The role of epithelial-mesenchymal interfacial tension in biological systems

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

The interfacial tension between two cell subpopulations in direct contact represents a key physical parameter responsible for the self-organization of tissues during biological processes such as: morphogenesis and the spreading of cancers. Higher interfacial tension: (i) reduces spreading of cancer-mesenchymal cells through the epithelial subpopulation, (ii) ensures efficient cell segregation in co-cultured systems, (iii) can induce extrusion of cancer-mesenchymal cells along the biointerface with the epithelial subpopulation, and (iv) results in the generation of higher mechanical stress along the biointerface. Inhomogeneous distribution of the interfacial tension leads to the Marangoni effect, which further facilitates the rearrangement of cells. The formation of mobile stiffness gradients (i.e., durotaxis) under in vivo conditions is directly related to an inhomogeneous distribution of the interfacial tension. As the product of homotypic and heterotypic cell-cell interactions, the interfacial tension depends on the distance between the subpopulations, which is a time-dependent parameter.

This review: (1) summarizes biological aspects related to the homotypic and heterotypic cell-cell interactions along the biointerface, together with the viscoelasticity of cell subpopulations caused by collective cell migration, and by compression (de-wetting)/extension (wetting) of the subpopulations; and (2) describes these same biological aspects from a bio-physical/mathematical perspective by pointing to the role played by the interfacial tension.

Key words: collective cell migration; cell residual stress accumulation, tissue surface tension, Marangoni effect; viscoelasticity; effects along the biointerface

1. Introduction

The interfacial tension between two cell subpopulations in direct contact is the one of the main physical parameters responsible for the self-organisation of tissues during the morphogenesis and spreading of cancers [1,2,3]. The primary objective of this review is to emphasize the significance of this parameter in the self-organization of diverse biological systems. More broadly, interfacial tension is characterized as the energy required to minimize the interface area between two immiscible phases. This energy is caused by homotypic and heterotypic interactions. The cumulative effects of these interactions influence the phases' cohesion and the adhesion between them. These interfacial interactions, which lead to a decrease in the interface area, result in volumetric alterations of the phases, manifesting as wetting (expansion) and de-wetting (compression). An inhomogeneous distribution of interfacial tension, driven by temperature and/or concentration gradients, generates shear flow of the phases along the interface, moving from areas of lower interfacial tension to those of higher interfacial tension. This process is referred to as the Marangoni effect, which represents an important physical phenomenon responsible for the phase rearrangement [4].

Two populations of cells that are in direct contact can be conceptualized as pseudo-phases. The determination of which cell pseudo-phase experiences compression (de-wetting) or extension (wetting) is influenced by the dynamics of two types of interactions: (i) homotopic interactions that promote cohesion among the pseudo-phases, and (ii) heterotypic interactions, which may be either attractive (such as adhesion) or repulsive, contingent upon the spatial relationship between the cell pseudo-phases. The degree of cohesion within a pseudo-phase is contingent upon the strength of the adhesion contacts between the cells. Furthermore, the interactions between homotopic and heterotypic cell-cell connections may be interdependent [2].

A decrease in the distance can result in repulsion caused by interplay between mechanical interactions and heterotypic contact inhibition of the locomotion (CIL) [2,5,6]. Contact inhibition of locomotion, caused by cell head-on interactions, induces cell repolarisation and the migration in opposite direction [7]. Taylor et al. [2] discuss the role of the Eph receptor and ephrin signalling in cell segregation and border formation in the context of heterotypic contact inhibition of locomotion. Lucia et al. [6] discuss the Eph/ephrin-induced repulsion along the biointerface between C2C12 cells and epithelial Madin-Darby canine kidney type II (MDCK), or HaCaT cells primarily related to myosin II activity. This repulsion results in an increase in the interfacial tension, which can destabilise the biointerface. The interfacial tension is therefore responsible for biointerface stability. While repulsive cell-cell interactions are dominant for shorter distances, over larger distances there are attractive interactions that tend to stabilise the biointerface. The interfacial tension for the stable regime of the biointerface is much lower than that for the unstable regime. Stability of the biointerface is a prerequisite for the maintenance of tissue surface and volumetric organization and homeostasis [1].

The tissue surface and volumetric organisation is induced by a decrease of the biointerface area. The surface reorganization, related to the change in surface cell packing density, occurs through two mechanisms: (i) surface compression and (ii) migration of the system constituents from the biointerface towards the bulk region [8,9]. Change of multicellular surface area caused by the work done by tissue surface tension has been measured by Mombach et al. [10] by considering cell aggregate rounding after uni-axial compression between parallel plates. In this case, the cell surface rearrangement, accompanied by the aggregate shape and surface changes, exhibits oscillatory behaviour suggesting oscillations in the tissue surface tension [10,11]. While the change of cell surface

packing density causes remodelling of cell-cell adhesion contacts on a time-scale of minutes, collective cell migration occurs on a time-scale of hours [11].

Alterations in cell volume through rearrangement, specifically the processes of wetting and de-wetting of cell pseudo-phases, can occur via both active and passive mechanisms. Active mechanisms are typically triggered by collective cell migration, whereas passive mechanisms are influenced by gradients in interfacial tension. The resultant compressive and tensile stresses influence the cell packing density, which in turn affects cell migration. Mechanical stress experienced by cells can either promote or inhibit their movement and may even lead to a transition into a cell-jamming (i.e., non-contractile) state, contingent upon the type of cell and the intensity of the stress [12,13]. The accumulation of compressive stress may also trigger a transition to a jamming state in epithelial cells [12,13]. Additionally, Marangoni effects have been experimentally validated within biological systems [14,15].

An inhomogeneous distribution of interfacial tension also induces the formation of mobile stiffness gradient along the biointerface, which in turn promotes directional cell migration, a process referred to as durotaxis [16]. Inhomogeneous distributions of the interfacial tension between the placode and neural crest subpopulations within Xenopus embryos arise from the formation of unstable, transient heterotypic cell-cell adhesion contacts, as discussed by Theveneau et al. [17] and Barriga et al. [18]. Additionally, collective durotaxis has been observed in vivo in Drosophila and zebrafish embryos [19]. Thus, heterotypic cell-cell interactions and signalling are critical physical factors that generate the mobile stiffness gradient within the placode boundary layer along the biointerface [16]. Furthermore, interfacial tension plays a significant role in the segregation of cell pseudo-phases in co-cultured systems under in vitro conditions, including multicellular spheroids and cell monolayers [6,20,21]. Heterotypic repulsion interactions along the biointerface are responsible for: (i) extrusion of cancer-mesenchymal cells along the biointerface with epithelial subpopulation and (ii) segregation of co-cultured epithelial-mesenchymal monolayers [6,22]. Detailed descriptions of these phenomena will be given below.

The main goal of this theoretical consideration is to point to: (i) the inter-relationship between homotypic and heterotypic cell-cell interactions and the interfacial tension, and (ii) the role played by the interfacial tension between two cell subpopulations in determining their surface and volumetric self-organisation. The relationship between the interfacial tension and other physical parameters is therefore discussed, including consideration of the surface tensions of the subpopulations and the generation of cell mechanical stress by collective cell migration along the biointerface. The review is also focused on the main properties of the interfacial tensions, such as: (1) the impact of cell homotypic and heterotypic interactions on the interfacial tension; and (2) the multi-time nature of the interfacial tension. The approach taken is to consider a variety of co-cultured epithelial-mesenchymal systems under *in vivo* and *in vitro* conditions. Direct contact of epithelial and mesenchymal subpopulations occurs during embryogenesis and the spreading of epithelial cancer in the early stage of the disease. Cells with varying degrees of mesenchymal character in terms of cell polarity, mobility, and strength of cell-cell adhesion contacts can appear within the epithelium itself as a product of the epithelial-to-mesenchymal transition (EMT) [23,24]. During this process, epithelial cells change their mechanical behaviour and their strength of cell-cell adhesion contacts [23]. The EMT can be induced by various biological and physical factors [23,24]. The main characteristics of epithelial-like cells are: cuboidal shape, reduced cell mobility, apical-basal polarity, and the establishment of strong E-cadherin mediated cell-cell adhesions. In contrast, mesenchymal-like cells can be characterized by: elongated cell shape, increased migratory propensity, establishment of front-rear cell polarity, and weak N-cadherin-mediated cell-cell adhesion [25].

This theoretical consideration aims to: (i) discuss biological aspects related to the homotypic and heterotypic cell-cell interactions along the biointerface by emphasizing practical implications, (ii) consider the viscoelasticity of cell subpopulations caused by their collective migration, and compression (de-wetting)/extension (wetting), (iii) formulate a biophysical model in order to point out the inter-connections between the physical parameters introduced, and (iv) explore potential methods for quantifying interfacial tension.

2. Cell-cell interactions along the biointerface

Homotypic and heterotypic cell-cell interactions along the biointerface significantly influence the interfacial tension between them. In further consideration, it is necessary to discuss the main characteristics of these interactions based on experimental data and theoretical considerations.

2.1 Homotypic cell-cell interactions

Epithelial cells establish strong E-cadherin-mediated cell-cell adhesion contacts, while mesenchymal cells establish weak N-cadherin-mediated cell-cell adhesion contacts [21]. The strength of the homotypic cell-cell adhesion contacts has an impact on: (1) how cells move; (2) viscoelasticity caused by collective cell migration; and (3) cohesiveness of the cellular systems. Epithelial cells migrate in the form of strongly-connected cell clusters, while mesenchymal cells migrate in cell streams [26,27]. The viscoelastic properties of migrating epithelial collectives resemble viscoelastic solids, whereas mesenchymal collectives exhibit viscoelastic liquid behaviour [20,26,27]. Epithelial collectives are more cohesive than mesenchymal ones. Heterotypic cell-cell interactions at the biointerface between two cell subpopulations have the potential to impact homotypic cell-cell interactions [2].

2.2 Heterotypic cell-cell interactions

Heterotypic cell-cell interactions along the biointerface between two cell subpopulations include: (1) attractive interactions characteristic of distances larger than the size of a single cell; and (2) repulsive interactions characteristic of distances smaller than the size of a single cell. Direct contact between the cell subpopulations stimulates secretion of signalling molecules, which has a feedback effect on the attractive/repulsive interactions and on the self-organisation of the subpopulations. We are interested here in the epithelial-mesenchymal interactions. Some epithelial-mesenchymal co-cultured systems can establish unstable heterotypic cell-cell adhesion contacts, while others do not establish adhesion contacts. If adhesion contacts exist, they are frequently N-cadherin-mediated [23]. Lucia et al. [6] emphasize that the segregation of co-cultured systems is more efficient when the subpopulations do not establish heterotypic cell-cell adhesions. However, they did not indicate the cause of this phenomenon. The main cause could be closely connected with the magnitude of the epithelial-mesenchymal interfacial tension, since the interfacial tension is a key physical parameter responsible for the segregation of cell subpopulations [20,21]. When the subpopulations are unable to establish heterotypic cell-cell adhesion contacts, the interactions occur via electrostatic, steric, and Van der Waals interactions between neighbouring cell surfaces. These interactions are mediated by the presence of the signalling molecules secreted by cells. In this case, the interfacial tension is larger, which is a prerequisite for efficient segregation. Detailed discussion about the role of the interfacial tension in the self-organisation of the subpopulations will be given in the next sections. Here, we will

describe some co-cultured cellular systems from the standpoint of the heterotypic cell-cell interactions.

MCF10A breast epithelial cells fail to form adhesion contacts with MDA-MB-231 mesenchymal cells in co-cultured spheroids [28]. Subpopulations that come into direct contact experience complete segregation [21]. The epithelial subpopulation is compressed and migrates towards the core region of the spheroid, whereas the mesenchymal subpopulation extends towards the surface region of the spheroid. The MDA-MB-231 cells release vesicles that induce the epithelial-to-mesenchymal transition of the MCF10A cells, whereas the MCF10A cells secrete proteins like fibronectin and laminin-5. These proteins play a crucial role in the development of focal adhesions (FAs) in MDA-MB-231 cells, thereby promoting their motility [29,30,31]. Lucia et al. [6] considered the dynamics along the biointerface between: (1) epithelial HaCaT and mesenchymal C2C12 subpopulations; and (2) Madin-Darby canine kidney type II (MDCK) epithelial and mesenchymal C2C12 subpopulations, which are unable to form heterotypic cell-cell adhesion contacts. Both types of co-cultured monolayer undergo partial segregation. For example, the epithelial subpopulation forms dispersed clusters surrounded by the mesenchymal subpopulation. The repulsion between two dissimilar cell subpopulations, characteristic of shorter distances between the epithelial and mesenchymal cell subpopulations, was recognised as being the main type of heterotypic cell-cell interaction responsible for the segregation [6]. This repulsion represents contact inhibition of locomotion (CIL) by heterotypic contact. In many subpopulations, ephrins are present in one cell population and Eph receptors in the other. When these cells come into contact, ephrins bind to their receptors, triggering signalling inside both cells that stops them from mixing [2,32]. Heterotypic CIL influences homotypic CIL along the epithelial-mesenchymal biointerface, and can reduce it [2].

The ability of epithelial and mesenchymal subpopulations to establish heterotypic cell-cell adhesion contacts causes a decrease in the interfacial tension along the biointerface between them. In normal epidermis, heterotypic melanocyte-keratinocyte (HaCaT) adhesions are mediated by E-cadherin [33]. However, during development of a melanoma, E-cadherin is downregulated and replaced by N-cadherin [34]. Schmitt et al. [35] pointed out that that desmoglein 2 (Dsg2) can indeed act as a heterotypic cell-cell adhesion molecule between keratinocytes and melanoma cells.

Collectively migrated mesenchymal Neural Crest subpopulation along the dorsoventral axis within Xenopus embryos (at the stage 20 of development) establishes a direct contact with the ectodermal placode subpopulation [19]. These cell subpopulations form heterotypic N-cadherin-mediated cell-cell adhesion contacts, which affects the rearrangement of them both [19,36,37].

After indicating the main characteristics of homotypic and heterotypic cell-cell interactions, it is necessary to discuss how these interactions influence the epithelial-mesenchymal interfacial tension.

3.Interfacial tension between epithelial and mesenchymal cell subpopulations

Interfacial tension between epithelial-like and mesenchymal-like cell subpopulations (i.e., the cell pseudo-phases) in direct contact depends on the surface tensions of both subpopulations, and their mutual interaction energy, which represents a product of cell-cell heterotypic interactions. The tissue surface tension indicates the level of cohesion present on a multicellular surface when in contact with a liquid medium. Whether the heterotypic cell-cell interactions along the biointerface are attractive or repulsive depends primarily on the distance between two subpopulations. Not only do the heterotypic cell-cell interactions influence the interfacial tension by influencing the interaction energy, but these interactions can have also a feedback effect on homotypic cell-cell interactions and

consequently influence the surface tensions of the sub-populations themselves [2]. The changes in the interfacial tension, like the changes in the tissue surface tensions, occur on two different time scales [11]. The time scale of minutes (i.e. the short-time scale) corresponds to the remodelling of cell-cell adhesion contacts, while the time scale of hours (i.e. the long-time scale) corresponds to collective cell migration. Proper formulation of the surface tensions of the epithelial and mesenchymal subpopulations is a prerequisite for deeper understanding the interfacial tension.

3.1 The surface tension of epithelial and mesenchymal subpopulations

The tissue surface tension is a measure of the energy of the tissue's surface when it comes into contact with a liquid medium [11,21,37]. This physical parameter is time- and space-dependent, i.e. is a dynamic tissue surface tension. The surface energy of a cellular system, depending on the strength of cell-cell adhesion contacts and cell contractility (i.e., the cortical tension), has been expressed in the form of 3D vertex model as [37,38]:

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

Where p is the cell pressure, dV_i is the volume change of single cells, A_{ceffi} is the single cell contribution to multicellular surface area, 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, and L_i is the perimeter of the *i*-th cell. Koride et al. [38] considered the first and second terms on the right-hand side as contributions to the passive energy, while the third term represented the active energy. The energy associated with the single cell-volume change (i.e., the first term of the right-hand side of eq. 1) plays a significant role in the context of osmotic stress. Additionally, increased compressive mechanical stress, which may contribute to alterations in cell volume, can be attributed to the core regions of multicellular aggregates rather than the aggregate surface regions.

The tissue surface tension represents the change of the tissue surface energy caused by changing the surface area and can be expressed as:

$$\gamma = \frac{\partial E_{ST}}{\partial A_{int}} \quad (2)$$

where A_{int} is the biointerface area. A frequently applied method for measurement of the tissue surface tension uses estimation of the cell aggregate shape change during aggregate rounding, after uni-axial compression between parallel plates [10,39]. Cell aggregate rounding results in a decrease in the aggregate surface area and a change of the aggregate shape caused by the work done by the tissue surface tension. This surface decrease causes an increase in the cell surface packing density and collective cell migration from the aggregate surface toward the bulk region [10]. An increase in the cell surface packing density intensifies cell-cell interactions, resulting in a remodeling of cell-cell adhesion contacts. While this remodeling occurs on a time-scale of minutes, collective cell migration occurs on a time-scale of hours. Both processes influence the surface cohesiveness, as well as the tissue surface tension. Experimental data pointed to a long-time oscillatory change of the aggregate shape and surface area caused by collective cell migration [10,11]. This result clearly pointed to a long-time oscillatory change of the tissue surface tension.

The surface tension of tissues is influenced by a combination of factors, including the contractility of cells, the adhesive strength of cell-cell contacts, and the stretching or compression of multicellular

surfaces [11, 21,40]. Epithelial cells are recognized for their ability to create strong cell-cell adhesion contacts via E-cadherin interactions, whereas mesenchymal cells tend to form weaker cell-cell adhesion contacts through N-cadherin interactions [23]. Devenny et al. [21] considered the segregation of co-cultured breast cell spheroids and pointed out that epithelial spheroids have significantly higher tissue surface tension than the spheroids made by mesenchymal cells, i.e. $\gamma_e \gg \gamma_m$ (where γ_e and γ_m are respectively the epithelial and mesenchymal surface tensions. The contractility of cells exerts varying effects on the surface tension of epithelial and mesenchymal spheroids. While it enhances the strength of epithelial cell-cell adhesion contacts, it induces a repulsion among mesenchymal cells [21]. Consequently, the surface tension of contractile epithelial surfaces is larger than that of non-contractile epithelial surfaces [21]. The mesenchymal surface tension is much lower and, additionally, decreases for contractile cells [21]. Extension of epithelial surfaces enhances the strength of E-cadherin-mediated cell-cell adhesion contacts leading to a rise in the epithelial surface tension [40]. In contrast to the extension, compression of an epithelial surface intensifies homotypic CIL, which induces weakening of cell-cell adhesion contacts and a decrease in the epithelial surface tension [11,41].

3.2. The formulation of the interfacial tension

Besides the surface tensions of the epithelial and mesenchymal subpopulations, the interaction energy between the subpopulations also influences the interfacial tension. Consequently, the interfacial tension can be expressed in the form of the surface energy balance as:

$$\gamma_{em}(\Re, t, \tau) = \gamma_e(\Re, t, \tau) + \gamma_m(\Re, t, \tau) - e_{int}^{\Re}(d_{int}) \quad (3)$$

where $\Re = \Re(x, y, z)$ is the coordinate of the biointerface, t is the time-scale of minutes, τ is the time-scale of hours, γ_e and γ_m are the surface tensions of epithelial and mesenchymal subpopulations, respectively, γ_{em} is the interfacial tension between them, and $e_{int}^{\Re}(d_{int})$ is the interaction energy per unit biointerface area, $d_{int}(\Re, \tau) = |\vec{\boldsymbol{u}}_{\boldsymbol{m}} - \vec{\boldsymbol{u}}_{\boldsymbol{e}}|$ is the distance between the subpopulations along the biointerface, and $\vec{\boldsymbol{u}}_{\boldsymbol{e}}(\Re, \tau)$ and $\vec{\boldsymbol{u}}_{\boldsymbol{m}}(\Re, \tau)$ are the displacement fields of the epithelial and mesenchymal subpopulations caused by collective cell migration and effects along the biointerface. The interaction energy can be repulsive, caused by heterotypic CIL, when the local distance $d_{int}(\Re, \tau)$ is lower than the minimum distance d_{min}, i.e. $d_{int}(\Re, \tau) < d_{min}$. For this case, the interaction energy is $e_{int\,R}^{\Re} < 0$ (where $e_{int\,R}^{\Re}$ is the repulsive interaction energy). The interaction energy is attractive and satisfies the condition $e_{int\,A}^{\Re} > 0$ for $d_{min} < d_{int}(\Re, \tau)$ (where $e_{int\,A}^{\Re}$ is the attractive interaction energy). The attractive interaction energy is larger when two cell subpopulations are able to establish heterotypic cell-cell adhesion contacts. Deforet et al. [42] proposed that $d_{min} \approx 8\ \mu m$. Consequently, the local interaction energy includes two contributions: attractive and repulsive. Kang et al. [43] considered cell rearrangement caused by collective cell migration and proposed the Lennard-Jones potential to describe cell-cell interactions in the form: $e_{int}^{\Re}(d_{int}) = \frac{1}{\Delta A_{int}} k \left[\left(\frac{d_{min}}{d_{int}}\right)^p - \left(\frac{d_{min}}{d_{int}}\right)^q\right]$ (where k is the strength of the interactions, ΔA_{int} is the part of the biointerface area, p and q are exponents). The proposed values of the exponents are $p = 6$ and $q = 3$ [43]. While the attractive interactions between two subpopulations (dominant at longer distances) result in decreased interfacial tension, and stabilisation of the biointerface, the repulsive interactions (dominant at shorter distances) cause an increase in the interfacial tension and can destabilize the biointerface. Consequently, the change of the epithelial-mesenchymal interfacial tension is time-dependent and should be considered within two regimes: (1) the regime of stable biointerface; and (2) the regime of unstable biointerface. Successive transitions from the stable-to-unstable regimes and *vice versa* can

induce oscillatory change of the interfacial tension. High values of the interfacial tension correspond to the unstable biointerface regime, while lower values of the interfacial tension correspond to that of a stable biointerface.

In accordance with fact that the surface tension of epithelial cells is much larger than that of mesenchymal cells, eq. 3 can be re-written in the form:

$$\gamma_{em}(\Re, t, \tau) = \gamma_e - e_{int}^{\Re}(d_{int}) \quad (4)$$

where γ_e is the epithelial surface tension, which also can depend on the energy of interactions $e_{int}^{\Re}$, i.e. $\gamma_e = \gamma_e(e_{int}^{\Re})$. The intracellular dynamics affects the interfacial tension. The contractility of epithelial cells, as demonstrated by Devanny et al. [21], plays a crucial role in strengthening E-cadherin-mediated cell-cell adhesion by elevating both the epithelial surface tension and the interfacial tension. Additionally, this contractility may influence the energy dynamics of cellular interactions. This observation aligns with the understanding that homotypic and heterotypic cell-cell interactions are inter-dependent [2]. Furthermore, the properties of cell contractility and polarization are interconnected. Notbohm et al. [44] emphasized that the dynamics of cell polarization is influenced by the concentration of phosphorylated myosin, which is fundamentally linked to cell contractility. The process of cell repolarization, triggered by heterotypic contact inhibition of locomotion within an unstable biointerface regime, affects: (i) the rate at which the distance d_{int} increases, thereby impacting the strength of heterotypic cell-cell interactions; and (ii) cell signalling [44]. In epithelial MDCK cell monolayers, the time required for repolarization during homotypic cell-cell interactions is approximately 1.2 hours [44]. The repolarization of cells along the biointerface disrupts the polarization of neighbouring cells by reducing the persistence of cell migration. This observation aligns with the tendency of cells to form head-to-tail contacts, a phenomenon referred to as contact following, which enhances the persistence of cell migration [45].

The epithelial surface tension, considered here for the stable regime of the biointerface, can be larger than that for the unstable regime. It is in accord with the fact that repulsive interactions along the biointerface within the unstable regime intensify homotypic CIL, which consequently results in a weakening of E-cadherin-mediated cell-cell adhesion contacts. Consequently, a change of the interfacial tension influences the epithelial surface tension, the rate of the biointerface area decrease, and the mechanical stress generation, which have a feedback effect on the rearrangement of the subpopulations.

In a further consideration, it is necessary to point to the role of the interfacial tension in the self-organisation of the subpopulations in relation to the wetting and de-wetting of the subpopulations.

4. The role of the interfacial tension in the tissue self-organisation: theoretical consideration

The interfacial tension does work as the biointerface area decreases. As a result, one subpopulation experiences compression (de-wetting), whereas the other experiences extension (wetting). The identification of which subpopulation is subjected to extension or compression is governed by the interplay between the adhesion and cohesion energies at the biointerface, which are indicated by the spreading factors of the respective subpopulations [12]. The spreading factor of the subpopulation k is equal to $S^k(d_{int}) = e_{int}(d_{int}) - e_c^k$ (where $e_{int}(d_{int})$ is the interaction energy between the subpopulations, l and k equal to $e_{int}(d_{int}) = \gamma_l + \gamma_k - \gamma_{lk}$, γ_l and γ_k are the surface tensions of the subpopulations, γ_{lk} is the interfacial tension between the subpopulations, and e_c^k is the cohesion energy of the subpopulation k, equal to $e_c^k = 2\gamma_k$). If $S^k > 0$, subpopulation k undergoes extension.

Otherwise, when the spreading factor $S^k < 0$, this subpopulation undergoes compression. Consequently, the spreading factor of the mesenchymal subpopulation is equal to: $S^m(d_{int}) = \gamma_e - (\gamma_m + \gamma_{em})$, while the spreading factor of the epithelial subpopulation is equal to $S^e(d_{int}) = \gamma_m - (\gamma_e + \gamma_{em})$. For distances larger than d_{min}, i.e. $d_{int} > d_{min}$, the biointerface is stable.

Because the surface tensions of the subpopulations satisfy the condition that $\gamma_e \gg \gamma_m$, the following conclusions can be drawn for the stable regime of the biointerface: (1) the epithelial subpopulation undergoes compression, i.e. $S^e < 0$; and (2) the mesenchymal subpopulation undergoes extension, i.e. $S^m > 0$. The following relations can be established between the surface tensions of the subpopulations and the interfacial tension between them: $\gamma_m \ll \gamma_e$, $\gamma_m < \gamma_{em}$, and $\gamma_e > \gamma_m + \gamma_{em}$. A schematic representation of two cell subpopulations in direct contact is presented in **Figure 1**:

Figure 1.

However, when the biointerface is unstable, the epithelial surface tension decreases, while the interfacial tension becomes large enough to satisfy the conditions: $\gamma_{em} \gg \gamma_m$ and $\gamma_{em} > \gamma_e$. In the context of the spreading factors of the subpopulations, it means that $S^e < 0$ and $S^m < 0$, i.e. both subpopulations undergo compression caused by the repulsion along the biointerface. Their compression, induced by heterotypic CIL in this case, results in an increase in the distance d leading again to biointerface stabilisation.

Thus, the biointerface transition from the stable to unstable regime and *vice versa*, via collective cell migration, causes a long-period oscillatory change of the epithelial-mesenchymal interfacial tension leading to an oscillatory decrease of the biointerface area, and oscillatory changes of the cell mechanical stress. It is necessary to discuss multi-time changes of the interfacial tension as the main cause of the cell mechanical stress generation.

4.1 Multi-time change of the interfacial tension

Consequently, the epithelial subpopulation undergoes a long-time oscillatory compression, while the mesenchymal subpopulation undergoes successive extension and compression caused by the successive transitions of the biointerface between the stable and unstable regimes. These stable/unstable transitions result in a long-time oscillatory variation in the interfacial tension, while the short-time change of the interfacial tension can be attributed to the cumulative effects of the remodelling of heterotypic and homotypic cell-cell adhesion contacts.

4.1.1 A long-time oscillatory change of the interfacial tension

The scenario of a long-time change of the epithelial-mesenchymal interfacial tension can be described as follows:

- A decrease in the distance d_{int} such that $d_{int} < d_{min}$ induces a repulsive interaction between the subpopulations along the biointerface.
- These repulsive interactions cause an increase in the interfacial tension (eq. 2). The increase in interfacial tension raises the rate of decrease of the biointerface area. Changes in the biointerface area, which occur on a long-time scale via collective cell migration, have a feedback effect on the interfacial tension itself.

- The main characteristic of this unstable biointerface regime is the compression of both subpopulations, leading to an increase in the distance d_{int}.
- This increase in d_{int} causes attractive interactions along the biointerface, resulting in a decrease in the interfacial tension again. A lower value of the interfacial tension slows down the rate of decrease in the biointerface area.
- The amplitude of these fluctuations depends on the mobility of the subpopulations expressed as: $\vec{\boldsymbol{v}}_{\boldsymbol{R}}(\Re,\tau) = \vec{\boldsymbol{v}}_{\boldsymbol{m}} - \vec{\boldsymbol{v}}_{\boldsymbol{e}}$ (where $\vec{\boldsymbol{v}}_{\boldsymbol{m}}$ and $\vec{\boldsymbol{v}}_{\boldsymbol{e}}$ are the velocities of the mesenchymal and epithelial subpopulations, respectively).
- An oscillatory decrease of the interfacial tension is caused by the permanent decrease in the biointerface area toward the equilibrium state during the segregation of the subpopulations, which results in an increase of the surface packing density of epithelial cells n_{Se} and enhances homotypic and heterotypic cell-cell interactions as well as the CIL. The equilibrium state obtained at $\tau = \tau_{eq}$ corresponds to the condition that: $\vec{\boldsymbol{v}}_{\boldsymbol{m}} \to 0$ and $\vec{\boldsymbol{v}}_{\boldsymbol{e}} \to 0$, while $n_{Se} \to n_{Se\,eq}$.

Consequently, the interfacial tension and the rate of decrease in the biointerface area are interconnected, exhibiting long-term oscillatory changes. Every change of the biointerface area provokes a short-time remodelling of the homotypic and heterotypic cell-cell interactions in the form of short-time relaxation cycles of the interfacial tension. The interfacial tension decreases from $\gamma_{em}(\Re, t_0, \tau)$ to $\gamma_{em}(\Re, t_{eq}, \tau) \equiv \gamma_{em}(\Re, \tau)$ within a single short-time relaxation cycle. The inferred multiscale nature of the dynamics of the interfacial tension is illustrated in **Figure 2**:

Figure 2.

The short- and long- time change of the interfacial tension can be discussed in the form of dilational viscoelasticity.

4.1.2 Short-time changes of the interfacial tension as short-time relaxation cycles

Dilational viscoelasticity refers to a surface characteristic that includes both reversible (elastic) and irreversible (viscous) alterations in structure that occur during the compression and extension of an interface [8]. We would like to discuss the main aspects of the dilatational viscoelasticity in this case, while the final form of a proper constitutive model can be formulated based on relevant experimental data. A decrease in the distance d_{int} intensifies cell-cell interactions, resulting in homotypic and heterotypic CIL [2,41]. The CIL leads to weakening of cell-cell adhesion contacts, which occurs on a time scale of minutes. An increase in the distance d_{int} induces a reinforcement of homotopic epithelial cell-cell adhesion contacts and attractive interactions between two cell subpopulations [12,21,40]. If two cell subpopulations are able to establish heterotypic cell-cell adhesion contacts, an increase in the distance d_{int} (for the stable biointerface regime) can also increase the strength of these heterotypic adhesions.

As a further consideration, it is necessary to postulate a constitutive model for the interfacial tension which satisfies the following conditions:

- As mentioned below, the change of the biointerface area occurs on a long-time scale, while changes in the interfacial tension occur on two time-scales.

- The interfacial tension: (1) causes a decrease in the biointerface area for the increment ΔA_{int} relative to the initial biointerface area A_0; and (2) influences its rate of decrease $\frac{d}{d\tau}\left(\frac{\Delta A_{int}}{A_0}\right)$ depending on the distance d_{int}. The rate $\frac{d}{d\tau}\left(\frac{\Delta A_{int}}{A_0}\right)$ is larger in the unstable regime compared to the stable regime.
- The decrease of the biointerface area $\frac{\Delta A_{int}}{A_0}$ and its rate of decrease $\frac{d}{d\tau}\left(\frac{\Delta A_{int}}{A_0}\right)$ have feedback effects on the interfacial tension γ_{em} and the rate of the interfacial tension change $\frac{d}{dt}(\gamma_{em})$ depending on the distance d_{int} [8]. This is in accord with the fact that various mechanisms influence the remodelling of cell-cell adhesion contacts in the stable and unstable regimes of the biointerface. The rate $\frac{d}{dt}(\gamma_{em})$ is determined by: (1) homotypic and heterotypic CIL in the unstable regime; and (2) reinforcement of epithelial adhesion contacts and attractive interactions between two cell subpopulations in the stable regime.

A long-period oscillatory change in interfacial tension causes oscillatory changes in: (1) the decrease in the biointerfacial area; and (2) the rate of decrease of the biointerface area. The change of the biointerface area results in the generation of mechanical stress. First, we will discuss the change of the biointerface area as a function of the distance d_{int} between the subpopulations and then, secondly, point to the role of the interfacial tension in generating cell mechanical stress.

4.2 Long-period oscillatory change of the biointerface area driven by the interfacial tension

Quantification of the changes in the biointerface area remains unaccomplished; however, the effects on the multicellular surface, driven by tissue surface tension, have been evaluated in simplified model systems. This includes the phenomenon of cell aggregate rounding that occurs subsequent to uniaxial compression, as referenced earlier [10]. In this case, changes of the cell aggregate shape and its surface, occurring via collective cell migration from the aggregate surface toward the bulk region, show long-period oscillations [10,11,46]. In the case considered here, the oscillatory change of the biointerface area between two subpopulations can be induced by successive transitions of the biointerface between its stable and unstable states, as discussed above (**Figure 2**).

The biointerface area between the subpopulations $A_{int}(\tau)$ can be expressed [47] as:

$$A_{int}(\tau) = f_{eS}(\tau) N_{Te} \langle A_{e\,eff} \rangle \qquad (5)$$

where $f_{eS}(\tau)$ is the fraction of epithelial cells in contact with mesenchymal cells, $\langle A_{e\,eff} \rangle$ is the average effective surface area of a single epithelial cell in contact with mesenchymal cells, and N_{Te} is the total number of epithelial cells which satisfies the condition that $N_{Te} \approx \text{const}$ during the tissue self-organisation. Cell division - as a possible cause of an increase in the number of cells - can be neglected on a time scale of hours, since it occurs on a much longer time scale (days) for many cell types including those considered here such as: epithelial MCF-10A cells, human keratinocytes, MDCK cells, breast mesenchymal MDA-MB-231 cells [48]. Cell doubling time is additionally prolonged under overcrowded conditions caused by an increase in cell packing density. The fraction of interfacial epithelial cells $f_{eS}(\tau)$ consists of cells, which have at least one contact with mesenchymal cells expressed as: $f_{eS}(\tau) = \int_1^{z^*} \rho_a(z_{em}, \tau) d\, z_{em}$ (where $\rho_a(z_{em}, \tau)$ is the distribution of coordination numbers z_{em} of epithelial cells which can be $z_{em} \geq 1$ for the interfacial epithelial cells, while the bulk epithelial cells satisfy the condition that $z_{em} = 0$, and z^* is the maximum coordination number of

epithelial cells in contact with mesenchymal cells corresponding to a movement of single epithelial cells through a mesenchymal subpopulation). The fraction of epithelial cells located within the bulk of the epithelium is equal to $f_{eV}(\tau) = 1 - f_{eS}(\tau)$.

The balance of these thermodynamic forces (affinities), which affect the variation in the coordination number of epithelial cells z_{em}, can be represented in the form of a Langevin-type equation as:

$$\frac{\partial z_{em}}{\partial \tau} = -\varphi_1(e_{int\,R}) + \varphi_2(e_{int\,A}) + \Gamma \qquad (6)$$

where $\varphi_1(e_{int\,R})$ represents a measure of repulsive interactions, leading to a decrease in the coordination number z_{em} [2], $\varphi_2(e_{int\,A})$ is the measure of attractive interactions which causes an increase in the coordination number z_{em}, and Γ is the stochastic driving force induced by the uncorrelated motility of the subpopulations along the biointerface.

A long-term change of the interfacial tension influences wetting and de-wetting of the subpopulations and consequently has an impact on their volumetric rearrangement of the subpopulations by altering the cell residual stress accumulation. The cell residual stress is a stress that remains in a system as a result of collective cell migration [46]. Its long-term oscillations have been confirmed within various experimental systems [44,46,49].

4.3 Long-time cell residual stress generation along the biointerface: the impact of the interfacial tension

Long-time oscillatory changes of the cell residual stress, cell velocity and resulted cell strain represent the hallmark of collective cell migration [44,46,49]. These oscillatory patterns, resembling mechanical waves, have been observed in several model systems: (1) the free expansion of epithelial monolayers [44,46,49,50]; (2) the rearrangement of confluent epithelial monolayers [44,46,51]; (3) fusion of two mono-cultured epithelial or cancer cell aggregates [20]; (4) wetting/de-wetting of cell aggregate on solid substrates [12,52]; and (5) segregation of co-cultured epithelial-cancer monolayers [6]. While the directional cell migration results in the generation of propagative waves, the cell swirling motion induces the generation of standing waves [46,51]. These oscillatory phenomena in collective cell migration are a product of low Reynolds turbulence that is also known as *elastic turbulence* [46,53]. It is caused by the system's viscoelasticity, and is induced by local changes of the system stiffness which can destabilize the flow even for low Reynolds numbers. The phenomenon has been observed in a range of soft matter systems, including solutions of flexible, long-chain polymers [54,55]. In the latter case, the local stiffening is induced by the stretching of polymer chains under flow. This stiffening has a feedback effect on the stress relaxation phenomenon which has been quantified by the Weissenberg number [54,55]. Multicellular systems, unlike other soft matter systems, exhibit active behaviour and possess the ability to self-reorganize, a phenomenon that has been characterized as a form of active turbulence [56]. Inhomogeneous distributions of cell residual stress resulting from collective cell migration, account for the variation in stiffness and its alterations over extended periods. These changes can produce mechanical waves, which have been interpreted as long-term inertial effects [42,44,48].

The important characteristics of mechanical stresses generated within epithelial systems due to collective cell migration are as follows: (1) the cell mechanical stress can be normal and shear [49,50]; (2) the stress can relax toward the cell residual stress, and the relaxation is exponential [39,57]; (3) the stress relaxation time occurs on a time-scale of minutes [39,57]; (4) the cell residual stress, accompanied by the corresponding strain changes, is oscillatory on a time-scale of hours [44,46,49];

and (5) the stress relaxes through many short-time relaxation cycles under constant strain per cycle (**Figure 3**). The maximum of the cell residual stress caused by collective cell migration is a few hundredPa [44,49,50,58].

The normal cell residual stress includes two contributions: isotropic and deviatoric. The isotropic contribution to the normal residual stress is induced by the work of the interfacial tension along the biointerface, while the deviatoric contribution is induced by collective cell migration. The interfacial tension influences directly the isotropic contribution, and indirectly the deviatoric contribution of the cell normal residual stress which will be discussed in this **Section** and the **Appendix.**

4.3.1 The impact of the interfacial tension in generation of normal cell stress: The Young-Laplace equation

The epithelial subpopulation undergoes compression (de-wetting), while the mesenchymal subpopulation undergoes extension (wetting) within the stable regime of the biointerface. Consequently, the cell normal residual stress for both subpopulations consists of: (i) an isotropic contribution caused by work of the interfacial tension and (ii) a deviatoric contribution caused by collective cell migration. It can be expressed as [11,53]:

$$\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rVi}}(r,\tau) = \pm\Delta p_{m\to e}\tilde{\boldsymbol{I}} + \widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rVi}}{}^{\boldsymbol{CCM}} \qquad (7)$$

where $i \equiv e, m$, $\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rVi}}(r,\tau)$ is the normal cell residual stress generation within the i-th subpopulation along the biointerface, r is the coordinate within the boundary layer between the subpopulations (**Figure 1**) which satisfies the condition that $r \in [0, \Re]$, $\Delta p_{m\to e} = -\gamma_{em}\left(\vec{\boldsymbol{\nabla}}\cdot\vec{\boldsymbol{n}}\right)$ is the isotropic part of the cell normal stress, expressed by the Young-Laplace equation, $\vec{\boldsymbol{n}}$ is the normal vector to the biointerface, $\tilde{\boldsymbol{I}}$ is the unity tensor, $\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rVi}}{}^{\boldsymbol{CCM}}$ is the deviatoric part of the cell normal residual stress. The '' $\pm$'' in front of the first term on the right hand side of eq. 7 means extension for ''+'' and compression for ''−''. Within the unstable regime of the biointerface, both subpopulations are compressed. The corresponding cell normal residual stress, in this case, is: $\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rVi}} = -\Delta p_{m\to e}\tilde{\boldsymbol{I}} + \widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rVi}}{}^{\boldsymbol{CCM}}$ **.** The deviatoric part of the cell normal residual stress depends on the viscoelasticity caused by collective cell migration. The viscoelasticity of the subpopulations is discussed in the form of the constitutive models proposed in the **Appendix**.

4.3.2 The impact of the gradient of the interfacial tension in generation of cell shear stress: The Marangoni effect

The cell shear residual stress can be generated either by either forced convection or natural convection [14,53]. The forced convection is related to collective cell migration along the biointerface. The natural convection is induced by an inhomogeneous distribution of interfacial tension along the biointerface caused by fluctuations in the separation of the two subpopulations. The interfacial tension gradient $\vec{\boldsymbol{\nabla}}_{\boldsymbol{s}}\gamma_{em}$ generated can induce cell spreading from the region of lower interfacial tension to the region of higher interfacial tension [14]. The phenomenon, known as the Marangoni effect, has been observed in various soft matter systems as a consequence of an inhomogeneous distribution of temperature or composition [4]. The total cell shear residual stress generated within the i-th cell subpopulation along the biointerface has been expressed as [53]:

$$\vec{\boldsymbol{n}}\cdot\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rSi}}(r,\tau)\cdot\vec{\boldsymbol{t}} = \vec{\boldsymbol{\nabla}}_{\boldsymbol{s}}\gamma_{em}\cdot\vec{\boldsymbol{t}} + \vec{\boldsymbol{n}}\cdot\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rSi}}{}^{\boldsymbol{CCM}}\cdot\vec{\boldsymbol{t}} \qquad (8)$$

where $i \equiv e, m$, $\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rSi}}(r,\tau)$ is the total cell shear residual stress generated within the i-th subpopulation, $\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rSi}}{}^{\boldsymbol{CCM}}$ is the shear stress generated by the forced convection (i.e. collective cell migration) which is discussed in the **Appendix**, and $\vec{\boldsymbol{t}}$ is the tangent vector on the biointerface. The first term on the right-hand side of eq. 8 represents the shear stress component generated by the natural convection. The cell residual stress generation caused by collective cell migration should be further characterized based on the constitutive models proposed in the **Appendix**. The total cell residual stress of the i-th sub-population is equal to $\widetilde{\boldsymbol{\sigma}}_{\boldsymbol{ri}}(r,\tau) = \widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rVi}} + \widetilde{\boldsymbol{\sigma}}_{\boldsymbol{rSi}}$. A schematic representation of the successive stress relaxation cycles for the epithelial subpopulation, based on the Zener constitutive models discussed in the **Appendix,** is shown in **Figure 3**:

Figure 3.

For deeper understanding of the successive changes of the biointerface state from stable-to-unstable and *vice versa,* which are responsible for the oscillatory change in the epithelial-mesenchymal interfacial tension, it is necessary to discuss the role played by relevant physical parameters in cell rearrangement along the biointerface, based on the biophysical model that will now be formulated.

4.3.3 The impact of the generated mechanical stress on the epithelial-to-mesenchymal cell state transition

Cells exhibiting varying degrees of mesenchymal characteristics, particularly in relation to cell polarity, motility, and the strength of cell-cell adhesion, can appear within the epithelium as a consequence of a partial epithelial-to-mesenchymal transition (EMT) induced by mechanical stress [23]. Specifically, compressive stress resulting from epithelial de-wetting, in conjunction with shear stress generated at the biointerface, can initiate EMT in certain epithelial subpopulations. A partial EMT can be induced by an applied compressive stress of approximately 600 Pa [13]. Additionally, during the reorganization of confluent MDCK epithelial monolayers, the peak compressive stress recorded was around 300 Pa, as noted by Notbohm et al [44]. Furthermore, a shear stress of 0.3 Pa has been shown to be sufficient to initiate partial EMT in epithelial ovarian cancer [59]. The shear stress resulting from active cell wetting is estimated to be in the range of several tens of Pa [49].

5. Inter-relationship between the interfacial tension and other physical parameters

The distribution of interfacial tension and its temporal changes are pivotal in shaping the self-organization of the subpopulations, impacting them both directly and indirectly. The direct influence is associated with (i) the decrease of the biointerface area, (ii) the rate of decrease of the biointerface area due to cell surface rearrangement caused by cell surface rearrangement and (iii) volumetric rearrangement arising from the wetting and de-wetting of the subpopulations quantified by their spreading factors. Indirectly, interfacial tension and its gradient play a significant role in the generation of residual mechanical stress along the biointerface, which further affects both cell velocity and packing density. The relationship between the interfacial tension and other physical parameters based on the theoretical considerations introduced above, is shown schematically in **Figure 4**.

Figure 4.

The inter-relationship between the interfacial tension and other physical parameters is discussed in the context of the modified biophysical model formulated by Pajic-Lijakovic et al. [12].

5.1 The biophysical model

The system of interest consists of two perturbed boundary layers of migratory epithelial and mesenchymal subpopulations along the biointerface as shown in **Figure 1**. The biointerfacial area decrease, caused by the work of the interfacial tension, results in a volumetric rearrangement of the subpopulations within the boundary layers. It is assumed that the perturbed layers possess thicknesses that are ten times greater than the dimensions of an individual cell.

Long-time changes in the separation of the subpopulations along the biointerface result from collective cell migration and can be expressed by the relative velocity $\vec{\boldsymbol{v}}_{\boldsymbol{R}} = \vec{\boldsymbol{v}}_{\boldsymbol{m}} - \vec{\boldsymbol{v}}_{\boldsymbol{e}}$ (where $\vec{\boldsymbol{v}}_{\boldsymbol{m}}$ and $\vec{\boldsymbol{v}}_{\boldsymbol{e}}$ are the velocities of the mesenchymal and epithelial subpopulations equal to $\vec{\boldsymbol{v}}_{\boldsymbol{m}} = \frac{d\vec{\boldsymbol{u}}_{\boldsymbol{m}}}{d\tau}$ and $\vec{\boldsymbol{v}}_{\boldsymbol{e}}(r,\tau) = \frac{d\vec{\boldsymbol{u}}_{\boldsymbol{e}}}{d\tau}$). Consequently, the relative velocity can be expressed as: $\vec{\boldsymbol{v}}_{\boldsymbol{R}}(r = \Re,\tau) = \frac{d\overrightarrow{\boldsymbol{d}_{\boldsymbol{int}}}(\Re,\tau)}{d\tau}$. The oscillatory changes in the velocities $\vec{\boldsymbol{v}}_{\boldsymbol{e}}$ and $\vec{\boldsymbol{v}}_{\boldsymbol{m}}$ are a product of the competition between: (1) driving forces such as the interfacial tension force; and (2) resistive forces such as a frictional force. The mixing and viscoelastic forces can be driving or restrictive forces depending on the stable/unstable regime of the biointerface and the cell type. Oscillatory changes of the velocities $\vec{\boldsymbol{v}}_{\boldsymbol{m}}$ and $\vec{\boldsymbol{v}}_{\boldsymbol{e}}$**,** obtained during the segregation of co-cultured epithelial-mesenchymal cell monolayers, were confirmed experimentally by Lucia et al. [6].

5.2 Force balances

The phenomenon of long-term oscillations of the velocities $\vec{\boldsymbol{v}}_{\boldsymbol{e}}$ and $\vec{\boldsymbol{v}}_{\boldsymbol{m}}$ have been discussed in the form of the effective inertia [42,44,48]. Inertial effects occur when the balance between driving forces and resistive forces is disrupted. Notbohm et al. [44] highlighted that effective inertia emerges from the interaction between cell contractility and strain, manifested as cell active stress, which is influenced by myosin concentration. Deforet et al. [42] conducted simulations of cell rearrangement driven by collective cell migration, incorporating effective inertia into the momentum conservation equation formulated at the cellular level. Pajic-Lijakovic and Milivojevic [48] addressed the emergence of effective inertia as a result of the viscoelastic characteristics of the system, considering the phenomenon at a supracellular level.

It is necessary to discuss the role of every force in the dynamics along the biointerface, and then formulate the force balances of the subpopulations by accounting for inertial effects. The interfacial tension force has been formulated by Pajic-Lijakovic et al. [12,20] and expressed as: $n_k \vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{k}} = n_k S^k \vec{\boldsymbol{u}}_{\boldsymbol{k}}$ (where $k \equiv e, m$, n_k is the packing density of the subpopulation k at the *k-l* biointerface, $\vec{\boldsymbol{u}}_{\boldsymbol{k}}$ is the displacement field caused by movement of the subpopulation k, and S^k is the corresponding cell spreading factor).

Besides the interfacial tension force, the mixing force has also to be accounted for as another driving/resistive force for the rearrangement of the subpopulations along the biointerface. The mixing force is formulated here as: $\vec{\boldsymbol{F}}_{\boldsymbol{mix}} = \frac{1}{h}\vec{\boldsymbol{\nabla}}_{\boldsymbol{s}} e_{int}\,(d_{int})$ (where h is the average size of a single cell and $\vec{\boldsymbol{\nabla}}_{\boldsymbol{s}}$ is the surface gradient). The mixing force depends on the distance between two subpopulations. In the stabile biointerface regime, established for $d_{int} > d_{min}$, two subpopulations undergo mixing and $\vec{\boldsymbol{F}}_{\boldsymbol{mix}} > 0$, while in the unstable biointerface regime, for $d_{int} \leq d_{min}$, the subpopulations undergo de-mixing and $\vec{\boldsymbol{F}}_{\boldsymbol{mix}} < 0$. The mixing force: (1) reduces compression of epithelial-like tissue and drives

extension of the mesenchymal-like tissue in the stabile regime; and (2) drives the compression of epithelial and mesenchymal subpopulations in the unstable regime.

The frictional force depends on the relative velocity between two subpopulations in direct contact. The frictional force has been expressed as: (1) $n_e\vec{F}_{FR}^{\,e} = n_e\xi_e(\vec{v}_m - \vec{v}_e)$ for the epithelial subpopulation; and (2) $n_m\vec{F}_{FR}^{\,m} = n_m\xi_m(\vec{v}_m - \vec{v}_e)$ for the mesenchymal subpopulation (where ξ_e and ξ_m are the frictional coefficients of the epithelial and mesenchymal subpopulations, and n_e and n_m are the volumetric cell packing densities of the subpopulations) [12]. The relative velocity $\vec{v}_R = \vec{v}_m - \vec{v}_e$ is lower in the stable biointerface regime (for $d_{int} > d_{min}$) than in the regime where it is unstable (for $d_{int} \leq d_{min}$). It is in accordance with fact that the subpopulations move in the opposite directions caused by the repulsion generated in the unstable regime.

The viscoelastic force results from the non-uniform distribution of cell residual stress and has been described by Murray et al. [60] in the form: $\vec{F}_{ve} = \vec{\nabla}(\tilde{\sigma}_e - \tilde{\sigma}_m)$. The residual stress characteristics of epithelial and mesenchymal tissues differ significantly; specifically, the residual stress in epithelial tissue is reversible (elastic), whereas that in mesenchymal cells is irreversible (viscous) (**Appendix**). Various cell types exhibit distinct responses to residual stress. For instance, while mechanical stress can impede the movement of certain cell types, it may have no impact on, or even promote, the movement of others. Notably, compressive stress of 773 Pa inhibits the movement of epithelial MCF-10A and MCF-7 cells, whereas it enhances the motility of mesenchymal cells such as 4T1, MDA-MB-231, and 67NR cells [13]. Riehl et al. [61] conducted a study to investigate and compare the responses of mesenchymal MDA-MB-231 and MDA-MB-468 cells alongside epithelial MCF-10A cells under a shear stress of 1.5 Pa. Their findings indicated that this level of shear stress facilitates the movement of MDA-MB-231 cells, has no effect on MDA-MB-468 cells and, interestingly, reduces the motility of MCF-10A epithelial cells. Additionally, the research revealed that increased compressive stress could inhibit the movement of epithelial MCF-10A cells and induce a transition into the cell-jamming state [62,63].

The modified force balances, formulated by Pajic-Lijakovic et al. [12], for the epithelial and mesenchymal subpopulations, in the stable and unstable regimes, are presented in **Table 2**:

Table 2.

The movement of the epithelial subpopulation from the biointerface toward the bulk during the compression is influenced by the interplay of mixing and interfacial tension forces, which act in opposition to the viscoelastic and frictional forces. The competition between these forces caused by the oscillation of physical parameters induces oscillatory compression of the epithelium accompanied by a change in the velocity $\vec{v}_e$. The compression is more intensive in the regime of unstable biointerface than in that of the stable biointerface. It is in accordance with fact that the mixing force is an additional driving force accompanied by the interfacial tension force in the unstable regime of the biointerface. The interfacial tension force of the epithelial subpopulation is larger in the unstable than in the stable regime of the biointerface, due to an increase in the interfacial tension. The scenario for the oscillatory change of the velocity $\vec{v}_e$ is as follows:

- The residual stress accumulation within the epithelial layer along the biointerface, accompanied by the viscoelastic and frictional forces, causes a decrease in the velocity of epithelial cells and an increase in the epithelial packing density. An induced decrease in the velocity $\vec{v}_e$ causes a decrease in the friction force.

- An increase in the epithelial packing density leads to intense homotypic cell-cell interactions. These interactions cause weakening of E-cadherin-mediated cell-cell adhesion contacts resulting in energy dissipation within the epithelial layer and, consequently, a decrease in: (1) the residual stress accumulation within the epithelium; and (2) a decrease in the epithelial surface tension.
- A decrease in the residual stress accumulated within the epithelium causes a decrease in the viscoelastic force. Consequently, a decrease in the both resistive forces, i.e. the viscoelastic and frictional forces, results in an increase in the velocity of epithelial cells again.

The scenario for the oscillatory change of the velocity $\vec{v}_m$ is as follows:

- The accumulation of residual stress within cells inhibits the motility of epithelial cells, whereas it promotes the mobility of mesenchymal cells. Consequently, the interfacial tension force, viscoelastic force, and mixing force drive an extension of the mesenchymal subpopulation against the frictional force within the stable regime of the biointerface.
- The extension velocity of the mesenchymal subpopulation is larger than the compression velocity of the epithelial subpopulations, i.e. $\vec{v}_m > \vec{v}_e$ [27] which leads to a decrease in the distance d_{int} .
- When the distance d_{int} becomes $d_{int} \leq d_{min}$, the mixing force increases rapidly, changes its sign, and becomes a resistive force. The mixing force then supresses extension of the mesenchymal subpopulation and drives its compression accompanied by the interfacial tension and viscoelastic forces within the unstable regime of the biointerface.
- The compression results in an increase in the distance d_{int} again. These successive extension-compression cycles result in an oscillatory change of the velocity $\vec{v}_m$.

The segregation of the subpopulations within a co-cultured epithelial-mesenchymal multicellular system is finished at the equilibrium state at $\tau = \tau_{eq}$ when the subpopulations finale the self-organisation and stop migration.

Besides the force balances for the subpopulations, it is necessary to formulate the mass balances for the perturbed boundary layers of the subpopulations along the biointerface.

5.3 Mass balances

The mass balance of the epithelial subpopulation within the boundary layer along the biointerface can be expressed as [12]:

$$\frac{\partial n_e(r,\tau)}{\partial \tau} = \vec{\nabla} \cdot (\vec{J}_{conv}^{\,e} \pm \vec{J}_{Me}) \qquad (9)$$

where $n_e(r,\tau)$ is the volumetric packing density of epithelial cells while the surface packing density of epithelial cells along the biointerface (from eq. 5) for $r = \Re$ is expressed as $n_{Se}(\Re,\tau) = hn_e(r = \Re,\tau)$, h is the average size of a single cell, $\vec{J}_{conv}^{\,e} = n_e\vec{v}_e$ is the convective flux of epithelial cells along the biointerface and $\vec{J}_{Me} = k_{Me}n_e\vec{\nabla}_s(\gamma_{em} - \gamma_e)$ is the Marangoni flux of the epithelial subpopulation (where k_{Me} is the measure of the mobility of epithelial cells along the biointerface) [12]. The Marangoni flux changes the direction from the stable-to-unstable regimes of the biointerface. It is in accordance with the fact that: the inter-relation between epithelial surface tension

and interfacial tension satisfies the condition $\gamma_e > \gamma_{em}$ in the stable regime and cells migrate toward the biointerface, while in the unstable regime $\gamma_e < \gamma_{em}$ and cells migrate away from the biointerface. Compression of the epithelial subpopulation leads to an oscillatory increase in the epithelial packing density along the biointerface toward the equilibrium state $n_e(r, \tau_{eq}) = n_{e\,eq}$ occurred at $\tau = \tau_{eq}$. An inhomogeneous distribution of epithelial packing density, caused by collective cell migration, was discussed by Tlili et al. [64]. It is directly connected to the accumulation of the cell normal residual stress and can induce the cell-jamming state transition [63]. The cell normal residual stress accumulated within the epithelium shows long-time oscillatory changes [44,49].

The mass balance of the mesenchymal subpopulation within the boundary layer along the biointerface can be expressed as:

$$\frac{\partial n_m(r,\tau)}{\partial \tau} = \vec{\nabla} \cdot (\pm \vec{J}_{conv}^{\,m} + \vec{J}_{Mm}) \qquad (10)$$

where $n_m(r, \tau)$ is the volumetric packing density of mesenchymal cells, $\vec{J}_{conv}^{\,m} = n_m \vec{v}_m$ is the convective flux of mesenchymal cells along the biointerface and $\vec{J}_{Mm} = k_{Mm} n_m \vec{\nabla}_s (\gamma_{em} - \gamma_m)$ is the Marangoni flux of the mesenchymal subpopulation (where k_{Mm} is the measure of the mobility of mesenchymal cells along the biointerface). In accordance with the fact that $\gamma_{em} > \gamma_m$, in the mesenchymal subpopulation, the Marangoni flux $\vec{J}_{Mm}$ is always directed toward the biointerface. In contrast, the convective flux, accompanied by the velocity $\vec{v}_m$, changes direction as the regime changes from the stable to the unstable state. The packing density of the mesenchymal subpopulation performs oscillatory increases and decreases caused by transitions of the biointerface between the stable and unstable regimes.

6. Practical implications of the interfacial tension for the self-organisation of the subpopulations

Maximum spreading of the mesenchymal subpopulation towards the epithelial subpopulation is achieved for: (i) a stable biointerface, (ii) high epithelial surface tension, and (iii) lower interfacial tension. These conditions maximise the spreading factor of the mesenchymal subpopulations [12]. The minimal interfacial tension is observed in scenarios characterized by strong attractive heterotypic cell-cell interactions that develop along the biointerface, as described by eq. 4.

The effectiveness of segregating co-cultured epithelial-mesenchymal systems is intricately linked to the interfacial tension value. A reduction in interfacial tension results in a partial segregation of these sub-populations. The occurrence of cell segregation has been qualitatively examined in various co-cultured spheroids [21] and monolayers [6].

An inhomogeneous distribution of the epithelial surface tension and the establishment of heterotypic cell-cell adhesion contacts influence the generation of the interfacial tension gradient. The interfacial tension gradient is responsible for the generation of a mobile stiffness gradient within the epithelial subpopulation [39]. This is caused by the parallel actions of compressive stress and shear stress accumulated within the epithelium. The phenomenon of durotaxis under *in vivo* conditions has been discussed on the placode-neural crest biointerface within Xenopus embryos by Bariga et al. [19], and Pajic-Lijakovic and Milivojevic [39].

An increase in the interfacial tension caused by heterotypic repulsive interactions between breast epithelial MCF-10A cells and cancer mesenchymal MDA-MB-231 cells in the unstable regime of the biointerface causes extrusion of cancer cells [24]. Given the significance of this physical parameter, it is essential to explore the methods available for its measurement.

7. Measurement of the interfacial tension

Despite the impact of the interfacial tension on tissue reorganisation, this parameter has yet to be measured. The surface tension of multicellular surfaces in contact with liquid medium (i.e. the tissue surface tension) has been measured under simplified, equilibrium conditions, yielding the static surface tension of the tissue. The static tissue surface tension has been measured by a variety of techniques, namely: cell aggregate compression between parallel plates [10,39], cell aggregate micropipette aspiration [40], and use of a magnetic force tensiometer [65]. The measured value depends not only on the type of cellular system, but also on the experimental technique being applied. Exposure of a cell aggregate to a magnetic field can additionally enhance the strength of cell-cell adhesion contacts as reported by Jafari et al. [66]. However, the influence of the measuring technique on the tissue surface tension has not yet been reported. The static tissue surface varies significantly for different cellular systems, from a few $\frac{\mathrm{mN}}{\mathrm{m}}$ to several tens of $\frac{\mathrm{mN}}{\mathrm{m}}$ [10,37,39,65].

The dynamic interfacial tension between epithelial and mesenchymal subpopulation remains experimentally unexplored. Resonant acoustic rheometry offers a viable method for quantifying this phenomenon. This technique has demonstrated success in measuring surface and interfacial tensions in soft matter systems, such as hydrogels [67].

8. Conclusion

The interfacial tension is the one of the main physical parameters responsible for the stability of a biointerface, which is necessary for the maintenance of tissue organization and homeostasis. Minimization of the interfacial tension by the establishment of heterotypic cell-cell adhesion contacts is a prerequisite for this biointerface stability. However, larger interfacial tension, obtained for the cell subpopulations that are not able to establish heterotypic cell-cell adhesion contacts, ensures more efficient cell segregation within co-cultured systems. The role played by the interfacial tension in cell rearrangement can be elaborated on a model system such as co-cultured epithelial-mesenchymal systems. Despite the importance of the parameter, the epithelial-mesenchymal interfacial tension has not yet been measured. The present paper has emphasised the importance of this parameter in cell rearrangement along the biointerface by considering: (1) the main properties of the interfacial tension; and (2) the relationship between interfacial tension and other physical parameters. The main results were obtained by combining constitutive models with biological and bio-mechanical experiments, and we can summarize them as follows:

- Repulsive interactions along the biointerface, caused by a decrease in the distance d_{int} between the subpopulations, result in an increase in the interfacial tension, while attractive interactions characteristic of larger distances d_{int}, result in a decrease in the interfacial tension. Heterotypic cell-cell adhesion contacts, if they exist, reduce the interfacial tension.
- The successive stable-to-unstable (and *vice versa*) transitions of the biointerface, caused by changes in the distance d_{int} induce long-time oscillatory changes of the interfacial tension. The short-time change of the interfacial tension is induced by cumulative effects of the remodelling of heterotypic and homotypic cell-cell adhesion contacts caused by changes in the biointerface area.

- The interfacial tension does work in decreasing the biointerface area. Consequently, this parameter also influences the rate of decrease of the biointerface area.
- The interfacial tension, accompanied by the surface tensions of the subpopulations, influences the compression (de-wetting)/extension (wetting) of the subpopulations via collective cell migration.
- The compression/extension of the subpopulations results in the generation of mechanical stress. The interfacial tension influences the isotropic normal residual stresses of the subpopulations. The cell shear residual stresses of the subpopulations are induced by the inhomogeneous distribution of the interfacial tension along the biointerface. The inhomogeneous distribution of cell residual stress generates a viscoelastic force.
- The interfacial tension depends on the distance d_{int} between the two subpopulations along the biointerface and, consequently, on the attractive/repulsive interactions. However, this parameter has a feedback effect on the long-time changes of the distance d_{int} by influencing the velocities of the subpopulations, which was discussed in terms of the corresponding force balances.
- The interfacial tension, in the form of the Marangoni flux, influences a change of the cell packing density within the boundary layers of the subpopulations along the biointerface which also perform long-period oscillations.

Acknowledgements:

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

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

Appendix

Cell residual stress generated by collective cell migration

The viscoelasticity, caused by collective cell migration, depends primarily on the strength of homotypic cell-cell adhesion contacts. Epithelial cells establish strong E-cadherin mediated cell-cell adhesion contacts and migrate in the form of strongly-connected cell clusters [12]. In contrast to epithelial cells, mesenchymal cells establish weak cell-cell adhesion contacts and migrate in the form of cell streams [27]. While migrating epithelial collectives have been treated as viscoelastic solids, migrating mesenchymal collectives have been treated as viscoelastic liquids. It is in accordance with the fact that coordinated movement of free or weakly-connected mesenchymal cells induces more energy dissipation [14]. In both cases, the viscoelasticity caused by collective cell migration has been described by linear constitutive models [20]. The Zener constitutive model, suitable for viscoelastic solids, (**Table 1**) has been confirmed experimentally in various model systems such as: (1) free expansion of epithelial monolayers [49]; (2) the rearrangement of confluent epithelial monolayers [44]; and (3) cell aggregate compression between parallel plates [39].

Table 1.

The main characteristics of the Zener model are that: (1) the stress can relax under constant strain conditions, (2) the strain can relax under constant stress conditions, and (3) the residual stress is purely elastic [68]. The stress relaxation time obtained after uni-axial compression corresponds to a time scale of minutes, while the strain relaxation time under constant compressive stress conditions corresponds to a time scale of hours [39,57]. Stress relaxation accounts for cumulative effects of the remodelling of cell-cell adhesion contacts, while the strain relaxation occurs via collective cell migration [10,39]. The residual stress correlates with the corresponding strain [49,44]. Consequently, the cell stress change occurs via many short-time relaxation cycles under constant strain per cycle, while strain change and the residual stress generation occur on a long-time scale as shown in **Figure 3**. It means that migrating epithelial systems pass through many equilibrium states characterized by the cell residual stress and the corresponding cell strain.

While the Zener model is suitable for describing the constitutive behaviour of viscoelastic solids, the Maxwell model is suitable for describing the constitutive behaviour of viscoelastic liquids. This constitutive model has been confirmed experimentally for a model system involving cell aggregate micropipette aspiration [40]. An externally applied force causes breaking of cell-cell adhesion contacts during collective cell movement toward the micropipette, resulting in the additional energy dissipation characteristic for movement of viscoelastic liquids. Guevorkian et al. [40] measured strain changes under constant stress conditions, while stress change was not measured in these experiments. The main characteristics of the Maxwell model are that: (1) stress can relax under a constant strain rate; (2) the strain and strain rates cannot relax; and (3) the cell residual stress is purely dissipative [68]. For this case, the cell stress relaxes within many short-time relaxation cycles under constant strain rate per cycle, while the strain rate and the cell residual stress change on a long-time scale. Although the stress can relax under constant strain for viscoelastic solids, the stress cannot relax under constant strain conditions for viscoelastic liquids [68].

References

1. E. Batlle E and D.G. Wilkinson, Cold Spring Harb. Persp. Biol. 4, a008227 (2012).
2. H.B. Taylor, A. Khuong, Z. Wu, Q. Xu, R. Morley, L. Gregory, A. Poliakov, W.R. Taylor, D.G. Wilkinson, Interface 14, 20170338 (2017).
3. F.R. Millar, Janes SM, A. Giangreco, Europ. Resp. Rev. 26, 160069 (2017).
4. A. Karbalaei, R. Kumar, H.J. Cho, Micromachines 7, 13 (2016).
5. R. Mayor and C. Carmona-Fontaine, Trends Cell Biol. 20, 319–328 (2010).
6. S.E. Lucia, H. Jeong, J.H. Shin, Mol. Biol. Cell 33, ar129, 1–12 (2022).
7. A. Roycroft and R. Mayor, Cell. Mol. Life Sci. 2016; 73:1119–1130.
8. R. Shuttleworth, Proc. Phys. Soc. A 63, 444-457 (1950).
9. V.G. Babak, J. Desbri`eres, V.E. Tikhonov, Coll. Surf. A: Physicochem. Eng. Aspects 255, 119–130 (2005).
10. J.C.M. Mombach, D. Robert, F. Graner, G. Gillet, G.L. Thomas, M. Idiart, J.P. Rieu, Phys. A 352, 525-534 (2005).
11. I. Pajic-Lijakovic, R. Eftimie, M. Milivojevic, S.P.A. Bordas, Adv. Coll. Int. Sci. 315, 102902 (2023).
12. I. Pajic-Lijakovic, R. Eftimie, M. Milivojevic, S.P.A. Bordas, Sem. Cell Dev. Biol. 147, 47-57 (2023).
13. J.M. Tse, G. Cheng, J.A. Tyrrell, S.A. Wilcox-Adelman, Y. Boucher, R.K. Jain, et al., PNAS 109(3), 911–49 (2012).
14. I. Pajic-Lijakovic and M. Milivojevic, Europ. Biophys. J. 51, 419-429 (2022).
15. S. Gsell, S. Tlili, M. Merkel, P-F. Lenne, bioRxiv (2023) doi.org/10.1101/2023.09.22.559003.
16. I. Pajic-Lijakovic and M. Milivojevic. Biosystems 237:105155 (2024).
17. E. Theveneau, B. Steventon, E. Scarpa, S. Garcia, X. Trepat, A. Streit, et al., Nat. Cell Biol. 15, 763–772 (2013).
18. Barriga, E.H., Franze, K., Charras, G., Mayor R. 2018. Nature 554(7693):523–527 (2018).
19. H.A. Le HA and R. Mayor, Biochem Soc Trans 51(4), 1733-1745 (2023).
20. I Pajic-Lijakovic, R. Eftimie, M. Milivojevic, S.P.A. Bordas, Quart. Rev. Biophys. 57(e5), 1-17 (2024).
21. A.J. Devanny, M.B. Vancura, L.J. Kaufman, Mol. Biol. Cell 32, ar24, 1-15 (2021).
22. L-Y Guan, S-Z Lin, P-C Chen, J-Q Lv, B Li, X-Q Feng, ACS Nano 17(24):24668-24684 (2023).
23. E.H. Barriga and R. Mayor, Sem. Cell Dev. Biol. 93, 55-68 (2019).
24. J. Yang, P. Antin, G. Berx, C. Blanpain, T. Brabletz, M. Bronner, K. Campbell, A. Cano, J. Casanova, G. Christofori, S. Dedhar, R. Derynck, et al., Nature Rev. (2020) doi.org/10.1038/s41580-020-0237-9.
25. A. Gandalovičová, T. Vomastek, D. Rosel, J. Brábek, Oncotarget 7(18), 25022-25049 (2016).
26. P. Friedl and S. Alexander, Cell 147, 992-1009 (2011).
27. A.G. Clark and D.M. Vignjevic, Curr. Op. Cell Biol. 36, 13-22 (2015).
28. P. Heine, J. Lippoldt, G.A. Reddy, P. Katira, J. Kaes, New J. Phys. 23, 043034 (2021).
29. N.W. Bateman, M. Sun, B.L. Hood, M.W.S. Flint, T.P. Conrads, J. Proteome Res. 9, 5311e24 (2010).
30. M.H. Lee, P.H. Wu, J.R. Staunton, R. Ros, G.D. Longmore, D. Wirtz, Biophys. J. 102, 2731–2741 (2012).
31. E. Leal-Orta, J. Ramirez-Ricardo, A. Garcia-Hernandez, P. Cortes-Reynosa, E.P. Salazar, J. Cell Comm. Signal 16, 531–546 (2022).
32. F. Fagotto, N. Rohani, A-S. Touret, R. Li, Dev. Cell 27, 1–16 (2013).

33. A. Tang, M.S. Eller, M. Hara, M. Yaar, S. Hirohashi, B.A. Gilchrest, J. Cell Sci. 107, 983–92 (1994).
34. M.Y. Hsu, M.J. Wheelock, K.R. Johnson, M. Herlyn, J. Investig. Dermatol. Symp. Proc. 1, 188–94 (1996).
35. C.J. Schmitt, W.W. Franke, S. Goerdt, B. Falkowska-Hansen, S. Rickelt, W.K. Peitsch, J. Investig. Dermatol. 127, 2191–2206 (2007).
36. B. Steventon, R. Mayor, A. Streit, Dev. Biol. 389(1), 28–38 (2014).
37. S. Alt, P. Ganguly, G. Salbreux, Phil. Trans. R. Soc. B 372:20150520 (2017).
38. S. Koride, A.J. Loza, S.X. Sun, APL Bioeng. 2, 031906 (2018).
39. P. Marmottant, A. Mgharbel, J. Kafer, B. Audren, J.P. Rieu, J.C. Vial, B. van der Sanden, A.F.M. Maree, F. Graner, H. Delanoe-Ayari, PNAS 106(41), 17271-17275 (2009).
40. K. Guevorkian, F. Brochard-Wyart, D. Gonzalez-Rodriguez, in Viscoelasticity and collective cell migration, eds. Pajic-Lijakovic I and Barriga E, (Academic Press, US, 2021), 2021; pp. 193.
41. J. Zimmermann, B.A. Camley, W.J. Rappel, H. Levine, PNAS 113(10), 2660-2665 (2016).
42. M. Deforet, V. Hakim, H. Yevick, G. Duclos, P. Silberzan, Nature Comm. 5(1), 3747 (2014).
43. W. Kang, J. Ferruzzi, C-P. Spatarelu, Y.L. Han, Y. Sharma, S.A. Koehler, J.A. Mitchel, A. Khan, J.P. Butler, D. Roblyer, M.H. Zaman, et al., iSci. 24, 103252 (2021).
44. J. Notbohm, S. Banerjee, K.J.C. Utuje, B. Gweon, H. Jang, Y. Park, J. Shin, J.P. Butler, J.J. Fredberg, M.C. Marchetti, Biophys. J. 110, 2729-2738 (2016).
45. R. Alert and X Trapet, Ann Rev Cond Mat Phys 11:77-101 (2020).
46. I. Pajic-Lijakovic and M. Milivojevic, Front. Phys. 8, (2020) doi: 10.3389/fphy.2020.585681.
47. I. Pajic-Lijakovic and M. Milivojevic, Appl. Phys. A 129, 553. (2023)
48. I. Pajic-Lijakovic, M. Milivojevic, P.V.E. McClintock, J. Biol. Eng. 18, 24 (2024).
49. X. Serra-Picamal, V. Conte, R. Vincent, E. Anon, D.T. Tambe, E. Bazellieres, J.P. Butler, J.J. Fredberg, X. Trepat, Nature Phys. 8(8), 628-634 (2012).
50. D.T. Tambe, U. Croutelle, X. Trepat, C.Y. Park, J.H. Kim, E. Millet, J.P. Butler, J.J. Fredberg, PLoS ONE 8(2), e55172 1-13 (2013).
51. V. Petrolli, T. Boudou, M. Balland, G. Cappello, in Viscoelasticity and collective cell migration: An Interdisciplinary Perspective Across Levels of Organization, eds. By I. Pajic-Lijakovic and E. Barriga, (Academic Press, United States, 2021), pp. 157.
52. G. Beaune, C. Blanch-Mercader, S. Douezan, J. Dumond, D. Gonzalez-Rodriguez, D. Cuvelier, T. Ondarçuhu, P. Sens, S. Dufour, M.P. Murrell, F. Brochard-Wyarta, PNAS 115(51), 12926-12931 (2018).
53. I. Pajic-Lijakovic and M. Milivojevic, Adv. App. Mech. 55, 393-424 (2022).
54. A. Groisman and V. Steinberg, Phys. Fluids 10(10), 2451-2463 (1998).
55. A. Groisman and V. Steinberg, Nature 405, 53-55 (2000).
56. R. Alert, J. Casademunt, J-F. Joanny, Annu. Rev. Condens. Matter Phys. 13, 143–70 (2022).
57. N. Khalilgharibi, J. Fouchard, N. Asadipour, A. Yonis, A. Harris, P. Mosaff, Y. Fujita, A. Kabla, B. Baum, J.J. Muñoz, M. Miodownik, G. Charras, Nature Phys. 15, 839-847 (2019).
58. X. Trepat, M.R. Wasserman, T.E. Angelini, E. Millet, D.A. Weitz, J.P. Butler, J.J. Fredberg, Nature Phys. 5, 426-430 (2009).
59. I. Rizvi, U.A. Gurkan, S. Tasoglu, N. Alagic, J.P. Cellia, L.B. Mensah, Z. Mai, U. Demirci, T. Hasan. PNAS E1974–E1983 (2013).
60. J.D. Murray, P.K. Maini, R.T. Tranquillo, Phys. Rep. 171(2), 59-84 (1988).
61. B.D. Riehl, E. Kim, J.S. Lee, B. Duan, R. Yang, H.J. Donahue, J.Y. Lim, J. Biomech. Eng. 142, 101001 (2020).
62. S. Grosser, J. Lippoldt, L. Oswald, M. Merkel, D.M. Sussman, F. Renner, P. Gottheil, E.W. Morawetz, T. Fuhs, X. Xie, et al., Phys. Rev. X 2021; 11, 011033 (2021).

63. I. Pajic-Lijakovic and M. Milivojevic, Europ. Phys. J. Plus 136, 750 (2021).
64. S. Tlili, E. Gauquelin, B. Li, O. Cardoso, B. Ladoux, H. Delanoë-Ayari, F. Graner, R. Soc. Open Sci. 5, 172421 (2018).
65. I. Nagle, A. Richert, M. Quinteros, S. Janel, E. Buysschaert, N. Luciani, H. Debost, V. Thevenet, C. Wilhelm, C. Prunier, F. Lafont, T. Padilla-Benavides, M. Boissan, M. Reffay, Front. Cell Dev. Biol. (2022) DOI 10.3389/fcell.2022.926322.
66. J. Jafari, X-I. Han, J. Palmer, P.A. Tran, A.J. O'Connor, ASC Biomat. Sci. Eng. 5, 2532-2542 (2019).
67. E.C. Hobson, W. Li, B.A. Juliar, A.J. Putnam, J.P. Stegemann, C.X. Deng, Biomat 269:120676 (2021).
68. I. Pajic-Lijakovic, in Viscoelasticity and collective cell migration, eds. I. Pajic-Lijakovic and E. Barriga, (Academic Press, US, 2021), 2021; pp. 21.

Figure captions

Figure 1. Epithelial and mesenchymal cell populations in direct contact: (a) the stable and (b) unstable regimes of the biointerface (Black arrows describe the direction of the populations extension/compression). Curved arrows (yellow and blue) represent the perturbation of collective cell migration caused by homotypic and heterotypic cell-cell interactions along the biointerface.

Figure 2. Schematic presentation of the multi time nature of the epithelial-mesenchymal interfacial tension. The interfacial tension performs oscillatory decrease and increase caused by successive changing the biointerface regime from stable to unstable state and *vice versa* within hours during the system evolution toward the equilibrium state. The short-time changes of the interfacial tension, in the form of successive relaxation cycles, occur as a consequence of: (1) homotypic and heterotypic CIL in the unstable regime; and (2) reinforcement of epithelial adhesion contacts and attractive interactions between two cell subpopulations in the stable regime. The biointerface area performs permanent oscillatory decrease toward the equilibrium state.

Figure 3. Schematic presentation of multi-time oscillatory change of cell mechanical stress generated during the free expansion of epithelial monolayers inspired by the experiments from Serra-Picamal et al. [49]. Successive stress relaxation cycles under constant strain per cycle for the Zener model suitable for describing the viscoelasticity of epithelial subpopulation.

Figure 4. The relationship between the interfacial tension and other relevant physical parameters which guide the self-rearrangement of the cell subpopulations.

Table captions

Table 1. Constitutive models for viscoelasticity caused by collective cell migration proposed for epithelial and mesenchymal subpopulations.

Table 2. Force balances for the mesenchymal collective and epithelial collective, formulated for the stable and unstable regimes of the biointerface

Figure 1.

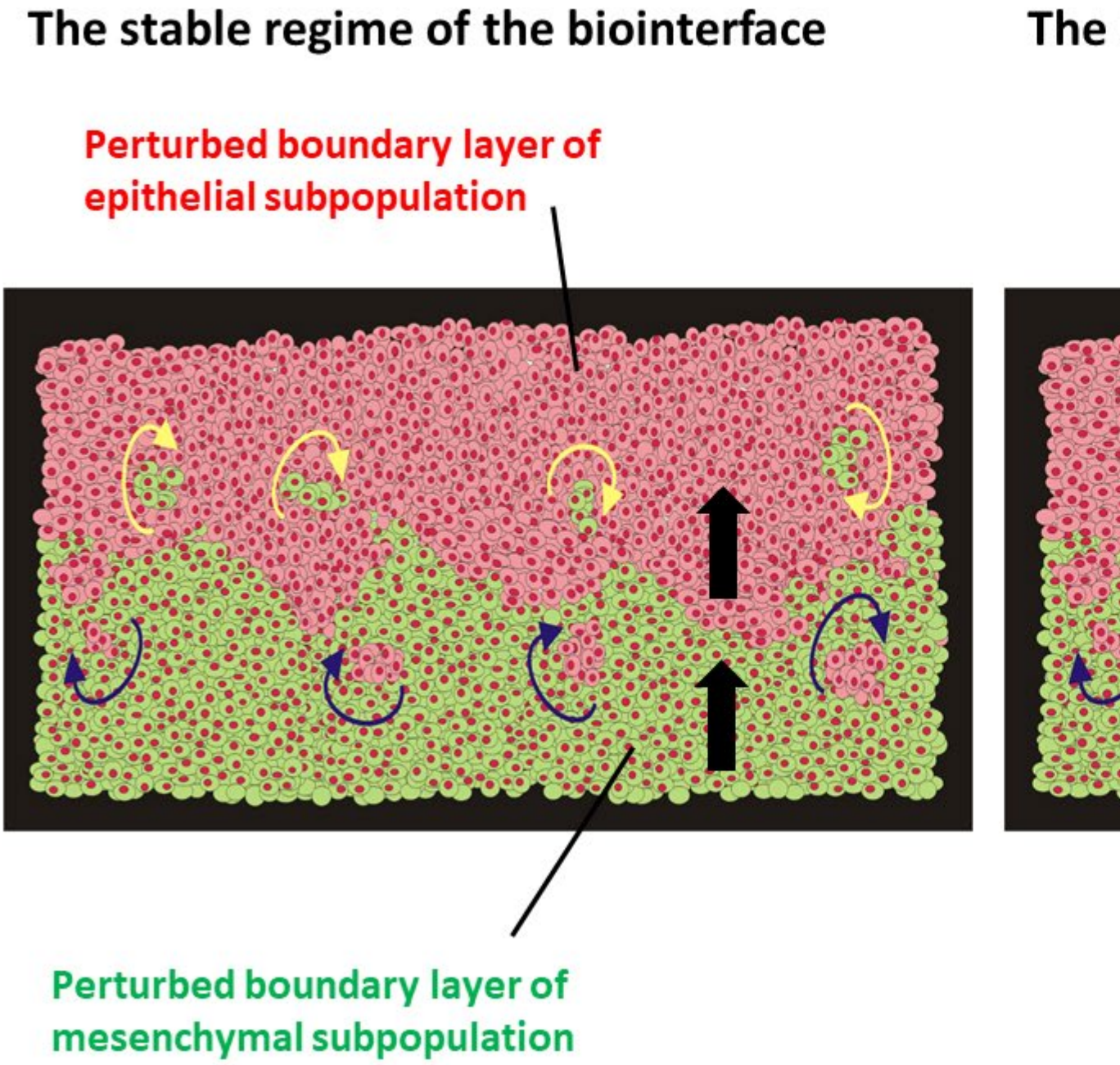
The stable regime of the biointerface
The
Perturbed boundary layer of epithelial subpopulation
Perturbed boundary layer of mesenchymal subpopulation

Figure 2.

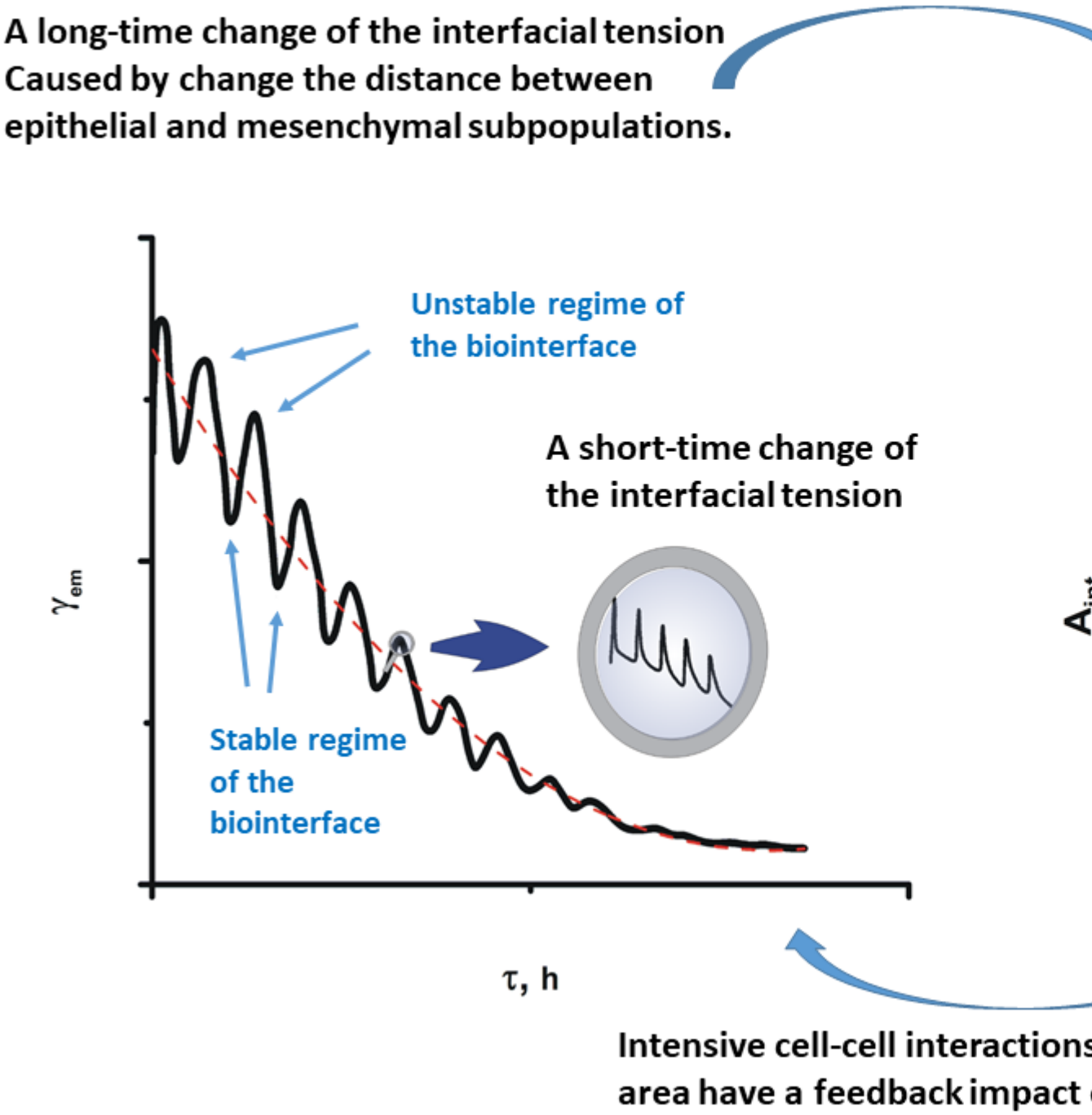
A long-time change of the interfacial tension
Caused by change the distance between
epithelial and mesenchymal subpopulations.
Unstable regime of
the biointerface
A short-time change of
the interfacial tension
Stable regime
of the
biointerface
γem
τ, h
Intensive cell-cell interactions
area have a feedback impact

Figure 3.

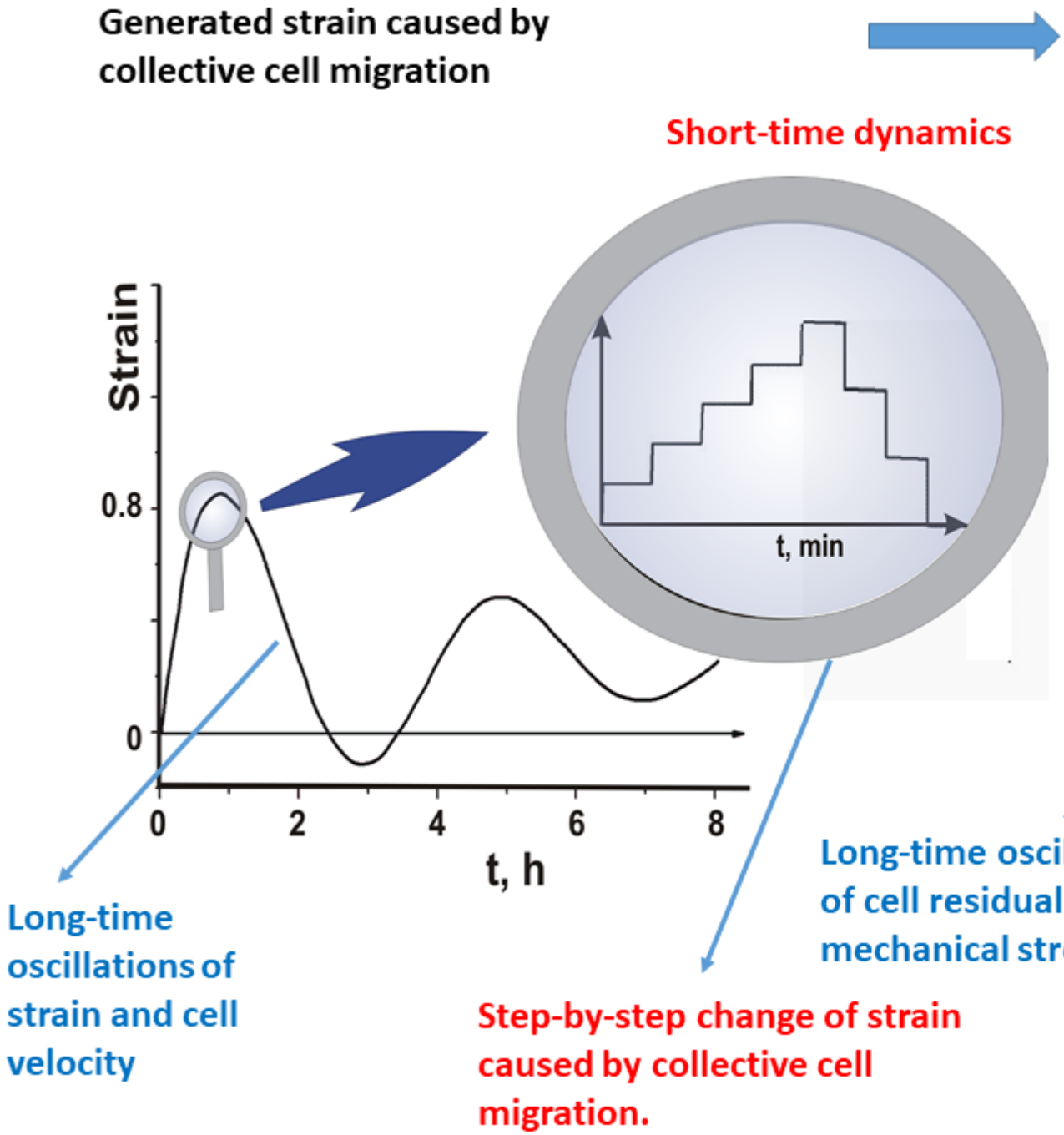
Generated strain caused by collective cell migration
Short-time dynamics
Strain
0.8
0
0
2
4
6
8
t, h
t, min
Long-time oscillations of strain and cell velocity
Step-by-step change of strain caused by collective cell migration.
Long-time oscillati
of cell residual
mechanical stress.

Figure 4.

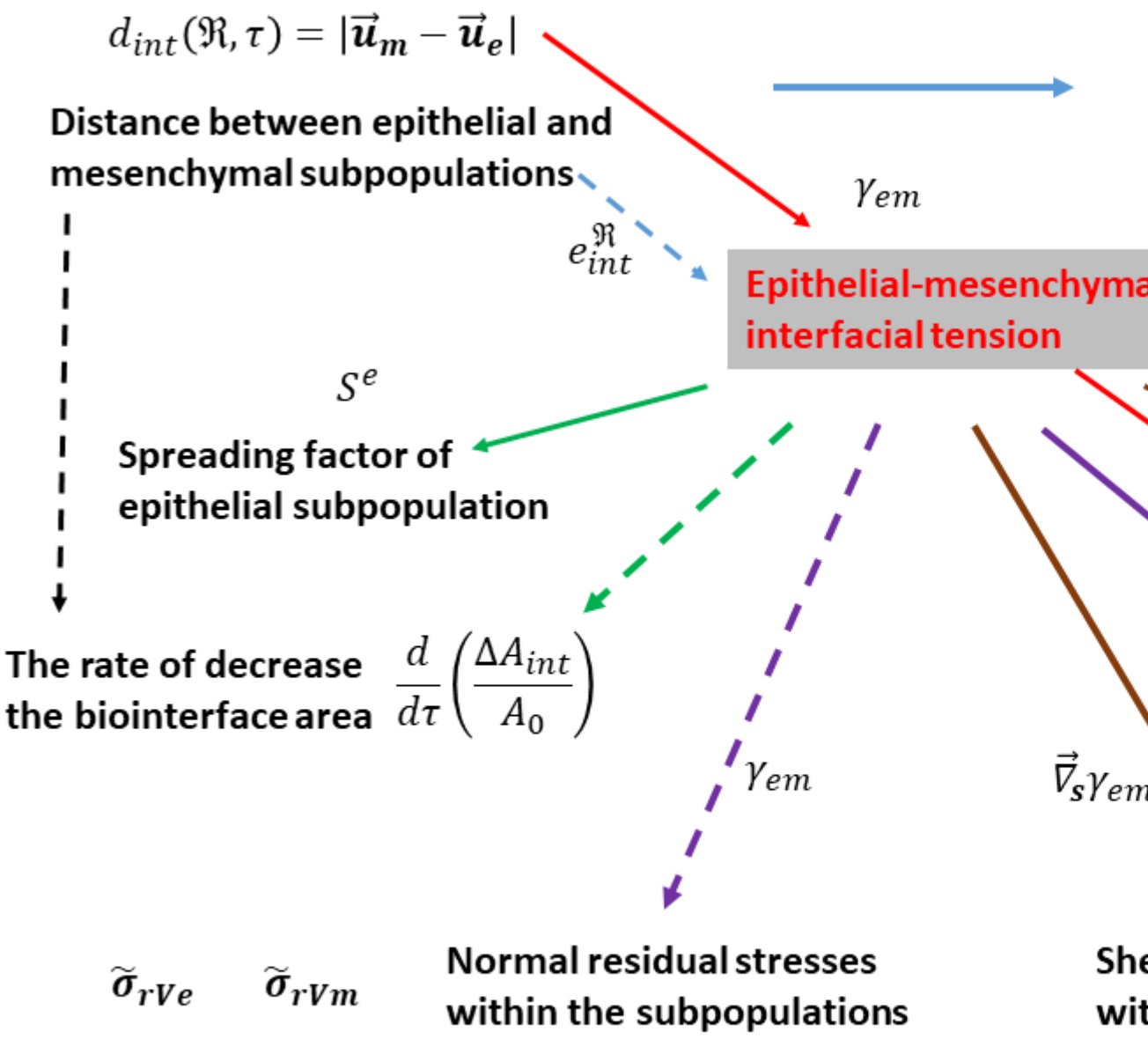
$d_{int}(\Re,\tau) = |\vec{u}_m - \vec{u}_e|$
Distance between epithelial and mesenchymal subpopulations
γ_{em}
$e_{int}^{\Re}$
Epithelial-mesenchyma
interfacial tension
S^e
Spreading factor of epithelial subpopulation
The rate of decrease the biointerface area $\frac{d}{d\tau}\left(\frac{\Delta A_{int}}{A_0}\right)$
γ_{em}
$\vec{\nabla}_s\gamma_{em}$
$\tilde{\sigma}_{rVe}$ $\tilde{\sigma}_{rVm}$
Normal residual stresses within the subpopulations
She
wit

Table 1. Constitutive models for viscoelasticity caused by collective cell migration proposed for epithelial and mesenchymal subpopulations.

	Constitutive model	Cell speed Cell packing density
Migrating mesenchymal cell collectives	The Maxwell model: $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{k}}(\Re, t, \tau) + \tau_{Rk}\, \dot{\tilde{\boldsymbol{\sigma}}}_{\boldsymbol{k}} = \eta_k \dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{k}}(\Re, \tau)$ Stress relaxation under constant strain rate $\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{0k}}$ per single short-time relaxation cycle: $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{k}}(\Re, t, \tau) = \tilde{\boldsymbol{\sigma}}_{\boldsymbol{0k}} e^{-\frac{t}{\tau_{Rk}}} + \tilde{\boldsymbol{\sigma}}_{\boldsymbol{rk}}(\Re, \tau)\left(1 - e^{-\frac{t}{\tau_{Rk}}}\right)$ Cell residual stress $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{rk}} = \eta_{\boldsymbol{k}} \dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{k}}$	$\|\vec{\boldsymbol{v}}_{\boldsymbol{c}}\| \geq 1\ \frac{\mu m}{min}$ $n \leq n_c$ n_c is the cell packing density at confluent state
Migrating epithelial cell collectives	The Zener model: $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{k}}(\Re, t, \tau) + \tau_{Rk}\, \dot{\tilde{\boldsymbol{\sigma}}}_{\boldsymbol{k}} = E_k \tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{k}}(\Re, \tau) + \eta_k \dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{k}}$ Stress relaxation under constant strain condition $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{0k}}$ per single short-time relaxation cycle: $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{k}}(\Re, t, \tau) = \tilde{\boldsymbol{\sigma}}_{\boldsymbol{0k}} e^{-\frac{t}{\tau_{Rk}}} + \tilde{\boldsymbol{\sigma}}_{\boldsymbol{rk}}(\Re, \tau)\left(1 - e^{-\frac{t}{\tau_{Rk}}}\right)$ Cell residual stress $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{rk}} = E_k\, \tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{k}}$	$0.1 < \|\vec{\boldsymbol{v}}_{\boldsymbol{c}}\| < \sim 1\ \frac{\mu m}{min}$ $n \leq n_c$

where $k \equiv S, V$, S is shear, V is volumetric, $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{k}}(\Re, t, \tau)$ is the cell stress (normal or shear), $\dot{\tilde{\boldsymbol{\sigma}}}_{\boldsymbol{k}}$ is the rate of stress change, $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{k}}$ is the corresponding strain shear or volumetric $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{S}}(\Re, \tau) = \frac{1}{2}\left(\vec{\boldsymbol{\nabla}}\vec{\boldsymbol{u}} + \vec{\boldsymbol{\nabla}}\vec{\boldsymbol{u}}^{\boldsymbol{T}}\right)$ and $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{V}}(\Re, \tau) = (\vec{\boldsymbol{\nabla}} \cdot \vec{\boldsymbol{u}})\tilde{\boldsymbol{I}}$, respectively, $\vec{\boldsymbol{u}}(\Re, \tau)$ is the local displacement field, $\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{k}}$ is the corresponding strain rate, $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{rk}}$ is the cell residual stress, $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{0k}}$ is the initial cell stress for every cycle, τ_{Rk} is the stress relaxation time, E_k is the Young's or shear elastic modulus, and η_k is shear or bulk viscosity, n is the packing density of epithelial/mesenchymal subpopulation.

Table 2. Force balances for the mesenchymal collective and epithelial collective, formulated for the stable and unstable regimes of the biointerface

Subpopulations in contact	Volumetric force balances
mesenchymal subpopulation Extension for $d_{int} > d_{min}$ $\vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{m}} > 0$ $\vec{\boldsymbol{F}}_{\boldsymbol{mix}} > 0$ Compression for $d_{int} \leq d_{min}$ $\vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{m}} < 0$ $\vec{\boldsymbol{F}}_{\boldsymbol{mix}} < 0$	$\langle m_m \rangle n_m \frac{D\vec{\boldsymbol{v}}_{\boldsymbol{m}}(r,\tau)}{D\tau} = n_m \vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{m}} + \vec{\boldsymbol{F}}_{\boldsymbol{mix}} + \vec{\boldsymbol{F}}_{\boldsymbol{ve}} - n_m \vec{\boldsymbol{F}}_{\boldsymbol{FR}}^{\ \boldsymbol{m}}$
epithelial subpopulation Compression for $d_{int} > d_{min}$ $\vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{e}} < 0$ $\vec{\boldsymbol{F}}_{\boldsymbol{mix}} > 0$ Compression for $d_{int} \leq d_{min}$ $\vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{e}} < 0$ $\vec{\boldsymbol{F}}_{\boldsymbol{mix}} < 0$	$\langle m_e \rangle n_e \frac{D\vec{\boldsymbol{v}}_{\boldsymbol{e}}(r,\tau)}{D\tau} = n_e \vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{e}} \pm \vec{\boldsymbol{F}}_{\boldsymbol{mix}} - \vec{\boldsymbol{F}}_{\boldsymbol{ve}} - n_e \vec{\boldsymbol{F}}_{\boldsymbol{FR}}^{\ \boldsymbol{e}}$

where $\frac{D\vec{v}_i}{D\tau} = \frac{\partial \vec{v}_i}{\partial \tau} + (\vec{\boldsymbol{v}}_{\boldsymbol{i}} \cdot \vec{\boldsymbol{\nabla}})\vec{\boldsymbol{v}}_{\boldsymbol{i}}$ is the material derivatives [48] and $\langle m \rangle_i$ is the average mass of single epithelial cells for $i \equiv e, m$ epithelial/mesenchymal cell).