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Dynamics along the epithelial-cancer biointerface: Hidden system complexities

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Abstract: The biointerface dynamics influence any cancer spreading through the epithelium since it is documented in the early stages some malignancies (like epithelial cancer). The altered rearrangement of epithelial cells has an impact on the development of cancer. Therefore, it is necessary to comprehend the underlying biological and physical mechanisms of this biointerface dynamics for early suppression of cancer. While the biological mechanisms include cell signaling and gene expression, the physical mechanisms are several physical parameters such as the epithelial-cancer interfacial tension, epithelial surface tension, and compressive stress accumulated within the epithelium. Although the segregation of epithelia-cancer co-cultured systems was widely investigated, the role of these physical parameters in cell reorganization is still not fully recognized. Hence, this review is focused on clarifying the role that some physical parameters have during cell reorganization within the epithelial cell clusters and cancer spread within co-cultured spheroids. We have applied the developed biophysical model to point out the inter-relations among physical parameters that influence cell reorganization within epithelial-cancer co-cultured systems. The main results of this theoretical consideration have been assessed by integrating the biophysical model with biological and bio-mechanical experiments from the available literature. The epithelial-cancer interfacial tension leads to the reduction of the biointerface area, which leads to an increase in the compressive residual stress within the epithelial clusters depending on the viscoelasticity of the epithelial subpopulation. This stress impacts epithelial rearrangement and the dynamics along the biointerface by influencing the epithelial surface tension and epithelial-cancer interfacial tension. Further, the interrelation between the epithelial surface tension and epithelial-cancer interfacial tension influences the spread of cancer cells.

Introduction

Cancer development entails several successive steps. The initial step of epithelial cancer development is cancer cell migration throughout the epithelium (Lee *et al.*, 2012; Millar *et al.*, 2017; Campbell *et al.*, 2019; Jo *et al.*, 2021; Riehl *et al.*, 2021; Pajic-Lijakovic *et al.*, 2023a). This contact with cancer cells causes epithelium reorganization, which further influences cancer cell spreading (Lucia *et al.*, 2022; Pajic-Lijakovic and Milivojevic, 2023d). This complex phenomenon has been assayed with model systems of co-cultured epithelial-cancer spheroids and studying the epithelial cell rearrangement within the dispersed epithelial clusters surrounded by the cancer subpopulation, which represents a continuum. Further, the spreading of a cancer subpopulation primarily depends on the dynamics along the biointerface (Batlle and Wilkinson, 2012; Lucia *et al.*, 2022; Pajic-Lijakovic *et al.*, 2023b). Biointerface dynamics is a product of homotypic and heterotypic interactions governed by the interplay among biological and physical mechanisms (Takeichi, 2023). The biological mechanisms include cell signaling and gene expression (Bateman *et al.*, 2010; Lee *et al.*, 2012; Leal-Orta *et al.*, 2022), while the physical mechanisms include the inter-relation between surface and interfacial tensions present in epithelial-cancer cellular systems (Pajic-Lijakovic *et al.*, 2023b).

The biological mechanisms that influence the rearrangement of epithelial and cancer cell subpopulations along the biointerface have been well elaborated using model systems such as MCF10A/ MDA-MB-231 co-cultured cellular systems. For example, Lee *et al.* (2012) revealed that the motility of MDA-MB-231 cancer cells is significantly increased when they are surrounded by the epithelial

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MCF10A cells. The release of macromolecules like fibronectin and laminin-5 by epithelial MCF10A cells facilitates the formation of focal adhesions (FAs) on MDA-MB-231 cells with these macromolecules (Bateman *et al.*, 2010). Additionally, the MDA-MB-231 cells secrete vesicles that stimulate the epithelial-to-mesenchymal transition of the MCF10A cells (Leal-Orta *et al.*, 2022). During the epithelial-to-mesenchymal transition of cells, several transformations occur. Cells lose their apicobasal polarity, obtain mesenchymal phenotypes, and the strength of cell-cell adhesion contacts and cell viscoelasticity are altered (Yang *et al.*, 2020). Further, Heine *et al.* (2021) pointed to an intensive neighbor exchange along the MCF10A/ MDA-MB-231 biointerface. Other epithelial-cancer cell co-cultured systems show similar inter-connected cellular responses induced by cell signaling (Lucia *et al.*, 2022).

While the biological mechanisms have been intensively studied, physical mechanisms are yet to be probed. In addition, while some physical parameters have been measured only under equilibrium conditions, other parameters have not been measured yet. It is well known that the tissue surface tension (also called "dynamic tissue surface tension") is a space-time dependent dynamic parameter. This varies due to the change of generated strain caused by collective cell migration (CCM), alterations in the cell surface packing density, variations in the strength of cell-cell adhesion contacts, and cell contractility (Pajic-Lijakovic *et al.*, 2023c). Although this parameter has a large impact on cell rearrangement and tissue shaping (Foty *et al.*, 1996), only the static (equilibrium) value of the tissue surface tension has been determined up to now. This included using various measuring techniques like uni-axial compression of cell aggregates between parallel plates, micropipette aspiration of cell aggregates, and the magnetic force tensiometer (Mombach *et al.*, 2005; Marmottant *et al.*, 2009; Stirbat *et al.*, 2013; Guevorkian *et al.*, 2021; Nagle *et al.*, 2022). In addition, the values of dynamic tissue surface tension have not been determined as of now (Pajic-Lijakovic *et al.*, 2023c). Further, a systematic comparative analysis of the static tissue surface tension for the same cellular system using different experimental techniques has not been performed. Hence, we still do not know which technique is the best for such measurements. Further, the values of several important physical parameters for biointerface dynamics, like epithelial-cancer interfacial tension, have not been measured yet as per records (Pajic-Lijakovic *et al.*, 2023b). Therefore, this review focuses on pointing out the role of the mentioned physical parameters in the segregation of the subpopulations and cancer spreading to inspire further biological experiments.

While the surface tension of cancer and epithelial subpopulations represent a product of the homotypic interactions, the epithelial-cancer interfacial tension is a product of the heterotypic interactions along the biointerface (Pajic-Lijakovic *et al.*, 2023b). The inter-relation between these parameters, called the spreading factor of the subpopulation, regulates the wetting/de-wetting process of the subpopulations and the size of the biointerface accompanied by the efficiency of the segregation process (Pajic-Lijakovic *et al.*, 2023a, 2023b). A minimum size of the biointerface is established for the case of complete segregation, while the size of the biointerface increases in the case of partial segregation. An increase in the biointerface area increases the number of epithelial cells that are in direct contact with the cancer cells (Pajic-Lijakovic and Milivojevic, 2023d). Epithelial-cancer interfacial tension exerts work to minimize the the biointerfacial area, due to which the compressive stress within the epithelium is increased. While the epithelial subpopulation undergoes de-wetting (compression), the cancer subpopulation undergoes wetting (extension) (Pajic-Lijakovic *et al.*, 2023a). Extension of cancer subpopulation is directly influenced by epithelial cell rearrangement especially, within the clusters which are under compressive stress (Lucia *et al.*, 2022). In spite of the extensive research examining the epithelial cancer segregation process, we still do not understand how this epithelial cell reorganization influences cancer spreading in light of introduced physical parameters. Therefore, this review is aimed to reveal the cause-consequence relations between (1) altered rearrangement of epithelium under compressive stress, (2) resulting change of the epithelial surface tension and epithelial-cancer interfacial tension, and (3) the influence of these changes on cancer spread. One of the main parameters that control epithelial cell rearrangement is the residual compressive stress accumulated within the epithelial clusters. Further, this stress is influenced by epithelial-cancer interfacial tension and epithelial viscoelasticity caused by collective cell migration (CCM). This complex cell segregation dynamics is discussed in this review using a developed biophysical model to emphasize the interplay among physical parameters.

The Segregation of Co-Cultured Cell Spheroids Due to Epithelial and Cancer-Mesenchymal Subpopulations

The segregation of co-cultured epithelial-cancer spheroids can undergo complete segregation or partial segregation (Kenny *et al.*, 2007; Carey *et al.*, 2013; Devanny *et al.*, 2021). The efficiency of the segregation process depends on the inter-relations among the macroscopic surface tension of the epithelial subpopulation in contact with a liquid medium, the macroscopic surface tension of the cancer subpopulation in contact with a liquid medium, and the interfacial tension between the subpopulations (Pajic-Lijakovic *et al.*, 2023b). These macroscopic quantities account for the cumulative effects of single-cell contributions along the biointerface. While the surface tension along the subpopulation biointerface is a measure of their cohesiveness, the epithelial-cancer interfacial tension reveals the level of adhesiveness between those subpopulations. A detailed discussion on the interrelation among these physical parameters and their impact on the segregation process itself is provided in the next section. The various model systems in the context of the segregation efficiency are now discussed.

The breast epithelial MCF-10A cells in co-cultured cellular systems perform partial or complete segregation depending on cancer-mesenchymal subpopulation surface tension and the epithelial-cancer interfacial tension. The surface tension of MCF-10A cells is very large; for instance,

Nagle *et al.* (2022) reported that the static surface tension of MCF-10A cells determined using a magnetic force tensiometer had a value of $45 \pm 18\ \frac{mN}{m}$. In contrast to this value, the surface tension values of other cellular systems have been listed. These include 1.6 ± 0.6 to $4.0 \pm 1.0\ \frac{mN}{m}$ within 9 days for embryonic neural retina aggregates (Mombach *et al.*, 2005), and $4.5\ \frac{mN}{m}$ for mouse embryonic carcinoma F9 wild-type cell aggregates (Stirbat *et al.*, 2013). Similar to the MCF-10A/MDA-MB-436, the MCF-10A/MDA-MB-231 co-cultured systems also perform complete segregation. However, in co-culture with the MDA-MB-468 cells, epithelial MCF-10A cells perform partial segregation. This result is expected when we take into account that the MDA-MB-468 cells can establish the cadherin-mediated adherens junctions to some extent rather than the MDA-MB-436 cells and the MDA-MB-231 cells. These could be additionally enhanced by the presence of the MCF-10A cells in their surroundings (Kenny *et al.*, 2007). Consequently, it is supposed that the value of surface tension for MDA-MB-468 cells is higher than for MDA-MB-231 cells or MDA-MB-436 cells, although the experimental values for these breast mesenchymal cell types have not been reported yet. The Madin-Darby canine kidney type II (MDCK) epithelial cells, like the keratinocytes (HaCaT), also perform partial segregation in contact with the mesenchymal C2C12 cells (Lucia *et al.*, 2022).

In order to recognize the complex dynamics of cell segregation and cancer spread, it is necessary to provide the biological and physical aspects of these complex phenomena.

The Biological Aspects of the Segregation of Epithelial-Cancer Co-Cultured Cell Spheroids

The epithelial and cancer subpopulation segregation depends primarily on heterotypic cell-cell collisions along the biointerface that are caused by the generation of shear stress (Lucia *et al.*, 2022). These collisions lead to the alignment of the epithelial cytoskeletal bundles, which has an influence on the cell cortical tension and the strength of cell-cell adhesion contacts (Lucia *et al.*, 2022). The strength of E-cadherin-mediated adhesion contacts is influenced by the number of cadherin molecules for a single contact and by inter-chain and intra-chain interactions (Sadhu *et al.*, 2021). Actin has a double function: it provides a protrusive force that pushes the membrane outwards and traction forces that augment the growth of adhesion complexes (Sadhu *et al.*, 2021). Consequently, cell-cell collisions have an impact on the traction forces (Lucia *et al.*, 2022). Cell signaling, caused by collisions, can also influence actomyosin-mediated contractions. Myosin II activation coincides with cadherin downregulation, suggesting a functional relationship between these molecules (Takeichi, 2023). In some cases, cell signaling accompanied by the generated mechanical stress along the biointerface can lead to the epithelial-to-mesenchymal transition (Leal-Orta *et al.*, 2022). Contrary to the epithelial cells, cancer-mesenchymal cells form weak cell-cell adhesion contacts and β1 integrin-mediated cell-matrix focal adhesions (Devanny *et al.*, 2021). Luctuations of the cell membranes are caused by cell-cell collisions which, result in the activation of various membrane receptors to further impact cell segregation (Takeichi, 2023).

Hence, the cell contractility, along with the strength of cell-cell adhesion contacts, affect the tissue surface tension, the epithelial-cancer interfacial tension, and cell stress generation.

Physical Conditions for the Complete and Partial Segregation of Co-Cultured Cell Spheroids

In a recent publication, Pajic-Lijakovic *et al.* (2023a, 2023b) showed that the reorganization of epithelial cells within the clusters surrounded by cancer-mesenchymal cells depends on the interplay among epithelial surface tension, cancer surface tension, and epithelial-cancer interfacial tension in the form of the spreading factors of the subpopulations. These spreading factors represent the difference between adhesion energy and cohesion energy of the subpopulation per unit area of the biointerface. These physical parameters are space-time dependent. They are approximately constant within multicellular mesoscopic domains within the biointerface and change from the domain to the domain. The inter-relation between macroscopic and local parameters can be expressed as: $\gamma_i(\tau) = \frac{1}{\Delta A}\int \gamma_i(\Re,\tau) d^2\Re$. Here, $i \equiv e, c, ce$, $\gamma_i(\tau)$ is the macroscopic value of the parameter and $\gamma_i(\Re,\tau)$ is the local value of the parameter, ΔA is the part of the biointerface area, $\Re = \Re(x,y,z)$ is the coordinate of the biointerface, τ is the long-time variable which corresponds to the time in hours in which CCM occurs (Serra-Picamal *et al.*, 2012; Notbohm *et al.*, 2016; Pajic-Lijakovic and Milivojevic, 2020b). $\gamma_e(\Re,\tau)$ is the local epithelial surface tension, $\gamma_c(\Re,\tau)$ is the local cancer-mesenchymal surface tension, and $\gamma_{ce}(\Re,\tau)$ is the local epithelial-cancer interfacial tension. Further, the biointerface domains are characterized by the local values of these parameters (Pajic-Lijakovic *et al.*, 2023a, 2023b). The local spreading factor of the epithelial subpopulation is $S^e(\Re,\tau) = \gamma_c(\Re,\tau) - (\gamma_e(\Re,\tau) + \gamma_{ce}(\Re,\tau))$, while the local spreading factor of cancer-mesenchymal subpopulation is $S^c(\Re,\tau) = \gamma_e(\Re,\tau) - (\gamma_c(\Re,\tau) + \gamma_{ce}(\Re,\tau))$. The distribution of the epithelial surface tension, cancer surface tension, and epithelial-cancer interfacial tension along the biointerface, as a function of time, depends on the strength of cell-cell adhesion contacts, cell contractility, and local strain caused by cell movement. The description of the main characteristics of epithelial and cancer surface tensions and the interfacial tension between them is shown in Table 1.

The surface tensions of the subpopulations, which are primarily determined by the strength of cell-cell adhesion contact, satisfy the condition that $\gamma_e(\Re,\tau) \gg \gamma_c(\Re,\tau)$ (Table 1). In this condition, the epithelial subpopulation undergoes compression (de-wetting) quantified by the expression $S^e < 0$, while the cancer subpopulation undergoes extension (wetting) quantified by $S^c > 0$

TABLE 1

The main characteristics of epithelial surface tension, cancer surface tension, and the epithelial-cancer interfacial tension

Surface tension of epithelial subpopulation	The surface tension of epithelial subpopulation depends on the strength of E-cadherin-mediated cell-cell adhesion contacts (Devanny *et al.*, 2021; Pajic-Lijakovic *et al.*, 2023c; Pajic-Lijakovic and Milivojevic, 2023e). It is in agreement with the fact that contractility increases the strength of E-cadherin-mediated cell-cell adhesion contacts, and it also generates increased epithelial surface tension. Consequently, the surface tension of active (contractile) cells is bigger than of non-contractile cells (Devanny *et al.*, 2021). An extension of the epithelial surface also generates increased epithelial surface tension (Guevorkian *et al.*, 2021).
Surface tension of cancer-mesenchymal subpopulation	The surface tension of cancer-mesenchymal cells is lower than the surface tension of the epithelial cells because cancer cells form weak cell-cell adhesion contacts, and some of them do not establish cell-cell adhesion contacts (Devanny *et al.*, 2021). Cell contractility induces a repulsion among cells that results in an additional decrease in the surface tension of cancer cells as opposed to the epithelial cells (Devanny *et al.*, 2021).
Epithelial-cancer interfacial tension	The epithelial-cancer interfacial tension is a function of the heterotypic interactions along the biointerface (Pajic-Lijakovic *et al.*, 2023b). These interactions account for physical collisions among cells along the biointerface and biochemical processes such as cell signaling and gene expression.

(Pajic-Lijakovic *et al.*, 2023a). The extent of cancer spread based on the proposed physical parameters is shown in Fig. 1.

The ratio of the local epithelial surface tension and epithelial-cancer interfacial tension or $X(\Re,\tau)=\dfrac{\gamma_e(\Re,\tau)}{\gamma_{ce}(\Re,\tau)}$ satisfies the condition that $X(\Re,\tau)\geq 1$ (Pajic-Lijakovic *et al.*, 2023b). This is in accordance with the fact that epithelial and cancer subpopulations do not establish heterotypic cell-cell adhesion contacts. However, the epithelial-cancer interfacial tension is dependent on cell signaling, which influences the movement of the subpopulations relative to each other (Lee *et al.*, 2012; Leal-Orta *et al.*, 2022). An increase in the ratio $X(\Re,\tau)$ induces a more intensive spread of cancer. Consequently, this ratio represents the efficiency of cancer spreading along the biointerface. For the hypothetical condition such that $\gamma_e(\Re,\tau)\sim\gamma_{ce}(\Re,\tau)$, the cancer spreading factor tends to zero, i.e., $S^c\rightarrow 0$. This means that under this condition, the cancer subpopulation behaves inertly in the segregation process and as a result, the cancer spread is suppressed. In some cases, cancer-mesenchymal cells can establish stronger cell-cell adhesion contacts which increases the cancer surface tension. The example below is related to behavior of MDA-MB-468 cells co-cultured with MCF-10A cells (Devanny *et al.*, 2021). An increase in the cancer surface tension $\gamma_c(\Re,\tau)$ in this case, reduces the compression (expressed as de-wetting) of the epithelial subpopulation and the extension (called wetting) of the cancer subpopulation, which leads to partial segregation. An increase in the epithelial surface tension, contrary to an increase in cancer surface tension, has a quite

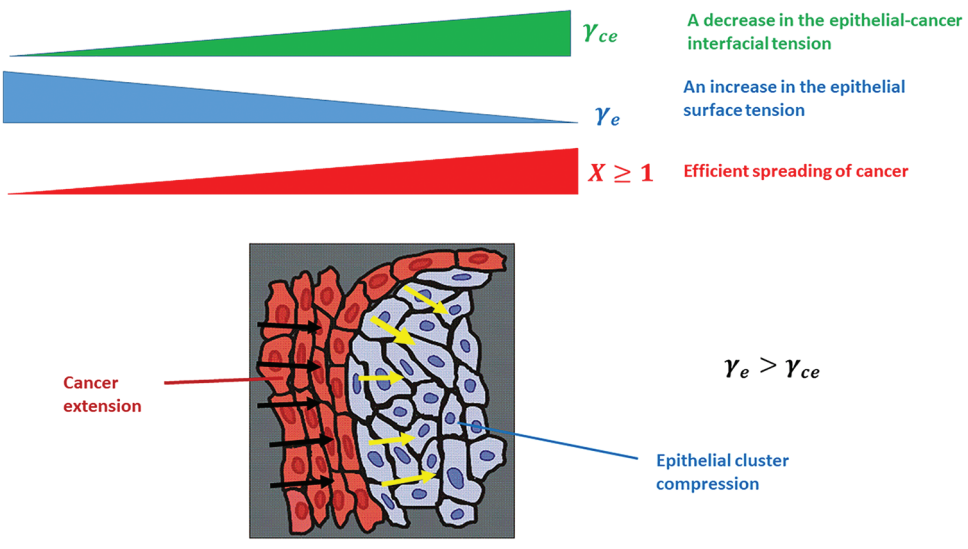

FIGURE 1. The efficiency of cancer spread depends on the epithelial surface tension and epithelial-cancer interfacial tension. Black arrows describe the spread of the cancer subpopulation, while yellow arrows describe the compression of the epithelial cluster. The physical parameters presented in the Fig. 1 are: γ_e is the epithelial surface tension, γ_{ce} is the epithelial-cancer interfacial tension, and X is the efficiency of cancer spreading.

different effect on the rearrangement of both subpopulations. The epithelial surface tension increases the $\gamma_e(\Re,\tau)$, produced by the establishment of a supracellular actin network-like structure along the biointerface (Lucia *et al.*, 2022). This enhances the compression of the epithelium and spread of the cancer subpopulation. An example for this is the keratinocytes (HaCaT) in contact with the mesenchymal C2C12 cells (Lucia *et al.*, 2022). Consequently, increased epithelia-cancer interfacial tension and decreased epithelial surface tension finally result in a reduction of the cancer spread. These two parameters are interrelated, as will be discussed in the next section. The work exerted by the epithelial-cancer interfacial tension induces a variation in the epithelial surface tension, which is in the function of the magnitude of the compressive stress accumulated within the epithelium.

The Compression of Epithelial Clusters Caused by the Epithelial-Cancer Interfacial Tension Work

The macroscopic interfacial tension is given by the expression $\gamma_{ce}(\tau)=\frac{1}{\Delta A}\int\gamma_{ce}(\Re,\tau)d^2\Re$ (where $\Delta A(\tau)$ is a change in the cluster biointerfacial area). The interfacial tension exerts work to minimize the biointerface area between the epithelial clusters and surrounding cancer cells which results in the compression of the clusters. Resistance effects to this compression depend on the viscoelasticity of the epithelial clusters. Consequently, a larger interfacial tension and lower resistance of the epithelial clusters are a prerequisite for the generation of the larger compressive stress within the clusters. The cell compressive stress is in the range from a few hundred Pa, generated within 2D cellular systems (Notbohm *et al.*, 2016), to a few kPa, generated within 3D epithelium in the presence of cancer cells (Kalli and Stylianopoulos, 2018; Cai *et al.*, 2022). This cause-consequence relation can be given in the form of the Young–Laplace equation (Pajic-Lijakovic and Milivojevic, 2022a) below:

$$\gamma_{ce}(\tau)\Delta A(\tau)=\Delta W_V(\tau) \tag{1}$$

where $W_V(\tau)=\int_V\widetilde{\sigma}_{erV}(r,\tau):\widetilde{\epsilon}_{eV}(r,\tau)d^3r$ is the strain energy density caused by collective cell migration during the compression, r is the radial coordinate within the epithelial clusters such that $r\in[0,\Re]$, $\widetilde{\epsilon}_{cV}$ is the local volumetric strain equal to $\widetilde{\epsilon}_{cV}(r,\tau)=\overrightarrow{(\nabla}\cdot\vec{u})\widetilde{I}$, $\vec{u}(r,\tau)$ is the local displacement field generated by CCM, and $\widetilde{\sigma}_{crV}(r,\tau)$ is the cell compressive residual stress. Hence, the extent to which some epithelial clusters can be compressed is governed by the magnitude of the interfacial tension and the viscoelasticity of epithelial clusters, which appears as a resistance factor. The generation of the compressive stress followed by an increase in cell packing density may intensify homotypic epithelial cell-cell interactions (Alert and Trepat, 2020; Pajic-Lijakovic *et al.*, 2023a). Consequently, compressive stress appears as one of the main control parameters that can decrease the strength of E-cadherin-mediated cell-cell adhesion contacts and even suppress the movement of epithelial cells within the clusters (Iyer *et al.*, 2019; Pajic-Lijakovic and Milivojevic, 2019).

Compressive Residual Stress Accumulation Inside the Migrating Epithelial Clusters

A local compressive stress inside the epithelial cell clusters depends on the effects along the biointerface and the CCM within the cell clusters. While the effects along the biointerface influence the isotropic part of compressive residual stress, the CCM within clusters influences the deviatoric part of the compressive residual stress. As a consequence, the compressive residual stress within the epithelial clusters has been expressed as given below (Pajic-Lijakovic *et al.*, 2023b):

$$\widetilde{\sigma}_{erV}(r,\tau)=+\Delta p_{c\to e}\widetilde{I}+\widetilde{\sigma}_{erV}{}^{CCM} \tag{2}$$

where $\widetilde{\sigma}_{erV}$ is the total compressive residual stress inside the epithelium, the first term on the right-hand side is an isotropic part of the residual stress equal to $\Delta p_{c\to e}=-\gamma_{ce}(\tau)\left(\vec{\nabla}\cdot\vec{n}\right)$, while $\widetilde{\sigma}_{erV}{}^{CCM}$ is the deviatoric part of the residual stress caused by CCM, $\vec{n}$ is the normal vector to the biointerface, and $\widetilde{I}$ is the unity tensor. The cell residual stress $\widetilde{\sigma}_{erV}{}^{CCM}$ generated by CCM can be produced based on the proper constitutive model of viscoelasticity (Pajic-Lijakovic, 2021). It is well known that the epithelial subpopulation migrates as inter-connected cell clusters (Serra-Picamal *et al.*, 2012; Nnetu *et al.*, 2012, 2013; Pajic-Lijakovic and Milivojevic, 2020a). The rheological behavior of these cellular systems may be considered similar to behavior of viscoelastic solids as they retain strong cell-cell adhesion contacts. Overall, the main characteristics of migrating epithelial collectives are listed below:

- Free expansion of epithelial monolayers (i.e., the expansion of epithelial monolayers toward the empty space) and swirling motion of confluent epithelial monolayers satisfy the condition that the cell's normal residual stress (extensional and compressive) correlates with the corresponding strain, which points to the elastic nature of the cell residual stress (Serra-Picamal *et al.*, 2012; Notbohm *et al.*, 2016).
- Cell stress can relax under the constant strain caused by (a) the uni-axial compression between parallel plates (Marmottant *et al.*, 2009) and (b) the external extension of cell monolayers (Khalilgharibi *et al.*, 2019). The ability of stress to relax under constant strain represents one of the main characteristics of viscoelastic solids (Pajic-Lijakovic, 2021).
- Cell strain can relax under a constant externally induced compressive stress of cell spheroids between parallel plates (Marmottant *et al.*, 2009). While the stress relaxation time is in a time scale of minutes, the strain relaxation time has a time scale of hours. These observations suggest that strain relaxation happens via CCM, and stress relaxation is caused by the remodeling of cell shapes and cell-cell adhesion

contacts (Marmottant *et al.*, 2009; Barriga and Mayor, 2019; Pajic-Lijakovic *et al.*, 2023c).

- The accumulation of the cell residual stress also falls in the time scale of hours (Marmottant *et al.*, 2009; Pajic-Lijakovic and Milivojevic, 2020b).

Consequently, experimental data on CCM of various epithelial-like model systems suggest the relevance of the Zener stress-strain model. The main characteristics of this constitutive model are: (1) a stress can relax under constant strain conditions, (2) a strain can relax under constant stress, (3) the cell residual stress is elastic. The Zener model is presented below (Pajic-Lijakovic and Milivojevic, 2022a):

$$\widetilde{\sigma}_{eV}{}^{CCM}(r,t,\tau)+\tau_R\dot{\widetilde{\sigma}}_{eV}(r,t,\tau)=E\widetilde{\epsilon}_{eV}(r,\tau)+\eta_V\dot{\widetilde{\epsilon}}_{eV}(r,\tau) \quad (3)$$

where $\widetilde{\sigma}_{eV}{}^{CCM}$ is the normal stress, $\dot{\widetilde{\sigma}}_{eV}=\frac{d\widetilde{\sigma}_{eV}}{dt}$, $\widetilde{\epsilon}_{eV}$ is the volumetric strain, $\dot{\widetilde{\epsilon}}eV=\frac{d\widetilde{\epsilon}_{eV}}{d\tau}$ is the strain rate, E is the Young's elastic modulus equal to $E(\tau)=k_BT_{eff}\langle n_e\rangle$, k_B is Boltzmann constant, T_{eff} is the effective temperature which is equal to $\left(k_BT_{eff}\right)^{1/2}\sim\langle s\rangle$, $\langle s\rangle$ is the average speed among cells within the cluster (Casas-Vazquez and Jou, 2003; Pajic-Lijakovic and Milivojevic, 2021a), $\langle n_e\rangle$ is the average cell packing density inside the cluster equal to: $\langle n_e(\tau)\rangle=\frac{1}{V}\int_V n_e(r,\tau)d^3r$, $n_e(r,\tau)$ is the local cell packing density, η_V is the bulk viscosity, and τ_R is the stress relaxation time.

The stress relaxation under constant strain $\widetilde{\epsilon}_{eV}(r,\tau)$ per single short-time relaxation cycle can be formulated starting from the initial condition $\widetilde{\sigma}_{eV}{}^{CCM}(r,t=0,\tau)=\widetilde{\sigma}_{e0V}$ as (Pajic-Lijakovic and Milivojevic, 2022a):

$$\widetilde{\sigma}_{eV}{}^{CCM}(r,t,\tau)=\widetilde{\sigma}_{e0V}e^{-\frac{t}{\tau_R}}+\widetilde{\sigma}_{erV}{}^{CCM}(r,\tau)\left(1-e^{-\frac{t}{\tau_R}}\right) \quad (4)$$

where $\widetilde{\sigma}_{erV}{}^{CCM}(r,\tau)$ is the normal residual stress equal to $\widetilde{\sigma}_{erV}{}^{CCM}=E\widetilde{\epsilon}_{eV}$. The stress relaxation represents the consequence of the remodeling of cell-cell adhesion contacts under strain caused by the movement of epithelial collectives (Pajic-Lijakovic and Milivojevic, 2019). The corresponding residual stress is purely elastic and is accumulated within the epithelium (Pajic-Lijakovic and Milivojevic, 2020b). The prerequisite of stress accumulation is that epithelial cells retain strong cell-cell adhesion contacts. The compression of the epithelial clusters induces an increase in the cell packing density and a decrease in the average relative speed of cells (Nnetu *et al.*, 2012, 2013; Pajic-Lijakovic and Milivojevic, 2021b). This change and increase in the cell packing density intensify cell-cell interactions and the contact inhibition of locomotion (Zimmermann *et al.*, 2016), which results in the weakening of cell-cell adhesion contacts. This weakening finally leads to energy dissipation (Pajic-Lijakovic and Milivojevic, 2019). When the cell packing density increases sufficiently, epithelial cells undergo the jamming transition for the condition $\langle s\rangle\rightarrow 0$ and consequently $T_{eff}\rightarrow 0$ which means that elastic modulus also tends to zero, i.e., $E\rightarrow 0$ (Pajic-Lijakovic and Milivojevic, 2021b). Under this condition, the cell stress cannot relax, i.e., the stress relaxation time $\tau_R\rightarrow\infty$ while the strain change is dampened and the cell movement corresponds to the sub-diffusion mechanism (Pajic-Lijakovic, 2021). The damped rearrangement of the system constituents near the jamming, which corresponds to the sub-diffusion mechanism, has been described mathematically by the fractional derivatives (Pajic-Lijakovic and Milivojevic, 2022d). Consequently, the compressive stress, in this case, includes dissipative (viscous) and elastic contributions and can be given in the form of the fractional constitutive model (Pajic-Lijakovic, 2021; Pajic-Lijakovic and Milivojevic, 2021b):

$$\widetilde{\sigma}_{erV}{}^{CCM}(r,\tau)=\eta_\alpha D^\alpha(\widetilde{\epsilon}_{eV}) \quad (5)$$

where η_α is the effective bulk modulus, $D^\alpha\tilde{\epsilon}$ are the fractional derivative, and α is the order of fractional derivatives which satisfy the condition $\alpha<0.5$. The fractional derivative corresponds to the Caputo's form expressed as:

$$D^\alpha\tilde{\epsilon}=\frac{1}{\Gamma(1-\alpha)}\frac{d}{d\tau}\int_0^\tau\frac{\widetilde{\epsilon}(r,\tau')}{(\tau-\tau')^\alpha}d\tau'$$

where $\Gamma(1-\alpha)$ is a gamma function (Podlubny, 1999). Depending on the magnitude of the accumulated compressive stress, epithelial cells respond in different ways by changing the state of viscoelasticity, mode of CCM (i.e., cell swirling motion, cell random motion, or cell jamming state transition), and, the surface characteristics described by the epithelial surface tension. The migration modes will be discussed in the next section and expressed by the corresponding fluxes.

Cell Rearrangement within the Epithelial Clusters Caused by the Compressive Residual Stress

The response of epithelial cell clusters depends on the magnitude of the accumulated compressive cell residual stress:

- When the compressive stress is low enough (i.e., less than a few hundred Pa) and is not able to induce a contact inhibition of locomotion, epithelial cells can establish supracellular actin cable aligned circumferentially in the form of a network along the biointerface which was shown schematically in Fig. 2a (Röper, 2013). This supracellular actin network protects epithelial clusters against the cell shear stress generated along the biointerface as a consequence of CCM (Lucia *et al.*, 2022).

This network induces the stiffening of epithelial cells that are in direct contact with cancer cells along the biointerface and as a result, stabilizes the biointerface (Lucia *et al.*, 2022). The contractility of this actin network can stimulate the cell swirling motion (Lucia *et al.*, 2022). This cell swirling motion causes an oscillatory change of the speed, velocity, and the compressive stress of cells (Notbohm *et al.*, 2016; Pajic-Lijakovic and Milivojevic, 2022c). Further, Peyret *et al.* (2019) pointed out at the cell swirling motion within the confluent HaCaT cell monolayers. Intensive cell-cell interactions cause an oscillatory mechanotransduction of yes-associated protein (YAP) in cells. The cell swirling motion depends on (1) the strength of cell-cell adhesion contacts and cell packing density, which influence the

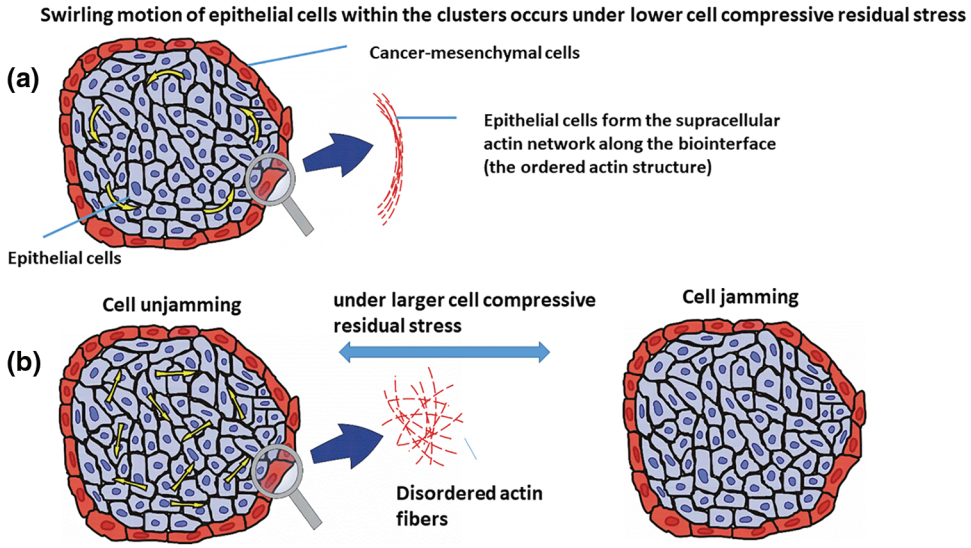

FIGURE 2. Epithelial cell rearrangement within the cell cluster under: (a) lower compressive residual stress, i.e., the cell swirling motion, and (b) larger compressive residual stress, which are the cell jamming/unjamming transitions. The velocity of epithelial cells within the cluster is presented by yellow arrows. The epithelial cells are shown in the blue color, surrounded by the cancer cells in red.

velocity correlation length, and (2) the size of the confinement (Doxzen *et al.*, 2013; Chen *et al.*, 2018; Wang and Xu, 2023). Various modes of the cell swirling motion have been considered depending on the interrelation between velocity correlation length and the size of the confinement (Wang and Xu, 2023). The overall rotation of the cellular system is characterized by a higher value of the velocity correlation length, while the appearance of local swirls points to more random cell movement characterized by a lower value of the velocity correlation length (Wang and Xu, 2023). An increased size of the confinement causes the induction of the local swirls for higher values of the velocity correlation length.

This oscillatory cell velocity change has been studied on the principles of mechanical standing waves in few reports (Notbohm *et al.*, 2016; Pajic-Lijakovic and Milivojevic, 2022c). The generated oscillations represent a part of the low Reynolds turbulence that has been generated during a flow of different soft matter systems (Pajic-Lijakovic and Milivojevic, 2022c). The low Reynolds turbulence is connected with long-time inertial effects, which appears due to system viscoelasticity (Pajic-Lijakovic and Milivojevic, 2020b, 2022c).

- When compressive stress is large enough (i.e., of about one kPa) and able to suppress the cell movement within the clusters, successive jamming, and unjamming transitions can be expected as given in Fig. 2b (Pajic-Lijakovic and Milivojevic, 2021b). Epithelial jamming has been recognized in various cellular systems such as (1) the development of a chicken embryo (Spurlin *et al.*, 2019), (2) the gastrulation of the developing fruit fly embryo (Bi *et al.*, 2016; Atia *et al.*, 2018, 2021), (3) the elongation of the body axis during the zebrafish development (Mongera *et al.*, 2018) and many others.

The development of these transitions includes several steps: (a) large compressive stress, accompanied by an increase in the cell packing density, intensifies the contact inhibition of locomotion (Zimmermann *et al.*, 2016; Roycroft and Mayor, 2016; Alert and Trepat, 2020), (b) the contact inhibition of locomotion reduces the cell movement and induces weakening of cell-cell adhesion contacts and consequently, cells undergo the jamming state transition such that the average cell speed tends to zero (Pajic-Lijakovic and Milivojevic, 2021b), i.e., $\langle s \rangle \rightarrow 0$, (c) the weakening of cell-cell adhesion contacts induces an energy dissipation which reduces the magnitude of the accumulated compressive stress and also causes a decrease in the epithelial surface tension, (d) this decrease in the compressive stress results in the cell unjamming and consequently, cells start random movement within the clusters, (e) the random migration induces an accumulation of the compressive stress $\widetilde{\sigma}_{erV}{}^{CCM}(r,\tau)$ again. These cell jamming/unjamming transitions are responsible for an oscillatory change of cell speed, cell velocity, corresponding strain, and cell compressive residual stress in this case (Pajic-Lijakovic and Milivojevic, 2020b).

Further, Lucia *et al.* (2022) studied the rearrangement of (1) Madin-Darby canine kidney type II (MDCK) epithelial cell clusters surrounded by mesenchymal C2C12 cells (i.e., immortalized mouse myoblast cell line) and (2) keratinocytes (HaCaT) epithelial cell clusters surrounded by mesenchymal C2C12 cells during the segregation process within spheroids. While both epithelial cell lines formed clusters surrounded by the same mesenchymal cells, their migration modes within the clusters were quite different, caused by compressive stress. The cell compressive stress was larger within the MDCK cell clusters in comparison to the HaCaT cell clusters. This is in agreement with the fact that the HaCaT cells provide larger resistance effects to the compression compared to MDCK cells. The MDCK cells performed random movement within the clusters, caused by the weakening of cell-cell adhesion contacts, while the HaCaT cells retained the strength of cell-cell adhesion contacts and underwent the swirling motion (Lucia *et al.*, 2022). Additionally, the HaCaT cells established a

supracellular actin structure along the biointerface with the mesenchymal cells. The actin fibers were aligned circumferentially, forming network-like structures along the biointerface (Lucia *et al.*, 2022). Circumferential actomyosin cables are likely to result in a centripetal tension which is responsible for the swirling motion of cells (Röper, 2013). These cables also frequently appear along the biointerface between epithelium and adjust tissue and represent local force generators (Röper, 2013). The supracellular actin cable is a hallmark of non-adhesive dynamics along the biointerface and can be established when cells retain their cell-cell adhesion contacts (Wei *et al.*, 2020). The appearance of the supracellular actin network along the biointerface causes an increase in the epithelial surface tension.

The MDCK cells can form the supracellular actin cable during 2D non-adhesive gap closure (Wei *et al.*, 2020). However, within the 3D MDCK cell clusters, the cells successively lost their cell-cell adhesion contacts and recovered them through the jamming/unjamming transitions in an oscillatory manner.

Overall, the common features of both modes of cell movement within the epithelial clusters, i.e., (1) the cell swirling motion and (2) the successive jamming/unjamming transitions are the oscillatory change in the cell velocity will be examined using a developed biophysical model in the next section.

The Biophysical Model

The biophysical model has been developed to describe the rearrangement of epithelial cells surrounded by cancer cells by focusing on the dynamics along the biointerface (Pajic-Lijakovic *et al.*, 2023b). The rearrangement of epithelial cells within clusters is characterized by long-time changes in cell packing density and relative cell velocity between epithelial and cancer subpopulations (Pajic-Lijakovic *et al.*, 2023a). The cumulative effects of epithelial-cancer interactions along the biointerface are characterized by epithelial-cancer interfacial tension, which causes the accumulation of compressive stress within the epithelial clusters. The accumulation of compressive stress has an effect on the epithelial surface tension that influences the rearrangement of epithelial cells and the spreading of cancer cells. The main goal of this modeling consideration is to describe the role of physical parameters in the segregation of epithelial-cancer co-cultured systems by observing the oscillatory trend of collective cell migration (Serra-Picamal *et al.*, 2012; Notbohm *et al.*, 2016; Pajic-Lijakovic and Milivojevic, 2022a). The model is created using the system of mass and force balances for the epithelial subpopulation. Additional calculations are not possible when we keep in mind that some parameters have not been measured yet, such as the epithelial-cancer interfacial tension, while others such as the epithelial surface tension have been measured under simplified conditions (i.e., in the form of the static epithelial surface tension). A detailed description of forces that influence the oscillatory change of the relative velocity between epithelial and cancer subpopulations will be listed first. After that, we will discuss the fluxes accompanied by physical mechanisms that influence long-time change of the epithelial packing density within the clusters.

The volumetric force balance accounts for the interplay among the inertial force $\vec{F}_{inert}{}^{e}$, mixing force $\vec{F}_{m}{}^{e}$, interfacial tension force $n_e\vec{F}_{it}{}^{e}$, viscoelastic force $\vec{F}_{Tve}{}^{c-e}$, and frictional force $n_e\vec{F}_{FR}{}^{e}$. While the mixing force and interfacial tension force drive epithelial compression, the viscoelastic force and frictional force act to reduce the cell movement (Pajic-Lijakovic *et al.*, 2023b). The expression of each of these forces accompanied by the role that they play in the cell rearrangement in epithelial clusters is shown in Table 2.

The force balance for the de-wetting of epithelial subpopulation along the biointerface has been given by a modified model put forth by Pajic-Lijakovic *et al.* (2023b).

$$\langle m\rangle_e n_e(r,\tau)\frac{D\vec{v}_{Re}(r,\tau)}{D\tau}=\vec{F}_{m}{}^{e}+n_e\vec{F}_{it}{}^{e}-\vec{F}_{Tve}-n_e\vec{F}_{FR}{}^{e} \quad (6)$$

The oscillatory change of the relative velocity of epithelial cells within the clusters is a product of the competition between the mixing force and interfacial tension force against the friction force and viscoelastic force. Cell movement increases the compressive stress followed by epithelial packing density increase within the clusters and overall affects the cell-cell interactions. There are different mechanisms for the oscillatory change of the epithelial velocity $\vec{v}_{Re}$, where the corresponding strain and cell residual stress exist depending on the magnitude of accumulated compressive stress:

- If the stress is large enough to reduce cell migration, the successive cell jamming/unjamming transitions (primarily caused by two processes such as the contact inhibition of locomotion and remodeling of E-cadherin adhesion contacts) are responsible for the oscillatory change of the epithelial velocity.
- If the stress is lower, cells retain their adhesion contacts and perform the swirling motion caused by contractions of the already established supracellular actin network distributed circumferentially along the biointerface. Cells inside a swirl start the rotational movement: (1) successive radial inward and outward flows and (2) azimuthal shear flow (Pajic-Lijakovic and Milivojevic, 2020b, 2022c). The radial inward flow and outward flow, caused by the action of centrifugal force, accompanied by the mixing force and interfacial tension force against viscoelastic force and frictional force, induce the 3D mechanical standing waves (Pajic-Lijakovic and Milivojevic, 2022c). The centrifugal force represents a part of the inertial force generated during the swirling motion of cells. The standing waves have also been obtained during the rearrangement of confluent epithelial MDCK monolayers (Notbohm *et al.*, 2016). Consequently, in this case, an oscillatory change of cell velocity is induced by the action of the centrifugal force

In addition to the force balance, it is also required to calculate the mass balance for the movement of epithelial cells within the clusters. The mass balance of the epithelial subpopulation can be expressed as:

TABLE 2

Forces that influence epithelial cell migration within cell clusters

Volumetric forces	Role	Reference
Inertial force $\vec{F}_{inert}{}^{e} = \langle m \rangle_e n_e(r,\tau)\frac{D\vec{v}_{Re}(r,\tau)}{D\tau}$ where $\vec{v}_{Re}$ is the velocity of epithelial cells relative to the velocity of cancer cells and $\frac{D\vec{v}_{Re}(r,\tau)}{D\tau}$ is the material derivative equal to $\frac{D\vec{v}_{Re}}{D\tau} = \frac{\partial \vec{v}_{Re}}{\partial \tau} + \left(\vec{v}_{Re}\cdot\vec{\nabla}\right)\vec{v}_{Re}$ (Bird *et al.*, 1960).	The long-time inertial effects are responsible for the oscillatory change of the epithelial velocity. The phenomenon represents a part of low Reynolds turbulence.	Pajic-Lijakovic *et al.* (2023a), Pajic-Lijakovic and Milivojevic (2020b)
Mixing force $\vec{F}_m{}^{e} = \frac{1}{l_e}\vec{\nabla}_s g_{ce}{}^{e}$ where $g_{ce}{}^{e}$ is Gibbs free energy of interactions between cells along the biointerface and is equal to $g_{ce}^{e} = \gamma_{ce} - \gamma_e$	Mixing effects between the subpopulations along the biointerface are expressed in the form of the gradient of the Gibbs free energy per biointerfacial area. Thermodynamics activity of the epithelial cells along the biointerface can be expressed as $a_s^e = e^{-\frac{g_{ce}^{e}\Delta A(\Re,\tau)}{k_B T_{eff}}}$ (where $\Delta A(\Re,\tau)$ is the local change of biointerface area generated by epithelial-cancer interactions.	Pajic-Lijakovic *et al.* (2023b)
Interfacial tension force $n_e\vec{F}_{it}{}^{e} = n_e S^e \vec{u}$ where S^e is the spreading factor of epithelial cells, which is equal to $S^e = \gamma_c - (\gamma_e + \gamma_{ce})$	The spreading of epithelial cells relative to the surrounding cancer depends on the magnitude of the spreading coefficient. When the epithelial spreading satisfies the condition of $S^e < 0$, epithelial cells undergo compression (de-wetting).	Pajic-Lijakovic *et al.* (2023a, 2023b)
Viscoelastic force $\vec{F}_{Tve} = \vec{\nabla}(\tilde{\sigma}_{erV})$	Viscoelastic forces are caused by the inhomogeneous distribution of the compressive stress within the epithelial clusters. This force reduces epithelial cell movement.	Murray *et al.* (1988), Pajic-Lijakovic et al. (2023a)
Frictional force $n_e\vec{F}_{FR}{}^{e} = n_e\xi_e\vec{v}_{Re}$	Frictional effects appear as a reduction factor of the movement of epithelial cells along the biointerface and influence the rearrangement of epithelial cells within the clusters.	Pajic-Lijakovic *et al.* (2023b)

Note: Key: $\langle m \rangle_e$ is the average mass of a single epithelial cell, $\vec{\nabla}_s$ is the surface gradient, $\vec{\nabla}$ is the volumetric gradient, l_e is the size of a single epithelial cell, $\gamma_{ce}(\Re,\tau)$ is the local epithelial-cancer interfacial tension, $\gamma_c(\Re,\tau)$ is the local cancer surface tension, $\gamma_e(\Re,\tau)$ is the local epithelial surface tension, ξ_e is the frictional coefficient caused by epithelial cluster movement within a cancer subpopulation, and $\vec{u}$ is the displacement field of epithelial cells relative to cancer caused by collective cell migration CCM.

$$\frac{\partial n_e(r,\tau)}{\partial \tau} = \vec{\nabla}\cdot\left(\overrightarrow{J_m}^{e} + \vec{J}_{Me}\right) \tag{7}$$

where the flux $\vec{J}_m{}^{e}$ describes the mode of cell movement that is in function of the magnitude of the compressive stress. For a lower cell compressive residual stress and cell packing density, which corresponds to a confluent state, the flux $\vec{J}_m{}^{e}$ corresponds to the convective flux, i.e., $\vec{J}_m{}^{e} \equiv \vec{J}_{conv}{}^{e}$ (where $\vec{J}_{conv}{}^{e} = n_e\vec{v}_{Re}$) (Pajic-Lijakovic and Milivojevic, 2021b). These conditions are satisfied for the established cell swirling motion within the clusters. For a larger cell

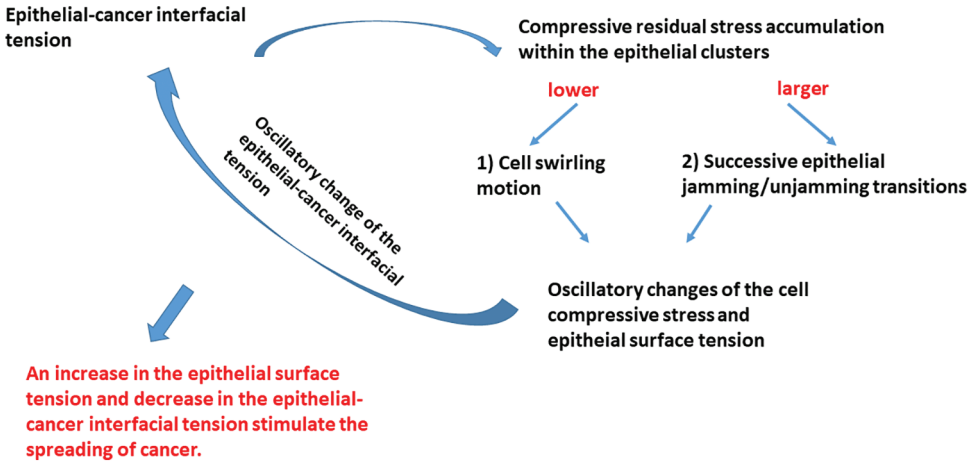

FIGURE 3. Schematic presentation of the impact that physical parameters have on the spreading of cancer cells.

compressive stress and cell packing density larger than the one for the confluent state, the flux $\vec{J}_m{}^e$ corresponds to the conductive flux, i.e., $\vec{J}_m{}^e \equiv \vec{J}_{cond}{}^e$, expressed as $\vec{J}_{cond} = -D_{eff}\vec{\nabla}n_e$ (where D_{eff} is the effective diffusion coefficient). This state corresponds to the random movement of loosely connected cells. When the compressive stress is large enough to induce the cell jamming state transition, the flux $\vec{J}_m{}^e$ corresponds to the damped conductive flux which corresponds to the sub-diffusion mechanism $\vec{J}_m{}^e \equiv \vec{J}_{d\ cond}{}^e$ and mass balance equation should be transformed to $D^\alpha n_e = \vec{\nabla}\cdot(\vec{J}_{d\ cond}{}^e + \vec{J}_{Me})$ (where $D^\alpha n_e$ is the fractional derivative expressed as $D^\alpha(n) = \frac{d^\alpha \boldsymbol{n}}{d\tau^\alpha}$, and α is the order of fractional derivative, i.e., the damping coefficient of the system structural changes which satisfy the condition $\alpha \leq 1/2$, the flux $\vec{J}_{d\ cond}{}^e = -D_\alpha\vec{\nabla}n_e$, and D_α is the damped-conductive diffusion coefficient which has the unit $\frac{m^2}{s^\alpha}$) (Pajic-Lijakovic and Milivojevic, 2021b).

The flux $\vec{J}_{Me}$ is the Marangoni flux which depends on the gradient of the epithelial-cancer interfacial tension and has been expressed as: $_{Me} = k_{Me}n_e\vec{\nabla}s\gamma_{ce}$ (where k_{Me} is the measure of the mobility of epithelial cells along the biointerface) (Pajic-Lijakovic and Milivojevic, 2022b). The Marangoni flux directs the movement of cells from the region of lower interfacial tension toward the region of larger interfacial tension. The Marangoni flux also exists in various soft matter systems, primarily as a consequence of the temperature distribution (Karbalaei *et al.*, 2016).

The Impact of Epithelial Cell Rearrangement within the Clusters on the Spread of Cancer

The main physical parameters related to the reorganization of epithelial cells within the clusters, which are responsible for the spreading of cancer cells, include the epithelial surface tension, epithelial-cancer interfacial tension, and compressive residual stress accumulation within the epithelial clusters. The interrelations between these parameters and the physical mechanisms that drive the spread of cancer are shown in Fig. 3.

The compressive residual stress accumulated within the epithelial clusters is one of the main parameters responsible for the rearrangement of epithelial cells within clusters. Two modes of the movement of epithelial cells are possible: (1) cell swirling motion occurring under lower values of the compressive stress and (2) cell jamming/unjamming transitions occurring under larger values of the compressive stress. Epithelial cells within the clusters can establish the supracellular actin network along the biointerface when two conditions are satisfied: (1) when epithelial cells do not establish heterotypic adhesion contacts and (2) the accumulated compressive stress is not high enough to induce the cell jamming. The establishment of the supracellular actin network along the biointerface results in an increase in the epithelial surface tension.

The common characteristics in both scenarios are oscillatory changes of the cell velocity, corresponding strain, and cell compressive stress. These oscillatory trends of the cell reorganization caused by cell movement, which represents an example of the low-Reynolds turbulence, have been obtained within various model systems. These include (1) the segregation of epithelial-cancer spheroids (Lucia *et al.*, 2022), (2) the rearrangement of confluent epithelial monolayers (Notbohm *et al.*, 2016), (3) free extension of epithelial monolayers (Serra-Picamal *et al.*, 2012), (4) cell aggregate rounding after uni-axial compression between parallel plates (Mombach *et al.*, 2005; Pajic-Lijakovic and Milivojevic, 2022a), and (5) fusion of two cell aggregates (Grosser *et al.*, 2021; Pajic-Lijakovic and Milivojevic, 2022a, 2023e). The oscillatory change of the cell stress causes the oscillatory change of the cell packing density (Trepat *et al.*, 2009), which leads to an oscillatory change of the strength of E-cadherin mediated cell-cell adhesion contacts along the biointerface followed by a change in the epithelial surface tension (Pajic-Lijakovic *et al.*, 2023b). The compressive stress oscillatory change also induces an oscillatory change of epithelial-cancer interfacial tension, as expressed by Eq. (1).

This oscillatory rearrangement of epithelial cells within the clusters results in oscillatory extension of the cancer subpopulation. The inter-relation between the efficiency of the segregation which primarily depends on the dynamics along the biointerface and the spreading of cancer cells is presented in Fig. 4.

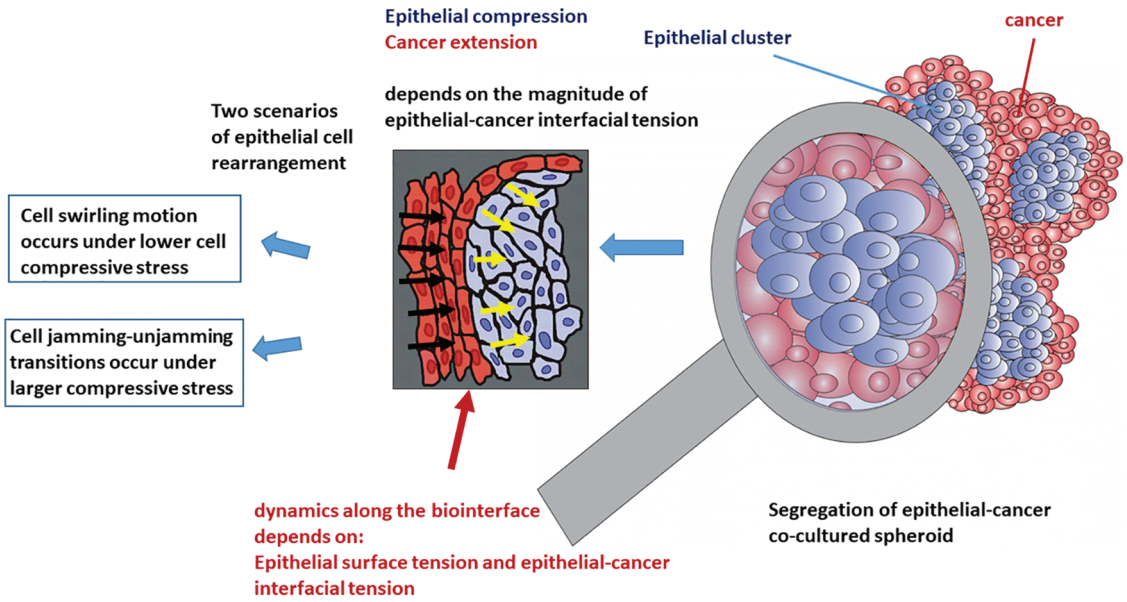

FIGURE 4. The efficiency of cell segregation and spreading of cancer depends on the dynamics along the epithelial-cancer biointerface.

The increase in the epithelial surface tension and decrease in the epithelial-cancer interfacial tension enhances the spreading of cancer. Based on all these findings, we can conclude that the spreading of cancer is more intensive when swirling motions of cells occur within the epithelial clusters under lower values of compressive stress.

Conclusion

This theoretical review is focused on the clarification of the role that physical parameters have on the segregation of co-cultured epithelial-cancer spheroids by highlighting the importance of the dynamics along the epithelial-cancer biointerface. These include the epithelial surface tension, the epithelial-cancer interfacial tension, and the generated stress caused by CCM, The main results were obtained by integrating the biophysical model with biological and bio-mechanical experiments from the literature, and we can summarize them as follows:

- The function of interfacial tension on the reduction of the biointerfacial area against the volumetric resistant effects of epithelial clusters is responsible for the generation of the compressive stress within the clusters. The volumetric resistance effects are connected with the viscoelasticity of epithelial clusters caused by CCM.
- The compressive stress, which depends on the system viscoelasticity, appears as one of the main control parameters, which influence the rearrangement of epithelial cells in the context of the mode of cell movement and the strength of cell-cell adhesion contacts. Lower compressive stress stimulates the epithelial cells to organize the supracellular actin network along the biointerface. This represents one of the factors responsible for the swirling motion of cells, where the cells retain the strong E-cadherin mediated cell-cell adhesion contacts. This actin network protects cells against the shear stress generated along the biointerface. However, larger compressive stress can also induce the cell jamming state transition. The cell jamming causes the weakening of cell-cell adhesion contacts, which causes a dissipation of energy and a decrease in the compressive stress and as a result cause the cell unjamming state transition. The swirling motion of cells, a characteristic of the lower compressive stress, and successive cell jamming/unjamming transition, a feature of the larger stress, cause an oscillatory change of the cell velocity, compressive stress, epithelial-cancer interfacial tension, and epithelial surface tension.
- Epithelial surface tension accompanied by the epithelial-cancer interfacial tension influences the compression (de-wetting) of the epithelial subpopulation and the extension (de-wetting) of the cancer subpopulation. The spreading of cancer is enhanced at lower values of the interfacial tension and larger values of the epithelial surface tension.

It is necessary to provide more experiments to measure an oscillatory change of these physical parameters and correlate the change of (1) the epithelial-cancer interfacial tension with the cell compressive residual stress, and (2) the epithelial-cancer interfacial tension with the epithelial surface tension.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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