Physics of cancer spreading through epithelium

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 and,

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

Ivana Pajic-Lijakovic, is a research professor at the University of Belgrade, focuses her research on biological physics, biophysics, biorheology, and biomechanics. Her work has been published in top international peer-review journals and one book entitled: ‘’Viscoelasticity and Collective Cell Migration: An Interdisciplinary Perspective Across Levels of Organization’’, (Elsevier), 2021. Her research covers topics such as viscoelasticity and collective cell migration, low Reynolds turbulence, multi scale nature of cell rearrangement, and effects along the biointerface between adjust tissues and between tissue and extracellular matrix. She is a subject editor for “Facets”, Canadian Science Publishing.

Milan Milivojevic is currently associate professor at the University of Belgrade. His research focuses on separation techniques, catalysis, polymer science, and biophysics applied in fields of chemical, environmental, pharmaceutical, biotechnological and biomedical engineering.

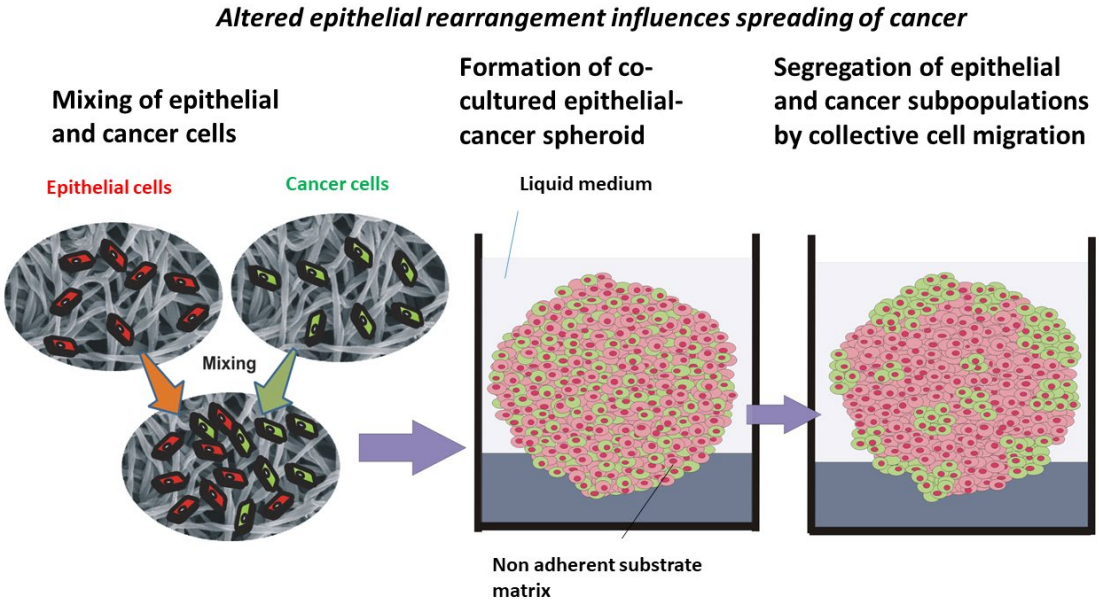
Peter V.E. McClintock is Research Professor of Physics (emeritus) at Lancaster University. After his education at Queen's University Belfast (BSc) and Oxford University (DPhil), followed by postdoctoral research at Duke University, he came to Lancaster in 1968. He was a SERC/EPSRC Senior Fellow, 1990–1995 and Head of Department 1997– 2003). He is Editor-in-Chief of *Fluctuation and Noise Letters* and CEO of Lancaster Helium Ltd. He has published 7 books and more than 500 papers. His research interests include low temperature physics, superfluidity, quantum turbulence, nonlinear dynamics, and the applications of nonlinear dynamics to biomedical problems.

Physics of cancer spreading through epithelium

Many cancers spread across the epithelium and physics is needed to describe the process. Cell dynamics at the normal epithelium/cancer biointerface play a crucial role in the progression of the disease. An altered arrangement of epithelial cells significantly impacts progression, so that a detailed understanding of the biological and physical mechanisms governing the interface dynamics provides a promising basis for cancer prevention. The biological mechanisms involve cell signalling and gene expression, while the physical mechanisms are associated with the interplay between physical properties such as the epithelium-cancer interfacial tension, the epithelial surface tension, and the compressive stress within the epithelium. Despite extensive *in vitro* research on epithelium/cancer co-cultured cell systems, the influence of these physical parameters on cell reorganization remains incomplete. This review provides an account of what is known about the physical processes involved in cell reorganization within epithelium/cancer cell clusters and the dissemination of cancer within co-cultured systems.

Keywords: collective cell migration; cell compressive residual stress; viscoelasticity; epithelial surface tension; epithelium/cancer interfacial tension; supracellular actin network

Graphical abstract



Glossary of terms

Adherens junctions (AJs): a type of cell-cell adhesion contact, which play an important rule for migrating cell collectives. Cells use AJs for mechanical coupling and as an important source of signalling that coordinates collective behaviour.

Amoeboid cells: migrate by sliding through narrow channels between other cells without forming cell-cell and cell-matrix adhesion contacts.

Apicobasal polarity of epithelial cells: a distinct form of cell polarity found in epithelial cells, characterized by the presence of a specialized apical membrane oriented towards the exterior of the body or the lumen of internal cavities, and a specialized basolateral membrane located on the opposite side, away from the lumen. Adherens junction complexes frequently serve as physical barriers that separate apical and basal domains.

Cell jamming state transition: the cell transition from the active (contractile) to the passive (non-contractile) state caused by an accumulation of cell compressive stress, leading to an increase in cell packing density. This process influences the viscoelasticity and surface characteristics of multicellular systems.

Cadherins: transmembrane glycoproteins containing an extracellular domain that mediates cell-cell adhesion via homophilic or heterophilic interaction, and an intracellular domain that controls signaling cascades involved in a variety of cellular processes, including polarity, gene expression, etc.

Constitutive models: stress-strain relationships.

Epithelial cells: exhibit cuboidal shape, limited mobility, apical-basal polarity, and strong E-cadherin-mediated cell-cell adhesions

Epithelium/cancer interfacial tension: a tension at the epithelium/cancer biointerface along the tangential direction.

Epithelial-to-mesenchymal transition: a process by which cells undergo a transition from an epithelial-like phenotype to a mesenchymal-like phenotype. This process influences the viscoelasticity of multicellular systems.

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

Mesenchymal cells: exhibit an elongated shape, increased migratory ability, front-rear cell polarity, and weak N-cadherin-mediated cell-cell adhesion.

Normal stress: a stress along the direction normal to the surface. If positive (in the outward direction normal to the surface), the stress is tensional. If negative (in the opposite direction), the stress is compressive.

Residual stress: stress that remains in a system in the absence of external forces. It can be normal or shear. The residual stress can be either dissipative (viscous) or elastic.

Segregation: self-organization of epithelial and cancer subpopulations within co-cultured spheroids such that epithelial cells migrate towards the spheroid core region, while cancer cells migrate towards the spheroid surface region.

Shear strain: deformation of a system in response to mechanical stress. applied tangentially.

Shear stress: stress that acts coplanar with the cross section of a system.

Solid stress: compressive stress caused by cell growth in a confined environment such as the spheroid core region. In contrast to pressure this stress includes isotropic and deviatoric components.

Spheroids: cell aggregates of size an order of magnitude or larger than the size of a single cell.

Stress relaxation: an observed, time-dependent, decrease in the stress of a system from an initial value towards the residual stress under constant strain, caused by structural ordering of the system under strain.

Strain rate: the change in strain per unit of time.

Tissue surface tension (epithelial or cancer): a tension at the external surface of the tissue, along the tangential direction.

Viscoelasticity: a property of material, which defines the elastic and viscous characteristics of the system when it undergoes deformation.

Viscoelastic liquids: liquids with viscoelastic behaviour satisfying the following conditions: (1) stress can relax under a constant strain rate, in some cases; (2) the strain rate can relax under a constant stress to a constant value, in some cases; and (3) the strain itself cannot relax.

Viscoelastic solids: solids with viscoelastic behaviour satisfying the following conditions: (1) stress can relax under a constant strain, in some cases; and (2) strain can relax under a constant stress, in other cases.

Volumetric strain: a strain that produces solely a change in volume. In linear analysis (small strains), it is computed as the trace of the strain tensor.

1. Introduction

Epithelial cancer is a highly lethal form of the disease that has significant impact on a global scale. The epithelium consists of either single layers or multiple layers (known

as stratified epithelium) that serve as a protective covering for the surfaces of organs and glands, as well as lining the cavities and hollow organs throughout the body. By targeting the early stages of the disease, there is potential for substantial improvements in the survival rates of cancer patients. The early stage of cancer invasion involves the spread of metastatic cancer cells from a primary tumour to the surrounding healthy epithelium [1-5]. Millar et al. [2] demonstrated that the migration of cancer cells through normal epithelium serves as an indicator of a pre-invasive state in lung cancer. The establishment and maintenance of precisely organized tissues rely on sharp biointerfaces between different cell populations, as highlighted by Batlle and Wilkinson [6]. The interaction between cancer cells and the epithelium leads to reorganization within the epithelium, which in turn influences the spreading of cancer cells [7,8]. As we will show, these processes are governed by physics as well as by biology.

Multicellular systems, such as three-dimensional (3D) cell spheroids and organoids, are widely utilized *in vitro* for studying fundamental processes in human pathophysiology and disease. Spheroids consist of cell aggregates grown in suspension or embedded in a 3D matrix through specialized culture techniques [9]. Organoids have more complex structures. Tumour organoids are self-organized collections of diverse cell types obtained from patient samples, replicating the essential histopathological, genetic, and phenotypic features of the original tumour [10]. However, to comprehend the segregation of distinct cells with epithelial and cancer properties within complex tumour organoids, it is imperative first to gain insights into the processes that result in cell segregation (**Glossary**), which can be achieved through studies of collective cell migration within simpler tumour spheroids. Possible complications arising from cell division may be disregarded, as this process unfolds over longer durations, typically spanning days. The separation of epithelial and cancer subpopulations in co-cultured

epithelial-cancer spheroids in a liquid medium provides an opportunity to assess the influence of epithelial cell self-organization on cancer spread. Such investigations of the biological and physical processes that drive the segregation of distinct cell types in a laboratory setting represent a crucial initial step in comprehending the intricate mechanisms governing cell segregation in a living organism. While biological aspects of the segregation are widely discussed, physical aspects are only starting to be elucidated. Of particular interest is the physics involved in the self-organization of the epithelial and cancer subpopulations within co-cultured spheroids, where the significance of various physical parameters and their inter-relationship can be described in terms of a mathematical model.

The majority of cancer cells in the primary tumour maintain their epithelial-like phenotype, while the metastatic subpopulation undergoes the epithelial-to-mesenchymal transition (EMT) [11,12] (**Glossary**). The EMT is a reversible process whereby cancer cells in the primary tumour change from an epithelial-like to a mesenchymal-like phenotype or *vice versa*. This transition is triggered by biological and physical factors such as cell signalling and the generation of mechanical stress within cells [13,14]. During the EMT process, cells experience several changes including a loss of apicobasal polarity, acquisition of mesenchymal characteristics, and alterations in cell-cell adhesion contacts and viscoelasticity [14]. Epithelial-like cells are characterized by their cuboidal shape, limited mobility, apicobasal polarity (**Glossary**), and strong E-cadherin-mediated cell-cell adhesions. On the other hand, mesenchymal-like cells exhibit an elongated shape, increased migratory ability, front-rear cell polarity, and weak N-cadherin-mediated cell-cell adhesion [12]. The EMT has been identified as a significant feature of breast cancer metastasis [11]. Nevertheless, it is important to note that this transition is not consistently realized in full, as cells within tumour spheroids exhibit varying levels of mesenchymal

traits. Healthy epithelial cells can also undergo the EMT caused by cell signalling, generation of cell mechanical stress, and the presence of cancerous mesenchymal-like cells in their vicinity. While epithelial-like cells can migrate collectively through strong E-cadherin-mediated cell-cell adhesion even without forming cell-matrix adhesion contacts, mesenchymal cells require the establishment of focal adhesions (i.e., cell-matrix adhesion contacts labeled as FAs) for migration. In cases where mesenchymal-like cells fail to establish FAs, they undergo a transition known as the mesenchymal-to-amoeboid transition (MAT) [12,15]. The amoeboid phenotype is a characteristic of cancer cells, enabling them to migrate by sliding through narrow channels between surrounding cells and/or cells and the extracellular matrix, without the need for cell-cell or cell-matrix adhesion contacts.

Healthy epithelial cells facilitate movement of cancer mesenchymal-like cells. Cancer cells exhibit coordinated movement by interacting with epithelial cells, whereas their movement through mono-cultured cancer systems is less consistent and less predictable [1]. In a related study, Heine et al. [16] highlighted the occurrence of intensive neighbour exchange along the MCF10A/MDA-MB-231 biointerface.

It is common knowledge that various physical factors, such as solid stress within the core region of cancer spheroids under *in vitro* conditions (**Glossary**), along with other biological factors, play significant roles in influencing the spread of cancer [17]. The solid stress present in the core of the spheroid and the tension on its surface are responsible for regulating the rearrangement of cells within mono-cultured spheroids through collective cell migration. The accumulated solid stress in the core region of multicellular spheroids is predominantly compressive and typically ranges from a few kPa [17]. The compressive residual stress experienced by cells in the core region of CT26 cancer cell spheroids (with

a diameter of 240 μm) is ~8 kPa, which is approximately eight times higher than the stress at the spheroid surface when subjected to an externally applied osmotic stress of 5 kPa [18]. The osmotic stress is generated through the application of large dextran molecules, characterized by a molecular weight of 500 kDa and an average radius of gyration measuring 15 nm. Due to their substantial size, these molecules are unable to permeate the cellular membrane. Instead, they promote an increase in the packing density of the cells, resulting in compressive mechanical stress, while the overall volume of individual cells remains relatively constant. This accumulated stress has a direct impact on cell shape and migration patterns, resulting in smaller and more rounded cells within the core region compared to those located on the surface of the spheroid [19].

The complex process of cancer spreading through epithelium has been evaluated by contrasting the self-organization of epithelial cells and cancer cells in mono-cultured spheroids with their rearrangement in co-cultured epithelial-cancer spheroids. Mono-cultured model systems are extensively utilized to explore various biological and physical aspects of cell rearrangement induced by collective cell migration in both normal and pathological conditions, including morphogenesis, wound healing, and cancer. Conversely, co-cultured epithelial-cancer spheroids are employed to simulate the dissemination of cancer through epithelial tissue [19,20].

Cancer spreading through epithelium within co-cultured spheroids is influenced by an interplay between physical parameters such as: (1) solid stress, (2) the surface tensions of the epithelial and cancer subpopulations, (3) the epithelium/cancer interfacial tension, and (4) the viscoelasticity of epithelial and cancer subpopulations caused by collective cell migration (**Glossary**). The surface tension of a multicellular system represents a measure of its surface cohesiveness in contact with the liquid medium. In

addition, while some physical parameters have been measured only under equilibrium conditions, other parameters such as the epithelium/cancer interfacial tension have not yet been measured. However, the tissue surface tension (also called "dynamic tissue surface tension'') is a dynamic parameter, which varies with time and space [21]. Its variations are due to the change of generated strain caused by collective cell migration, alterations in the cell surface packing density, variations in the strength of cell-cell adhesion contacts, and cell contractility [21]. Contractile cells are specially adapted to generate motile forces through the interaction of the (contractile) proteins actin and myosin. The movement of cells collectively within multicellular systems leads to the spontaneous emergence of mechanical waves [21-24]. The latter refer to periodic fluctuations in mechanical parameters, including cell velocity, strain rate, changes in surface, volume, and shape of multicellular systems, as well as cell mechanical stress. The frequency of the mechanical waves is $\sim 0.17 - 0.25\ \mathrm{h}^{-1}$ according to experimental data from Notbohm et al. [24]. These changes are accompanied by localized stiffening and softening of multicellular systems during collective cell migration. The oscillatory dynamics of collective cell migration is driven primarily by effects at the epithelium/cancer biointerfaces for co-cultured spheroids and the multicellular surface for mono-cultured spheroids [5,8].

The purpose of this review is to explore the relationships between collective cell migration, viscoelasticity in the context of cell mechanical stress generation, and the surface properties of epithelial and cancer cells. These relationships are measured by the surface tensions, interfacial tension between the subpopulations, and interfacial tension gradients of the different sub-populations. In doing so, we will consider mono-cultured epithelial and cancer spheroids, as well as co-cultured epithelium/cancer spheroids. Furthermore, we will emphasize the role of viscoelasticity in the physics governing the

spread of cancer. We will also mention some unresolved issues and potential opportunities that can be explored using these tools.

2. Collective migration of epithelial and cancer cell subpopulations: physical properties of the subpopulations

The cell rearrangement in spheroids is the result of collective cell migration, and so it is essential to explore the physical properties of the migrated epithelial and cancer subpopulations in greater depth. Collective cell migration can be characterized by coordination and cooperation [25]. Coordination refers to the ability of cells to move in a specific direction, while cooperation is determined by the interactions between cells through adhesion. As a result, the level of intracellular organization plays a crucial role in determining physical properties such as the viscoelasticity and surface characteristics of a multicellular system. The viscoelastic behaviour of a soft-matter system results from an interplay between energy storage and energy dissipation as the system undergoes structural changes in response to different stress or strain conditions. Two types of viscoelastic behaviour can be distinguished: (i) viscoelastic solid behaviour occurs when the stored energy is higher than the dissipated energy and (ii) viscoelastic liquid behaviour occurs when the stored energy is lower than the dissipated energy. The characterization of viscoelasticity encompasses: (i) the ability of stress to relax under constant strain, (ii) the ability of strain to relax under constant stress, and (iii) the residual stress that reflects either viscous or elastic characteristics. The relaxation phenomena inherent in viscoelastic systems can be investigated through experimental approaches in both the time and frequency domains, namely: (1) measurements of strain relaxation under constant stress (creep experiments) and of stress relaxation under constant strain; and (2) the assessment of storage and loss moduli under conditions of low oscillatory strain.

One typical experimental setup is shown in **Figure 1**:

Figure 1.

Epithelial and cancer subpopulations can undergo partial or total segregation depending on the interplay between physical parameters such as the epithelial and cancer surface tensions, the interfacial tension between them, and the viscoelasticity of the subpopulations. In partial segregation, the epithelial subpopulation forms clusters surrounded by the cancer subpopulation. When the epithelial surface tension is much higher than that of cancer, the subpopulations undergo total segregation such that epithelial cells move towards the spheroid core, while cancer cells migrate towards its surface [19].

The key features of collective migration in epithelial and cancer subpopulations are as follows:

- Epithelial cells exhibit a remarkable degree of coordination and cooperation in that they migrate in tightly connected clusters. In contrast, cancer cells migrate in loosely connected streams, displaying a lower level of coordination and a medium-to-high level of cooperation [25,26].

- The main characteristics of the movement of epithelial cells are the inhomogeneous distribution of the strengths of cell-cell adhesion contacts, of the cell packing density, and of the cell mechanical stress [27], while the migration of cancer cells is more homogeneous, owing to the dissipative nature of cancer cell migration [28].

- The movement of cancer cells corresponds to that of a viscoelastic liquid [28] (**Glossary**). In contrast, the movement of epithelial cells corresponds to that of a

viscoelastic solid and it results in the accumulation of additional residual mechanical stress in the cells (**Glossary**) [28].

- The residual stress is the stress remaining in a system in the absence of external forces. It can be either normal (compressive or tensional) or shear (**Glossary**). Furthermore, residual stress can be categorized as either dissipative, characterized by its viscous nature, or elastic [29].

- Accumulated cell compressive residual stress within the epithelium induces an increase in cell packing density, which can further reduce cell movement [28,30]. The epithelial movement reduction under cell compressive residual stress leads to a change in the state of viscoelasticity and can result in the cell jamming state transition (**Glossary**) [24,31].

- While the majority of epithelial cells migrate, some cell clusters have undergone the jamming state, and therefore cannot migrate. These cell clusters remain arrested during an interval, and then undergo the unjamming transition. These jamming/unjamming transitions occur many times during epithelial self-organization [32]. Consequently, during the process of cell segregation, only one part of the epithelial cells migrates, while the other part remains stationary in the cell-jammed state [28,31].

- The migrating and resting epithelial subpopulations show significant differences in their physical parameters such as cell stiffness and surface tension. Contractile (migrating) epithelial-like cells are much stiffer than non-contractile (resting) ones due to the accumulation of contractile energy [28,33,34].

- While epithelial cells undergo the jamming state transition, this is not the case for

cancer cells which remain unjammed on account of their mesenchymal and/or amoebic properties [35].

- Changes in cell velocity, packing density, and residual stress occur on a time scale of hours, whereas stress relaxation, if it happens, occurs on a time scale of minutes [36].

- The main characteristics of migrating epithelial collectives are long-time oscillations in cell velocity, together with corresponding changes in strain, residual stress, and packing density. The phenomenon has been discussed in the context of mechanical waves [22,24,36]. While free expansion of epithelial monolayers generates propagating waves [22], cell swirling motion within confluent epithelial monolayers creates standing waves [24]. The migration of cancer cells is also oscillatory in character [8].

The epithelial and cancer subpopulations respond differently to mechanical stress, which has a feedback impact on their self-organization.

3. Response of cells to various stress conditions

Mechanical stress can have varying effects on different cell types. For instance, while some cancer cell types experience enhanced movement under mechanical stress, epithelial cell types may have their movement reduced, or they may be unaffected [4,30]. Additionally, solid stress caused by cell growth in number due to reproduction can accumulate within the spheroid core region, leading to compressive stress of a few kPa [17]. Normal mechanical stress in the range of several hundred Pa, both compressive and tensional, can also be generated by collective cell migration [23]. Increased compressive

stress can inhibit the movement of epithelial MCF-10A cells and can trigger a transition to the jammed state [35]. Therefore, it is essential to take into account the mechano-sensitivity of co-cultured multicellular systems when analyzing their behavior.

Parameters related to cell mechano-sensitivity have been discussed extensively in the literature. They include the stiffness of individual cells, the level of E-cadherin, and the mechanism of cell movement. Tse et al. [30] conducted a study and reported that cells with higher stiffness exhibit lower mechano-sensitivity. On the other hand, Rudzka et al. [37] and Riehl et al. [4] highlighted a correlation between the stiffness of cancer cells and their invasiveness. The stiffness of individual cells is influenced by both mechanical and biochemical interactions with their surrounding environment. These interactions trigger a range of internal molecular mechanisms within the cells themselves, leading to adaptations such as rearrangement of the cytoskeleton and changes in the strength of cell-cell adhesion contacts.

Furthermore, Mohammed et al. [38] identified E-cadherin-mediated cell-cell adhesion contact as the primary factor contributing to the reduction in movement of breast epithelial cells and the transition to a jamming state under compressive stress (**Glossary**). These findings shed light on the complex interplay between cell mechanics, biochemical factors, and cell behavior, providing valuable insights into the understanding of cell mechano-sensitivity.

Understanding the self-organization of the epithelial and cancer subpopulations within mono-cultured and co-cultured cell spheroids requires a thorough examination of the physical parameters that govern collective cell migration. They encompass the epithelial and cancer surface tensions, along with the epithelium/cancer interfacial tension, and the viscoelasticity of the subpopulations.

4. Physical parameters which influence self-organization of epithelial and cancer subpopulations within co-cultured spheroids

In the context of the surface characteristics of multicellular systems, there are three parameters that are commonly considered to be of crucial importance: the surface tensions of the epithelial and cancer subpopulations, and the epithelium/cancer interfacial tension (**Glossary**). Following a discussion of the main characteristics of these parameters, we will explore the viscoelastic properties of the subpopulations resulting from collective cell migration.

4.1 Epithelial and cancer surface tensions: comparative analysis

Tissue surface tension can be expressed as the change in the surface energy with change of the multicellular surface area (**Glossary**). The surface energy can be written as [39]: $E_{ST} = \sum_i \frac{K}{2}\left(A_{ceffi} - A_0\right)^2 + \sum_{i,j} \Lambda l_{ij} + \sum_i \frac{T_{con\,i}}{2} {L_i}^2$, where where A_{ceffi} is the effective surface area of the *i*-th cell, K is the effective modulus of the cell around its preferred surface area A_0, l_{ij} is the interface length between the *i*-th and *j*-th cells, Λ is the line tension per unit interface length between two cells, $T_{con\,i}$ is the contractility coefficient, and L_i is the perimeter of the *i*-th cell. The tissue surface tension represents the change of the tissue surface energy caused by changing the surface area and can be expressed as: $\gamma = \frac{\partial E_{ST}}{\partial A_s}$ where A_s is the external multicellular surface area in contact with a liquid medium.

4.1.1 Surface tensions of the epithelial and cancer subpopulations: comparative analysis

The surface tensions of the epithelial and cancer subpopulations depend on an interplay between the strength of the cell-cell adhesion contacts and the cell contractility. Epithelial cells establish strong E-cadherin-mediated adherens junctions (AJs), which are

inhomogeneously distributed along multicellular surfaces. In contrast to epithelial cells, metastatic cancer cells frequently establish weak cell-cell adhesion contacts [13,19]. Cell contractility plays contrasting roles in the strengths of cell-cell adhesion contacts in epithelial and cancer cells. Contraction of epithelial cells enhances the strength of AJs [19]. On the other hand, the contractility of cancer mesenchymal-like cells leads to an increase in cell-cell repulsion, thereby reducing the cancer surface tension [19]. Therefore, the surface tension of epithelial and cancer cells is determined by an interplay between these factors. Summarising:

- The surface tension of active contractile epithelial cells is higher than the surface tension of passive non-contractile cells [19].
- The surface tension of active epithelial cells is much higher than that of active cancer cells [19].
- An inhomogeneous distribution of the strengths of E-cadherin-mediated cell-cell adhesion contacts causes an inhomogeneous distribution of the epithelial surface tension [27].
- Changes in the surface tension of multicellular systems, i.e., the dynamic surface tension [21] occur on two time-scales. A short time-scale (minutes) corresponds to remodeling of cell-cell adhesion contacts, while a long-time-scale (hours) corresponds to collective cell migration.
- Cohesive multicellular aggregates exhibit oscillations in their dynamic surface tension over a few hours [21]. This finding was inferred from the observation of shape oscillations in the aggregates during the rounding process following uni-axial compression [40]. Note that the latter authors specifically focused on measuring the static (equilibrium) surface tension of the cell aggregate after it had been rounded between parallel plates following compression.

It is interesting and relevant to discuss how the surface tension was measured.

4.1.2 Measurement of a surface tension of multicellular systems

Although the surface tension of multicellular systems is known to vary with time and space, there are currently no empirical data supporting the notion of temporal fluctuations in this physical property. Thus far, only a static (equilibrium) value for surface tension has been observed and measured.

The literature reveals that the static surface tension reported for multicellular systems depends, not only on the cell type, but also on the measurement technique used. Cell aggregates typically exhibit a static surface tension of a few mN/m when measured through cell aggregate compression between parallel plates, which is significantly less than the surface tension of water [40,41] (72 mN/m at room temperature). However, when employing a magnetic tensiometer to measure the static surface tension of breast epithelial MCF-10A cell aggregates, it amounts to a few tens of mN/m, as observed by Nagle et al. [42]. It is widely acknowledged that the strong cell-cell adhesion contacts formed by MCF-10A cells influence the surface tension. Furthermore, Jafari et al. [43] reported that exposure of cell aggregates to a magnetic field can further enhance the strength of these cell-cell adhesion contacts. There does not appear to be a comprehensive review about the impact of the measuring technique on the value of the tissue surface tension determined.

Besides the surface tensions of the subpopulations, the epithelium/cancer interfacial tension influences significantly the self-organisation of the subpopulations in direct contact.

4.2 Epithelium/cancer interfacial tension

The interfacial tension observed between epithelial and metastatic cancer cell

subpopulations when they are in direct contact is found to be a dynamic parameter that varies with time and space. This dynamic interfacial tension is determined by the interplay between the surface tensions of the two subpopulations and the adhesion energy present between them:

$$\gamma_{ec}(r,\tau) = \gamma_e(r,\tau) + \gamma_c(r,\tau) - \omega_a(r,\tau) \quad (1)$$

where r is the coordinate along the epithelium/cancer biointerface, τ is the long (hours) time constant, and $\gamma_{ec}(r,\tau)$ is the epithelium/cancer interfacial tension, while $\gamma_e(r,\tau)$ and $\gamma_c(r,\tau)$ are the epithelial and cancer surface tensions, which satisfy the condition $\gamma_e(r,\tau) \gg \gamma_c(r,\tau)$, and $\omega_a(r,\tau)$ is the adhesion energy between the epithelial and cancer subpopulations when in direct contact. Accordingly, the epithelium/cancer interfacial tension can be expressed as: $\gamma_{ec}(r,\tau) = \gamma_e(r,\tau) - \omega_a(r,\tau)$. The adhesion energy $\omega_a(r,\tau)$ is the energy that can be released when the epithelial and cancer multicellular surfaces come into direct contact. This energy depends on the epithelial-cancer interactions along the biointerface. Three types of interaction should be considered. They are all facilitated by signalling molecules released by the cells. When the subpopulations fail to form heterotypic cell-cell adhesion contacts, interactions take place through electrostatic, steric, and Van der Waals forces between adjacent cell surfaces. Some epithelial cell types are able to establish direct heterotypic cell-cell adhesion contacts with mesenchymal cells. These heterotypic cell-cell adhesion contacts are weak and unstable [44]. Additionally, specific epithelial cell types have the ability to secrete proteins like fibronectin and laminin-5 along the biointerface with cancer cells [45]. In such instances, both cell populations are inclined to establish focal adhesions with proteins, leading to what can be described as protein-mediated heterotypic cell-cell interactions. The interfacial tension is highest when epithelial and cancer cells are not able to establish

heterotypic cell-cell adhesion contacts. Consequently, the interfacial tension increases, playing a crucial role in the effective separation of subpopulations within co-cultured epithelial-cancer spheroids.

The measurement of dynamic interfacial tension among subpopulations within cellular systems remains unexplored. Resonant acoustic rheometry stands as a potential technique for quantifying this phenomenon. This method has been utilized successfully in determining surface and interfacial tensions in soft matter systems such as hydrogels [46].

4.3 Efficiency of spreading of cancer cells in co-cultured spheroids

The extension or compression of a subpopulation along the biointerface is determined by a balance between the adhesion and cohesion energies at the biointerface, as indicated by the spreading factors [5]. The spreading factor of the subpopulation k in contact with the subpopulation l is defined as $S^k = \omega_a^{k-l} - \omega_c^k$, where ω_a^{k-l} is the adhesion energy between the subpopulations l and k and is given by $\omega_a^{k-l} = \gamma_l + \gamma_k - \gamma_{lk}$, γ_l, and γ_k are the surface tensions of the subpopulations, γ_{lk} is the interfacial tension between them, and ω_c^k is the cohesion energy of the subpopulation k, equal to $\omega_c^k = 2\gamma_k$. If the $S^k > 0$, the subpopulation k undergoes extension. Otherwise, when the spreading factor is $S^k < 0$, this subpopulation undergoes contraction. Consequently, the spreading factor of the mesenchymal-like cancer subpopulation is equal to: $S^c = \gamma_e - (\gamma_c + \gamma_{ec})$, while the spreading factor of the epithelial subpopulation is equal to $S^e = \gamma_c - (\gamma_e + \gamma_{ec})$. Because the surface tensions of the subpopulations satisfy the condition that $\gamma_e \gg \gamma_c$, the epithelial spreading factor $S^e < 0$, while the cancer spreading $S^c > 0$. This means that the epithelial subpopulation contracts, while the cancer subpopulation extends.

The efficiency with which cancer spreads into the epithelium can be quantified by the ratio of the epithelial surface tension to the epithelium/cancer interfacial tension, denoted as $X = \frac{\gamma_e}{\gamma_{ce}}$. The ratio X satisfies the condition that $X \geq 1$ which is in accordance with eq. (1) [8]. Effective cancer spreading along the biointerface is therefore promoted by higher epithelial surface tension, characteristic of migrating (contractile) epithelium [15], and lower epithelium/cancer interfacial tension. This implies that stronger E-cadherin-mediated cell-cell adhesion contacts, and increased protein-mediated heterotypic epithelial-cancer interactions at the biointerface, facilitate the spread of cancer.

4.4 Cell mechanical stress generation caused by collective cell migration of the subpopulations

Cell mechanical stress resulting from the self-organization of different cell subpopulations encompasses both normal (tensional and compressive) and shear stress components (**Glossary**). This stress is induced by interactions between cells along the biointerface and the coordinated migration of cells in a specific direction. The epithelial subpopulation experiences compression, whereas the cancer subpopulation undergoes extension along the biointerface. The compression (indicated by a "-" sign) or extension (indicated by a "+" sign) is quantified by the cell's isotropic normal residual stress, which is described by the Young-Laplace equation. Consequently, the normal stress within the epithelial subpopulation is compressive and comprises both isotropic and deviatoric contributions. The isotropic contribution arises from the interfacial tension γ_{ce}, which decreases the interface area, while the deviatoric contribution is the anisotropic, direction-dependent part of the normal stress, resulting from collective cell migration. The normal residual stress within the epithelium can be expressed as [5,47]:

$$\tilde{\boldsymbol{\sigma}}_{erV} = -\Delta p_{c\to e}\tilde{\boldsymbol{I}} + \tilde{\boldsymbol{\sigma}}_{erV}{}^{CCM} \qquad (2)$$

where $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{erV}}$ is the normal residual stress within the epithelium. The first term on the right-hand side is the isotropic part of the residual stress, $\Delta p_{c\to e} = -\gamma_{ce}\left(\vec{\boldsymbol{\nabla}} \cdot \vec{\boldsymbol{n}}\right)$, while $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{erV}}{}^{\boldsymbol{CCM}}$ is the deviatoric part of the residual stress caused by collective cell migration; $\vec{\boldsymbol{n}}$ is the vector normal to the biointerface, and $\tilde{\boldsymbol{I}}$ is the unity tensor. The normal residual stress within the cancer subpopulation is extensional and expressed as [5,47]:

$$\tilde{\boldsymbol{\sigma}}_{\boldsymbol{crV}} = +\Delta p_{c\to e}\tilde{\boldsymbol{I}} + \tilde{\boldsymbol{\sigma}}_{\boldsymbol{crV}}{}^{CCM} \tag{3}$$

where $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{crV}}{}^{CCM}$ is the deviatoric part of the normal residual stress caused by the collective cell migration of cancer cells.

The shear stress experienced by the epithelial and cancer subpopulations at their biointerface results from two main factors: natural convection and forced convection. Natural convection is driven by the gradient of the interfacial tension along the biointerface, leading to cell movement from areas of lower interfacial tension to those of higher tension, known as the Marangoni effect [5]. This phenomenon is observed in various soft matter systems due to changes in temperature or constituent distribution [48]. On the other hand, forced convection-induced shear stress is attributed to collective cell migration. Unlike cell normal stress, cell shear stress does not affect the cell packing density within the epithelial subpopulation. The shear stress within the epithelial subpopulation $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{erS}}$ is expressed as [5,47]:

$$\vec{\boldsymbol{n}} \cdot \tilde{\boldsymbol{\sigma}}_{\boldsymbol{erS}} \cdot \vec{\boldsymbol{t}} = \vec{\boldsymbol{\nabla}}\gamma_{ce} \cdot \vec{\boldsymbol{t}} + \vec{\boldsymbol{n}} \cdot \tilde{\boldsymbol{\sigma}}_{\boldsymbol{erS}}{}^{CCM} \cdot \vec{\boldsymbol{t}} \tag{4}$$

where $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{erS}}$ is the shear residual stress within the epithelial subpopulation, $\vec{\boldsymbol{\nabla}}\gamma_{ce}$ is the interfacial tension gradient, $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{erS}}{}^{CCM}$ is the shear stress generated by collective migration of the epithelial subpopulation, and $\vec{\boldsymbol{t}}$ is the unit tangential vector. The first term on the right-hand side of eq. (4) is the shear stress contribution caused by natural convection while the second term is the shear stress contribution caused by the forced convection

(i.e. collective cell migration). The shear stress within the cancer subpopulation $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{crS}}$ may be expressed [5,47] as:

$$\vec{\boldsymbol{n}} \cdot \tilde{\boldsymbol{\sigma}}_{\boldsymbol{crS}} \cdot \vec{\boldsymbol{t}} = \vec{\boldsymbol{V}}\gamma_{ce} \cdot \vec{\boldsymbol{t}} + \vec{\boldsymbol{n}} \cdot \tilde{\boldsymbol{\sigma}}_{\boldsymbol{crS}}{}^{CCM} \cdot \vec{\boldsymbol{t}} \quad (5)$$

where $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{crS}}$ is the shear residual stress within the cancer subpopulation and $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{crS}}{}^{CCM}$ is the shear stress generated by collective migration of the cancer subpopulation. As in the previous case, the first term on the right-hand side of eq. (5) is the shear stress contribution caused by natural convection while the second term is the shear stress contribution caused by the forced convection (i.e. collective cell migration). We can develop appropriate constitutive models of viscoelasticity for each subpopulation by utilizing experimental data obtained from the existing literature.

The migration of epithelial cells induces an accumulation of cell mechanical stress due to the establishment of strong cell-cell adhesion contacts. In contrast, the migration of cancer cells induces more intensive dissipation of the mechanical energy due to the establishment of weak and unstable cell-cell adhesion contacts. The magnitude of residual stress, whether shear or normal, arising from the collective migration of cells depends on the particular migration mechanism, which is influenced by the density of cell packing.

Epithelial cell migration is influenced by three distinct mechanisms: (1) the convective mechanism for the cell packing density $n_e \leq n_{conf}$ [29], (2) the diffusion mechanism for the cell packing density $n_{conf} < n_e < n_j$, and (3) the sub-diffusion mechanism for the cell packing density $n_e \sim n_j$, where n_{conf} is the cell packing density in the confluent state equal to $\sim 2.5x10^5 \ \frac{\text{cells}}{\text{cm}^2}$ [49] and n_j is the cell packing density in the jamming state $\sim 1x10^6 \ \frac{\text{cells}}{\text{cm}^2}$ [50]. Cancer cell migration is characterized by the convective mechanism, with the density of cancer cells showing fluctuations around the confluent

state. The constitutive models (**Glossary**) for the various mechanisms of epithelial cell migration are discussed in the **Appendix**.

A lot of research on the reorganisation and migration of epithelial cells has used Madin-Darbvy canine kidney (MDCK) cell monolayers for a cell packing density $n_e \leq n_{conf}$ as was investigated by Serra-Picamal et al. [22] and by Notbohm et al. [24]. Their findings revealed a direct correlation between the cell residual stress, and the corresponding strain (**Glossary)**. This discovery suggests that these cells can be classified as viscoelastic solids (see **Glossary)**. The presence of strong cell-cell adhesion contacts, mediated by E-cadherin in epithelial cells, further supports this observation. Another noteworthy characteristic of epithelial monolayers within this range of cell packing densities is their ability to relax stress towards the cell residual stress.

Stress relaxation was found to occur over minutes by Khalilgharibi et al. [51], while the accumulation of cell residual stress was observed to take several hours. Marmottant et al. [52] also detected stress relaxation in cell aggregates under uni-axial compression, providing additional support for stress relaxation in these systems. Pajic-Lijakovic and Milivojevic [21] concluded that changes in cell stress occur within minutes through successive short-term stress relaxation cycles, while cell strain and the corresponding cell residual stress change over hours. The Zener model, described in the **Appendix**, could be a suitable constitutive model that meets the criteria of stress relaxation within minutes and the correlation between cell residual stress and strain, indicating long-term elastic behaviour [21]. Energy dissipation, which is characteristic of the viscoelastic behaviour of multicellular systems, occurs over minutes due to the rearrangement of cell-cell adhesion contacts [5]. The cell stress relaxes towards the elastic cell residual stress, while the cell residual stress, cell velocity, and strain oscillate over hours, as discussed in the context of mechanical waves [21,22,24]. Conversely, in their

collective migration, cancer cells behave as viscoelastic liquids (**Glossary**) for cell packing density $n_c \leq n_{conf}$ compared to epithelial cells, with the Maxwell model being more suitable for such systems [29]. Experimental validation of the Maxwell model for characterizing the viscoelasticity of multicellular systems through micropipette aspiration has been reported [53]. The movement of cells during this process leads to the disruption of cell-cell adhesion contacts, resulting in significant energy dissipation. The Maxwell model delineates the purely dissipative nature of cell residual stress, while the Zener model describes the purely elastic component of residual stress (**Appendix**).

When the density of epithelial packing increases further, specifically within the range of $n_{conf} < n_e < n_j$, it leads to the inhibition of stress relaxation within the cells. The presence of intense friction between cells, which is a characteristic feature of higher cell packing densities, causes the dissipation of energy over an extended period during the rearrangement of cells. Since the movement of cells is governed by a linear diffusion mechanism, it is essential to utilize a corresponding constitutive model that is also linear. In this specific regime, Pajic-Lijakovic [29] proposed the Kelvin-Voigt constitutive model (**Appendix**), which considers both the elastic and viscous contributions to the long-term change in cell stress.

Cell movement in an overcrowded environment is damped and is regulated by a sub-diffusion mechanism, which has been elucidated through the utilization of fractional derivatives. In this particular scenario, Pajic-Lijakovic [29] introduced a fractional constitutive model for effective portrayal of the viscoelastic properties of cell monolayers (**Appendix**).

4.4.1 Measurement of cell mechanical stress caused by collective cell migration

Various experimental methods have been utilized to quantify the cell stress induced by collective cell migration. Tambe et al. [23] employed monolayer stress

microscopy to measure the 2D distribution of cell stress. This technique relies on the assumptions that cell monolayers exhibit linear, uniform, and isotropic elasticity, as well as uniform thickness, which do not align well with the actual conditions. Measuring the 3D stress distribution poses a greater challenge. To address this, several studies have introduced different inclusions in the form of microbead/droplet-based stress sensors with well-defined mechanical properties into 3D cellular systems to overcome the complexities associated with 3D traction force microscopy [54]. Incompressible micro-droplet sensors can be utilized to measure the anisotropic normal stress component [55]. Dolega et al. [18] developed elastic microbead sensors to quantify the isotropic compressive stress resulting from tumour growth within the matrix (i.e., solid stress). These inclusion-based experimental approaches enable the measurement of stress values in close proximity to the inclusion.

5. Compaction of co-cultured epithelial-cancer spheroids by collective cell migration

The volume and surface of a spheroid can: increase, decrease, or remain relatively constant. To gain a comprehensive understanding of epithelial and cancer self-organization, it is useful to conduct a comparative analysis of the behaviors of mono-cultured epithelial and cancer spheroids, as well as of co-cultured epithelium/cancer spheroids. These analyses will provide valuable insights into the mechanisms underlying self-organization in these cellular structures:

- Epithelial surface tension induces cell migration from the spheroid's surface region towards its core, leading to a decrease in the spheroid volume and surface and, correspondingly, an increase in the average cell packing density [19,20]. The phenomenon of epithelial spheroid compaction shown in **Figure 2a** is pronounced for epithelial cells, which have relatively high epithelial surface tension.

Accumulated cell residual stress caused by collective cell migration, along with cell solid stress accumulated with the spheroid core region, can suppress cell movement.

Figure 2a,b.

- The migration of cancer cells differs from that of epithelial cells, as it is primarily directed from the core region towards the surface, driven by cell solid stress as shown in **Figure 2b**. This movement is facilitated by the lower cancer surface tension compared to epithelial surface tension. Consequently, this migration process can result in an expansion of the spheroid surface and volume, with a corresponding decrease in cell packing density. In the particular case of breast MDA-MB-468 cancer cells, however, the surface and volume of the spheroid remain relatively constant.
- Compaction of the epithelial subpopulation is reduced by the presence of cancer cells in co-cultured epithelium/cancer spheroids. In this case, compaction of the epithelial subpopulation involves work done by the epithelium/cancer interfacial tension [8,47]. While epithelial cells migrate towards the spheroid core region, cancer cells migrate towards the spheroid surface by the establishment of complete or partial segregation of the subpopulations [28] as shown in **Figure 3**:

Figure 3.

Following the segregation process, the co-cultured spheroid exhibits greater surface area and volume when compared to the mono-cultured epithelial spheroid, yet smaller than the mono-cultured cancer spheroid [19].

6. Oscillations of collective cell migration

Oscillatory patterns of collective cell migration, in the form of mechanical waves, have been documented in a variety of multicellular systems, for example during the

expansion of epithelial MDCK cell monolayers [22], and in their rearrangement [24]. These mechanical waves are indicative of prolonged oscillations in cell velocity, cell strain, and cell mechanical stress. Furthermore, the phenomenon of collective cell migration within mono-cultured and co-cultured spheroids also exhibits oscillatory behaviour, as highlighted by Pajic-Lijakovic and Milivojevic [8] and Lucia et al. [7].

The compaction of cohesive mono-cultured cell spheroids via collective cell migration exhibits oscillatory behaviour. Pajic-Lijakovic et al. [21] explored the oscillations in the shape of cell spheroids as they round up under uni-axial compression, a phenomenon previously observed by Mombash et al. [40]. The change in the shape of the cell aggregate was expressed as: $\frac{d\Delta AR(\tau)}{d\tau} = -k\Delta AR(\tau)$ (where the aspect ratio difference is $\Delta AR = AR - 1$ and k is the specific rate of the cell aggregate shape change while k^{-1} is the relaxation time of cell aggregate shape during the aggregate rounding) [56]. Pajic-Lijakovic and Milivojevic [56] discovered that there are two to three values of k that satisfy certain conditions: (i) $k^I \to 0$, (ii) $k^{II}, k^{III} \gg k^I$, and (iii) $\frac{k^{III}}{k^{II}} \approx 2 - 3$. These relaxation rates are associated with different scenarios of surface cell rearrangement: (i) k^I corresponds to the majority of cells being in a jamming state, (ii) k^{II} indicated that some cells migrate, while the others remain jammed, and (iii) k^{III} suggested that majority of cells migrate. Considering that it is the tissue surface tension that governs surface changes, the oscillations in the spheroid's surface point towards oscillations in the surface tension [21].

The collective migration of cancer cells in mono-cultured multicellular systems also exhibits oscillatory behaviour. This phenomenon has been studied in model systems, such as the fusion of two cancer spheroids. Grosser et al. [35] pointed to oscillations of cell velocity during the fusion of two breast MDA-MB-436 cancer spheroids. Pajic-Lijakovic et al. [57] pointed to oscillations of the rate of expansion in the radius of the

contact region between these spheroids, calculated from experiments by Dechristé et al. [58].

Cell self-organisation within co-cultured epithelium/cancer systems via collective cell migration also shows oscillatory behaviour, similarly to that of mono-cultured systems. Lucia et al. [7] investigated the rearrangement of two types of co-cultured monolayers: epithelial MDCK/cancer C2C12 spheroids and epithelial HaCaT/cancer C2C12 spheroids. The velocities of the epithelial and cancer subpopulations were measured and found to oscillate during the segregation process. These co-cultured systems undergo partial segregation, wherein clusters of epithelial HaCaT or MDCK cells are surrounded by C2C12 cancer cells. The rearrangement of the epithelial cells within these clusters is influenced by an accumulation of cell compressive stress and shear stress along the epithelium/cancer biointerface. The magnitudes of the interfacial tension and viscoelasticity of the epithelial cells, as expressed by eq. (4), determine the level of compressive stress experienced. The compressive stress accumulated within the MDCK cell clusters is found to be higher than that within the HaCaT cell clusters. This observation aligns with the fact that HaCaT cells exhibit greater resistance to compression than MDCK cells. The latter display a sequence of cell jamming/unjamming transitions, while the HaCaT cells maintain strong cell-cell adhesion contacts and exhibit swirling motion [7]. Furthermore, the HaCaT cells form a supracellular actin structure along the biointerface with the cancer cells. This network serves as a shell around the cluster, which causes stiffening of the epithelial cells along the biointerface and, consequently, an increase in the resistance effects. It protects epithelial clusters against cell compressive and shear stress components. Supracellular contractions of a cell cluster caused by the presence of the actin network lead to swirling motion of cells within the clusters. These induce successive radial extension and compression of the clusters, involving work by

the centrifugal force. A common feature of cell movement in epithelial clusters, including both the swirling motion and jamming/unjamming transitions, is an oscillatory variation of cell velocity [8].

A biophysical model can be used to analyse these oscillatory variations.

6.1 Oscillations of collective cell migration: the biophysical model

The oscillations of cell velocity result from oscillations of driving forces or resistive forces. These forces depend on physical parameters such as: the epithelial and cancer surface tensions, interfacial tension between them, solid stress, and cell residual stress generation. The inter-relationship between these physical parameters and their impact on the spreading of cancer is shown in **Figure 4**:

Figure 4.

While epithelial cells in mono- and co-cultured spheroids undergo compression, cancer cells undergo extension. The force balance for the surface region of mono or co-cultured spheroids [57], can be expressed as:

$$\langle m\rangle_k \frac{D[n_k(r,\tau)\vec{v}_k(r,\tau)]}{D\tau} = \sum_i \vec{F}_{di}^{k} - \sum_i \vec{F}_{Ri}^{k} \qquad (6)$$

where the subscript $k \equiv e, c$ for epithelial and cancer cells respectively, $\langle m\rangle_k$ is the average mass of a single cell, n_k is the epithelial or cancer packing density, $\vec{\boldsymbol{v}}_{\boldsymbol{k}}(r,\tau) = \frac{d\vec{\boldsymbol{u}}_{\boldsymbol{k}}}{d\tau}$ is the velocity (i.e., the rate of displacement change) of the epithelial/cancer subpopulations, $\vec{\boldsymbol{u}}_{\boldsymbol{k}}$ is the epithelial/cancer displacement field, $\sum_i \vec{F}_{di}^{k}$ is the sum of driving forces, and $\sum_i \vec{F}_{Ri}^{k}$ is the sum of resistive forces. The material derivative presented on the right-hand side of eq. (6) is: $\frac{D[n_k(r,\tau)\vec{\boldsymbol{v}}_{\boldsymbol{k}}(r,\tau)]}{D\tau} = n_k\left[\frac{\partial\vec{\boldsymbol{v}}_{\boldsymbol{k}}}{\partial\tau} + (\vec{\boldsymbol{v}}_{\boldsymbol{k}}\cdot\vec{\boldsymbol{\nabla}})\vec{\boldsymbol{v}}_{\boldsymbol{k}}\right] + \vec{\boldsymbol{v}}_{\boldsymbol{k}}\left[\frac{\partial n_e}{\partial\tau} + (\vec{\boldsymbol{v}}_{\boldsymbol{k}}\cdot\vec{\boldsymbol{\nabla}})n_k\right]$ [59]. Multicellular systems are compressible. However, a small change in the cell packing density induces a significant change in the cell velocity.

Epithelial speed can be correlated with cell packing density in the form of $\|\vec{v}_e\| \sim {n_e}^b$ (where b is the scaling exponent) based on experimental data obtained for 2D multicellular systems [31,60]. The scaling exponent is: (i) $b = 1.85$ for a free expansion of MDCK cells [60], or (ii) $b = 2.35$ for a free expansion of MCF-10A cells [31]. The packing density of metastatic cancer cells is more homogeneous in comparison with epithelial cells due to the dissipation of cell residual stress in this case (see **Appendix**). The driving and resistive forces, which influence cell rearrangement within mono-cultured epithelial and cancer spheroids, as well as co-cultured epithelial-cancer spheroids are shown in **Table 1**:

Table 1.

The scenario accounting for the oscillatory change of the epithelial velocity $\vec{v}_e$ in mono-cultured spheroids is as follows:

- The epithelial surface tension drives cells from the spheroid surface towards its core. This increases the cell compressive residual stress and the cell packing density, and therefore increases the viscoelastic force tending to reduce cell movement.
- The increase in cell packing density intensifies cell-cell interactions thereby weakening E-cadherin-mediated cell-cell adhesion contacts, which can induce the cell jamming state transition. The weakening of cell-cell adhesion dissipates energy, decreasing both the cell residual stress and the viscoelastic force. The cells then resume their migration.
- Successive remodelling of the cell-cell adhesion contacts results in oscillations of the epithelial surface tension force, as well as the spheroid surface area.

The scenario for the oscillatory change of the cancer velocity $\vec{v}_c$ in mono-cultured spheroids is as follows:

- The solid stress within the spheroid's core region (**Glossary**) and the viscoelastic force drive cancer cells towards the surface. This migration of cancer cells is dissipative and leads to a decrease in the residual stress accumulated within the spheroid core region by reducing further movement of cancer cells [47].
- The cancer surface tension reduces the movement of cancer cells to some extent by increasing the mechanical stress within the core, accompanied by the cancer viscoelastic force [8]. It is well known that the cancer surface tension is much lower than that of the epithelium [19]. However, the spontaneous formation of cancer spheroids under *in vitro* conditions needs some cohesion among cancer cells [19].
- An increase in the cancer viscoelastic force stimulates cell movement leading to an increase in the spheroid volume and surface area. This increase in the surface area of the spheroid causes an increase in the surface tension force capable of reducing movement of cancer cells.
- Oscillations of the viscoelastic force and the cancer surface tension force cause oscillations of cancer velocity.

Thus, while the migration of the epithelial subpopulation within a co-cultured spheroid is directed from the surface towards the core, driven primarily by the epithelial interfacial tension force, the migration of cancer cells is directed from the core towards the surface driven by the cancer interfacial tension force and viscoelastic force [5,47]. The epithelial interfacial tension force induces compression of the epithelial subpopulation, while the cancer interfacial tension force causes extension of the cancer subpopulation towards: (i) the epithelium and (ii) the spheroid's surface region. It is in accordance with fact that these interfacial tension forces depend on the spreading factors of the subpopulations. These movements of the subpopulations in opposite directions generate frictional forces,

reducing cell movement along the epithelium/cancer biointerface. These frictional forces reduce cell movement depending on the relative velocity between the subpopulations $\vec{v}_R = \vec{v}_c + \vec{v}_e$. The thermodynamic mixing force in a co-cultured spheroid is caused by mixing of the subpopulations and depends on the epithelium/cancer adhesion energy, as a product of cell-cell heterotypic interactions along the biointerface [5]. Oscillatory migration of the subpopulations during the segregation can be explained in a few steps:

- Directed movement of epithelial cells driven primarily by the epithelial interfacial tension force induces accumulation of cell compressive stress, leading to an increase in the viscoelastic force capable of suppressing cell movement.
- Epithelial cells have the ability to regulate both the interfacial tension force and the viscoelastic force through the remodeling of cell-cell adhesion contacts. Two mechanisms of remodeling for E-cadherin-mediated cell-cell adhesion contacts have been observed within co-cultured spheroids: (i) jamming/unjamming transitions; and (ii) formation of a supracellular actin network along the epithelium/cancer biointerface [7,8].
- Successive cell jamming/unjamming transitions induce weakening/reinforcement of cell-cell adhesion contacts, leading to a decrease/increase: (i) in the compressive stress accompanied by the viscoelastic force within the epithelial bulk; and (ii) in the epithelial surface tension accompanied by the epithelial interfacial tension force along the epithelium/cancer biointerface. Another mechanism is associated with the formation of a contractile supracellular actomyosin network along the biointerface that acts as a protective barrier against the build-up of compressive and shear stress components within epithelial clusters [7,62]. The swirling motion of cells within the cluster leads to successive cell radial inwards/outwards flows, resulting in oscillations in the cluster surface area

and volume. These oscillations induce oscillations in the epithelial surface tension, and in the epithelial interfacial tension force, and in the compressive stress accompanied by the viscoelastic force within the clusters.

- The oscillation of the epithelial surface tension causes an oscillation of the cancer spreading factor and hence of the interfacial tension force of the cancer cells.
- The viscoelastic force undergoes successive increases and decreases, which in turn affect the movement of both subpopulations. The movement of epithelial cells leads to the storage of mechanical energy (and epithelial stress) resulting in an increase in cell residual stress accompanied by the viscoelastic force, while the movement of cancer cells causes energy dissipation, resulting in a decrease in the viscoelastic force.

These oscillations in the physical parameters, along with the corresponding driving and resistive forces, give rise to oscillations in the velocities of both the epithelial and cancer subpopulations.

Conclusion

This review has sought to elucidate the role of physical factors, particularly viscoelasticity and the surface characteristics of epithelial and cancer subpopulations, in the progression of cancer through epithelial tissues. The interplay between the physical/mechanical properties of cancer and its surroundings is increasingly recognised as providing major regulatory cues affecting the spreading of cancer. The discussion is focused on model systems, especially the separation of epithelial and cancer subpopulations using co-cultured spheroids. The development of three-dimensional spheroid and organoid cancer models that accurately simulate the microenvironment of cancer tissues is becoming increasingly important as a preclinical model system. These physical factors emerge from

both homotypic and heterotypic cell-cell interactions, as well as the contractility of the cells. As a result, interactions between cells, shaped by biochemical signalling, exert a reciprocal influence on the cohesiveness of subpopulations, their adhesiveness, and the generation of mechanical stress. These physical parameters, defined on a mesoscopic scale, in turn affect the cell-cell interactions themselves. Thus, while the biological factors influencing the spreading of cancer are reasonably well understood, an appreciation of how physical factors affect the spreading of cancer is still in its infancy. The known biology includes the cell signalling and gene expression governing cell contractility, and the remodelling of both the cell-cell and cell-matrix adhesion contacts, influencing the homotypic and heterotypic interactions. In striking contradistinction the physical picture is only now starting to come into focus. A full understanding will obviously require these two complementary pictures to be brought together and reconciled.

Cancer cells exhibit two distinct modes of collective migration. They extend towards the epithelium and engage in directional movement from the core of the spheroid to its outer surface. The directional migration of cancer cells is driven by the compressive residual stress that generates within the spheroid's core, whereas the expansion towards the epithelium is governed by the interplay between epithelium-cancer interfacial tension and epithelial surface tension in the form of cancer spreading factor.

The compressive residual stress is composed of three components: solid stress, the stress caused by work of interfacial tension on reduction the interface area, and the stress resulting from the collective migration of the cells. The stress associated with collective migration is contingent upon the viscoelastic properties of the various subpopulations. While the migration of epithelial cells leads to an increase in compressive mechanical stress, the spreading of cancer cells is characterized as dissipative, ultimately resulting in a reduction of the residual stress within the spheroid core.

The efficiency of cancer spreading along the biointerface increases with: (i) an increase in epithelial surface tension relative to interfacial tension and (ii) establishment of the interfacial tension gradient.

Cumulative effects of physical cell-cell interactions appear in the form of various driving and resistive forces that govern spreading of cancer. Cancer invasion through epithelium was explored through a framework of the system of force balance equations, which merge surface and volumetric effects. This methodology facilitates an understanding of how the interplay of diverse physical parameters affects the velocities of epithelial and cancer subpopulations.

Further investigations are required to: (i) establish the relationship between the surface tension of the epithelium and the interfacial tension between epithelial and cancer cells; (ii) examine the gradients of interfacial tension in relation to the shear stress experienced by cells along the biointerface; and (iii) evaluate the effects of the swirling motion of epithelial clusters on the dynamics of the biointerface. A combination of experimental studies and complementary theoretical analyses is needed.

The approach that we have described has brought insights that promise new ways of inhibiting cancer metastasis, e.g. through the development of drugs to reduce the epithelial surface tension and to enhance the epithelium/cancer interfacial tension by the remodelling of cell-cell adhesion contacts.

Conflict of interest: The authors report that there are no conflicts of interest.

Funding: 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).

Authors' contributions: The authors contributed equally to the preparation of the manuscript.

Ivana Pajic-Lijakovic: Orcid https://orcid.org/0000-0001-9663-6916

Appendix

The viscoelasticity of the subpopulations depends primarily on the strength of cell-cell adhesion contacts and cell contractility. Metastatic cancer cells establish weak cell-cell adhesion contacts and migrate as a stream. Their migration corresponds to a convective mechanism. In contrast to cancer cells, epithelial cells establish strong cell-cell adhesion contacts and migrate in the form of cell clusters. Consequently, the migration of epithelial cells induces inhomogeneous storage of mechanical stress and corresponding inhomogeneous epithelial packing density, while the migration of cancer cells induces dissipation of mechanical stress resulting in a more homogeneous distribution of cancer packing density around n_{conf}. An increase in epithelial packing density, caused by inhomogeneous accumulation of compressive stress, triggers a transition in the mechanism of cell movement from convective, through diffusive, to sub-diffusive movement, resulting in a decrease in epithelial velocity. This change in migration mechanism also affects the viscoelastic properties of the epithelial subpopulation described by the particular constitutive model. As a result, a single constitutive model has been suggested to explain the movement of cancer cells, whereas the migration of epithelial cells has been characterized by three distinct constitutive models.

The linear constitutive model used for describing the viscoelasticity of the cancer cell subpopulation in the convective regime of cell movement (for $n_c \leq n_{conf}$) is the Maxwell model, which may be written in the form [29]:

$$\tilde{\boldsymbol{\sigma}}_{\boldsymbol{ci}}(r,t,\tau)+\tau_{Ri}\,\dot{\tilde{\boldsymbol{\sigma}}}_{\boldsymbol{ci}}(r,t,\tau)=\eta_{ci}\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ci}}(r,\tau) \qquad \text{(A1)}$$

where: $i \equiv S,V$; S is shear and V implies volumetric; t is the short time of minutes; τ is the long-time of hours; $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{ci}}$ is the cancer mechanical stress (shear or normal); $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ci}}$ is the cancer strain (shear or volumetric); $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{cS}}=\frac{1}{2}\left(\vec{\boldsymbol{\nabla}}\vec{\boldsymbol{u}}_c+\vec{\boldsymbol{\nabla}}\vec{\boldsymbol{u}}_c^{\,\boldsymbol{T}}\right)$ is the cancer shear strain and $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{cV}}=(\vec{\nabla}\cdot\vec{\boldsymbol{u}}_c)\tilde{\boldsymbol{I}}$ is the cancer volumetric strain; $\vec{\boldsymbol{u}}_c$ is the displacement field of the cancer subpopulation; $\tilde{\boldsymbol{I}}$ is the unity tensor; $\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ci}}$ is the cancer strain rate; $\dot{\tilde{\boldsymbol{\sigma}}}_{\boldsymbol{ci}}$ is the rate of cancer stress change; η_{ci} is the shear or bulk viscosity of the cancer subpopulation; and τ_{Rei} is the stress relaxation time of the cancer subpopulation.

This model for viscoelastic liquids describes stress relaxation from the initial value towards the cancer residual stress expressed as $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{cri}}=\eta_{\boldsymbol{ci}}\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ci}}$, under constant strain rate conditions $\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ci}}$, while the strain rate itself cannot relax. The stress relaxes within many short-time stress relaxation cycles under constant strain rate per cycle, while the strain rate change and residual stress generation occur on a long-time scale. The stress includes viscous and elastic contributions. The main characteristic of the Maxwell model is that elastic contribution to stress vanish during the stress relaxation, whereas the corresponding residual stress is purely dissipative.

The linear constitutive model, suitable for describing the viscoelasticity of the epithelial subpopulation in the convective regime of cell movement (for $n_e \le n_{conf}$), is the Zener model written in the form [29]:

$$\tilde{\boldsymbol{\sigma}}_{\boldsymbol{ei}}(r,t,\tau)+\tau_{Rei}\,\dot{\tilde{\boldsymbol{\sigma}}}_{\boldsymbol{ei}}(r,t,\tau)=E_{ei}\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}(r,\tau)+\eta_{ei}\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ei}}\,(r,\tau) \qquad \text{(A2)}$$

where: $i \equiv S,V$; S is shear and V is volumetric; $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{ei}}$ is the epithelial mechanical stress (shear or normal); $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}$ is the epithelial strain (shear or volumetric); $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{eS}}=\frac{1}{2}\left(\vec{\boldsymbol{\nabla}}\vec{\boldsymbol{u}}_e+\vec{\boldsymbol{\nabla}}\vec{\boldsymbol{u}}_e^{\,\boldsymbol{T}}\right)$

is the epithelial shear strain; $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{eV}} = (\vec{\nabla} \cdot \vec{\boldsymbol{u}}_e)\tilde{\boldsymbol{I}}$ is the epithelial volumetric strain; $\vec{\boldsymbol{u}}_c$ is the displacement field of the cancer subpopulation; $\tilde{\boldsymbol{I}}$ is the unity tensor; $\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ei}}$ is the epithelial strain rate; $\dot{\tilde{\boldsymbol{\sigma}}}_{\boldsymbol{ei}}$ is the rate of epithelial stress change; η_{ei} is shear or bulk viscosity of the epithelial subpopulation; E_{ei} is the Young's or shear modulus of the epithelial subpopulation; and τ_{Rei} is the stress relaxation time of the epithelial subpopulation.

This model for viscoelastic solids describes stress relaxation from its initial value towards the residual stress of $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{rei}} = E_{ei}\,\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}$ under constant strain $\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}$ and strain relaxation under constant stress. Stress relaxation takes place through multiple short-time cycles under a constant strain per cycle, while strain change and residual stress generation happen over a longer time period. The ability of strain to relax is a key characteristic of viscoelastic solids. The main characteristic of the Zener model is that viscous contribution to stress vanishes during the stress relaxation, whereas the corresponding residual stress is purely elastic.

The linear constitutive model, suitable for describing the viscoelasticity of the epithelial subpopulation in the conductive (diffusion) regime of cell movement (for $n_{conf} < n_e < n_j$), is the Kelvin-Voigt model written in the form [29]:

$$\tilde{\boldsymbol{\sigma}}_{\boldsymbol{ei}}(r,\tau) = E_{ei}\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}} + \eta_{ei}\,\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ei}}. \qquad \text{(A3)}$$

This model for viscoelastic solids characterizes the relaxation of strain under conditions of constant stress, with the stress itself unable to relax. The alteration in stress resulting from the collective migration of cells takes place over an extended period and encompasses both viscous and elastic components.

The non-linear sub-diffusive regime of movement of epithelial cells near the cell-jamming transition (for $n_e \to n_j$) can be described by the fractional model in the form

[29]:

$$\tilde{\boldsymbol{\sigma}}_{\boldsymbol{ei}}(r,\tau) = \eta_{\alpha i} D^{\alpha}(\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}) \quad \text{(A4)}$$

where: $\eta_{\alpha i}$ is the effective modulus; $D^{\alpha}(\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}})$ is Caputo's fractional derivative expressed as $D^{\alpha}\tilde{\boldsymbol{\varepsilon}} = \frac{1}{\Gamma(1-\alpha)}\frac{d}{dt}\int_0^t \frac{\tilde{\varepsilon}(r,\tau')}{(\tau-\tau')^{\alpha}} d\tau'$; $\Gamma(1-\alpha)$ is a gamma function [63] and α is the order of the fractional derivative (i.e., the damping coefficient) in the range $0 < \alpha \leq 0.5$. When $\alpha \to 0$, the stress becomes purely elastic and is $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{ei}} = E_{ei}\,\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}$, where $\eta_{0i} \equiv E_{ei}$ and the derivative $D^{0}(\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}) \equiv \tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}$. Otherwise, when $\alpha \to 1$, the stress is purely viscous, i.e., $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{ei}} = \eta_{\boldsymbol{ei}}\dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ei}}$, where $\eta_{1i} \equiv \eta_{\boldsymbol{ei}}$ and the derivative is $D^{1}(\tilde{\boldsymbol{\varepsilon}}_{\boldsymbol{ei}}) \equiv \dot{\tilde{\boldsymbol{\varepsilon}}}_{\boldsymbol{ei}}$.

This model of non-linear viscoelastic solid behaviour is primarily characterized by the inability of stress and strain to relax.

References:

1. Lee MH, Wu PH, Jack Rory Staunton JR, Ros R, Longmore GD, Wirtz D. (2012). Mismatch in Mechanical and Adhesive Properties Induces Pulsating Cancer Cell Migration in Epithelial Monolayer. Biophys J 2012; 102:2731–2741.
2. Millar FR, Janes SM, Giangreco A. Epithelial cell migration as a potential therapeutic target in early lung cancer. Europ Resp Rev 2017; 26:160069.
3. Campbell K, Rossi F, Adams J, Pitsidianaki I, Barriga FM, Garcia-Gerique L, Batlle E, Casanova J, Casali A. (2019) Collective cell migration and metastases induced by an epithelial-to-mesenchymal transition in Drosophila intestinal tumours. Nature Comm 2019; 10:2311.
4. Riehl BD, Kim E, Bouzid T, Lim YJ. The Role of Microenvironmental Cues and Mechanical Loading Milieus in Breast Cancer Cell Progression and Metastasis. Front Bioeng Biotechnol 2021; 8:608526.
5. Pajic-Lijakovic I, Eftimie R, Milivojevic M, Bordas SPA. The dynamics along the biointerface between the epithelial and cancer mesenchymal cells: Modeling consideration. Sem Cell Dev Biol 2023; 147:47-57.

6. Batlle E and Wilkinson DG. Molecular Mechanisms of Cell Segregation and Boundary Formation in Development and Tumorigenesis. Cold Spring Harb Persp Biol 2012; 4:a008227.
7. Lucia SE, Jeong H, Shin JH. (2022) Cell segregation via differential collision modes between heterotypic cell populations. Mol Biol Cell 2022; 33:ar129.
8. Pajic-Lijakovic I and Milivojevic M. Dynamics along the epithelial-cancer biointerface: hidden system complexities. Biocell 2023; 47(11):2321-2334.
9. Katt ME, Placone AL, Wong AD, Xu ZS, Searson PC. In Vitro Tumor Models: Advantages, Disadvantages, variables, and Selecting the Right Platform. Front Bioeeng Biotechnol 2016; 4(12), http://dx.doi.org/10.3389/fbioe.2016.00012.
10. Gunti S, Hoke ATK, Vu KP, Jr London NR. (2021). Organoid and spheroid tumour models. Techniques and applications. Cancers 2021; 13:874.
11. Wang Y and Zhou BP. Epithelial-mesenchymal transition – A hallmark of breast cancer metastasis. Cancer Hallm 2013; 1(1):38- 49.
12. Gandalovičová A, Vomastek T, Rosel D, Brábek J. Cell polarity signaling in the plasticity of cancer cell invasiveness. Oncotarget 2016; 7(18):25022-25049.
13. Barriga EH, Mayor R. 2019. Adjustable viscoelasticity allows for efficient collective cell migration. Sem Cell Dev Biol 2019; 93:55-68.
14. Yang J, Antin P, Berx G, Blanpain C, Brabletz T, Bronner M, Campbell K, Cano A, Casanova J, Christofori G, Dedhar S, Derynck R, et al. Guidelines and definitions for research on epithelial–mesenchymal transition. Nature Rev 2020; doi.org/10.1038/s41580-020-0237-9.
15. Wu JS, Jiang J, Chen BJ, Wang K, Tang YL, Liang XH. Plasticity of cancer cell invasion: Patterns and mechanisms. Translat Oncol 2021; 14:100899.
16. Heine P, Lippoldt J, Reddy GA,Katira P, Kaes J. Anomalous cell sorting behaviour in mixed monolayers discloses hidden system complexities. New J Phys 2021; 23:043034.
17. Kalli M and Stylianopoulos T. Defining the Role of Solid Stress and Matrix Stiffness in Cancer Cell Proliferation and Metastasis. Front Oncol 2018; doi: 10.3389/fonc.2018.00055.
18. Dolega ME, Delarue M, Ingremeau F, Prost J, Delon A, Cappello G. Cell-like pressure sensors reveal increase of mechanical stress towards the core of multicellular spheroids under compression, Nature Comm 2017; 8:14056 1-9.

19. Devanny AJ, Vancura MB, Kaufman L.J. Exploiting differential effects of actomyosin contractility to control cell sorting among breast cancer cells. Mol Biol Cell 2021; 32:ar24, 1-15.
20. Carey SP, Starchenko A, McGregor AL, Reinhart-King CA. Leading malignant cells initiate collective epithelial cell invasion in a three-dimensional heterotypic tumor spheroid model. Clinic Exp Metast 2013; 30:615–630.
21. Pajic-Lijakovic I, Eftimie R, Milivojevic M, Bordas SPA. Multi-scale nature of the tissue surface tension: theoretical consideration on tissue model systems. Adv Coll Int Sc 2023; 315:102902.
22. Serra-Picamal X, Conte V, Vincent R, Anon E, Tambe DT, Bazellieres E, Butler JP, Fredberg JJ, Trepat X. Mechanical waves during tissue expansion. Nature Phys 2012; 8(8):628-634.
23. Tambe DT, Croutelle U, Trepat X, Park CY, Kim JH, Millet E, Butler JP, Fredberg JJ. Monolayer Stress Microscopy: Limitations, Artifacts, and Accuracy of Recovered Intercellular Stresses. PLoS ONE 2013; 8(2):e55172 1-13.
24. Notbohm J, Banerjee S, Utuje KJC, Gweon B, Jang H, Park Y, Shin J, Butler JP, Fredberg JJ, Marchetti MC. Cellular Contraction and Polarization Drive Collective Cellular Motion. Biophys J 2016; 110:2729-2738.
25. Shellard A and Mayor R. Supracellular migration – beyond collective cell migration. J Cell Sci 2019; 132:jcs226142.
26. Clark AG and Vignjevic DM. Modes of cancer cell invasion and the role of the microenvironment. Curr Op Cell Biol 2015; 36:13–22.
27. Pérez-González C, Alert R, Blanch-Mercader C, Gómez-González M, Kolodziej T, Bazellieres E, Casademunt J, Trepat X. Active wetting of epithelial tissues. Nature Phys 2019; 15:79-88.
28. Pajic-Lijakovic I, Eftimie R, Milivojevic M, Bordas SPA. Segregation of co-cultured multicellular systems: review and some modeling consideration. Quart Rev Biophys 2024, 57(e5):1-17.
29. Pajic-Lijakovic I. Basic concept of viscoelasticity, in Viscoelasticity and collective cell migration: In Pajic-Lijakovic I and Barriga E (Eds), Viscoelasticity and collective cell migration: An Interdisciplinary Perspective Across Levels of Organization, Academic Press, US, 2021; p. 21.

30. Tse JM, Cheng G, Tyrrell JA, Wilcox-Adelman SA, Boucher Y, Jain RK, et al. Mechanical compression drives cancer cells toward invasive phenotype. PNAS 2012; 109(3):911–49.
31. Nnetu KD, Knorr M, Pawlizak S, Fuhs T, Kaes J. Slow and anomalous dynamics of an MCF-10A epithelial cell monolayer. Soft matter 2013; 9:9335-9341.
32. Atia L, Bi D, Sharma Y, Mitchel JA, Gweon B, Koehler S, DeCamp SJ, Lan B, Hirsch R, Pegoraro AF, Lee KH, Starr J, Weitz DA, Martin AC, Park JA, Butler JP, Fredberg JJ. Universal geometric constraints during epithelial jamming. Nature Phys 2018; DOI: 10.1038/s41567-018-0089-9.
33. Kollmannsberger P, Mierke CT, Fabry B. Nonlinear viscoelasticity of adherent cells is controlled by cytoskeletal tension. Soft Matter 2011; 7:3127-3132.
34. Schulze KD, Zehnder SM, Urueña JM, Bhattacharjee T, Sawyer WG, Angelini TE. Elastic modulus and hydraulic permeability of MDCK monolayers. J Biomech 2017; 53:210-213.
35. Grosser S, Lippoldt J, Oswald L, Merkel M, Sussman DM, Renner F, Gottheil P, Morawetz EW, Fuhs T, Xie X. et al. Cell and Nucleus Shape as an Indicator of Tissue Fluidity in Carcinoma. Phys Rev X 2021; 11:011033.
36. Pajic-Lijakovic, I., Milivojevic, M. Mechanical oscillations in 2D collective cell migration: the elastic turbulence. Front Phys 2020; 8:585681, doi: 10.3389/fphy.2020.585681.
37. Rudzka DA, Spennati G, McGarry DJ, Chim YH, Neilson M, Ptak A, Munro J, Kalna G, Hedley A, Moralli D, Green C, et al. Migration through physical constraints is enabled by MAPK induced cell softening via actin cytoskeleton re-organization. J Cell Sci 2019; 132:jcs224071.
38. Mohammed D, Park CY, Fredberg JJ Weitz DA. Tumorigenic mesenchymal clusters are less sensitive to moderate osmotic stresses due to low amounts of junctional E-cadherin. Sci Rep 2021; 11:16279.
39. Koride S, Loza AJ, Sun SX. Epithelial vertex models with active biochemical regulation of contractility can explain organized collective cell motility. APL Bioeng. 2018; 2:031906.
40. Mombach JCM, Robert D, Graner F, Gillet G, Thomas GL, Idiart M, Rieu JP. Rounding of aggregates of biological cells: Experiments and simulations. Phys A 2005; 352:525-534.

41. Stirbat, T.V., Mgharbel, A., Bodennec, S., Ferri, K., Mertani, H.C., Rieu, J.P., Delanoe¨-Ayari, H. Fine Tuning of Tissues' Viscosity and Surface Tension through Contractility Suggests a New Role for a-Catenin. PLOS ONE 2013; 8(2):e52554.
42. Nagle I, Richert A, Quinteros M, Janel S, Buysschaert E, Luciani N, Debost H, Thevenet V, Wilhelm C, Prunier C, Lafont F, Padilla-Benavides T, Boissan M, Reffay M. Surface tension of model tissues during malignant transformation and epithelial–mesenchymal transition. Front. Cell Dev. Biol 2022; DOI 10.3389/fcell.2022.926322.
43. Jafari J, Han X-I, Palmer J, Tran PA, O'Connor AJ. Remote Control in Formation of 3D Multicellular Assemblies Using Magnetic Forces. ASC Biomat Sci Eng 2019; 5:2532-2542.
44. Le HA and Mayor R. Cell–matrix and cell–cell interaction mechanics in guiding migration. Biochem Soc Trans 2023; doi.org/10.1042/BST20230211.
45. Bateman NW, Sun M, Hood BL, Flint MS, Conrads TP. Defining central themes in breast cancer biology by differential proteomics: conserved regulation of cell spreading and focal adhesion kinase. J Proteome Res 2010; 9(1):5311-24.
46. Hobson EC, Li W, Juliar BA, Putnam AJ, Stegemann JP, Deng CX, Resonant acoustic rheometry for non-contact characterization of viscoelastic biomaterials. Biomat 2021; 269:120676.
47. Pajic-Lijakovic I, Eftimie R, Milivojevic M, Bordas SPA. 2023d. The rearrangement of co-cultured cellular model systems via collective cell migration. Sem Cell Dev Biol 2023; 147:34-46.
48. Karbalaei A, Kumar R, Cho HJ. Thermocapillarity in Microfluidics—A Review. Micromach 2016; 7:13.
49. Petitjean L, Reffay M, Grasland-Mongrain E, Poujade M, Ladoux B, Buguin A, Silberzan P. Velocity fields in a collectively migrating epithelium. Biophys J 2010; 98(9):1790-1800.
50. Kaliman S, Hubert M , Wollnik C, Nuić L, Vurnek D, Gehrer S, Lovrić J, Dudziak D, Rehfeldt F, Sunčana Smith A. Mechanical Regulation of Epithelial Tissue Homeostasis. Phys. Rev. X 2021; 11:031029.
51. Khalilgharibi N, Fouchard J, Asadipour N, Yonis A, Harris A, Mosaff P, Fujita Y, Kabla A, Baum B, Muñoz JJ, Miodownik M, Charras G. 2019. Stress

relaxation in epithelial monolayers is controlled by actomyosin. Nature Phys. 2019; 15:839-847.

52. Marmottant P, Mgharbel A, Kafer J, Audren B, Rieu JP, Vial JC, van der Sanden B, Maree AFM, Graner F, Delanoe-Ayari H. The role of fluctuations and stress on the effective viscosity of cell aggregates. PNAS 2009; 106(41):17271-17275.
53. Guevorkian K, Brochard-Wyart F, Gonzalez-Rodriguez D. Flow dynamics of 3D multicellular systems into capillaries, in Viscoelasticity and collective cell migration (eds. Pajic-Lijakovic I and Barriga E) 2021; pp. 193 Academic Press, US.
54. Zhang Y, Xu G, Lee RM, Zhu Z, Wu J, Liao S, Zhang G, Sun Y, Mogilner A, Losert W, et al. Collective cell migration has distinct directionality and speed dynamics. Cell Mol Life Sci 2017; 74:3841–3850.
55. Campàs O, Mammoto T, Hasso S, Sperling RA, O'Connell D, Bischof AG, Maas R, Weitz DA, Mahadevan L, Ingber DE. Quantifying cell-generated mechanical forces within living embryonic tissues. Nature Meth 2013; doi:10.1038/nmMeth.2761.
56. Pajic-Lijakovic I, Milivojevic M. Successive relaxation cycles during long-time cell aggregate rounding after uni-axial compression. J Biol Phys 2017; 43(2):197-209.
57. Pajic-Lijakovic I, Milivojevic M., McClintock PVE. Role of viscoelasticity in the appearance of low-Reynolds turbulence: Considerations for modelling. J Biol Eng 2024; 18:24.
58. Dechristé G, Fehrenbach J, Griseti E, Lobjois V, Clair Poignar C. Viscoelastic modeling of the fusion of multicellular tumor spheroids in growth phase. J Theor Biol 2018; 454:102–109.
59. Bird RB, Stewart WE, Lightfoot EN. Transport Phenomena. New York, London, John Wiley & Sons INC, 1960.
60. Tlili S, Gauquelin E, Li B, Cardoso O, Ladoux B, Delanoë-Ayari H, Graner F. Collective cell migration without proliferation: density determines cell velocity and wave velocity. R. Soc. Open Sci 2018; 5:172421.
61. Murray JD, Maini PK, Tranquillo RT. Mechanochemical models for generating biological pattern and form in development. Phys Rep 1988; 171(2):59-84.
62. Röper K. (2013) Supracellular actomyosin assemblies during development. BioArchitect 2013; 3(2):45–49.

63. Podlubny I. Fractional Differential Equations, Mathematics in Science and Engineering. London Academic Press, 1999; 198, pp. 78.

Table 1. The driving and resistive forces, which influence cell rearrangement within mono-cultured epithelial and cancer spheroids, as well as co-cultured epithelial-cancer spheroids.

Figure 1. Process of segregation of epithelial and cancer subpopulations within co-cultured spheroids via collective cell migration

Figure 2a,b. Rearrangement of cells within: (a) mono-cultured epithelial spheroids and (b) mono-cultured cancer spheroids. Arrows indicate the direction of movement in each case.

Figure 3. Schematic illustration of the segregation in a co-cultured epithelium/cancer spheroid. Blue and yellow arrows show the directions of movement of cancer and epithelial cells, respectively).

Figure 4. Schematic diagram illustrating the influence of physical parameters on the spread of cancer cells.

Table 1. The driving and resistive forces, which influence cell rearrangement within mono-cultured epithelial and cancer spheroids, as well as co-cultured epithelial-cancer spheroids

Multicellular system	Driving forces	Resistive forces
Mono-cultured Epithelial spheroids	$n_e\vec{\boldsymbol{F}}_{\boldsymbol{st}}^{\ \boldsymbol{e}} = n_e\vec{\boldsymbol{F}}_{\boldsymbol{st}}^{\ \boldsymbol{e}} = n_e\gamma_e\vec{\boldsymbol{u}}_{\boldsymbol{e}}$ The epithelial surface tension force [36] drives cells from the spheroid surface towards the core region.	$\vec{\boldsymbol{F}}_{\boldsymbol{Tve}}^{\ \boldsymbol{e}} = \boldsymbol{\nabla}\cdot\left(\tilde{\boldsymbol{\sigma}}^{\,SD} + \tilde{\boldsymbol{\sigma}}_{\boldsymbol{er\,T}}\right)$ The epithelial viscoelastic force [47,61], reduces cell movement caused by an accumulation of cell residual stress $\tilde{\boldsymbol{\sigma}}_{\boldsymbol{er\,T}} = \tilde{\boldsymbol{\sigma}}_{\boldsymbol{er\,S}} + \tilde{\boldsymbol{\sigma}}_{\boldsymbol{er\,V}}$ and solid stress $\tilde{\boldsymbol{\sigma}}^{\,SD}$.
Mono-cultured cancer spheroids	$\vec{\boldsymbol{F}}_{\boldsymbol{Tve}}^{\ \boldsymbol{c}} = \boldsymbol{\nabla}\cdot\left(\tilde{\boldsymbol{\sigma}}^{\,SD} - \tilde{\boldsymbol{\sigma}}_{\boldsymbol{cr\,T}}\right)$ The cancer viscoelastic force drives movement of cancer cells from the spheroid core region towards its surface [61]. This collective movement of cancer cells reduces solid stress accumulated in the spheroid's core region.	$n_c\vec{\boldsymbol{F}}_{\boldsymbol{st}}^{\ \boldsymbol{c}} = n_c\gamma_c\vec{\boldsymbol{u}}_{\boldsymbol{c}}$ The cancer surface tension force, formulated by Pajic-Lijakovic et al. [5] reduces expansion of cancer cells to some extent depending on the magnitude of the cancer surface tension.
Epithelial subpopulation in co-cultured spheroids	$n_e\vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{e}} = n_eS^e\vec{\boldsymbol{u}}_{\boldsymbol{e}}$ The epithelial interfacial tension force of the epithelial subpopulation [5] drives epithelial compression within a co-cultured spheroid (where S^e is the epithelial spreading factor). $\vec{\boldsymbol{F}}_{\boldsymbol{mix}}^{\ \boldsymbol{c-e}} = \frac{\mathbf{1}}{l}\vec{\boldsymbol{\nabla}}\omega_a$ The epithelium/cancer mixing force drives the compression of epithelial cells and depends on the epithelium/cancer adhesion energy ω_a (where l is the average size of a single cell).	$\vec{\boldsymbol{F}}_{\boldsymbol{Tve}}^{\ \boldsymbol{c-e}} = \boldsymbol{\nabla}\cdot\left(\tilde{\boldsymbol{\sigma}}^{\,SD} + \tilde{\boldsymbol{\sigma}}_{\boldsymbol{er\,T}} - \tilde{\boldsymbol{\sigma}}_{\boldsymbol{cr\,T}}\right)$ The viscoelastic force within epithelium/cancer co-cultured spheroids [47], reduces movement of the epithelial subpopulation in a co-cultured spheroid. $n_e\vec{\boldsymbol{F}}_{\boldsymbol{FR}}^{\ \boldsymbol{e}} = n_e\xi_e(\vec{\boldsymbol{v}}_{\boldsymbol{c}} + \vec{\boldsymbol{v}}_{\boldsymbol{e}})$ The epithelial friction force [5], reduces movement of epithelial cells along the epithelium/cancer biointerface (where ξ_e is the friction coefficient of epithelial cells and the relative velocity between the subpopulation $\vec{\boldsymbol{v}}_{\boldsymbol{R}}$ is equal to $\vec{\boldsymbol{v}}_{\boldsymbol{R}} = \vec{\boldsymbol{v}}_{\boldsymbol{c}} + \vec{\boldsymbol{v}}_{\boldsymbol{e}}$).
Cancer subpopulation in Co-cultured spheroids	$\vec{\boldsymbol{F}}_{\boldsymbol{Tve}}^{\ \boldsymbol{c-e}} = \boldsymbol{\nabla}\cdot\left(\tilde{\boldsymbol{\sigma}}^{\,SD} + \tilde{\boldsymbol{\sigma}}_{\boldsymbol{er\,T}} - \tilde{\boldsymbol{\sigma}}_{\boldsymbol{cr\,T}}\right)$ The viscoelastic force stimulates movement of the cancer subpopulation in the co-cultured spheroid. $n_c\vec{\boldsymbol{F}}_{\boldsymbol{it}}^{\ \boldsymbol{c}} = n_cS^c\vec{\boldsymbol{u}}_{\boldsymbol{c}}$ The cancer interfacial tension force, formulated by Pajic-Lijakovic et al. [5], drives cancer extension within a co-cultured spheroid (where S^c is the cancer spreading factor). $\vec{\boldsymbol{F}}_{\boldsymbol{mix}}^{\ \boldsymbol{c-e}} = \frac{\mathbf{1}}{l}\vec{\boldsymbol{\nabla}}\omega_a$ The epithelium/cancer mixing force, formulated here, drives the extension of cancer cells.	$n_c\vec{\boldsymbol{F}}_{\boldsymbol{FR}}^{\ \boldsymbol{c}} = n_c\xi_c(\vec{\boldsymbol{v}}_{\boldsymbol{c}} + \vec{\boldsymbol{v}}_{\boldsymbol{e}})$ The cancer friction force, formulated by Pajic-Lijakovic et al. [5], reduces movement of cancer cells along the epithelium/cancer biointerface (where ξ_c is the friction coefficient of cancer cells).

Figure 1. Process of segregation of epithelial and cancer subpopulations within co-cultured spheroids via collective cell migration

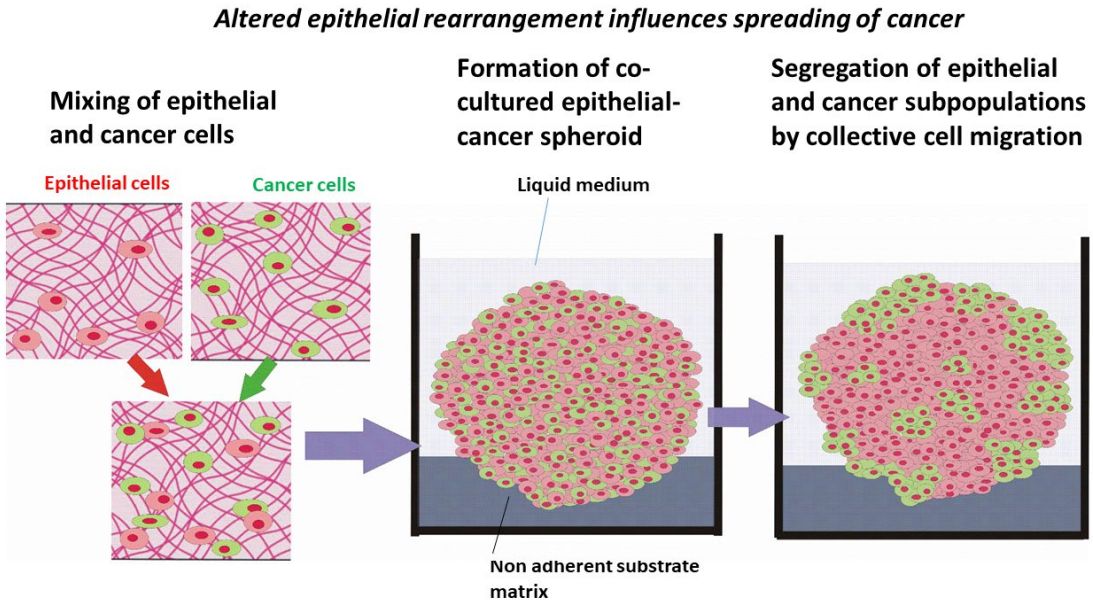


Figure 2a,b. Rearrangement of cells within: (a) mono-cultured epithelial spheroids and (b) mono-cultured cancer spheroids. Arrows indicate the direction of movement in each case.

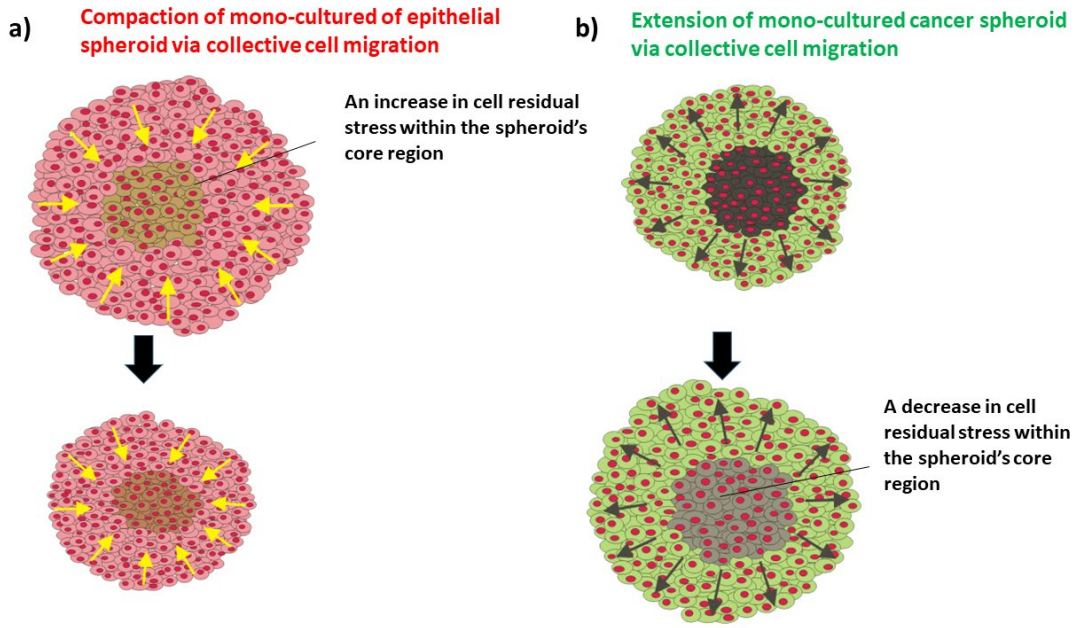


Figure 3. Schematic illustration of the segregation in a co-cultured epithelium/cancer spheroid. Blue and yellow arrows show the directions of movement of cancer and epithelial cells, respectively).

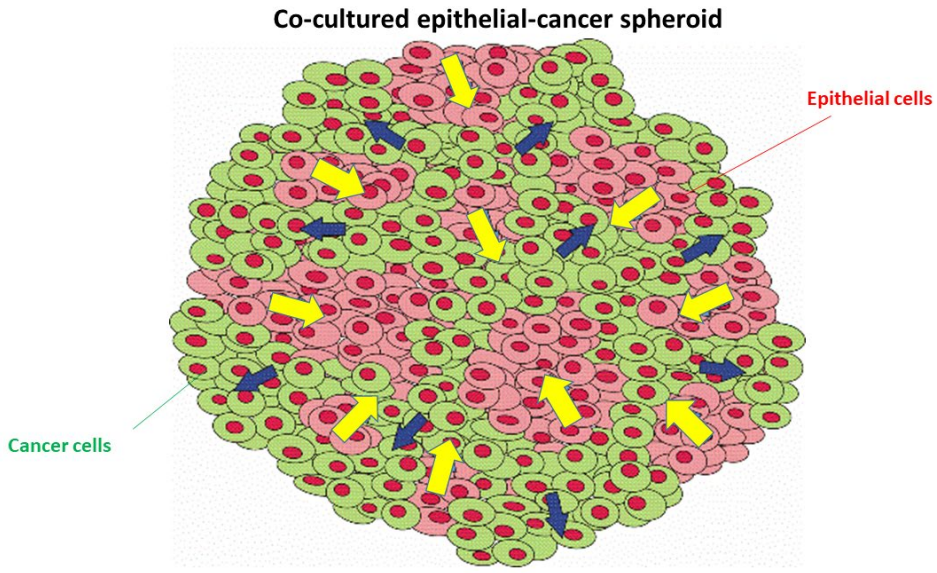


Figure 4. Schematic diagram illustrating the influence of physical parameters on the spread of cancer cells.