Collective Cellular Phase Transitions in Cancer

Adrian F. Pegoraro, Thien-Khoi N. Phung, and Jennifer A. Mitchel

Abstract The growth and metastasis of tumors are increasingly recognized to be an inherently collective, multiscale problem, wherein understanding at the genetic and molecular level is necessary but is not sufficient; the mechanical response of cells must also be accounted for to understand collective behavior in cancer. Like glassy, granular, and colloidal materials, cells exist in a fundamentally crowded and disordered environment and are capable of undergoing collective phase transitions between states resembling the material phases of solid, liquid, and gas. By mapping concepts from material science to cell motion, it becomes possible to better predict and understand how macroscopic properties of the cellular system – fluidity and rigidity – emerge from physical cellular-scale interactions. These cellular interactions, though enormously complex and variable from a biological standpoint, can be abstracted to generalized state variables, including density, cell shape constraints, and fluctuations, which allow phase diagrams to be constructed to aid in predicting behavior. In this chapter, we review both experimental evidence and theoretical frameworks toward understanding multicellular collectives as material systems, exploring both the power and the limitations of comparisons between biological and non-living soft matter systems. We conclude with how these lessons are being applied to develop a more holistic understanding of how physical constraints affect collective migration and invasion in cancer.

Keywords Cell rheology · Dynamic heterogeneity · Emergence · Flocking · Glass transition · Jamming · Phase transition · Soft matter

A. F. Pegoraro
Nanoscale measurement, Metrology Research Center, National Research Council, Ottawa, ON, Canada

T.-K. N. Phung
Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

J. A. Mitchel (✉)
Department of Biology, Wesleyan University, Middletown, CT, USA
e-mail: jmitchel@wesleyan.edu

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1 Introduction

Cancer is not one disease but a range of conditions which are characterized by aberrant, uncontrolled growth. Tumor growth can occur in any organ and leads to cell populations that are highly heterogeneous [1]. Tumor growth and eventual spreading through metastasis involve changes in cell mechanics, cell signaling, drug resistance, and a host of other properties measured at the single-cell level [2–4]. Understanding these changes is important for diagnosis and treatment of cancer, but measurements of single-cell properties alone are insufficient to understand cancer behavior. Tumor cells necessarily interact with each other and their environment, and understanding these interactions is needed for a holistic picture of disease progression [5].

Recent approaches to understanding the mechanics of cellular collectives have established that physical models can both aid interpretation and predict behaviors and properties of these collectives, many of which are not apparent at the single-cell level. Instead of focusing on individual cells, these frameworks treat the collective as a *material system* and aim to predict average behavior [6–9]. While it is possible to point to examples of different material phases in the body – including solids in the form of bones and cartilage, liquids in the form of blood and lymph, and gas in the lungs – the majority of our cell and tissue systems are better described as disordered materials. In everyday life, disordered materials are everywhere, from glass in our windows to pastes and colloids like toothpaste and yogurt to granular materials like coffee beans in a dispenser or sand on the beach. These diverse systems share the ability to behave either as a solid or liquid, or, in some cases, a gas. These systems exist in metastable states far away from a thermodynamic equilibrium, yet we can still make predictions about their behavior and mechanics.

The distinguishing feature of these systems is their lack of order at both the local and long-range scale. This has made it challenging to develop straightforward metrics to characterize local behavior, although ongoing developments in machine learning may allow the determination of such metrics [10–13]. Nonetheless, average behavior does change in predictable ways in disordered systems; for example, as a glass cools from a melted, viscous state, particle motion slows down and eventually arrests, and the glass transitions from a fluid to a solid material. This slowing and eventual arrest are accompanied by a change in the mechanical properties of the bulk system. The macroscopic stiffening, or solidification, corresponds at the microscopic level to an increase in molecular coordination, as the individual constituents of the glass coordinate their motion over longer time and length scales. That molecular coordination gives rise to bulk stiffness is an example of an *emergent mesoscopic phenomenon* (see Box 1). Emergent phenomena occur across all scales of life and embody the notion that “more is different”: that is, one cannot understand the behavior of the group by investigating the individual in ever-higher detail, while at each level of increasing scale or complexity, entirely new and unpredictable properties emerge [14–16]. While biological systems are more complex than inert materials, due to the presence of energy consumption,

biochemical signaling, biological coordination, and heterogeneity, through adapting our understanding of the physics of disordered media it becomes possible to relate the measurement of single-cell properties to many types of collective behavior.

Box 1: Terms and Concepts Originating in Physics and Materials Science
When describing cell collectives as a material, it is natural to draw upon the language of material science, but the correspondence of ideas is not exact. The concepts and terms are used as analogies, or behavior is described as being *like* a material phase. It is thus helpful to understand what a term means both in reference to inert material and in reference to living systems. Some of the most commonly used terms in the description of collective cell motion are:

Active matter: Material where each constituent particle consumes energy [203]. While this energy consumption is often tied to motility, it is not necessarily so. For example, motors in the cellular cytoskeleton consume energy and change the behavior of the cellular interior. Active matter systems are by definition not in thermal equilibrium; however, they can reach a meta-stable state.

Adhesion: This is the tendency of cells to attach to other materials. This can include adhesion to the substrate or extracellular matrix, but in the context of collective cell motion often refers to cell–cell adhesion. Cell–cell adhesion is controlled by a raft of proteins collectively referred to as the adhesome [204]. When compared to inert materials, adhesion is somewhat akin to the binding energy or attractive potential [6].

Agitation: Refers to the forces which tend to disturb the collective. In inert materials like glasses, this property is dominated by thermal fluctuations and is measured in units of k_BT where k_B is Boltzmann's Constant and T is temperature [60, 205]. For granular materials where the constituent particles are large, thermal forces are insufficient to displace the particles. Instead, external forces such as gravity or shear moves the particles; this is why granular systems are said to be athermal [29]. Despite this, by analogy with the effect of temperature in molecular systems, we can define an effective temperature for granular materials which reflects the degree to which agitation displaces the constituent particles [206]. Similarly, for active systems, which include self-propelled particles and cells, we can also define an effective temperature [207]. In this case, however, the effective temperature is related to the self-motility of the constituents and not related to external forces.

Attractive energy: When discussing the phase behavior of inert, particulate matter, this describes the tendency of particles to attract or repel each other. For a system of attractive solid particles to fluidize, the amount of agitation must be sufficient to overcome this barrier [30]. In cellular systems, the

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attractive energy is determined by balance of cell adhesion and contractility [41].

Colloidal systems: Inert materials where one insoluble phase is dispersed in another. The insoluble phase is small and remains dispersed in the supporting medium. A commonly used system is polymer microspheres dispersed in solution. In this system, the colloids are used as model atoms to help understand standard bulk materials [208]. Similarly, analogies are made between cellular systems and colloidal suspensions [209].

Contractility: The cytoskeleton of a cell is contractile. When a cell adheres to an outside material, it then pulls on its surroundings. The ability of a cell to contract is resisted by both its own compressibility and its adhesion to its surroundings. When combined with adhesion, contractility can be used to define an effective binding energy of a cell with its neighbors [41, 66].

Density: For a traditional condensed phase material, density is understood in the standard thermodynamic sense. In jamming parlance, density is better understood in terms of volume fraction; that is, the portion of free space taken up by the particles [31, 32]. In cell systems, density more typically refers to number density [33]. A confluent cell monolayer can cover all free space yet can still accommodate new cells when divisions occur; this changing number density can affect behavior [33, 85].

Dynamic heterogeneity: As a glass cools, transitioning from a liquid to a solid, it undergoes structural rearrangements [61]. The distribution of these rearrangements is non-Gaussian with very long tails. Practically speaking, this means that for particles to move, they must coordinate with their neighbors. As the system cools further, the length scale of this coordination becomes longer. The presence of dynamic heterogeneity in a system is considered a hallmark of glassy dynamics [60, 61, 205]. In cellular systems, both the distribution shape and an increasing length scale of collective phenomena have been used to identify dynamic heterogeneity in cells [33, 85].

Elastic: Materials which are elastic store energy when they are deformed from their resting state. This energy can be recovered if the force deforming them is removed. This is in contrast to viscoelastic materials.

Equilibrium: When discussing changes in material state, equilibrium can mean either a thermodynamic equilibrium or, as is more typical in disordered material, a local metastable equilibrium. The term equilibrium is also used to refer to different scale objects in the same system. For example, a monolayer that is in equilibrium typically means that large scale motion has arrested, but is not referring to internal cellular activity.

Flocking: A collective transition where groups of particles or cells move as a cohort [136]. Flocking effects can be seen in self-propelled systems, including cells [121, 144].

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Fluid versus solid: In inert materials, the distinction between these phases is obvious. In cell systems, the distinction is less clear and is partly dependent on the time-scale of observation [72]. In many cases, a relative comparison is more apt with cell collectives being described as more fluid-like or more solid-like in comparison to another grouping of cells. The character of the collective can be described in terms of order parameters or dynamic measurements of rearrangements. It is worth noting that in soft matter systems, which are condensed phases, that "fluid" is often synonymous with "liquid" despite the fact that gasses are also fluids. Because many of the concepts of soft matter have been adopted to discuss collective cellular motion, fluid is often used to describe systems which are flowing even if they are strictly speaking behaving more like a liquid state.

Fragile glasses: When a glass melts from a solid to a liquid, it can either change its viscosity rapidly or slowly, in which case it is called either fragile (rapid change) or strong (slow change) [210]. For molecular systems, evidence suggests these changes in macroscopic behavior are related to the underlying structure [211]. For colloidal glasses where the glass formers are particles themselves, instead the behavior of the glass being either fragile or strong is related to the stiffness of the colloids, with stiff particles forming fragile glasses and soft particles forming strong glasses [48, 212]. In biological systems, isolated cells appear to undergo a glass transition when being osmotically compressed that is consistent with soft particles forming a strong glass [209], although this may be dependent on metabolic activity as well [213]. It has also been proposed that collective cell behavior is consistent with the formation of strong glasses made of deformable particles [6, 214].

Glass transition: For inert materials, glass formation and jamming have some commonalities, but also differences, with the primary difference being that jamming occurs in athermal systems, whereas glass transitions happen in thermal ones [60]. In cellular systems, these terms are occasionally used interchangeably to describe the transition from a more fluid-like configuration to a more solid-like one.

Glassy material: As an amorphous liquid cools, it can solidify by undergoing crystallization. Glasses, however, solidify while remaining amorphous. This temperature-driven transition is accompanied by slowing dynamics yet increasing dynamic heterogeneity [60, 61, 205]. Both cell collectives and the interior of a cell can be described as glassy materials [33, 209].

Granular material: A collection of macroscopic particles that are big enough that they do not move due to thermal motion [29]. Common examples of granular material are sand, coffee beans, or rice. Energy dissipation in these systems is often due to friction. Jamming transitions were first

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hypothesized for granular systems where arrest is largely due to geometric confinement [31, 32]. Because it is easy to visualize granular material and its jamming transition, the language used to describe cell collectives has borrowed extensively from these concepts [6].

Emergent phenomenon: Broadly defined, emergent phenomena are behaviors that cannot be predicted solely by looking at individual units of a whole [215]. Collectivity or collective behavior connote similar ideas. In disordered media, there are many such phenomena. For example, a glass transition where disordered particles transition from a liquid to a solid is an emergent property; looking at any one particle, it would not be possible to predict whether this system is solid-like and only by looking at the collective can this property be determined. An example from living material is flocking; the motion of a single cell may look persistent, but when compared to its neighbors, it becomes more obviously collective behavior. Conveniently, in reference to both inert and living materials, emergent maintains a consistent definition.

Jamming transition: A collective effect that helps explain how disordered systems transition from fluid-like to solid-like behavior, it was initially proposed as a potential explanation for behavior in a wide range of systems such as glasses, colloids, and particles [30, 31]. It is possible to develop a rigorous definition of jamming, which is due to geometric confinement and occurs in the absence of any activity or agitation [60]. In practice, however, jamming is often used to describe the emergence of a range of collective phenomena in disordered materials and used to describe systems ranging from microscopic particles to traffic jams. For many systems, a jamming transition is associated with the transition from a fluid-like state to a solid-like state where the material resists deformation. Somewhat confusingly, some of the same language is used to describe flocking formation as well [216]. When discussing cells, the term jamming transition is used to describe both types of behavior in cells; jamming is used to describe motion arrest [33] and flock formation [121, 144].

Mesoscale: A "middle" length scale where the actual length scale is dependent on the system being studied. Weather patterns [217], cell motion [7, 218], and individual polymers [219] all have an effective mesoscale. In some sense, the mesoscale of a system is related to emergent phenomena and collectivity; it is a length scale that spans multiple individual units and where new physics may appear. Like emergent phenomena, the definition of mesoscale is maintained for inert and living systems.

Order parameter: An order parameter is any system parameter which can be used to distinguish an ordered phase from a disordered one. It describes the long-range order of a material. It can be structural, such as density or crystallographic phase, or thermodynamic, such as magnetic or dielectric

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susceptibility [220]. Because order parameters are used to distinguish phases of material, there is not an exhaustive list of them and new ones, such as recent ones proposed for some disordered materials [10–12, 221], can be developed over time to help better understand material properties.

Percolation: Given a series of nodes, it is possible to form links between them, be they fluid channels, electrical connections, bonds, or some other connection. As links are randomly added, the network can grow rapidly at a critical density of links, and the network spans the extent of the system. Under these conditions, macroscopic changes in system behavior can occur. In both inert [222] and cellular [40] systems, percolation refers to the formation of system spanning networks which give rise to changing material properties.

Phase transition: For transitions where order parameters change, such as solid to liquid, a phase transition is a clear change in the state of matter. For disordered materials, the transition from a fluid to a solid is less clear. For jamming and glass transitions, these transitions are often not associated with a distinct change in an order parameter and are instead identified by changing correlation lengths and time scales and might more correctly be described as kinetic arrest [32]. Flock formation, however, is more akin to freezing with the nucleation and growth of a flock behaving solid freezing in a liquid bath yet the system remains disordered [136, 216].

Rigidity transition: Rigidity transitions can be ascribed to numerous causes, but are characterized by a change in the macroscopic material properties of the system being studied. In cell systems, the term rigidity transition is used to describe both resistance to neighbor swapping [66] and changes to the elastic modulus of a tissue [40, 202]. The opposite of the rigidity transition is the yielding transition, a term which is more frequently used in reference to condensed matter systems [223, 224].

Soft matter: Broadly defined as systems where interparticle bond strength is on the same order as thermal energy.

Strong glasses: See fragile glasses.

Viscoelastic: Materials which are viscoelastic dissipate some of the energy used to deform them. If a force is applied and then removed, the system does not return completely to its original state. Nonetheless, some energy is stored by these materials and can be recovered. Many soft matter systems, and biological systems, are viscoelastic.

One of the most commonly described, and intuitively accessible, collective emergent phenomena is **jamming**, a concept simply illustrated (and best characterized) with inert materials. Imagine a box containing a relatively low number of spheres. These spheres are free to move around if the box is tilted or jostled. As more

and more spheres are placed in the box, their ability to roll freely is restricted. Eventually, as a critical number of spheres is added, the system becomes crowded and motion stops; this is a jamming transition from a fluid-like state where motion can occur to a solid-like state where motion stops. As discussed in this chapter, in cell systems, a similar type of transition occurs despite the fact that cells are active, soft particles which can replicate and adapt to their environment. By recognizing this parallel between inert, disordered materials and cellular collectives – because cellular collectives exhibit transitions between collectively mobile and immobile states that are reminiscent of transitions between fluid-like and solid-like states in inert materials – we can apply the insights from materials science and soft matter physics to understand complex biological phenomena, including cancer. Of course, there are differences between the collective transitions observed in simple inert matter systems and in cells. Nonetheless, the overall concept – motion changing in response to neighbors – has led to much of the terminology of jamming, and phase transitions more broadly, being adapted to cellular systems. Definitions of terms and concepts which originated in the world of materials science, and which have since been adopted for use in describing cellular systems, are presented in Box 1, which covers how the different terms are used at the intersection of these different fields.

There are three core ideas underpinning the experimental results and theoretical frameworks discussed in the rest of the chapter. First, as was highlighted with the example of spheres in a box, density, defined as the number of particles in a given space, can affect whether or not those particles are free to move. This is true for both inert materials and living systems. Second, how those particles interact also affects behavior. Returning to spheres in a box, we can imagine the behavior of the system will be different if the particles stick to each other after a collision or bounce off one another like billiard balls. In inert systems, this behavior is described as an inter-particle attraction (or repulsion). For living systems, "particle-particle," or more accurately, "cell–cell" adhesion is determined by an array of proteins, collectively called the adhesome [17]. Because cell–cell adhesion is controlled by the cells themselves and changes over time, it is a critical parameter in understanding collective cell motion, especially in the context of cancer where adhesome changes may drive cancer progression [18]. The third key axis for understanding collective material transitions is agitation. In our example of spheres in a box, the box is tilted and shaken to impart energy to the particles. This is an example of a granular system in which external energy is needed to move the particles, in contrast to soft matter systems, where thermal energy is sufficient to displace the particles, like in the example of cooling glass. In living systems, it is clear that thermal agitation is not the main driver of cell motion. Instead, agitation comes from the cells themselves which exist out of thermal equilibrium and expend energy to migrate, grow, and rearrange their environment. In the language of soft matter/jamming, cells are active matter that consumes energy. Despite all the differences, these systems all exhibit similar behavior at transition points where the collective material physics changes between fluid-like and solid-like or vice versa. Said another way, when density, particle interactions, and agitation balance, emergent collective phenomena appear

across a diverse range of systems that nonetheless bear a striking similarity to one another.

Within the framework of phase transitions of cellular systems, when cells jam, collective motion slows (i.e., becomes more solid-like) with reduced ability for cells to rearrange themselves, whereas when they unjam, collective motion increases (i.e., becomes more fluid-like) with more frequent rearrangements. By recognizing the existence of these material phases in cellular systems, solid-like versus fluid-like (using the terminology of soft matter and jamming) and solid versus liquid versus gas (if using the terminology of traditional condensed matter), it is possible to construct phase diagrams to describe cellular behavior. Many such phase diagrams have been constructed as theoretical and experimental predictions and evidence have evolved (Fig. 1). Each phase diagram is not definitive, but instead is a tool for thought that aids in the interpretation of experimental data and making predictions about how changes at the single-cell level will be reflected in the behavior of the collective. The evolution of the proposed phase diagrams depicted in Fig. 1 also highlights that cellular jamming occurs in a multidimensional space. Early phase diagrams focused on specific state parameters, while later ones have transitioned to classes of state parameters which encompass several different cellular and environmental properties. To understand how these concepts apply to cancer, it is first necessary to understand how these concepts apply to cellular systems in general, both in terms of the underlying physics and the experimental evidence to support these models. It is only thus armed that it is possible to discuss how aberrant processes in cancer fit into this framework.

2 Cell Jamming: From Sand to Cells

In cell systems, many experiments have shown that both motion and rate of division slow as cell density increases, through the phenomena of contact inhibition of locomotion and contact inhibition of proliferation, respectively [19–21]. While the role of biochemical signaling has long been recognized in these processes [22–25], the role of cell mechanics and physical forces has only more recently been explored. Experimental evidence demonstrates that mechanical forces could play an important role in determining behavior in collective systems [26], with several different models from soft matter physics being used to help explain collective cell properties [27, 28]. The related phenomena of jamming and glass transitions, which are collective phase transitions between motile and arrested states, offered new possibilities for understanding both contact inhibition and cell motion [29–32]. A hallmark of classical jamming transitions – kinetic arrest over time as the density of the constituent particles rises – has been observed in living systems in vitro [33–38] and in vivo [39, 40].

In each of these diverse examples, the cellular collective can transition between two states: a lower-density state, where cells collectively behave in a gas-like or liquid-like manner and a higher density state, where cells collectively behave in

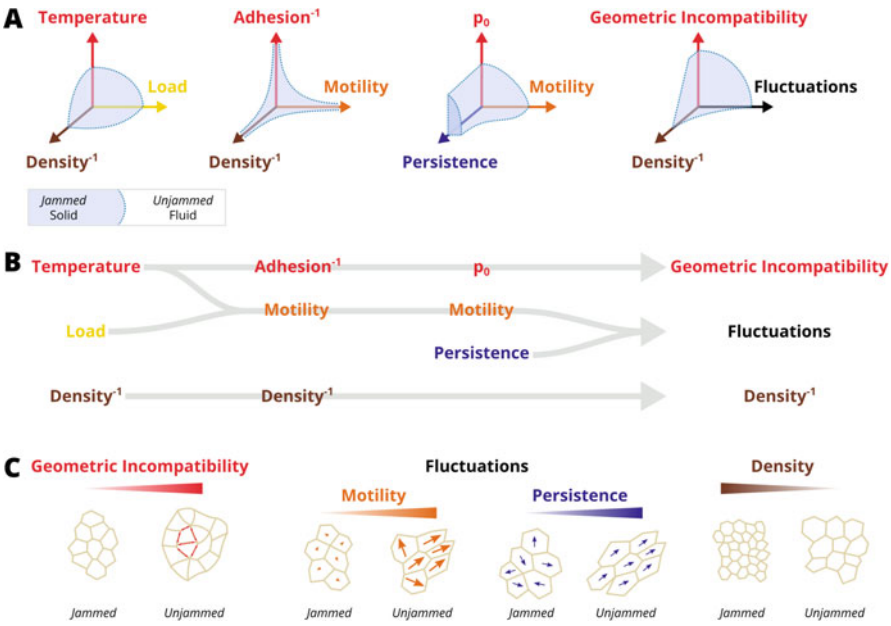

Fig. 1 Evolution of phase diagrams for living systems. (**A**) Phase diagrams for cell jamming were adapted from established work in soft matter physics (left-most panel), where close to the origin the system is solid-like (shaded in blue) and beyond the phase boundary the system is fluid-like [30, 31]. Proposed diagrams for cells introduced analogous variables describing more complex cell behaviors. A first proposed phase diagram for cell jamming (second panel) added inherent cell motility as a possible state variable [6]. At constant density, cell motility can be further decomposed into inherent motility and directional persistence, while adhesion is subsumed into a preferred shape parameter p_0 (third panel) [41]. Because many cell parameters can contribute to collective cell motion, it has been proposed to recast the cell jamming phase diagram in terms of broad classes of parameters to facilitate predictions of collective motion (right-most panel) [42]. (**B**) Through-lines connecting the state variables for cell jamming. In inert materials, temperature and interparticle attraction are in opposition, so a natural evolution of that variable is cell–cell adhesion. External loads for inert materials can in turn be replaced by internal forcing, as can be caused by active, motile particles. Motility itself can be decomposed into different components such as absolute speed and its persistence, that is, the tendency of cells to continue migrating in a given direction. Adhesion, in turn, can be viewed as a component of intercellular force balance which can be represented as a preferred shape parameter. (**C**) The evolution of the cell jamming phase diagram has resulted in generalized parameters which encompass several different cell properties. Geometric incompatibility accounts for force balance between cells due to contractility, cell–cell adhesion, cytoskeletal elasticity, and related parameters. Fluctuations account for active cellular processes and its derivatives such as inherent motility, persistence, and others. Density for cell systems typically reflects number density, since area fraction is often unity

a solid-like manner. In the low-density state, cells are less constrained by their neighbors and may have less contact with them, thus allowing greater freedom to move; and, by contrast, cells in the high-density state have greater contact with their neighbors, restricting the ability of single cells to move. A multicellular system

that is increasing in density over time, for example due to the proliferation of the constituent cells, has thus been hypothesized to undergo a *jamming transition*, analogous to the observation of an increase in viscosity with increased particle density observed in granular, colloidal, or glassy materials (Box 1, Fig. 1). In some cases, density, per se, may not be increasing, but rather the contact between neighboring cells increasing [40] or extracellular space between them decreasing [39]; in all of these closely related, but physically distinct, situations, individual cells are increasingly constrained or caged by their neighbors.

As will become clear, many of the hallmarks of cell jamming arise from making analogies to inert materials. It is thus not surprising that early proposed phase diagrams for cell jamming [6] (Fig. 1A, second panel) drew upon already established work in soft matter physics (Fig. 1A, left panel) [30, 31]. However, as the field of collective cell jamming has matured, it has become clear that while the concept of phase transitions and state variables holds, the details are quite different between inert and living matter. Cells have active machinery to maintain their shape and can engage in persistent directed motion as opposed to random thermal motion of inert materials (Fig. 1A, third panel) [41]. More generally, cells are active matter which consume energy and can agitate their surroundings while simultaneously resisting changes due to their external environment (Fig. 1A, right panel) [42]. The recognition of this more complex behavior is reflected in the evolution of the proposed phase diagrams and state variables for cell jamming (Fig. 1B) resulting in general classes of variables as opposed to focusing on specific parameters (Fig. 1C). However, certain through-lines have remained, in particular that cell density is important, and also that developing metrics to recognize and characterize a change in phase is needed to establish that the material state has changed. Thus it is necessary to be familiar with the various changes in motion and mechanics that accompany changes in collective cell phase.

While the arrest of motion is the most easily recognizable feature of a cellular system undergoing a phase transition from a more fluid-like state toward a more solid-like state, several other system properties are expected to, and indeed have been observed to, change during this transition and impact biological function. In addition to the arrest of motion, these include changes in bulk rheology, dynamic heterogeneity, and structural changes; evidence of each of these in cellular systems is discussed below. Beyond that, we discuss how these hallmarks of phase transitions manifest in living systems in response to changing biologically relevant variables.

2.1 Hallmarks of Collective Cellular Phase Transitions

2.1.1 Arrest of Motion

In 2011, Angelini and colleagues demonstrated that, as cell density rises in a confluent layer of model epithelial cells (see Fig. 2A), the average migration speed of cells within the layer decreases in a manner that is consistent with a jamming or

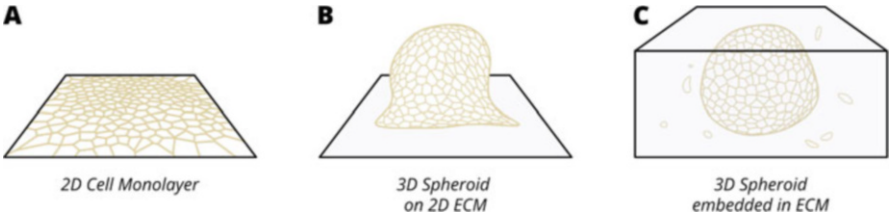

Fig. 2 In vitro models for studying phase transitions. Collective cellular phase transitions have been most commonly studied using (**A**) 2D monolayer cell culture. However, more complex 3D models have also been developed to study migration phase transitions. For example, spheroids can be used to study changes in cell phase by (**B**) placing them on 2D ECM [27, 28, 163], or (**C**) embedding them in 3D ECM [121, 130, 131]

glass transition [33]. In this experiment, all available surface area of the substrate was filled with cells – that is, observation of the system began at area fraction 1; subsequently, the number density of cells increased as cells proliferated (Fig. 1). As the number density of cells increased over time, individual cells necessarily decreased in their cross-sectional area (Fig. 1C, right panel, "density"). The authors drew parallels between the observed slowing of movements within the cellular collective with the behavior of a particulate system as it becomes increasingly crowded and approaches a glassy, or jamming, transition [33]. This seminal study was performed using the Madin-Darby Canine Kidney (MDCK) model epithelial line. The observation that system dynamics slow toward the total arrest of motion as cellular crowding increases due to the proliferation of cells in a confluent monolayer has since been shown in MDCK cells [37, 43, 44], immortalized and primary human bronchial epithelial cells (HBECs) [34, 45], and in cell lines derived from epithelial breast cancers [38, 46].

This density-dependent phase transition – relatively slower motions at relatively higher cell number densities – was later demonstrated to be due directly to differences in cell density and not a confounding effect occurring due to aging of the system such as depletion of nutrients. Saraswathibhatla and colleagues directly compared confluent monolayers of MDCK cells seeded at low versus high densities [36]. Consistent with previous reports in systems where cell density differences resulted from proliferation, here the low-density cell layers were considerably more motile than the high-density layers; therefore, the higher-density cell layers are interpreted to be closer to a jammed, solid-like state.

Changes in cell density giving rise to system-slowing toward arrest have also been observed in vivo during developmental processes [47]. During axis elongation in the developing zebrafish embryo, a solid-to-fluid transition was observed in the extending end of the axial tissue [39]. In this system, the posterior end of the embryo elongates, and cells at the mesodermal progenitor zone – the extending tip – initially display fluid-like movements; as elongation continues, these cells become incorporated into the presomitic mesoderm, which is more solid-like. The transition between the fluid-like and solid-like states is correlated with a gradient in the activity

of N-cadherins, such that increases in cell–cell adhesion gives rise to a decrease in extracellular space, solidifying the presomitic mesoderm. This change in behavior is confirmed both by observing cellular rearrangements and measuring the change in yield stress of the tissue.

2.1.2 Changes in Bulk Rheology

The change in yield stress measured during axis elongation of the developing zebra fish [39] highlights another key expectation of cell jamming transitions: if a phase change occurs, we expect to observe changes in bulk rheology of the system. In both jamming and glass transitions for inert materials, as the system becomes more solid-like, the viscosity increases and a bulk rigidity emerges [29–31, 48]. In 2D cell systems, measuring bulk rheology has been challenging, as the majority of the available techniques for measuring cell mechanics were developed to probe molecular or cellular-level properties. Local cellular mechanics can be measured using techniques such as AFM [49], optical tweezers [50], magnetic twisting cytometry [51, 52], Brillouin scattering [53], and more. Single-cell stiffness measurements may reveal evidence of dynamic heterogeneity [54], as discussed below, but bulk measurements are more traditional hallmarks of phase transitions. Though experiments have been limited in 2D contexts, Nnetu and colleagues showed that, for an expanding monolayer, the local elastic modulus of cells in lower-density areas was lower compared to in high-density areas, with the relationship between density and elastic modulus scaling as a weak offset power law [46]. Recent works have demonstrated that it is possible to directly measure the mechanics of cellular sheets by removing them from the underlying substrate [55], although it is unclear if a fluid-like layer could survive the process or how cell migration would proceed after removal.

Measurements of changing bulk rigidity have instead largely been used with 3D geometries (see Fig. 2B for an example system). For model spheroid systems, multiple techniques take advantage of the relatively large size and ease of handling to measure bulk rheology of the entire tissue [28]. These techniques include, amongst others, parallel-plate compression, micropipette aspiration, aggregate centrifugation, aggregate fracture, aggregate fusion, and laser ablation [28]. Micropipette aspiration in particular has found success in measuring collective phase changes in 3D cell systems [40, 56]. In micropipette aspiration, suction is applied to a material and its subsequent displacement through the pipette tip can be used to infer its mechanical properties. By changing the size of the pipette opening and pressure applied, it is possible to measure the mechanical properties of a range of living systems, from soft cells, which can have stiffness on the order of a few Pa [57], to tissues with stiffness up to the order of 10s of kPa [58]. When applied to a developing embryo, the Hannezo and Heisenberg groups find that the blastoderm undergoes fluidization and solidification by directly measuring the changes in bulk rheology that occur during this process using micropipette aspiration [56]. This collective transition is found to be driven by changes in cell–cell connectivity:

initially, cell–cell junctions are disrupted and the volume of the interstitial space increases, reducing the packing fraction of cells while the tissue fluidizes. As junctions reform and the interstitial volume is reduced, the tissue solidifies. Modeled as a rigidity percolation transition akin to what occurs in granular material [40], this provides clear evidence that collective transitions occur in biological systems.

That bulk rheology is tuned by interstitial volume in developing tissues had previously been found by measuring the local yield stress in the mesodermal progenitor zone [39] through the technique of ferrofluid deformation [59]. By implanting a force actuator inside the tissue, the authors were able to measure local yield stresses and found that the tissues with lower yield stresses had larger interstitial volume. Interestingly, this change in yield stress was correlated with changes in cellular motion, which is in contrast to the change in stiffness observed by micropipette aspiration of the developing blastoderm, where there was no obvious correlation with motion. This difference highlights that collective cell phase transitions are complex and can demonstrate some, but not all, features of glassy transitions, depending on the underlying mechanism which drives the transition.

2.1.3 Dynamic Heterogeneity

As inert systems undergo a density-dependent jamming transition – as the volume becomes more packed and each particle becomes caged by its neighbors – they exhibit dynamic heterogeneity, large fluctuations, and collectivity [32, 60–62]. These physical hallmarks have been observed in epithelial monolayer forces, stresses, migration patterns, and structure [6, 37]. Dynamic heterogeneity refers to the spatial correlations of dynamic properties; typically this has been applied to motion and velocity, but can also be applied to forces in cellular systems (see Box 1). Velocities are straightforward to measure in many cases but revealing the forces at work is more challenging. To measure the forces exerted by the cells on their matrix, traction force microscopy can be used. If the cell layer is confluent and assumed to be roughly uniform, it can be treated like a continuous material and forces inside the monolayer can also be calculated [63, 64]. Interestingly, dynamic heterogeneities in cellular systems can be observed using either forces [63, 64] or velocities [33], indicating the broad relevance of this physical hallmark in multicellular phase transitions.

Early evidence of dynamic heterogeneities of forces in cells was collected by Trepat and colleagues using MDCK cells on soft substrates [63]. The traction forces within an advancing monolayer are highly dynamic with large fluctuations that are not related to the structure of the underlying substrate or the presence of leader cells [63]. Interestingly, when the strength of these fluctuations is observed over time, they are found to not be normally distributed as is expected for random fluctuations but instead show evidence of exponentially decaying tails, a feature often associated with jamming behavior and dynamic heterogeneity. To further explore this behavior, the traction forces can be integrated in space; because the monolayer is not accelerating across the substrate, any unbalanced forces are necessarily balanced by

stress in the monolayer itself. Calculation of this monolayer stress reveals that cell monolayers primarily exist in a state of constant tension [63] that varies dramatically in time and space [64]. Indeed, these variations are remarkably similar to those observed in other glassy systems where spatial correlations spontaneously arise and fall within the layer. Here, Tambe and colleagues found that not only do packs form when looking at spatial correlations of the forces but also the average size of these packs grows as the system approaches arrest, as would be expected for glassy systems.

That monolayer stress demonstrates evidence of dynamic heterogeneity was a beautiful complement to the work by Angelini and colleagues discussed above [33]. In that study, beyond showing that MDCK cells arrested motion as density increased, they also showed the formation of packs of cells by segmenting based on velocity. By looking at the distribution of the fastest 20% of cells, they found that as cell density increased, the fast cells tended to cluster into packs; however, these packs were not tied to specific structural changes and were instead simply transient grouping that formed. Together these studies provided compelling initial evidence that dynamic heterogeneities occur in living systems [65].

Dynamic heterogeneity has also been observed in cell stiffness when Fujii and colleagues used AFM to study cell stiffness in an MDCK monolayer near the jamming transition [54]. Despite variations in individual cell stiffness, stiffness was correlated over long ranges among neighboring cells. These correlated mechanical properties depended on a long range actin filament network that formed within the collective. Interestingly, the length scale of the correlation in cellular stiffness was quite different from what had been observed when looking at monolayer stress. The formation of more permanent bonds via a long-range actin network raises questions as to whether these stiffness networks meet the traditional definition of dynamic heterogeneity in inert materials. Determining how these networks evolve in time will help clarify this in the future.

2.1.4 Structural Changes

Historically, hallmarks of jamming transitions relied on dynamic measurements because single snapshots in time were not thought to provide sufficient information to predict the material state. As mentioned above, recent works using machine learning have revealed that this is not necessarily the case. By testing a wide range of inert materials systems and simulations of collective material, the Liu group revealed that structural parameters could be derived which predict where particle rearrangements will occur [10–13]. Known as the “softness parameter,” this metric is based using many structural measurements and multidimensional fits. Interestingly, recent work has shown that this same parameter can be applied to models of cellular monolayers [13]. The vertex model, and the closely related Voronoi model, have been used extensively to model cellular monolayers by treating cells as space-filling polygons where each unit incurs an energy cost as it departs from its preferred perimeter and area [26, 41, 66, 67]. By applying their machine

learning approach to an in silico model of cells, Tah and coworkers were able to show that rearrangements and "cellular" motion can be predicted solely by looking at stationary images of the system.

Interestingly, the vertex model itself has been used to predict structural changes in living cell monolayers that are indicative of jamming in cell systems. For example, Bi et al. used the vertex model to predict that as cell shape, as measured by the cell shape parameter q, defined as the ratio of cell perimeter to the square root of cell area, decreases toward the cell shape of a pentagon ($q \sim =3.81$), energy barriers to motion would arise and cell monolayers would jam (Fig. 1A) [66]. The importance of the pentagonal cell shape comes from the shape change required for a T1 transition: while hexagonal cells ($q \sim =3.72$) *cannot* undergo a T1 transition, those cells which adopt a more elongated shape are able to rearrange freely. The vertex model predictions by Bi and colleagues rely on the assumption that cells have a preferred value of their cell shape, denoted p_0, and that rearrangements will occur as cells attempt to adopt this p_0; experimentally, we can attempt to estimate p_0 by measuring q. This prediction was subsequently confirmed in primary asthmatic cells in culture [45]. Subsequent works have demonstrated that in more complex systems – with active propulsion of the cells [41], heterogeneity of the constituents [68], or active fluctuations caused by cell division [44] – the absolute prediction of the critical shape index can change, but the overall trend that as the shape index decreases, cells become more jammed, holds.

The success of the shape index in marking transitions in collective cell motion inspired other structural markers of behavior to be investigated such as cellular aspect ratio [69]. Much like shape index, the average aspect ratio of the cells in a collective monolayer decreases as the cells approach jamming. While not necessarily directly predictive for comparisons across cell types, changes in aspect ratio are predictive of cell jamming across a range of systems, including inert materials [69], asthmatic and healthy lung cells [70], and cell lines of different metastatic potential [38]. Beyond aspect ratio, other order parameters have been shown to be indicative of collective cell behavior as well, with changes in cell volume indicating transitions from gas-like to liquid-like behavior and changes in cell shear indicating transitions from liquid-like to solid-like behavior [43].

The length scale over which different order parameters are predictive of cell behavior remains unclear. For example, in 3D model tumors (Fig. 2C), cell shape changes from the core to the periphery [71] and appears to be predictive of how dynamic, or fluid-like, the cells are [72]. This suggests that changes in structural parameters could be used to assess mesoscopic shifts in material phase on the length scale of several cells. However, these measurements, as with the others highlighted here for living cells, largely quantify average as opposed to local behavior and are often best understood as characterizing relative changes in state. Softness measurements can predict local behavior but have not yet been applied to living cell systems, only model ones where the individual constituents are homogeneous [13]. Whether local behavior is universally predictable from structural measurements in living systems remains unclear.

2.2 *Understanding Collective Cell Dynamics Through Phase Transitions*

In the jamming phase diagram of inert materials, changing the density or effective volume fraction is a conceptually straightforward method of affecting system behavior with clear spatial signatures of the change, yet it is not the only route to inducing a jamming/unjamming transition [30, 31]. In particular, temperature or agitation, along with applied or external load, have been suggested as two important control parameters for inert materials (Fig. 1A, left panel). Similarly, we expect in cell systems that it should be possible to induce fluidization and solidification by changing system parameters other than cell density. Indeed, the first proposed phase diagram for cell jamming was made by analogy to the ones for inert materials and suggested that cell–cell adhesion and cell motility could be alternative control parameters for cell jamming (Fig. 1A, second panel) [6]. As will be illustrated below, these parameters and many others can be used to describe cell jamming in what is effectively a multi-dimensional phase space [42, 72]. Despite the increase in complexity of working with biological systems, it is still possible to make predictions about collective cell behavior using effective phase diagrams and measurements of cellular properties.

2.2.1 The Complex Roles of Cell Density, Division, and Maturation

The most intuitive instance of multicellular phase transitions occurs when increasing cell number leads to arrest of motion, in an easily recognizable analogy to jamming of inert materials [6, 73]. However, the role of density and the impacts of cell crowding have been shown to be much more complicated than initially predicted. While the intuitive conception, in which an increase in cell number leads to arrest of motion, is a powerful framework for interpreting and predicting cellular phase transitions, it is also context-dependent, such that studies have shown an unexpected relationship between the phase state of the system and parameters, including density, cell division, and system maturation. For cellular systems, density can change in two distinct ways: first, volume fraction can change, as cells transition between a sub-confluent state, in which gaps between cells exist, to a confluent state, in which there are no gaps between cells (illustrated in Fig. 3A); second, a confluent layer of cells can change in density as cells divide and die, with cells modulating their volume and spread area to maintain coverage of the surface (illustrated in Fig. 1C).

At low density, where cells are sub-confluent, the interactions between cells can be broadly understood to fall into one of two categories: contact inhibition of locomotion (CIL), or cell–cell adhesion or aggregation [74, 75]. During CIL, cells make an initial contact which actively repolarizes the motility machinery such that cells reverse direction and migrate away from each other [74]; a sped-up time-lapse movie of two cells undergoing CIL is reminiscent of two billiard balls colliding. Importantly, the molecular mechanisms at play in CIL – namely, the collapse of

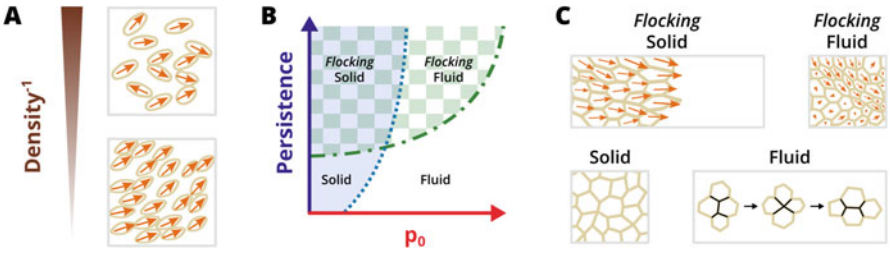

Fig. 3 Flocking phase transitions. (**A**) Increasing density of cells can trigger a kinetic phase transition, such that while cells at lower densities migrate individually, at higher densities they migrate as a collective cluster or flock [8, 137, 139]. (**B**) At constant density, varying cell preferred perimeter (p_0) or directional persistence leads to solid-fluid transitions with or without flocking behavior [121, 144]. (**C**) Solid-like cellular collectives do not feature motion or rearrangements, while fluid-like collectives exhibit prominent local rearrangements characterized by T1 transitions throughout the system. Flocking solids and flocking fluids exhibit large-scale movement of collective clusters; however, flocking fluids include local rearrangement of individual cells whereas solid flocks do not demonstrate neighbor swapping

the leading-edge of a migrating cell in response to meeting a neighboring cell – are essential for guidance of collective migration in morphogenetic processes such as migration of the neural crest [74–78]. However, many epithelial cells will not undergo CIL when meeting; rather, they will aggregate together, forming cell–cell junctions [75, 79]. This process appears reminiscent of two soap bubbles meeting. Therefore, the nature of the transition from sub-confluent to confluent may depend in part on the inherent properties of the constituent cells: whether they are individualistic cells that will tend to undergo CIL vs. inherently communicable cells that will tend to aggregate. Cells which undergo CIL are able to form "supercells," in which only the cells at the leading edge of the cell cluster have lamellipodia, and thus exhibit a fascinating and rich form of emergent collective behavior [80].

Regardless of whether cells tend to undergo CIL or adhere to their neighbors upon collisions, as groups of cells transition from sub-confluent to confluent, whether by proliferation, migration, or confinement, emergent multi-cellular phenomena occur. In cells that are inherently individualistic, such as sarcoma or mesenchymal cells, crowding can cause cells to behave as a collective [6, 81–83]. Friedl and colleagues demonstrated that melanoma and fibrosarcoma cells will switch from individual to collective migration when confined by their extracellular environment [81], despite the lack of cell–cell adhesion machinery expressed by these cells. The effect of confinement on collective migration in cancer is discussed below (Sect. 3.1). Similarly, cells with inherent tendency to aggregate (epithelial cells) also become increasingly collective under confinement or as density shifts from sub-confluent to confluent [34, 35, 84]. At subconfluent densities, epithelial cells can move freely and motion tends to be diffusive, indicating gas-like behavior, but as they become more crowded, they become caged by their neighbors and eventually transition from gas-like to liquid-like motion [34]. Further, as cell density increases, epithelial cells tend to cluster into ever-larger groups [43], where

intermediate densities and cluster sizes exhibit the fastest overall migration [35]. The transition from gas-like to liquid-like as epithelial cells proliferate from sub-confluent to confluent is captured by a volumetric order parameter [43].

Once a cell layer is confluent, such that there is no free space between neighboring cells, cell density can change considerably, generally on the order of ~2– to 4-fold from "low" to "high" density, all while maintaining confluence and intact intercellular junctions [33, 36, 37, 43, 44, 69, 85, 86]. As described above (Sect. 2.1.1), a hallmark of increasing cell density through proliferation within a confluent monolayer is arrest of motion and a transition from a fluid-like to a solid-like state. Another hallmark of phase transitions that occurs in systems with variable density is emergence of cooperative groups of cells, referred to as clusters, packs, or flocks; these are discussed in detail below (Sect. 2.2.4). Importantly, changes in density concur with structural changes, as cells become less elongated and variable in shape and become more rounded and isotropic in shape [36, 37, 43, 44, 69].

Depending on the experiment performed and the outcome measured, studies have reported both gradual and sharp changes in motion, correlation, structural signature, or material properties of the collective as a function of crowding and cell–cell contact [34, 40, 43, 45, 56, 69]. Importantly, in these experiments where cell density changes, whether the changes are moderate or extreme, other control parameters are observed to change in tandem. During growth in culture systems or during morphogenesis in development, cellular collectives mature and individual cells differentiate. During these processes, cell-substrate and cell–cell contacts mature, and cells change their expression profiles and cellular identities; these processes are occurring concurrently with cell division and arrest of motion [34, 45, 69]. Importantly, maturation and differentiation of epithelial layers have received attention as being drivers of a jamming transition, such that delayed maturation in the case of diseased cells corresponds to a delay in the jamming transition [45, 69]. Another control parameter predicted to play an important role in cellular phase transitions is active motility [41, 66, 70], or "cell jiggling," [8] which can take the form of fluctuations at the cell–cell interface or be generated by traction forces exerted by cells (Fig. 1). As cell density increases or as cell–cell contacts increase, the concurrent arrest of motion may in some cases be due to a reduction in generation of active movements by the cells [39, 44]. While some progress has been made in teasing out the relative contributions of density changes in tandem with active force generation and system maturation, this remains a complex area with many open questions.

Though many studies have demonstrated the arrest of cell motion and other hallmarks of a jamming transition with increasing cell density or cell–cell contact, there are fewer studies investigating the inverse process. That is, how do collective properties such as motion, rheology, heterogeneity, and local coordination change when cell density or cell–cell contact are *reduced* throughout an initially jammed multicellular collective? There are limited studies relevant to this scenario, but the expectation is that reduced density or reduced cell–cell contact would cause an unjamming transition or tissue-level fluidization.

Mitotic rounding during cell divisions, extrusion of cells due to crowding, and loss of cells due to apoptosis can all disrupt cell–cell contacts or reduce the number of cells in a crowded monolayer. Though cell division, and the concomitant increase in cell number, tends to be associated with tissue solidification and jamming, cell division has also been shown to drive local rearrangements during normal gastrulation [87]. The stresses associated with these events have been predicted by physical models [88] and found experimentally [44] to induce local fluidization. During gastrulation in the chick embryo, a model of amniote morphogenesis, cell division is in fact necessary for cell movement, such that inhibition of cell division stabilizes the tissue, keeping it in a solid-like state without local movements [87]. The effect of reduced cell–cell contact without large changes in cell density has been investigated as well. During epiboly in the developing zebrafish, cells in the central blastoderm experience a loss of cell–cell contact that causes a sharp decrease in tissue viscosity; tissue-level fluidization is spatially restricted to this region [40, 56]. Together these data indicate that while in many circumstances, increases in cell number will promote collective arrest, cell divisions themselves can introduce active fluctuations which can fluidize a group of cells, pointing toward the complexity of understanding the role of even the most seemingly straightforward control parameters.

Cell extrusion is a widespread phenomenon used by both developing and homeostatic systems to maintain proper cell density. Cell extrusion and concomitant reduction in cell density occur when individual cells in the layer undergo apoptosis; in this case, the dying cell signals to its neighbors to contract around it, thus preventing disruption of monolayer integrity [89]. Cell extrusion can also occur in response to overcrowding in an apoptosis-independent manner, in which a fraction of cells undergo a progressive loss of junctions and are pushed out of the epithelium [90]. Importantly, extrusion of single cells or small groups promotes migration of nearby cells [91]. The effect of large-scale apoptosis on monolayer integrity and dynamics was recently investigated through use of an inducible-apoptosis system, in which epithelial cells expressing an inducible apoptosis-inducing construct were mixed with wild-type cells [92]. In this study, large-scale defects occurred, as one-third of the cells present in the monolayer expressed the apoptosis-inducing construct. In response to this large-scale disruption, cells spread out and exhibited migratory dynamic heterogeneities and structural hallmarks of an unjamming transition. Further work is needed to fully illuminate the behavior of density-dependent fluidization, to determine how reduction in cell numbers or contacts results in a solid-to-fluid phase transition across a wide variety of systems and circumstances.

2.2.2 Phase Transitions at Boundaries

In the examples discussed up to this point, cell density has changed relatively gradually, for example, through cell proliferation. These experiments logically lead to a related question: what happens when cell density changes dramatically or

suddenly, for example, in response to the creation of a wound or the removal of a barrier? It has been recognized for over a century that epithelial sheets will migrate into an empty space in an attempt to re-epithelialize and heal a perceived wound [93]. Collective movement of a group of cells into an open space or across a boundary occurs in the context of wound healing, development, and cancer.

Injury of the epithelium causes the formation of a free, unconstrained edge. The resulting asymmetry in both mechanical constraints and in engagement of mechanosensitive junctional complexes of cells at the wound boundary induces the formation of a leading edge [94]. Cells at the leading edge of a wound quickly adopt a front-back polarization, including the formation of a lamellipodium – a flat, spread cellular structure filled with branched actin and typically associated with cellular motility [95]. Though wounds in vivo involve a range of molecular and cellular mechanisms, including cell death and the release of damage-associated molecular patterns and reactive oxygen species [96], studies in vitro have revealed that the presence of a free edge itself, for example, created by removal of an inert, non-adhesive barrier, is sufficient to trigger leading edge repolarization and subsequent migration into the free space [97–99]. As the leading edge cells detect and begin to migrate into the newly open space, leader cells or leader groups (sometimes called fingers) can emerge [99]. In many cases these leader cells are designated by the environment, as geometry can determine where leader cells emerge, with areas of high curvature promoting formation of leader cells in a cytoskeletal tension-dependent manner [100], and leader cells can be "selected" by followers, with regions of high stress behind the leading edge preceding the emergence of a leader cell [101].

In response to the creation of a wound or gap, the initial movement of the cell monolayer occurs only at the wound boundary, with the first row of cells beginning to move and the bulk of monolayer remaining immobile [98]. Because these leading edge cells are mechanically and biochemically coupled to their neighbors [94], cellular movement begins to penetrate progressively deeper into the layer as each row of cells pulls on those behind them. This process manifests as a wave of movement that travels back into the layer from the front, where the initiation and propagation of these waves require both intact intercellular junctions and formation of cellular structures essential to migration such as lamellipodia [98, 102, 103]. After many rows of cells become migratory and begin to travel into the wound as a coordinated group, continuous mechanochemical waves of cell-stretching and contraction function to polarize the cell collective in the direction of movement [103, 104]. The migrating monolayer has characteristics of a glassy or fluid-like system, including spatial dynamic heterogeneity [37, 105], and can vary in density significantly from the leading edge into the bulk [102, 106, 107]. Importantly, changes in self-propulsion or alignment have also been found to be sufficient to unjam the leading edge of a wound, independent of density changes [108]. Together these data support a framework in which the advancing front of a monolayer can be understood to be fluid-like, whereas the immobile bulk of the collective remains solid-like, thus indicating that wound-healing can occur as a wave of unjamming.

As wounds heal, the two epithelial layers meet, whereupon barrier function and layer integrity can be restored. When these two cell sheets meet, they can either mix, with cells from each side crossing the collision line into the other layer, or a boundary can form, with each cell remaining adjacent to its original neighbors and not crossing that line. Mesenchymal cells readily mix following monolayer collision, while epithelial cells tend to form stable, long-lived boundaries [105, 109–111]. When two migratory epithelial monolayers collide, and do not mix, this is a form of a jamming transition [105] and indeed, just as a wave of unjamming propagates in response to a wound, a wave of jamming occurs in response to the collision with a dense monolayer [109]. Interestingly, in heterotypic collisions between epithelial monolayers of different densities, higher-density layers are able to displace lower-density layers with a speed proportional to the density gradient, moving the boundary until a new equilibrium is reached [110].

2.2.3 Density-Independent Phase Transitions

We have extensively discussed the evidence for, and circumstances leading to, phase transitions in multicellular systems in which cell density changes and thus drives the behavior of the system. However, in a vast array of biological systems, proliferation is balanced with apoptosis and extrusion, such that homeostatic balance is maintained [90, 112–114]. Both theoretical predictions [26, 115, 116] and experiments [90, 113] support the model that mechanical stresses essentially monitor cell density, orchestrating cell division when cells are too large or are stretched [117–119], and on balance, triggering extrusion of dead or living cells when cells are too compressed [89, 90, 112, 113, 119]. These observations therefore prompt the question: in these crowded cellular systems where overall density does not change, is it possible to see phase transitions between fluid-like and solid-like behaviors?

First theoretically, and then confirmed experimentally, such transitions have been found for cell layers. Theoretical models largely derive from the vertex model and subsequent improvements [26, 41, 66, 67]. To picture cell rearrangements, imagine that cells are randomly distributed in a layer, yet attached to their neighbors; these cells may be far from their preferred shape p_0, due to being either stretched or compressed. As cells begin to move toward their ideal shape, they necessarily push and pull on their neighbors; this push and pull is determined by the elasticity of the cell itself, by the strength and fluidity of its adhesions to its neighbors, and its ability to contract and pull against those constraints. As the strain increases on a cell–cell junction, it becomes energetically favorable to swap neighbors instead of maintaining the current neighbors. This neighbor swap is known as a T1 transition in the parlance of soft matter physics and is also known as intercalation in the language of developmental biology (see Fig. 3C). Intercalation is recognized as being critical to embryonic development and is controlled by the cytoskeletal structural machinery of the cells; moreover, in these developmental systems, intercalations can occur at constant density and lead to large scale rearrangements of the cell layer [120]. In

terms of fluid-like versus solid-like behavior, neighbor swapping facilitates motion toward a ground state where cells are able to better match their preferred shape. As the cells approach this ground state, the question of whether motion continues then becomes a question of energy balance. For example, if the density is low, cell–cell junctions are strained and it is easier to swap neighbors, whereas at high density the strain on cell–cell junctions is reduced and it requires more energy to swap neighbors. This balance of forces and striving toward a preferred shape is an intuitive reason why cell shape itself has emerged as a useful predictor of cell jamming dynamics in a wide range of systems.

With this physical picture in mind, it then becomes possible to investigate neighbor swapping both theoretically and experimentally to see how changes to cell properties can lead to transitions between fluid-like and solid-like states at nearly constant density. The phase diagram proposed for a constant-density system has evolved over time [45, 66, 67]; a popular, and as shown below, useful version uses axes of preferred cell shape, motility, and persistence (Fig. 1A) [41, 70, 121]. Preferred shape, denoted p_0 and discussed in Sect. 2.1.4 above, is hypothesized to be set by a ratio between the cell-intrinsic properties of adhesion and contractility [26]. Experimental evidence for the role of these parameters individually in cell shape determination has shown that adhesion between neighboring cells tends to elongate cell boundaries, not unlike the lengthening that occurs when two strips of Velcro meet; cortical tension or contractility, on the other hand, has been shown to shorten cell–cell contact boundaries, as individual cells tend to become more rounded [26, 122–124]. Motility, or self-propulsion, is also hypothesized as a control parameter, as is directional persistence, or the tendency of an individual cell the move in a given direction [41].

One of the first demonstrations of the success of this framework, and in particular of the utility of measuring cell shape, came from studying primary human bronchial epithelial cells (HBECs) where cell motion slowed and cells approached a more uniform preferred shape over time [45, 69]. Interestingly, in this same cell system using well-differentiated HBECs, solid-like to fluid-like transitions can be provoked at constant density, triggered by the application of mechanical compression or exposure to ionizing radiation [45, 69, 70, 125, 126]. The biophysical characteristics of this collective fluidization were shown to be consistent with predictions from theoretical models in which cells that have a higher motility can more easily overcome energy barriers to rearrangements [41, 70]. Importantly, when these cells are triggered to unjam by either ionizing radiation or mechanical compression, they concurrently remodel their basal actin stress fibers [126], a process hypothesized to generate the propulsive forces responsible for the higher cellular motility that allows these cells to overcome the energy barriers to rearrangement [36, 127]. Furthermore, in this system, the HBEC layers fluidize without the characteristic weakening of cell–cell junctions that is observed in the epithelial-to-mesenchymal transition [70, 125].

The biochemical signals that initiate fluidization in cell collectives are still under investigation and may be disease or cell-type specific. For example, recent studies have found that in idiopathic pulmonary fibrosis, activation of EGFR,

Yap, and IL-6 can all promote collective fluidization in differentiated, previously stationary airway epithelial cells [128, 129]. However, these same studies found these pathways did not appear to be active in fluidization related to chronic obstructive pulmonary disease. Density-independent collective fluidization of an intact epithelial cell layer has been found to occur in other cell types, as well. For example, in MCF10A breast epithelial cells, overexpression of the small GTPase Rab5a, a regulator of endocytosis, induces collective fluidization in both 2D cell layers and 3D spheroids (Fig. 2A, C) [121, 130, 131]. Further, pharmacological activation of the GTPase RhoA fluidizes high-density confined monolayers of MDCK cells through reorganization of the actin cytoskeleton and generation of elevated tractions [36]. Importantly, the theoretical models utilized here essentially rely on force balance and, as such, we would expect to see changes in monolayer stress associated with changes in cell motion if the models of cell jamming hold. While not measured for all cellular systems where jamming/unjamming occurs at constant density, several of the systems highlighted here have had cell forces measured directly, or have examined a proxy for cellular forces, and they do indeed change dramatically through the observed phase transition [36, 121, 126, 130]. Together, current theoretical and experimental evidence supports the conclusion that, when cell density is roughly constant, it is through changes in cell force balance that cells can transition from one material state to another.

The variety of biological details across these studies demonstrates the usefulness of a unifying jamming framework, while also raising questions about its limits. Multiple distinct triggers – compression, ionizing radiation, elevated expression of Rab5a, and activation of RhoA, EGFR, YAP, or IL-6 – all result in qualitatively similar behaviors: initially jammed layers of epithelial cells become fluidized, wherein cells migrate in cooperative multicellular packs and swirls, while maintaining constant density. While it is unknown whether this range of triggers activates a common set of targets or converges on a single pathway, multiple studies have established a role for EGFR and ERK signaling in collective fluidization [70, 121, 129, 130], which have also been found to be essential in coordinated cell migration in other contexts [103, 132, 133].

Recently, it has been proposed that the density-dependent and density-independent phase diagrams could be unified into a single phase diagram where both density and cell-intrinsic properties are represented as control parameters (Fig. 1A, right panel) [42]. Here, motility and persistence, along with other forms of active movement such as tension fluctuations, are collapsed into the parameter "fluctuations," while the constituent elements of preferred cell shape, adhesion, and cortical tension, are generalized to geometric incompatibility [134]. Future studies will have to explicitly explore this phase space, to understand how collective phase transitions depend on complex changes across multiple axes.

2.2.4 Local Coordination and Flocking

Emergent collective behavior is exemplified by dynamic heterogeneities of motion, characterized by transient clustering of cells into flocks or packs (Fig. 3). Coordinated motion within groups is found at all scales of life, from bacteria to cells of the neural crest during development or from cancer invasion to schools of fish and flocks of birds and beasts [135, 136]. Experimental and theoretical results suggest that three simple physical rules are sufficient to predict and interpret collective motions across multiple scales: first, individuals cannot occupy the same space as each other and therefore exhibit some degree of repulsion when close together; second, individuals are attracted to come close together when far apart or exhibit some degree of cohesion when in close proximity to each other; and third, neighboring individuals exhibit some degree of alignment with each other. This framework has been used to understand collective motions in both active matter and living systems [135]. Within the context of phase transitions in multicellular systems, phase space variables hypothesized or experimentally tested to control flocking in crowded multicellular systems include density, alignment, persistence, motile force, and cell–cell interactions. Though experimental details vary widely between studies, the emerging physical picture is one in which cellular crowding and the resulting physical constraint from neighboring cells causes cells to self-coordinate into locally aligned, locally cooperative, and locally coordinated migratory packs [33, 35–38, 70, 121, 137]. In practice, this has been measured by tracking cellular motions in a crowded system and measuring clusters of cells grouped by speed [33] or orientation [38, 70, 85], or by measuring the difference between each cell's displacement and that of its nearest neighbors [36, 138].

Collective or cooperative motions in crowded multicellular systems have been explored both as a consequence of increased density, and also within constant-density confluent systems as a function of multiple control parameters (Fig. 3A, B). Multiple terms have been used to describe this transient clustering of cells and their usage is not always consistent across the literature. For our purposes, coordinated or collective movement refers to similarity in trajectory between neighboring cells, while cooperative movement connotes common purpose or an effect on behavior beyond what would occur in the absence of these clusters. Therefore, inert materials can exhibit collective behaviors, as multiple units act together through purely physical means, but it is only in living systems where we observe truly cooperative behaviors, as physical interactions are reinforced by biological responses. For example, groups of cells can form an actin-based superstructure across multiple cells, and groups of cells can behave as "supercells." [80] Importantly, though cellular groupings are cooperative over the time scale that such a superstructure exists, membership in the group can change over time and therefore the group also remains dynamic. Further, in cellular systems where processes are not necessarily reversible, and a spontaneously formed grouping may not dissipate with the same kinetics as its formation, the difference between collective and cooperative could become meaningful.

Cooperativity has been observed, where beyond the emergence of clusters themselves, the group appears to work together as a cohesive whole. For example, several papers have found that the formation of clusters leads to higher migration speeds than would occur in the absence of clustering [37, 38, 70]. More broadly, whether the formation of clusters is determined by looking at the monolayer stress [37], velocity alignment [38, 70], or velocity magnitude [33], their presence seems to enhance the ability of cells to migrate in a dense environment (see Sect. 2.2.3). Moreover, as these clusters increase in size, the effect becomes more pronounced, wherein larger clusters lead to greater migration [38, 70].

In a foundational study published in 1995, Vicsek and colleagues theoretically explored emergent self-ordered motions characteristic of a kinematic phase transition in a system of self-propelled particles [139]. By modeling particles moving with a constant velocity, which align their direction of motion to neighboring particles within a certain radius, the authors found a strong role for both persistence (in the form of stochastic reorientation of the velocity vector) and for density. The result from this work with the largest impact on understanding cellular collectives was the observation that, at high density, the particles spontaneously formed flocks that moved collectively. This prediction was directly tested a decade later when different densities of highly migratory keratocytes were observed over time. Primary fish keratocytes have been shown to migrate with fast and highly persistent motion [140] and are classically used as a model to understand individual cell motion. As density was increased from sparse to crowded, a sharp transition from random motility to collective migration of clusters of cells was observed (Fig. 3A) [137].

Classic Vicsek style systems are different from those considered previously because they lack cell–cell adhesion and collective motion is, instead, largely driven by orientational coordination as opposed to direct force contact. Given the similarity of these collective packs to groups of animals, they are often called flocks and the transition is known as a flocking transition [141–143]. Interestingly, flocking transitions have recently been identified in epithelial layers as well, where cell adhesion does play a role [121]. In these types of transition, the preference of cells to orient with their neighbors helps control the overall phase. In work by Giavazzi et al., the authors extended existing self-propelled Voronoi models to include a preference of neighbors to mutually align; under these conditions, alignment preference, along with factors such as density and preferred cell shape, can define new types of phases of collective motion [144]. Interestingly, under these conditions, cells can form a *flocking solid* where the entire assembly moves as one unit, but neighbor exchange is rare or a *flocking liquid* where a subset of the cells form a flock, but still exchange neighbors with those cells outside of the flock (Fig. 3B, C). These types of orientationally induced alignment are part of a broad category of geometric constraints, also called topological constraints, which lead to collective phenomena in a broad range of soft matter, and in particular active matter, systems [145].

Though the overarching picture established above, wherein cellular crowding and resulting physical constraints promote the emergence of cell clusters, holds across many systems, the relationship between changing densities and details

of flock characteristics varies between systems. For example, increasing density due to proliferation can lead to larger, slower packs, as might be expected by analogy to glassy dynamics [6, 33, 34]. However, in other systems, higher density corresponds to smaller, slower packs [36] and reduced correlations in movement [46]. Similarly, increasing maturity and differentiation of the system, which can occur with relatively small increases in cell density, also results in packs that are smaller and slower [34, 45]. Therefore, while density increases or system maturation can both result in a jamming transition, as characterized by arrest of motion, the precise nature of the dynamic packs that form and dissipate during this process varies across experimental systems.

In addition to the emergence of collective and cooperative behaviors as a function of density changes, clusters of coordinated cells can form in already-crowded systems at a constant density (Fig. 3B, C) [45, 70, 121, 125, 130]. In epithelial or endothelial cell layers where cell–cell adhesion is present, cell migration within a crowded multicellular system can occur by multiple distinct regimes, as follows: (1) as local rearrangements (the simplest form being intercalation, also known as a T1 transition [146]); (2) as larger scale motions characterized by dynamic heterogeneity and formation of cooperative but temporary packs of coordinated cells; (3) as large scale motions where coordination is longer-lived and local rearrangements are rare or non-existent. Large-scale motile packs can form in the presence or absence of orientational effects and, as such, are referred to as dynamics heterogeneity, swirls, packs, or flocks, depending on the model used to describe the motion. One framework for comparing these distinct forms of motion is to construct a phase diagram that distinguishes between a flowing solid (also known as a flocking solid) and flowing fluid in addition to solid-like and fluid-like phases [121, 144]. In this conception, fluids have no barrier to local rearrangements and exhibit local T1 transitions freely, while flowing solids and flowing fluids both exhibit large-scale movement, with flowing solids moving as a bulk system with no local rearrangements, while flowing fluids exhibit packs and dynamic heterogeneities. A phase transition from solid-like to a flowing fluid has been demonstrated in multiple constant-density systems, as described above (Sect. 2.2.3) [45, 70, 121, 125, 128]. As with density-dependent phase transitions, the nature of dynamic packs can be variable. In particular, in differentiated primary human bronchial epithelial cells, which undergo a density-independent fluidization in response to mechanical compression [45, 69, 70], over time, packs of cells were observed to grow larger and move more quickly [70]. Experimental and theoretical work suggests that, in constant-density systems, cells can move more efficiently if they do so in groups, in a manner reminiscent of a peloton [38, 70].

3 Phase Transitions in Cancer

In recent years, it has become apparent that collective cellular movements play essential roles in cancer, during both invasion and dissemination. Histological evidence shows multicellular invasive strands and streams in cancers of both

mesenchymal and epithelial origin [147–155], and recent experiments with tissue explants and organoids directly demonstrate invasive dynamics of cancer cell collectives extending from the tumor mass into the surrounding ECM or stroma [72, 156, 157]. In addition to collective invasion, cellular collectives have been demonstrated to both exist in circulation and to be more effective than single cells at initiating metastases [158, 159]. Although the relationship between collective invasion from tumors and circulating tumor cell clusters has not been entirely elucidated, current evidence suggests that rather than forming by aggregation in circulation, that tumor cell clusters both leave the original tumor and subsequently seed a metastatic tumor through collective migration [159–161].

Together, these experiments paint a picture wherein collective cellular movements are central to cancer invasion, dissemination, and metastasis, and raise important questions about the role of phase transitions in cancer. How does the presence of the ECM change collective cell behavior? Or phrased another way, what is the role of soft or variable confinement as opposed to the hard barriers typically used in studies of collective motion. Another question is how do the phenotypic changes of individual cancer cells affect collective behavior? For example, if cells undergo either full or partial EMT, can we still apply the lessons of collective cell motion and jamming to these systems? Assuming phase transitions are occurring, what is their role in cancer? Beyond that, we can also ask what insights we can gain by applying the metrics and perspectives of physical phase transitions to studying, diagnosing, and treating cancer. Recent progress toward answering these questions is addressed in the following sections.

3.1 Tumor Invasion: Force Balance

Three-dimensional models of tumor invasion, including tissue explants, spheroids, and organoids, have been used as in vitro models that recapitulate many aspects of in vivo tumor formation and invasion. Though details vary across a range of experimental systems, these methods generally consist of a multicellular aggregate, which is capable of self-organization that can be precisely manipulated to adopt a particular spatial organization [162]. These multicellular aggregates are generally embedded in a 3D extracellular matrix, where their invasive behavior can be studied with greater temporal and spatial resolution than is possible with in vivo cancer models. In recent years, the lens of phase transitions has been applied to understand the invasion of cells from a tumor into the surrounding matrix. Taking together evidence from multiple studies, discussed below, a picture has emerged wherein tumors are composed of a core that exists in a solid-like phase, while the phase state of the periphery depends on the surrounding environment.

The structure, composition, and organization of the ECM around a spheroid play a significant role in determining the phase of the peripheral cells of a spheroid. One of the clearest demonstrations of this effect is from spheroids dropped on a flat surface as opposed to embedded in a matrix (Fig. 2B, C). In a series of papers

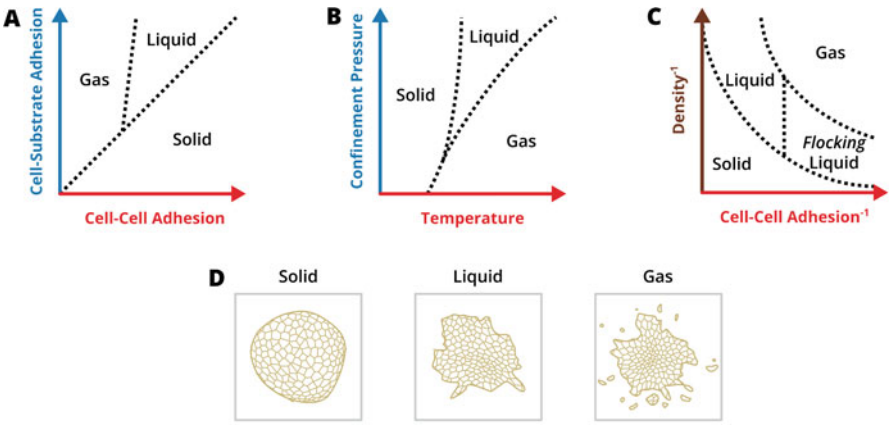

Fig. 4 Phase transitions in cancer. (**A**) For a spheroid on top of a 2D substrate, interactions between cell–cell adhesions and cell-substrate adhesions govern the solid, liquid, and gas phases describing the invasion of cells into the substrate [28]. (**B**) For spheroids embedded in 3D ECM, confinement pressure and temperature (encompassing structural, migratory, mechanical, and metabolic factors) govern the phase transitions [72]. (**C**) In vivo, the role of cell density has been shown to govern the phase transitions of collective invasion [82]. (**D**) A solid-like phase features no invasion. A liquid-like phase features tracks of cells collectively migrating away from the spheroid. And a gas-like phase features individual cells migrating and escaping from the spheroid

from the Brochard-Wyart group [27, 28, 163], spheroids of different cell types were placed on substrates of varying physical properties. By varying substrate stiffness or surface chemistry, it was then possible to change the resultant spheroid spreading on the surface and observe solid-like (no spreading), liquid-like (monolayer spreading) or gas-like (individual cell escape) behavior (Fig. 4A, D). The phase transition at the interface of the spheroid and the substrate depended on the force balance between cell–cell and cell-substrate adhesion. If cell-substrate adhesion was weak, then the cells remained as a solid-like collective and did not spread. If cell-substrate interactions supported adhesion of cells onto the substrate, then spreading could occur. In this case, whether the phase of the motile cells spreading at the interface was liquid-like (migrating collectively) or gas-like (migrating individually) was determined by the difference between cell-substrate and cell–cell adhesion; liquid-like monolayer spreading occurred if cell–cell adhesion was dominant whereas gas-like individual cell escape was dominant if cell-substrate adhesion was dominant.

For spheroids which are embedded in a 3D ECM, the fundamental concept of force balance between cell-substrate and cell–cell adhesion remains a powerful framework for predicting and interpreting cellular behaviors. However, in 3D, additional complications can arise. Even at the single-cell level, migration speed of cells through collagen matrices is controlled by parameters including cell tractions, cell stiffness, collagen density, and proteolytic capacity [157, 164, 165]. These same single-cell parameters in turn can affect collective migration. For example, the role of collagen properties has been studied by several groups [72, 82, 166]. Valencia et

al. [166] found that changing the collagen density surrounding a spheroid changed the rate at which cells infiltrated the ECM from a spheroid. Low-density collagen was more permissive to invasion, while higher-density collagen slowed the rate of invasion. While this study showed this effect using a single-cell type, Kang et al. [72] showed that by varying both the cell type comprising the spheroid and the collagen concentration, it was possible to observe solid-like, liquid-like, and gas-like phases in the embedded spheroid and surrounding invasion front (Fig. 4B). Importantly, in this 3D study, the cells behaved in a manner that is consistent with the lessons learned from the Brochard-Wyart group's work at the 3D/2D interface. A similar balance of cell–cell adhesion and confinement effects was observed by Ilina and colleagues, even for in vivo measurements (Fig. 4C) [82]. However, unlike in the studies led by Valencia and Kang where the collagen matrix was homogeneous, Ilina et al. observed invasion in collagen-based systems that were structured to match those found in vivo, as well collagen seeded with fibroblasts. When fibroblasts were embedded in the collagen, they oriented collagen fibers and created micro-tracks through the collagen. Strikingly, they observed that invasion occurred along the structural features of collagen in a manner very reminiscent of their in vivo findings. Interestingly, they also found that invasion can arrest depending on ECM structure and lead the cells to "rejam" in a new location, even if the cells are highly invasive and non-epithelial in character. This is consistent with previous findings from the same group [81] and highlights the diverse effects that the ECM can have on directing and controlling collective cell motion in cancer.

Beyond the structure of collagen itself, cancer cells can remodel their surrounding ECM in a manner which changes the collective cellular phase. For example, Carey et al. [167] found that when spheroids of MCF10A cells were embedded in collagen, they did not invade and remained relatively stationary and solid-like, whereas MDA-MB-231 cells would degrade and remodel collagen and thus were able to invade and migrate into the surrounding ECM. Interestingly, when the two cell types were co-cultured in a spheroid, the MCF10A cells became "invasive" because they were able to migrate along the tracks formed by the MDA-MB-231 cells. In a follow-up study [168], the same authors demonstrated that there are also signaling changes that occur during this invasion process; as epithelial cells transit from the epithelial compartment to the stromal compartment, the change in their local ECM promotes biochemical changes which lead to the epithelial cells adopting a more invasive phenotype. Moreover, when the stromal ECM was stiffened, such as might occur by cellular remodeling during cancer, this invasive phenotype was further reinforced, highlighting how changes in the collagen network can lead to complex cell–cell interactions. Beyond single-cell effects, the ECM can also be deformed by collective cellular effects. If cells within a relatively stationary spheroid embedded in collagen begin to overexpress Rab5a, large-scale collective motion can be triggered at the surface of the spheroid [130]. This motion in turn deforms the collagen matrix due to the applied stress of the motile spheroid. Ilina et al. [82] similarly observed that, invasion can more easily proceed along structured as opposed to randomly oriented ECM.

3.2 Cell Jamming as Cell Phenotype Changes

As was illustrated above with the changing invasiveness of co-cultured versus monocultured spheroids in Carey et al. [167], the phenotype of the cells which comprise a spheroid or tumor can dramatically change the collective phase. Interestingly, recent results have demonstrated that the jamming process itself may contribute to new phenotypes arising spontaneously in the system. Building on their work showing that overexpression of Rab5a can fluidize the outer monolayer of a spheroid and subsequently rearrange the surrounding collagen [130], the Scita group has demonstrated that the resultant stresses on the cells provoke changes in nuclear properties such as increased nuclear stiffening and DNA damage [131]. In this system, when the cells continue to be subjected to mechanical stresses, they take on traits of malignant cells, suggesting that mechanically driven phenotype changes can contribute to cancer development.

The impact of collective mechanics and jamming on cell phenotypes has also been investigated by Han et al. [71], where the authors grew spheroids in situ in a collagen matrix. As the spheroids evolved over time, a solid core developed while cell motion was more fluid-like in the invasive protrusions. Additionally, the cells in the invasive front were softer and more active, and, as such, had a phenotype more consistent with cancerous cells. Interestingly, the phenotype was not a permanent change in cell behavior and instead was due to geometric location; if cells swapped places with neighbors, they swapped phenotypes as well. The authors hypothesized that intratumor stress was driving fluid flow from core cells to peripheral ones, which in turn led to changes in phenotype.

While the role of mechanically induced changes in phenotype is a relatively new concept, the most commonly cited change in cell behavior during cancer is EMT. EMT, the epithelial-to-mesenchymal transition, is a transcriptionally driven process whereby epithelial cells progressively lose epithelial characteristics, including apical-basal polarity and barrier function, while simultaneously progressively gaining mesenchymal characteristics, including front-back polarity and individual migratory capacity [169, 170]. EMT was initially conceptualized to explain a phenotypic switch from epithelial to mesenchymal, whereupon single cells could be generated and gain the ability to migrate long distances [171–173], and gained wide recognition for its potential to explain cancer invasion and metastasis [174]. However, both understanding of underlying mechanisms and recognition of the nuance of this process have vastly increased in recent years, and EMT is now viewed as a dynamic process used by a range of cell types to interact with and respond to their environment. Importantly, there exists a spectrum of hybrid epithelial–mesenchymal states with a range of phenotypes, migratory capacity, adhesion, and collectivity [175–177]. As our understanding of EMT has evolved, it has become widely recognized that partial EMT allows collective cell migration without full individualization to mesenchymal cells [178–180], and has thus become a central framework for understanding collective phase transitions in development, wound healing, and cancer [177]. As such, early theoretical identification of cellular phase

transitions came with speculation that collective fluidization might be explained biologically by the EMT [41, 66].

The relationship between collective phase transitions and EMT remains an active area of investigation with many open questions, including around the role of adhesion in general and E-cadherin in particular. During EMT, as cells transition from epithelial to mesenchymal phenotypes, they change their adhesion protein profiles, with a notable loss of E-cadherin. Under such conditions, individual cell migration becomes more likely, and confinement of the collective is reduced. The hypothesis that loss of adhesion is critical for cancer migration is supported by evidence showing that diminished E-cadherin is associated with increased metastasis [181, 182]. However, E-cadherin is not solely responsible for cell–cell adhesion, while the loss of it leads to a host of downstream transcriptional changes, pointing toward a complex role in cancer [181, 182]. Moreover, recent evidence has found that both the presence of E-cadherin and the lack of observed EMT may not be barriers to the development of metastases [183–185]. That collective motion is not dependent on the loss of E-cadherin or initiation of an EMT program has also recently been confirmed in healthy tissues. For example, in the developing embryo, a fluid-like state with local rearrangements through T1 transitions can occur without evidence of EMT and with maintenance of intact junctions [186–188], while in vitro studies have shown that unjamming transitions can be triggered without EMT by multiple stimuli [70, 125]. Therefore, it has been suggested that the unjamming transition and the epithelial-mesenchymal transition are two distinct but complementary gateways to collective migration through fluidization [70, 125, 189–191]. Whether, and how, these transitions act together, in cooperation or in opposition, remains an open question.

3.3 What Is the Role of the Jammed State in Cancer?

The physics of cell jamming and collective cell phases changes appear to be universal; current evidence indicates that, all cells can undergo some degree of jamming behavior. For example, Kim et al. investigated collective cell motion in 2D for a panel of breast cancer cells that included both epithelial and mesenchymal lines as well as ones that could and could not metastasize in live mice; in all cases the cells became more collective over time and demonstrated evidence of jamming [38]. In 3D samples, mesenchymal cells have been found to jam if collagen density is sufficiently high to confine motion [72, 81, 82]. The question then arises, what predictive power does jamming provide in terms of understanding, diagnosing, or treating cancer?

In terms of providing new understanding, the development of phase diagrams and models can provide a framework for interpreting how interventions at the cellular level can result in changes of collective cell dynamics (Figs. 1 and 4). Weaver et al. [192] had previously demonstrated that interventions targeting cell–cell adhesion led to changes of the 3D structure of healthy and cancerous cells; these

changes were later reinterpreted by Oswald et al. [193] as evidence for the role of jamming in cancer and its ability to shape tissue structure. While this particular example uses retrospective reasoning, it is now becoming increasingly clear that some behaviors in cancer cannot be understood without taking jamming into context such as those highlighted in Sect. 3.1. Going forward, it is possible that jamming may be needed to help resolve ongoing problems in understanding cancer. For example, EMT is thought to be critical to cancer development [194], but recent studies have questioned whether it is strictly necessary [82, 183–185]. While it was illustrated in Sect. 3.2 that EMT and unjamming are both routes to collective motion, it has also been demonstrated that mixtures of mesenchymal and epithelial cells are more likely to unjam [35]. Given that recent works have shown that unjamming increases metastasis in vivo [195], and that unjamming can precipitate damage to the cell nucleus [131], could EMT and unjamming work in concert to promote metastatic behavior? This is an open and ongoing area of investigation.

Despite the fact that the mechanism of unjamming in cancer remains unclear, the evidence that unjamming is associated with increased invasiveness can already provide new tools for diagnosing cancer. Cell shape, nuclear shape, and other morphological parameters are used to grade the severity of tumor biopsies; using tools from collective cell motion it should be now possible to better understand how these changes arose and whether or not they are predictive of cell migration and possible escape; indeed recent works have already found that unjamming in cancer models is associated with changes in cell shape [196]. This could be particularly attractive in the assessment of tumor margins where using cell shape to predict where, and to what degree, unjamming has occurred could prove predictive of disease state. Beyond shape indicators, cell stiffness is known to decrease during cancer development. This change was first identified in isolated cells [197, 198], and has recently been confirmed in patient samples [199]. While measurements of cell stiffness have already been proposed as a clinical marker of cancer [200], these measurements could similarly be incorporated into models of collective cell motion to better predict motile regions of tumors.

If unjamming is associated with increased metastasis and increased DNA damage, could the jammed state be tumor suppressive, as some authors suggest? [131] And if so, could inducing jamming help treat cancer? The answers to these questions are currently unclear, but a broad-based treatment that induces jamming is unlikely to be viable given that unjamming is critical in normal biological processes like wound healing. Moreover, it is unclear how jamming is related to the establishment of secondary metastasis. To date, experiments have focused on the primary tumor and those changes which lead to cell escape, but it is plausible that jamming could play a role in the establishment of metastases. For example, when discussing the role of EMT in the spread of cancer, the simplest description of EMT is that it leads to cell escape, while the reverse process, the mesenchymal to epithelial transition (MET), plays a role in the establishment of a new cancer site [201]. Is it possible that analogous processes could be described for cell jamming? If unjamming leads to cell escape, then does jamming lead to the establishment of metastases? Given that changes in the local environment can cause mesenchymal, invasive cells to rejam

[82], it is at least conceivable. A global description of the role of jamming in cancer is still lacking, but that does not preclude a more limited application of these ideas to treatment. For example, low levels of irradiation, such as might be experienced at the periphery of a target tumor, can induce unjamming in cell systems [125]. Would pharmacological treatment to induce local jamming mitigate some immediate term damage of treatment in healthy tissue? While such questions are still open, it is exciting to consider them and will undoubtedly lead to further research in this area.

4 Perspective for the Future

Cells exist in a crowded environment surrounded by extracellular matrices and other cells. These systems are typically highly regulated in terms of their biological function and tissue-level organization, yet are highly disordered at a local multicellular scale, exhibiting a structure reminiscent of disordered glassy or granular materials. This disordered structure could be treated as inevitable noise and changes in cell motion ascribed only to biochemical signaling effects, but, as we have highlighted in the chapter, cells undergo collective transitions that are highly reminiscent of those found in disordered materials. Moreover, transitions in both inert and living materials can be described using similar key state variables: density, cell shape constraints, and agitation. This new framework of treating tissues and cells as a collective material phase is still a relatively recent development but is already leading to new understanding in biological function [202]. In healthy tissues, operating near a jamming or critical transition allows for large-scale changes in tissue properties with relatively small local-scale changes [189]. In cancer, where normal cell processes are disrupted, these types of phase transitions still occur and can still be modeled using concepts from material science, but the role of such phase transitions remains less clear in terms of biological function or clinical implications. Future research will continue to explore if, for example, inducing jamming in a primary tumor could suppress metastasis in the short term? This line of thinking leads to further questions, including whether there would be long-term consequences if/when cells break free? Because it is clear that changes in the collective phase of multicellular systems play a crucial role in the development and progression of cancer, work toward answering these questions is active and ongoing.

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