive strain, than ε_{xx} is also compressive.

4.1.4 Viscoelastic stress-strain constitutive model for the monolayer buckling/folding

Wyatt et al. [15] considered buckling of MDCK and HaCaT epithelial monolayers and revealed that the buckling threshold corresponds to a compressive strain of ~ -0.35. In the buckling regime, in-plane stress relaxes exponentially under constant strain toward the residual stress, which points to a linear constitutive model (see **Glossary of Terms)**. In accordance with the fact that epithelial cells establish strong E cadherin-mediated cell-cell adhesion contacts, their viscoelastic behaviour corresponds to that of a viscoelastic solid (see **Glossary of Terms)**. The Zener model satisfies these experimental requirements. The constitutive model is expressed as [57]:

$$\tilde{\boldsymbol{\sigma}}^{ve} + \tau_\sigma \dot{\tilde{\boldsymbol{\sigma}}}^{ve} = E_e \tilde{\boldsymbol{\varepsilon}} + \eta_e \dot{\tilde{\boldsymbol{\varepsilon}}} \quad (8)$$

where $\tilde{\boldsymbol{\sigma}}^{ve}(x,y,z,t)$ is the stress of the multicerllular system, $\dot{\tilde{\boldsymbol{\sigma}}}^{ve}$ is the rate of stress change equal to $\dot{\tilde{\boldsymbol{\sigma}}}^{ve} = \frac{d\tilde{\boldsymbol{\sigma}}^{ve}}{dt}$, $E_e(x,y,z)$ is the Young's modulus, η_e is the viscosity, and τ_σ is the stress relaxation time. The latter depends on the strength of the AJs [58,59] and corresponds to a time scale of minutes [60]. Higher AJ strengths result in shorter stress relaxation times. The relaxation of the normal stress component σ_{xx} can be estimated by introducing eq. 7 into eq. 8:

$$\sigma_{xx}{}^{ve}(x,y,z,t) = \sigma_0 e^{-\frac{t}{\tau_\sigma}} + E_e \varepsilon_{xx\,r}\left(1 - e^{-\frac{t}{\tau_\sigma}}\right) + \frac{\tau_\varepsilon}{\tau_\sigma + \tau_\varepsilon}\left[\eta_e \frac{1}{\tau_\varepsilon} - E_e\right] \varepsilon_{xx\,r}\left(e^{-\frac{t}{\tau_\varepsilon}} - e^{-\frac{t}{\tau_\sigma}}\right) \quad (9)$$

where σ_0 is the epithelial pre-stress, which is tensional (i.e., $\sigma_0 > 0$) and equal to 240 ± 30 Pa [15]. While the stress relaxation is primarily the result of the remodelling of the AJs, the strain relaxation time is caused by equilibration of the in-plane and out-of-plane force balances, which depends on the distribution of epithelial surface tension, and the in-plane and out-of-plane stress components along the monolayers, which will be discussed in **Section 4.2**. Two scenarios can be considered:

(i) If $\tau_\varepsilon \leq \tau_\sigma$ the boundary condition specifies that for $t \to \infty$, the *x*-component of normal residual stress is $\sigma_{xxr}{}^{ve} \to E_e \varepsilon_{xx\,r}$ (where the normal residual strain in the *x*-direction is $\varepsilon_{xx\,r} < 0$). The compressive residual stress calculated for a compressive strain of $\varepsilon_d = -0.30$, which corresponds to the buckling regime, and Young's modulus of 640 Pa, for the case that $\varepsilon_d \approx \varepsilon_{xx\,r}$ is equal to -213 Pa. The normal stress component in the *y*-direction is tensional and relaxes toward the residual stress $\sigma_{yyr}{}^{ve} = -\nu(x,y,z,t) E_e \varepsilon_{xx\,r}$ (see **Glossary of Terms)**.The experiments from Wyatt et al. [15] show that the stress and strain relaxation times satisfy the condition that $\tau_\varepsilon \sim \tau_\sigma$.

(ii) When $\tau_\varepsilon \gg \tau_\sigma$ then, as $\tau_\varepsilon \to \infty$, $\sigma_{xxr}{}^{ve} \to 0$. Harris et al. [34] considered strain relaxation under instantaneous application of tensional stress of 3 kPa to the monolayers. The strain relaxation time is more than 10 min.

Besides the viscoelastic component $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{r}}^{\boldsymbol{ve}}$ the residual stress includes another component which depends on epithelial surface tension.

4.1.5 Cell residual stress accumulation within the monolayers

The cell residual mechanical stress also depends on the epithelial surface tension and the surface tension gradient. The normal cell residual stress $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{Nr}}$ has been expressed as [57]:

$$\tilde{\boldsymbol{\sigma}}_{\boldsymbol{Nr}} = \Delta p_e \tilde{\boldsymbol{I}} + \tilde{\boldsymbol{\sigma}}_{\boldsymbol{Nr}}^{\boldsymbol{ve}} \quad (10)$$

where an isotropic part of the residual stress has been expressed in the form of the Young-Laplace equation as $\Delta p_e = \frac{1}{2}\gamma_e \nabla^2 w$, $\tilde{\boldsymbol{I}}$ is the unit tensor, while the deviatoric part of the normal residual stress $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{Nr}}^{\boldsymbol{ve}}$ is caused by the distribution of the strain energy caused by the viscoelastic response of the monolayer. If the monolayer is curved, the surface tension γ_e generates an additional pressure tending to flatten it.

The cell shear stress accounts for two contributions, i.e., the shear stress induced by: (i) the gradient of surface tension and (ii) the distribution of strain energy. The shear stress within the epithelial subpopulation $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{erS}}$ is expressed as [57]:

$$\vec{\boldsymbol{n}} \cdot \tilde{\boldsymbol{\sigma}}_{\boldsymbol{Sr}} \cdot \vec{\boldsymbol{t}} = \vec{\boldsymbol{V}}\gamma_e \cdot \vec{\boldsymbol{t}} + \vec{\boldsymbol{n}} \cdot \tilde{\boldsymbol{\sigma}}_{\boldsymbol{Sr}}{}^{ve} \cdot \vec{\boldsymbol{t}} \quad (11)$$

where $\vec{\boldsymbol{n}}$ and $\vec{\boldsymbol{t}}$ are the normal and tangential vectors to the direction of the monolayer deformation, respectively. The first term on the right hand side corresponds to the shear stress contribution caused by the gradient of surface tension, i.e., the Marangoni effect, while the second term represents the shear stress contribution caused by the distribution of strain energy. When an epithelial monolayer is attached to a substrate matrix, the epithelial-matrix interfacial tension contributes to the generation of a part of the cell residual stress rather than epithelial surface tension [57].

Cell stress generates an internal bending moment, which resists the buckling/folding of epithelial monolayers. The internal bending moment per unit length caused by compression of the epithelial monolayer depends on the total mechanical stress, which includes in-plane and out-of-plane contributions, and can be expressed as [60]:

$$M_k(x,y,t) = \int_{-h/2}^{h/2} z\sigma_k(x,y,z,t)dz \quad (12)$$

where $k = xx, yy, xy$, $M_k(x,y,t)$ is the component of the bending moment per unit length and h is the thickness of the monolayer. While the bending moment resists epithelial buckling/folding, the components of the in-plane force tensor $\widetilde{\boldsymbol{N}}$ can be expressed as $N_k(x,y,t) = h\,\sigma_k^{in-plane}$ and the gradient of epithelial surface tension $\vec{\boldsymbol{\nabla}}\gamma_e$ drives buckling/folding.

4.2 In-plane and out-of-plane force balances

Change in the displacement field $\vec{\boldsymbol{u}}(x,y,t)$**,** which includes compression in the *x*-direction and extension in the *y*-direction, can be expressed in terms of the in-plane force balance by accounting for inertial effects. They arise as a consequence of fast, stepped, uni-axial compression of the monolayer. The corresponding force balance can be formulated by the modified model of Audoly and Pomeau as [37] by including the impact of epithelial surface tension on the velocity $\vec{\boldsymbol{v}}_{\boldsymbol{e}}$**.** It is expressed as:

$$\langle m_e\rangle\rho_e \frac{d\vec{\boldsymbol{v}}_{\boldsymbol{e}}(x,y,t)}{dt} = \rho_e\gamma_e^{in-plane}\vec{\boldsymbol{u}} - h\boldsymbol{\nabla}\tilde{\boldsymbol{\sigma}}^{in\ plane} \quad (13)$$

where $\vec{\boldsymbol{v}}_{\boldsymbol{e}} = \frac{d\vec{\boldsymbol{u}}}{dt}$ is the rate of change of the in-plane displacement field, $\langle m_e\rangle$ is the average mass of a single cell, $\tilde{\boldsymbol{\sigma}}^{\boldsymbol{in\ pane}}(x,y,t)$ is the in-plane stress, ρ_e is the surface packing density of the epithelial monolayer, and $\gamma_{e0}(x,y,t)$ is the pre-buckling epithelial surface tension. While the surface tension force (i.e., the first term on the right-hand side of eq. 13) drives cell rearrangement by reducing the surface area of the monolayer, internally generated in-plane stress resists the monolayer compression. Imbalance between these two forces caused by instantaneously applied uni-axial compression is the main cause of the inertial effects. Although the internal in-plane stress opposes the creation of in-plane displacement, it facilitates the occurrence of out-of-plane displacement.

The out-of-plane force balance, which accounts for inertial effects, can be expressed through use of the modified model proposed by Audoly and Pomeau as [37] by including the impact of the epithelial surface tension and the surface tension gradient on changes in the rate of change of the out-of-plane displacement. It is expressed as:

$$\langle m_e\rangle\rho_e \frac{\partial^2 w(x,y,t)}{\partial t^2} = \vec{\boldsymbol{\nabla}}\gamma_e\vec{\boldsymbol{t}} + \nabla\cdot\left(\nabla\widetilde{\boldsymbol{N}}\right)w - \frac{1}{h}\nabla\cdot\left(\nabla\widetilde{\boldsymbol{M}}\right) - \frac{1}{h}\gamma_e \quad (14)$$

where the gradient of the epithelial surface tension, along with the spatial distribution of the in-plane force tensor—specifically the first and second terms on the right side of eq. 14—drives out-of-plane displacement, while the bending moment and the epithelial surface tension itself resist buckling/folding. The force balances presented by eqs. 13-14 is presented in scalar notation in the Appendix. Change of the out-of-plane displacement can be considered within three regimes:

(i) **Buckling regime**: An increase in the out-of-plane displacement w is driven by the in-plane stress expressed in the form of the in-plane force tensor $\widetilde{\boldsymbol{N}}$**,** such that the rate $\frac{dw}{dt}$ is positive, i.e. $\frac{dw}{dt} > 0$. Wyatt et al. (2020) pointed out that the critical in-plane stress

necessary for the buckling of epithelial monolayers is low and equal to ~ 7 Pa [15]. It means that epithelial buckling appears after a few seconds of monolayer uni-axial compression. An increase in the displacement w causes increases in both the epithelial surface tension γ_e and the surface tension gradient $\vec{\nabla}\gamma_e$. While the epithelial surface tension resists folding, the surface tension gradient destabilises the monolayer, which further stimulates buckling. Further increase in the displacement w leads to the **buckling-to-folding transition**.

(ii) **Folding regime**: The displacement w increases for the rate $\frac{dw}{dt} > 0$ and reaches a maximum value w_{max}. In this state, driving and resistive forces are equilibrated such that the net rate $\frac{dw}{dt}$ is zero, i.e., $\frac{dw}{dt} = 0$.

(iii) **Relaxation regime**: The relaxation of in-plane stress causes a decrease in the driving force $\nabla \cdot \left(\nabla \widetilde{\boldsymbol{N}}\right)$ by perturbing the equilibrium state. Consequently, the displacement w starts decreasing, such that the rate $\frac{dw}{dt} < 0$. A decrease in the displacement w over a few minutes causes a decrease in both the epithelial surface tension and the surface tension gradient $\vec{\nabla}\gamma_e$ by reducing the out-of-plane displacement. When the in-plane stress relaxes toward the residual stress, a new equilibrium between the driving and resistive forces is established such that the rate $\frac{dw}{dt} = 0$. This state can be characterized by minimum buckling, such that the out-of-plane displacement is $w = w_{min}$. In some cases, for lower in-plane residual stress and the corresponding externally applied strain, the epithelial monolayer is able to recover an initial state such that $w \to 0$.

Consequently, the relaxation of in-plane stress is a key process responsible for the dynamics of buckling/folding. This relaxation phenomenon is closely connected with the viscoelasticity of epithelial monolayers. When the monolayer is attached to a substrate matrix it is necessary to account for the in-plane and out-of-plane traction forces.

The cascade of relaxation phenomena related to the relaxation of: (i) strain component ε_{xx} under externally applied uni-axial compressive strain ε_d, (ii) strain component ε_{yy} on the strain component ε_{xx} depending on the viscoelastic Poisson's ratio, (iii) epithelial surface tension under change in the monolayer surface area, (iv) mechanical stress under internal strain, and (v) epithelial bending described by the bending modulus govern the buckling/folding of epithelial monolayers. Spatio-temporal changes of epithelial surface tension influence the in-plane and out-of-plane displacement fields and the cell residual stress accumulation, which have a feedback impact on the epithelial surface tension itself. An increase in the out-of-plane displacement stimulates cell contractility [4]. Consequently, cells are able to actively self-regulate their cohesiveness, as well as energy storage and dissipation, during the buckling of epithelial monolayers: they do so by remodelling the cell-cell adhesion contacts, as a part of their adaptation to micro-environmental conditions [25]. The inter-relationships between the relevant physical parameters are shown in **Figure 2**:

Figure 2

The relaxation of in-plane stress reduces the out-of-plane displacement, such that the equilibrium level of epithelial buckling after the stress relaxation process depends on the residual stress accumulation. The residual stress includes two components, i.e., the viscoelastic component and a component that depends on the epithelial surface tension (eqs. 10, 11).

5. Wavelength and the modes of epithelial buckling/folding

We can propose the out-of-plane displacement in the *x*-direction at constant *y* as a function of time based on experiments by Wyatt et al. [15]: it must satisfy the conditions that, at $t = 0$, the displacement is $w(0, y = const, 0) = 0$ and $w(L, y = const, 0) = 0$ (where L is the distance between opposite edges of the monolayer after the applied uni-axial compression). It is expressed as:

$$w(x, y = const, t; n) = \sum_n A(x, y = const, t; n)\, sin\left(\frac{2\pi}{\lambda_n} x\right) \quad (15)$$

where $A(x, y = const, t; n)$ is the amplitude, which decreases exponentially with time, corresponding to mechanical stress relaxation. The decrease of amplitude can be expressed as: $A(x, y = const, t; n) = A_0 e^{-f(n)t}$ (where $A_0(x, y = const, t = 0)$ is the initial value of the amplitude and $f(n)$ is the rate of decrease in amplitude with time, which depends on the magnitude of the externally induced strain) [37]. The mode with the largest $f(n)$ dominates as time progresses. The wavelength of the curvature can be expressed by supposing discrete buckling/folding modes such that the evolution of wavelength means jumping between modes $n(t)$. Consequently, the wavelength can be expressed as: $\lambda_n = \frac{2L}{n}$ (where $n \in N$). During the evolution of the out-of-plane displacement, the mode $n(t)$ is changed to $n \to n_{min}$ which satisfies the condition that F_{en} is minimal [37] (where F_{en} is the free energy functional of the monolayer, which accounts for the sum of surface energy $e_A(w(n))$, bending energy $e_B(w(n))$, and strain energy $e_s(w(n))$, expressed in **Section 3**). Consequently, the evolution of modes during the out-of-plane displacement relaxation can be expressed as [14,61]:

$$\frac{dn}{dt} = -\Gamma \frac{\partial F_{en}}{\partial n} \quad (16)$$

where Γ is the kinetic constant, which quantifies the relaxation phenomenon. Higher modes n correspond to higher energetic states of epithelial monolayers.

6. Conclusion

Our theoretical analysis indicates that epithelial cells are capable of regulating their degree of folding under uniaxial compression along the *x*-direction. Uniaxial compression initiates a series of interconnected relaxation processes. The externally applied compressive strain ε_d leads to the development of an internal compressive strain in the *x*-direction, denoted as ε_{xx}, along with its subsequent relaxation. This internal strain component along *x* results in an extensional strain component in the *y*-direction ε_{yy}, which is influenced by the strain ε_{xx} and the viscoelastic Poisson's ratio ν, and which varies in space and time. The induced strain generates mechanical stress and its relaxation.

Mechanical stress exerts a reciprocal influence on the relaxation of strain by affecting both in-plane and out-of-plane displacements. The divergence of in-plane stress opposes in-plane displacement while simultaneously promoting out-of-plane displacement, which can result in the buckling or folding of epithelial monolayers. Additionally, epithelial surface tension works to minimize the surface area of these monolayers, thereby inducing in-plane compression. Conversely, an increase in epithelial surface tension, triggered by buckling or folding, counteracts further out-of-plane displacement. This indicates that, not only do physical factors such as mechanical stress and epithelial surface tension play a role in epithelial buckling or folding, but the heterogeneous

distribution of these factors also has a feedback effect on the mechanical stability of the monolayer, as well as on energy storage and dissipation. The key findings of this research were obtained through the integration of physical models and experimental observations within the fields of mechanobiology and biological physics. These findings can thus be summarized as follows:

- Remodelling of cell-cell adhesion contacts, accompanied by cell contractility in response to external mechanical forces, can modulate the processes of energy storage and dissipation within cellular monolayers.
- A distribution of the strength of cell-cell adhesion contacts, caused by cell signalling, induces an inhomogeneous distribution of epithelial cohesiveness and mechanical stress. These are quantified by the divergence of mechanical stress and the gradient of epithelial surface tension that drive epithelial buckling/folding.
- A monolayer that exhibits increased disorder or heterogeneity, attributable to the non-uniform distribution of the strengths of cell-cell adhesion contacts, may experience folding for a lower mechanical force, and to a greater degree than for a monolayer that is highly structured.
- The thickening of epithelial monolayers, resulting from the structural changes of cell cytoskeletons and an increase in Young's modulus due to enhanced epithelial cohesiveness and contractility, contributes to a rise in the bending modulus, thereby diminishing the occurrence of epithelial buckling or folding.

Further experiments are required to gain a more comprehensive understanding of epithelial folding, particularly regarding the spatiotemporal distribution of physical parameters, including cellular mechanical stress, epithelial surface tension, and Poisson's ratio.

Conflict of interest: The authors report there is no conflict of interest.

Acknowledgments: This work was supported in part by the Engineering and Physical Sciences Research Council, UK (grant number EP/X004597/1) and by the Ministry of Science, Technological Development and Innovation of the RS (Contract No. 451-03-65/2024-03/200135).

Appendix

Scalar notation of the force balance equations are:

In-plane force balance expressed from eq. 13:

$$\langle m_e \rangle \rho_e \frac{dv_{ex}(x,y,t)}{dt} = \rho_e \gamma_e^{in-plane} u_x - h\left(\frac{\partial}{\partial x}\sigma_{xx}^{in-plane} + \frac{\partial}{\partial y}\sigma_{xy}^{in-plane}\right) \quad \text{(A1)}$$

$$\langle m_e \rangle \rho_e \frac{dv_{ey}(x,y,t)}{dt} = \rho_e \gamma_e^{in-plane} u_y - h\left(\frac{\partial}{\partial y}\sigma_{yy}^{in-plane} + \frac{\partial}{\partial x}\sigma_{xy}^{in-plane}\right) \quad \text{(A2)}$$

Out-of-plane force balance expressed from eq. 14:

$$\langle m_e \rangle \rho_e \frac{\partial^2 w(x,y,t)}{\partial t^2} = \left(\frac{\boldsymbol{\partial}}{\boldsymbol{\partial x}}\gamma_e + \frac{\boldsymbol{\partial}}{\boldsymbol{\partial y}}\gamma_e\right) + \left(\frac{\partial^2 N_{xx}}{\partial x^2} + 2\frac{\partial^2 N_{xy}}{\partial x \partial y} + \frac{\partial^2 N_{yy}}{\partial y^2}\right) w - \frac{1}{h}\left(\frac{\partial^2 M_{xx}}{\partial x^2} + 2\frac{\partial^2 M_{xy}}{\partial x \partial y} + \frac{\partial^2 M_{yy}}{\partial y^2}\right) - \frac{1}{h}\gamma_e \quad \text{(A3)}$$

References

1. Shyer AE, Tallinen T, Nerurkar NL, Wei Z, Gil ES, Kaplan DL., Tabin CJ, Villification LM. How the gut gets its villi. Science 2013; 342:212–218. doi:10.1126/science.1238842.
2. Pispa J, and Thesleff I. Mechanisms of ectodermal organogenesis. Dev. Biol. 2003; 262:195–205, doi:10.1016/s0012-1606(03)00325-7
3. Sumigray KD, Terwilliger M, Lechler T. Morphogenesis and compartmentalization of the intestinal crypt. Dev. Cell. 2018; 45:183–197, doi:10.1016/j.devcel.2018.03.024.
4. Wang Y, Stonehouse-Smith D, Cobourne MT, Green JBA, Seppala M. Cellular mechanisms of reverse epithelial curvature in tissue morphogenesis. Front Cell Dev Biol 2022; 10, https://doi.org/10.3389/fcell.2022.1066399
5. Izquierdo E, Quinkler T, De Renzis S. Guided morphogenesis through optogenetic activation of Rho signalling during early Drosophila embryogenesis. Nat. Commun. 2018; 9:2366. doi:10.1038/s41467-018-04754-z.
6. Krueger D, Tardivo P, Nguyen C, De Renzis S. Downregulation of basal myosin-II is required for cell shape changes and tissue invagination. EMBO J. 2018; 37:e100170. doi:10.15252/embj.2018100170.
7. Pajic-Lijakovic I, Eftimie R, Milivojevic M, Bordas SPA. Multi-scale nature of the tissue surface tension: theoretical consideration on tissue model systems. Adv Coll Int Sci 2023; 315:102902, DOI: 10.1016/j.cis.2023.102902.
8. Lancaster MA, Renner M, Martin C.-A, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA. Cerebral organoids model human brain development and microcephaly. Nature 2013; 501:373–379, DOI: 10.1038/nature12517.
9. Timoshenko SP, Gere JM. Theory of Elastic Stability. 2nd ed., McGraw-Hill, New York, 1961.
10. Davidson LA. Epithelial machines that shape the embryo. Trends Cell. Biol. 2012; 22:82–87. doi:10.1016/j.tcb.2011.10.005
11. Tan DW and Barker N. Intestinal stem cells and their defining niche. Curr. Top. Dev. Biol. 2014; 107:77–107. doi:10.1016/B978-0-12-416022-4.00003-2
12. Pearl EJ, Li J, Green JB. Cellular systems for epithelial invagination. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2017; 372:20150526. doi:10.1098/rstb.2015.0526.
13. Li J, Economou AD, Vacca B, Green JBA. 2020. Epithelial invagination by a vertical telescoping cell movement in mammalian salivary glands and teeth. Nature Comm 11:2366, https://doi.org/10.1038/s41467-020-16247-z.
14. Hannezo E, Prost J, Joanny J-F. Instabilities of monolayered epithelia: shape and structure of villi and crypts. Phys Rev Lett 2011; 107:078104, http://dx.doi.org/10.1103/PhysRevLett.107.078104.
15. Wyatt TPJ, Fouchard J, Lisica A, Khalilgharibi N, Baum B, Recho P, Kabla AJ, Charras GT. Actomyosin controls planarity and folding of epithelia in response to compression. Nature Mat 2020; 19:109–117, https://doi.org/10.1038/s41563-019-0461-x
16. Guillot C and Lecuit T. Mechanics of epithelial tissue homeostasis and morphogenesis. Science 2013; 340(6137):1185–1189, DOI: 10.1126/science.1235249.
17. Lecuit T and Yap AS. E-cadherin junctions as active mechanical integrators in tissue dynamics. Nature Cell Biol 2015; 17(5):533–539, DOI: 10.1038/ncb3136.
18. Blonski S, Aureille J, Badawi S, Zaremba D, Pernet L, Grichine A, Fraboulet S, Korczyk PM, Recho P, Guilluy C, Dolega ME. Direction of epithelial folding defines impact of mechanical forces on epithelial state. Devel Cell 2021; 56:3222–3234, https://doi.org/10.1016/j.devcel.2021.11.008.

19. Youn J, Kim D, Kwak H, Lee A, Kim DS. Tissue-scale in vitro epithelial wrinkling and wrinkle-to-fold transition. Nature Comm 2024; 15:7118, https://doi.org/10.1038/s41467-024-51437-z.
20. Pajic-Lijakovic I. Basic concept of viscoelasticity, in Viscoelasticity and collective cell migration, eds. I. Pajic-Lijakovic and E. Barriga, Academic Press, United States, 2021; pp.21.
21. Lecuit T and Lenne P-F. Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. Nature Rev Mol Cell Biol 2007; 8(8):633–644, https://doi.org/10.1038/nrm2222.
22. Wang H, Liu Y-Q, Hang J-T, Xu G-K, Feng X-Q. Dynamic high-order buckling and spontaneous recovery of active epithelial tissues. J. Mech. Phys. Solids 2024; 183:105496, https://doi.org/10.1016/j.jmps.2023.105496.
23. Storgel N, Krajnc M, Mrak P, Strus J, Ziherl P. Quantitative Morphology of Epithelial Folds.Biophys J. 2016; 110:269–277, http://dx.doi.org/10.1016/j.bpj.2015.11.024.
24. Nelson CM. On buckling morphogenesis. J Biomech Eng 2016; 138(2):021005, https://doi.org/10.1115/1.4032425.
25. Vasquez CG and Martin AC. Force transmission in epithelial tissues. Dev Dyn 2016; 245:361–371, DOI: 10.1002/DVDY.24384.
26. Fouchard J, Wyatt TPJ, Proag A, Lisica A, Khalilgharibi N, Recho P, Suzanne M, Kabla A, Charras G. Curling of epithelial monolayers reveals coupling between active bending and tissue tension. PNAS 2020; 117(17):9377-9383, doi/10.1073/pnas.1917838117.
27. Pajic-Lijakovic I, Milivojevic M, McClintock PVE. Compressibility of biological systems: the viscoelastic Poisson's ratio. Advances in Physics X 2025; 10(1), DOI: 10.1080/23746149.2024.2440023.
28. Valencia-Expósito A, Khalilgharibi N, Martínez-Abarca Millán A, Mao Y, Martín-Bermudo MD. Local weakening of cell-extracellular matrix adhesion triggers basal epithelial tissue folding. EMBO J 2025; 44(7):2002 – 2024; https://doi.org/10.1038/s44318-025-00384-6.
29. Heermann S, Schütz L, Lemke S, Krieglstein K, Wittbrodt J. Eye morphogenesis driven by epithelial flow into the optic cup facilitated by modulation of bone morphogenetic protein. eLife 2015; 4:e05216. https://doi.org/10.7554/eLife.05216.
30. Bedzhov I and Zernicka-Goetz M. Self-organizing properties of mouse pluripotent cells initiate morphogenesis upon implantation. Nature Cell Biol 2014; 16(6), 517–525. https://doi.org/10.1038/ncb2928
31. Smith JL and Schoenwolf GC. Neurulation: coming to closure. Trends Neurosci 1997; 20(11), 510–517. https://doi.org/10.1016/S0166-2236(97)01121-1.
32. Ban E, Wang H, J. Franklin M, Liphardt JT, Janmey PA, Shenoy VB. Strong triaxial coupling and anomalous Poisson effect in collagen networks. PNAS 2019;116(4):6790-6799, DOI:10.1073/pnas.1815659116.
33. Nam S, Hu KH, Butte MJ, Chaudhuri O. Strain-enhanced stress relaxation impacts nonlinear elasticity in collagen gels. PNAS 2016;113:5492 LP – 5497, doi:10.1073/pnas.1523906113.
34. Harris AR, Peter L, Bellis J, Baume B, Kabla AJ, Charras GT. Characterizing the mechanics of cultured cell monolayers. PNAS 2012; 109(41):16449–16454, https://doi.org/10.1073/pnas.1213301109.
35. Kobiela T, Milner-Krawczyk M, Pasikowska-Piwko M, Bobecka-Wesołowska K, Eris I, Święszkowski W, Dulinska-Molak I. The Effect of Anti-aging Peptides on Mechanical and Biological Properties of HaCaT Keratinocytes Int. J Pept. Res. Ther. 2018; 24(4):577-587, doi: 10.1007/s10989-017-9648-7.

36. Nestor-Bergmann A, Johns E, Woolner S, Jensen OE. Mechanical characterization of disordered and anisotropic cellular monolayers. Phys Rev E 2018; 97(5-1):052409. doi: 10.1103/PhysRevE.97.052409.
37. Audoly B and Pomeau Y. Elasticity and Geometry: From Hair Curls to the Non-linear Response of Shells. Oxford University Press Inc., New York pp. 192, 2010.
38. Devanny AJ, Vancura MB, Kaufman L.J. Exploiting differential effects of actomyosin contractility to control cell sorting among breast cancer cells. Molecular Biology of the Cell. 2021; 32:ar24, 1-15, DOI: 10.1091/mbc.E21-07-0357.
39. Guevorkian K, Brochard-Wyart F, Gonzalez-Rodriguez D. Flow dynamics of 3D multicellular systems into capillaries, in Viscoelasticity and Collective Cell Migration, eds. I. Pajic-Lijakovic and E. Barriga, (Academic Press, US, 2021), 2021; p. 193.
40. Iyer KV, Gomez RP, Paijmans J, Julicher F, Eaton S: Epithelial viscoelasticity Is regulated by mechanosensitive E-cadherin turnover. Curr Biol 2019; 29:1–14, DOI: 10.1016/j.cub.2019.01.021.
41. Pajic-Lijakovic I, Milivojevic M, McClintock PVE. Epithelial cell-cell interactions in an overcrowded environment: jamming or live cell extrusion. J Biol Eng 2024; 18(1), DOI: 10.1186/s13036-024-00442-3.
42. Barriga EH and Mayor R. Adjustable viscoelasticity allows for efficient collective cell migration. Sem Cell Dev Biol 2019; 93:55-68, DOI: 10.1016/j.semcdb.2018.05.027.
43. Sumi A, Hayes P, D'Angelo A, Colombelli J, Salbreux G, Dierkes K, Solon J: Adherens Junction Length during Tissue Contraction Is Controlled by the Mechanosensitive Activity of Actomyosin and Junctional Recycling. Dev Cell 2018; 47(4):453-463e3, DOI: 10.1016/j.devcel.2018.10.025.
44. Liu Z, Tan JL, Cohen DM, Yang MT, Saidecki NJ, Ruiz SA, Nelson CM, Chen CS. Mechanical tugging force regulates the size of cell–cell junctions. PNAS 2010; 107(22):9944-9949, DOI: 10.1073/pnas.0914547107.
45. Charras G and Yap AS: Tensile Forces and Mechanotransduction at Cell–cell junctions. Curr Biol 2018; 28:R445–R457, DOI: 10.1016/j.cub.2018.02.003.
46. Stirbat TV, Mgharbel A, Bodennec S, Ferri K, Mertani HC, Rieu JP, Delanoe¨-Ayari H. Fine Tuning of Tissues' Viscosity and Surface Tension through Contractility Suggests a New Role for a-Catenin, PLOS ONE 2013; 8(2):e52554, DOI: 10.1371/journal.pone.0052554.
47. Alt S, Ganguly P, Salbreux G. Vertex models: from cell mechanics to tissue morphogenesis Phil. Trans. R. Soc. B 2017; 372:20150520, DOI: 10.1098/rstb.2015.0520.
48. Koride, S, Loza AJ, Sun SX. Epithelial vertex models with active biochemical regulation of contractility can explain organized collective cell motility. APL Bioeng. 2018; 2:031906, doi.org/10.1063/1.5023410.
49. Mombach JCM, Robert D, Graner F, Gillet G, Thomas GL, Idiart M, Rieu JP. Rounding of aggregates of biological cells: Experiments and simulations. Physica. A 2005; 352, 525-534, doi:10.1016/j.physa.2005.02.008.
50. Nagle I, Richert A, Quinteros M, Janel S, Buysschaert E, Luciani N, Debost H, Thevenet V, Wilhelm C, Prunier C, et al. 2022. Surface tension of model tissues during malignant transformation and epithelial–mesenchymal transition. Front. Cell Dev. Biol. 2022; DOI 10.3389/fcell.2022.926322.
51. Tolman RC. The Effect of Droplet Size on Surface Tension. J Chem Phys 1949; 17 (3):333–337, doi:10.1063/1.1747247.
52. Binysh J, Wilks TR, Souslov A. Active elastocapillarity in soft solids with negative surface tension. Sci. Adv 2022; 8:eabk3079, DOI: 10.1126/sciadv.abk3079.

53. Trushko A, I. Di Meglio I, Merzouki A, Blanch-Mercader C, Abuhattum S, Guck J, Alessandri K, Nassoy P, K. Kruse K, Chopard B, Roux A, Buckling of an epithelium growing under spherical confinement. Dev Cell 2020; 54:655-668, doi.org/10.1016/j.devcel.2020.07.019.
54. Pajic-Lijakovic, I and Milivojevic, M. Marangoni effect and cell spreading. Europ Biophys J 2022; 51:419–429, doi.org/10.1007/s00249-022-01612-1.
55. Karbalaei A, Kumar R, Cho HJ, Thermocapillarity in microfluidics—A review. Micromachines 2016; 7:13, doi:10.3390/mi7010013.
56. Moisdon E, Seez P, Molino F, Marcq P, Gay C. Mapping cell cortex rheology to tissue rheology and vice versa. Phys. Rev. E 2022;106(3-1):034403, doi = {10.1103/PhysRevE.106.034403}.
57. Pajic-Lijakovic I, Milivojevic M, McClintock PVE. Role of viscoelasticity in the appearance of low-Reynolds turbulence: Considerations for modelling. J Biol Eng 2024; 18:24, https://doi.org/10.1186/s13036-024-00415-6.
58. Etournay R, Popovic M, Merkel M, Nandi A, Blasse C, Aigouy B, Brandl H, Myers G, Salbreux G, Frank Julicher F, Eaton S. Interplay of cell dynamics and epithelial tension during morphogenesis of the Drosophila pupal wing. eLife 2015; 4:e07090, DOI: 10.7554/eLife.07090.
59. Ladoux B and Mège R-M. Mechanobiology of collective cell behaviours. Nat Rev Mol Cell Biol 2017; 18(12):743-757, doi: 10.1038/nrm.2017.98.
60. Khalilgharibi N, Fouchard J, Asadipour N, Yonis A, Harris A, Mosaff P, Fujita Y, Kabla A, Baum B, Muñoz JJ, Miodownik M, Charras G. Stress relaxation in epithelial monolayers is controlled by actomyosin. Nat. Phys. 2019; 15:839-847, DOI: 10.1038/s41567-019-0516-6.
61. Cerda E and Mahadevan L. Geometry and physics of wrinkling. Phys Rev Lett 2003; 90(7):074302. DOI: 10.1103/PhysRevLett.90.074302.
62. Serra-Picamal X, Conte V, Vincent R, Anon E, Tambe DT, Bazellieres E, et al. Mechanical waves during tissue expansion. Nat Phys. 2012;8(8):628–34, https://doi.org/10.1038/nphys2355.

Glossary of terms

Anisotropy: The property of a system where its physical characteristics vary depending on the direction they are measured in. This means a system can exhibit different properties, like strength or stiffness, along different axes.

Compressibility of multicellular systems: Multicellular systems are compressible. The porous structure of the cell membranes and extracellular spaces ensures an outflow of intracellular and extracellular liquid even under relatively modest physiological strain conditions [27,56].

Epithelial surface tension: A measure of the cohesiveness of epithelial surfaces, which depends on cell contractility and the strength of cell-cell adhesion contacts. The cohesiveness of epithelial surfaces is space-time dependent [7,51,52].

Mechanical stress: A physical quantity that describes the magnitude and direction of forces per unit area that cause a system deformation.

Remodelling of cell-cell adhesion contacts: include change in (i) the number of adhesion complexes per single cell (ii) the number of E-cadherin molecules per adhesion complex and (iii) strength of bond between two cadherin molecules between neighbor cells. These processes are governed by cell signaling and gene expression [42].

Residual stress: A stress that remains in a system in the absence of external forces. The residual stress can be either dissipative (viscous) or elastic. This stress consists of shear and normal (tensional or compressive) components.

Strain: The deformation of a system in response to mechanical stress.

Viscoelasticity: A time-dependent response of cellular systems under externally or internally applied forces, which includes energy storage and dissipation during cell rearrangement. Mechanism of energy storage and dissipation is closely connected with the stress and strain relaxation phenomena and can be described in the form of proper constitutive model [20].

Types of viscoelastic behaviour: linear/non-linear viscoelastic liquids and viscoelastic solids

Viscoelastic liquids: The main characteristics of this type of systems are that: (i) strain cannot relax under constant stress conditions, (ii) stress can relax under constant strain rate conditions, and (iii) strain rate can relax under constant strain rate in some cases [20].

Viscoelastic solids: The main characteristics of this type of systems are that: (i) strain can relax under constant stress conditions, (ii) stress can relax under constant strain conditions in some cases [20].

Viscoelasticity of epithelial monolayers: Epithelial monolayers behave as linear viscoelastic solids. This behaviour, confirmed in various experimental systems, is primarily resulted by ability of epithelial cells to establish strong E-cadherin mediated cell-cell adhesion contacts [15,60,62].

Figure captions:

Figure 1. Schematic representation of two scenarios for relaxation of out-of-plane displacement of epithelial monolayers under uni-axial compression. The yellow arrows represent the directions of uni-axial compression. An epithelium (depicted in red) is positioned between two coverslips (illustrated in grey); when the right coverslip is shifted, it causes deformation of the tissue.

Figure 2. The inter-relationship between relevant physical parameters, which drive and resist epithelial buckling/folding. Dashed-line box indicate interrelationship between various strain components that further contribute to the generation of mechanical stress.