Glass-like dynamics in the cell and in cellular collectives

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Prominent fluctuations, heterogeneity, and cooperativity dominate the dynamics of the cytoskeleton as well as the dynamics of the cellular collective. Such systems are out of equilibrium, disordered, and remain poorly understood. To explain these findings, we consider a unifying mechanistic rubric that imagines these systems as comprising phases of soft condensed matter in proximity to a glass or jamming transition, with associated transitions between solid-like versus liquid-like phases. At the scale of the cytoskeleton, data suggest that intermittent dynamics, kinetic arrest, and dynamic heterogeneity represent mesoscale features of glassy protein–protein interactions that link underlying biochemical events to integrative cellular behaviors such as crawling, contraction, and remodeling. At the scale of the multicellular collective, jamming has the potential to unify diverse biological factors that previously had been considered mostly as acting separately and independently. Although a quantitative relationship between intra- and intercellular dynamics is still lacking, glassy dynamics and jamming offer insights linking the mechanobiology of cell to human physiology and pathophysiology. © 2014 Wiley Periodicals, Inc.

INTRODUCTION

The body is a collective of cells each of which is a living factory with specific forms and special functions. Each cell is now understood to respond to internal and external mechano-physio-chemical stimuli. There is, however, a long path ahead before we obtain a reasonable understanding of the mechanisms underlying the dynamics of the individual cell, the cellular collective, or the impact of these processes upon the physiology of wound healing, growth, and remodeling, or the pathophysiology of disease processes such as cancer, glaucoma, and asthma. Studies on the role of physical forces inside cells or between cells have led to the emergence of a field called

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mechanobiology.[1] Physical forces of interest are those that cause static or dynamic compression, expansion, elongation, or shear, all of which play significant roles in cellular contraction, spreading, crawling, invasion, and division. Individual and cooperative responses of cells to mechanical forces manifest themselves in sensing, communicating, and interacting of cells with each other and with their local microenvironment.

The nature of glassy materials has been said to be among the 125 most compelling unanswered questions in all science, and recent advances firmly establish that intracellular and intercellular dynamics falls within that framework.[2–11] A signature of glassy behavior in an otherwise homogeneous material is the spontaneous emergence of heterogeneous dynamics.[9] A trail of evidence has led to the hypothesis that the dynamics within the intracellular microenvironment, including the abilities of an individual cell to deform, to flow, and to remodel, shows these and other signatures of soft glassy behavior. Also, at the intercellular level cells move collectively and demonstrate features of cooperative heterogeneous dynamics. The transition of the cellular collective from a liquid- to

a solid-like behavior is of interest because it may impact the final morphology, alignment, aggregation, anisotropy, and deformability of the tissue, thus making study of the dynamics of this transition attractive both fundamentally and practically.

Here we briefly review these features of soft glassy materials[12–15] and then address the observed patterns of glassy behavior for dynamics inside living cells as well as cellular collectives comprising epithelial or endothelial monolayers. We address the possibility that a jamming phase diagram might help to unify understanding of intracellular and intercellular nonequilibrium dynamics in living systems much as it does for inert glass formers. We conclude by discussing insights that glassy dynamics and jamming hypothesis can provide to biology and medicine. Moreover, we raise the question whether there is a missing link between the intracellular and intercellular glassy behaviors.

SOFT GLASSY MATERIALS

Glasses are poorly understood and lie at an intersection of open questions in disciplines previously thought of as being distinct. Current understanding suggests the following: a glass arises in a quenched liquid that is rapidly cooled or compressed such that the formation of an ordered array and a corresponding solid state is suppressed. Even though the state of least free energy would be ordered, the interactions between elements in a glass are too complicated and too weak to form ordered structures spontaneously.[16,17] Instead, as the system is rapidly quenched, each individual element finds itself trapped within an energy landscape containing many energy wells with different well depths. These energy traps or cages are created by the constraints imposed by neighboring elements. As the elements are trapped away from energy minima, the system is not at a thermodynamic equilibrium.

Activated by thermally driven random fluctuations, a microscale element can escape its current cage and fall into a nearby cage.[18,19] Therefore, unlike the crystalline material, each element of a glass does not oscillate about a fixed spatial location. Rather, the existence of ongoing hopping events implies that the links between elements are impermanent (metastable) and the system experiences a never-ending evolution, also called aging. The elements find themselves in a never–never land of metastability that is kinetically stable enough to exhibit the structural rigidity of a solid, yet not truly thermodynamically stable. If individual elements can hop, then the matrix of elements as a whole can reorganize and reorder its internal structures. Hopping events are driven by

local fluctuations. In isothermal conditions, as in much of biology, these fluctuations can be as small as the thermal agitation or can be as large as the energy released by hydrolysis of one molecule of adenosine triphosphate (ATP), which is about 20–25 thermal units, or more. However, if these local fluctuations become small enough, energy barriers impede reorganization into states of lower free energy, and therefore the rate of hopping slows. And if these fluctuations become small enough, or the energy barriers grow large enough, that hopping events become rare, then individual elements freeze in place, the matrix can no longer reorganize, and the material becomes simply elastic; this is called the glass transition.[18–20]

With application of a mechanical stress that is sufficiently large an ordinary glass will fracture. A so-called soft glassy material (SGM), however, will merely flow. The class of SGMs comprises a collection of substances that is astounding in its diversity; the group includes foams, pastes, colloids, concentrated emulsions, slurries, and, interestingly, a variety of living cells. All these materials, however, are composed of elements that are discrete, numerous, and aggregated with one another via weak interactions.[21] These materials exist far away from thermodynamic equilibrium and are arrayed in a microstructural geometry that, at some level or other, express cooperative rearrangements that are inherently disordered.[12,21,22] According to Sollich,[13,15,23] generic structural organization in these nonergodic materials leads to the common mechanical behavior in the form of local activated yielding.

In soft glasses the structural rearrangement is not dominated by thermal agitation, but rather by an effective temperature that represents the amount of nonthermal agitation imparted by interactions with neighboring elements in the immediate microenvironment.[12,14,23] In other words, the effective temperature governs the jump rate out of local traps.[13] A clear physical interpretation of the effective temperature remains an open question. To escape its cage, an element must get kicked over energy barriers that are large compared with thermal energies.[13]

INTRACELLULAR DYNAMICS

The cytoskeleton is responsible for the integrated mechanical properties of the cell and as such includes scaffolding proteins and the contractile apparatus. The cytoskeleton of the living cell is crowded, disordered, and far from equilibrium.[24] The high volume fraction of macromolecules within the cell can alter the intermolecular interactions by orders of magnitude.[25] Furthermore, the nonequilibrium nature of the
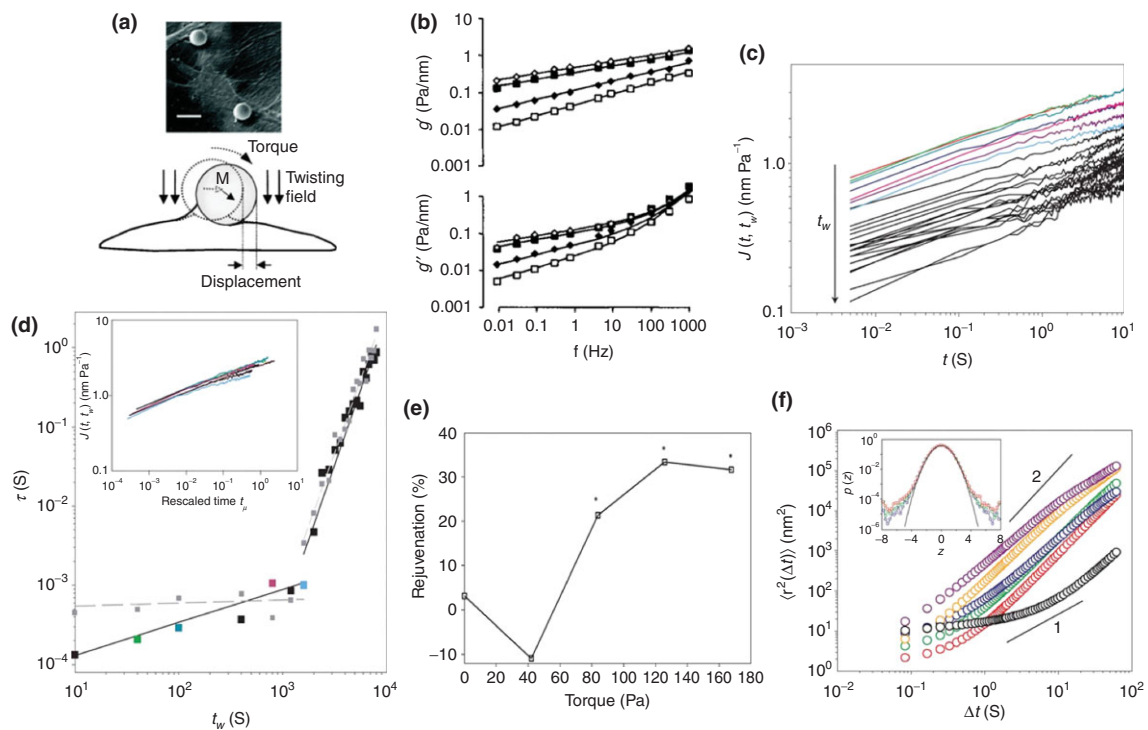
FIGURE 1 | Aging and rejuvenation: (a) in optical magnetic twisting cytometry (OMTC), to measure the cytoskeleton properties, a torque induced by a magnetic field rotates and displaces beads which are tightly bound on cell surface, (b) HASM cell with a series of biological perturbations shows power law responses, (c) creep behavior indicates stiffening of the cytoskeleton with increasing t_w, (d) behavior of τ with waiting time t_w. Solid lines are best fits by a power law, the exponent of which gives the aging exponent $\mu = 0.4$ for $t_w \leq 1600$ s. Grey symbols (data) and dashed lines (best fits) correspond to time controls (no shear applied). Inset, $J(t, t_w)$ (for $t_w = 1600$ s) collapsed onto a master curve using the rescaled time with $\mu = 0.4$. (e) Rejuvenation was quantified as the per cent change between $J(t, t_w)$ at $t = 1$ s, $t_w = 10$ s after shear, and the creep before shear, at $t = 1$ s. (f) The MSD of the spontaneous bead motions exhibited subdiffusive behavior for small Δt ($\beta < 1$) and superdiffusive behavior for large Δt ($\beta > 1$) and non-Gaussian probability distribution. (Reprinted with permission from Ref 4. Copyright 2003 American Physical Society; and Ref 29. Copyright 2005 Nature Publishing Group)

cytoskeleton stimulates a nonstop structural evolution within the cell mainly through the hydrolysis ATP.[25]

The complexity of the cytoskeleton has made it difficult to characterize intracellular dynamics in a tidy way. In addition to traditional biochemical explanations, these dynamics have been thoroughly studied for isolated systems of cytoskeletal proteins, as well as one-to-one molecular interactions such as that between myosin and actin. Furthermore, an extensive literature has developed using dilute model systems involving reconstituted actin filament gels, which use a single crosslinker and are usually studied at thermodynamic equilibrium.[26,27] These approaches overlook the important features of the cytoskeleton, i.e., complex structure, extreme crowding, and the sustained departure from thermodynamic equilibrium. The details of these reconstituted systems are therefore beyond the scope of this review. Instead, we will discuss the intracellular deformability, dynamics, and remodeling from the perspective of glassy dynamics and SGMs, in which all these messy features are

considered. The perspective of glassy dynamics does not supersede established biochemical mechanisms but rather suggest that the rate at which these biochemical processes progress might be significantly slowed.[6,28]

Living Cell as a Soft Glassy System

The first evidence suggesting an analogy between the dynamics of cytoskeleton and the relaxation behavior of SGMs goes back to Fabry et al.'s measurements of dynamic moduli for the cytoskeleton in different types of living adherent cells.[3,4] Using optical magnetic twisting cytometry (Figure 1(a)) to probe five decades of frequency in different biological conditions, they found that dynamics of the cytoskeleton cannot be characterized by any specific relaxation time. Similar to SGMs, the storage and loss moduli of the cytoskeleton follow power-laws in frequency with a small power law exponent (Figure 1(b)). This similarity in dynamics raised the idea that like inert SGMs, the cytoskeleton may exhibit aging as well

as rejuvenation and could undergo discrete out-of-equilibrium remodeling events.

The physical aging[29–31] process for a glassy system may happen when it evolves in a complicated energy landscape containing numerous local energy minima. If the system's energy is not sufficient to push its state over the local energy barrier, the system finds itself trapped in a subdomain of its configuration space. Because within the laboratory time scale the structural rearrangement process is too slow to let the system reach its equilibrium state, the system physically ages and relaxes slowly over time. However, in the presence of mechanical stress or strain, the system may experience rejuvenation (the reverse of aging) where agitation energy may be sufficient to move the system into other configuration subspaces with higher levels of energy. Motivated by Fabry et al.'s findings, such out-of-equilibrium dynamics have been extensively studied to explore more deeply the analogy between the dynamic characteristics of the living cytoskeleton and inert SGMs.[14,16–20,32]

Measurements of the creep response of the cytoskeleton revealed that for a given waiting time after the cessation of a large oscillatory shear, the compliance follows a weak power-law dependence which increases with time without showing any distinct characteristic time.[29] However, with increase in waiting time, the compliance decreases and such stiffening of the cytoskeleton demonstrates similarity to physical aging in inert SGMs. Another indication of glassy behavior[33] is the collapse of mechanical measurements onto a master curve with exponent 0.4. Applying a large shear partially rejuvenates the system and the cytoskeleton matrix becomes progressively softer with increase in the shear amplitude (Figure 1(c)–(e)).

Further support to the hypothesis of glassy cytoskeleton dynamics was provided by studying the spatiotemporal remodeling of cytoskeleton. An et al.[34] employed ligand coated magnetic microbeads tightly bound to the cytoskeleton. The spontaneous motion of those beads is driven by internal cellular forces associated with the dynamics of cytoskeleton's rearrangement.[29] The mean square displacement (MSD) of microbeads in a variety of cells over different lag times Δt shows a general behavior of MSD $\sim (\Delta t)^{\beta}$ with different dynamical regimes. They observed a subdiffusive behavior ($\beta < 1$) for small Δt associated with cage-trapping and a superdiffusive regime ($\beta > 1$) for large Δt associated with hopping events. Also, the probability distribution of microbead displacements followed a non-Gaussian behavior (Figure 1(f)). Interestingly, similar intermittent dynamics and associated non-Gaussian statistics have been observed in dense colloidal suspensions approaching a kinetic arrest regime and glass transition.[19,35]

In a variety of cell types, Trepat et al.[6] studied structural rearrangements within the cytoskeleton in response to external transient elongational stress. Upon stretching, the cytoskeleton fluidizes promptly and thus accelerates structural rearrangement. However, the system then slowly resolidifies and the structure reenters a solid-like state. The larger the magnitude of stretch, the greater is the extent of fluidization and the faster is its subsequent resolidification (Figure 2(a) and (b)). The robustness and generality of this cytoskeleton response was verified by repeating the stretch experiment over a wide range of cell types including pre-treated cells with an extensive set of mechanistically distinct drugs. Although disparate in magnitude and time scale, the prompt fluidization and the subsequent resolidification of the cytoskeletons showed similar behavior. Moreover, the prompt stiffness reduction data versus the pre-stretch value of the phase angle collapse onto a unifying master curve (Figure 2(c)). Again, this kind of behavior is reminiscent of inert SGMs, which can flow when sheared but are soft amorphous solids when the shear stress is lowered. In these athermal systems, thermal energy is insufficient to cause particle rearrangements and therefore, some driving forces like external shear are necessary to induce any motion.

Subsequently, Zhou et al. reported shear stiffness of the living cell subjected to an osmotically induced compressive stress.[24] Their results confirmed that the osmotically compressed cell is under continuous remodeling and relaxation, which dramatically slows with increasing osmotic compression, much as is observed in the colloidal glass transition. The volume fraction dependency of the intracellular viscosity shows that the osmotically compressed cell behaves very differently from suspensions of hard spheres; whereas the latter behaves as a fragile glass-former, the eukaryotic cell is reminiscent of a strong glass-former.[36] In this regard, the behavior of a cell under compressive stress resembles the behavior of a concentrated system of repulsive, soft microparticles[37] which exhibit more gradual increase in viscosity than those found for hard sphere colloids as they approach the colloidal glass transition.[38]

New insights into the underlying physical origin of motor-driven fluctuations in the cytoskeleton have been obtained using endogenous cytoskeletal microtubules as probes. As these filaments are physically linked to the other components of the cytoskeleton[39] their motion also reflects fluctuations of the network and is a measure of the applied force.[40,41] Analyzing
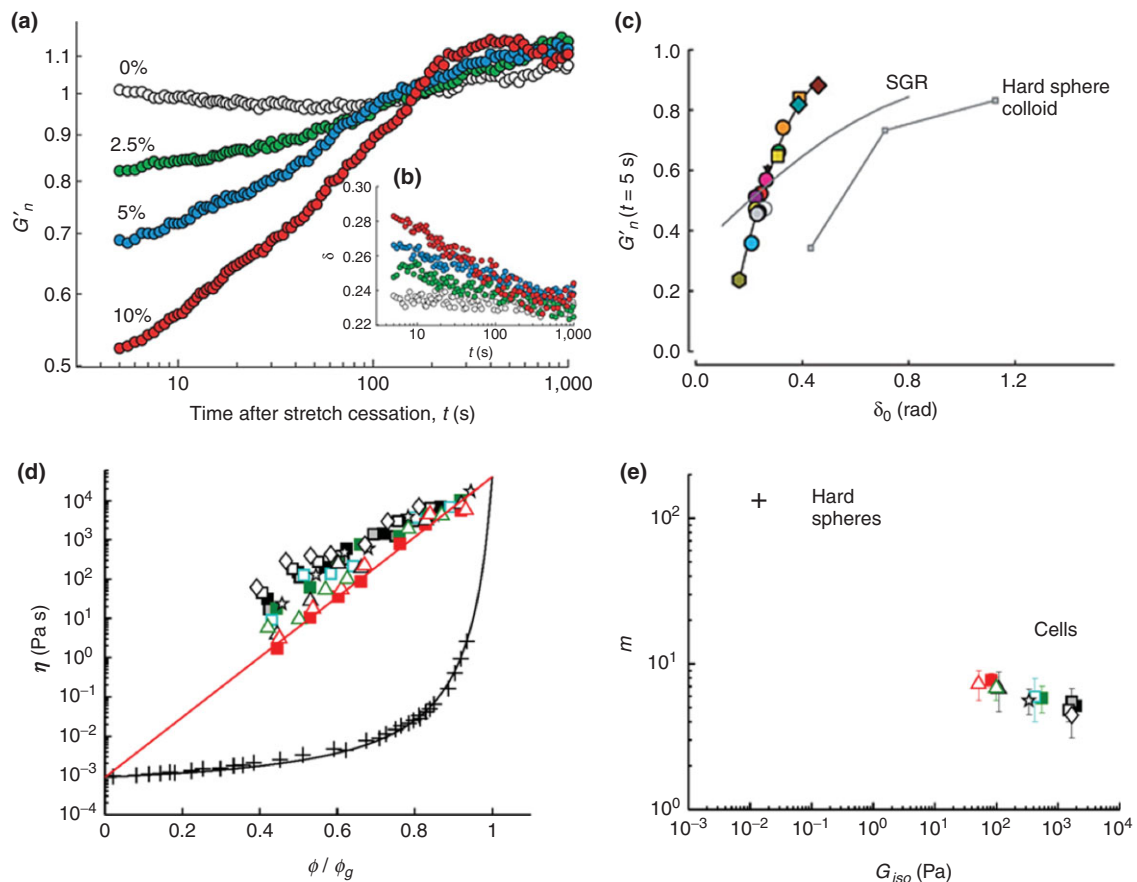
FIGURE 2 | Cells behave as strong colloidal glass formers. (a) Evolution of relative stiffness, G'_n (stiffness after stretch relative to stiffness immediately before) of HASM cells after different magnitude of a single transient stretch. (b) Evolution of the phase angle ($\delta = \tan^{-1}(G''/G')$ after stretch. (c) Phase angle changes between 0 and $\pi/2$ for a Newtonian fluid and a Hookean solid, respectively. This places cells closer to the solid-like state. In response to the transient stretch of similar magnitude, cells show a fluidization response comparable to but to the left of hard sphere colloids. (d) Estimation of the viscosity of the colloidal phase of cells. The exponential growth of viscosity for cells is sharply contrasted by the stronger increase in viscosity for hard spheres, which greatly accelerates as the volume fraction increased toward the glass transition (pluses). In the x axis the volume fraction has been normalized by that at the glass transition, defined as the point at which viscosity reaches an arbitrarily chosen high value, 40,000 Pa s. Data for the hard spheres are fitted with Mooney's equation for hard-sphere viscosity (the black curve), and the red line is the Arrhenius equation with $1/T$ replaced by ϕ. (e) The fragility as $m = d\log_{10}(\eta)/d(\phi/\phi_g)|_{\phi=\phi g}$, was quantified and plotted against the isotonic stiffness. This stiffness for hard spheres was estimated at the volume fraction of 0.3. The fragility of the hard spheres is >1 order of magnitude higher, whereas their ''isotonic stiffness' is a few orders of magnitude lower, than the corresponding values for cells. Data points with error bars represent median values and interquartile ranges. (In panels (d) and (e), symbol shape represents cell type: square, diamond, triangle, and star correspond to HASM cells, lung fibroblasts, MDCK cells, and neurons, respectively; different colors stand for different treatments: no treatment (black), ATP depletion (green), cytochalasin D (cyan), and latrunculin A (red). (Reprinted with permission from Ref 6. Copyright 2007 Nature Publishing Group; and Ref 24. Copyright 2007 National Academy of Sciences)

the intracellular dynamics by this approach the role of thermal and motor-induced fluctuations has been distinguished within the cell.[40,42,43] Motor-induced, nonequilibrium cellular activities have shown to rise to the diffusion-like intracellular motions which are significantly larger in magnitude than those developed by thermal agitations.[42]

Recently, quantifying mechanical properties of cancer cells with different metastatic potential has shown that enhancing cancer cell motility and invasion shifts the cytoskeleton toward a more fluid-like state.[44]

Glassy Dynamics in Idealized Cytoskeleton Models

A theoretical framework that incorporates nonlinear elasticity of individual filamentous polar polymers as the main structure of the cytoskeleton, steric constraints (crowding), and active crosslinking has been presented by Wang et al.[45–48] In their model, the cytoskeleton is thought as a motorized 'cat's cradle' that links glassy dynamics to network architecture (density and concentration) and nonequilibrium motor processes at crosslinking points. Driven

by spatially anticorrelated motor-driven events, the model cytoskeleton behaves as if it were at an effective equilibrium with a nontrivial effective temperature.[47] Their theory predicts a trend consistent with previous observations[24] on the behavior of the cytoskeleton as a strong colloidal glass former; the reconfiguration barrier height raises with packing fraction, which implies that molecular motors in the cytoskeleton tend to resist the imposed mechanical load.[48]

Evolution of a dynamically frozen phase in an active system has been recently investigated using a two-dimensional system of highly concentrated actin filaments crosslinked by fascin. The system can be easily realized as long flexible rods propelled by nonprocessive motor proteins.[49,50] In the absence of crosslinking molecules, when motor-driven events combine with steric constraints due to crowding, dynamic patterns in the forms of swirls, clusters, and density waves appear.[51] Continuous input of energy at the scale of an individual filament, however, leads to successive built-up and destruction of these structures, which is a feature of nonequilibrium liquid states. Upon addition of passive crosslinking proteins, they found a structural arrest accompanied by a nonequilibrium phase transition.[50] As the filaments still move under this condition, the system therefore carries the hallmarks of both an active system and a frozen absorbing state, i.e., the state to which the configurations can be reached by the dynamics but cannot be left.[49,50,52]

Recently, the glassy dynamics in the presence of nonthermal driving forces and energy dissipations has been theoretically investigated by Berthier and Kurchan.[53] Trying to answer the question of whether a glass transition can occur in an active crowed system far from thermal equilibrium, they have shown that dramatic changes in dynamic properties of the materials driven by nonthermal fluctuations resemble the important signatures of the glass transition in simple fluids at thermal equilibrium.[54] The similarities include dynamic heterogeneity, caging effect, and in particular the behavior of time correlation functions and the existence of effective thermal dynamics at long time scales. This new finding confirms that although nonthermal fluctuations drive the system far from equilibrium, steric constraints induce a kinetic arrest and glass transition in the system; the location of the transition, however, shift to larger density with increasing activity.[55]

Integrative Frameworks

Altogether, these observations establish that the cytoskeleton of the living cell shows many of the same features as inert soft glassy dynamics. These include not only power law rheology but also aging and rejuvenation, intermittent dynamics, shear fluidization, compressive shear stiffening, structural arrest, and dynamic heterogeneity.[6,24,29,41,45,50,53] But compared with out-of-equilibrium inert soft materials, the living cell is more complicated. In the living cell, for example, additional interacting factors that are driven by metabolism of ATP prevent attainment of thermodynamic equilibrium, including active forces, active motions, and active remodeling.

Can these behaviors be interpreted in terms of phase transitions from a solid- to a liquid-like state of the cell, which are known to be important in basic cell functions such as spreading, crawling, and dividing? From the literature of inert soft matter,[18,56] these effects in the living systems might be imagined to play out within the jamming phase diagram that considers the possibility of unjamming by decreasing interparticle attractive potential by decreasing the volume fraction occupied by particles or by applying appropriately large stress.[57] In the living cell, ATP-dependent processes are clearly capable of modulating both the attractive potential (by a variety of cytoskeleton crosslinkers, pH, and ionic strength) and the volume fraction of macromolecules (by polymerization and depolymerization). The volume fraction can also be altered by osmotically-induced compressive stresses.[24] The stress coordinate could be thought of as comprising two distinct components; the externally imposed stress, which acts to fluidize the matrix, and the internally generated active stress. Active stresses would appear to have dual but opposing roles, being able both to fluidize the matrix and to solidify it. On the one hand, motor proteins and actin polymerization/depolymerization can create active stresses that act to shear the local matrix and fluidize it[58] in much the same way as does the externally imposed stress. But on the other hand, motor proteins can create prestresses[59] that make the matrix more solid-like.[60]

At the multicellular level, interestingly, the motion of cells is known to be collective, depending on the presence of their nearest neighbors.[61] During collective migration, cell layers behave like a fluid but remain topologically unchanged at short time scales as the motion of each cell is constrained by the crowding.[7,61,62] In the next section, beginning with considering the role of intercellular physical forces, we speculate about collective cellular migration within the perspective of glassy dynamics. In particular, we address dynamic heterogeneity, cooperativity, and kinetic arrest, and then discuss the jamming hypothesis as an integrative framework to merge these largely unappreciated properties.

INTERCELLULAR DYNAMICS

Coordinated motion of cellular collectives is an essential step for many physiological and pathological processes. Embryonic development, organ regeneration, and wound healing as well as cancer metastasis, all depend upon collective cellular migration. [7,8,62–71] Instead of moving independently, cells comprising tissue tend to migrate as a part of a collective.[1,72] Collective cellular migration is poorly understood, however, and has been listed as one of the 10 major unsolved mysteries in biology.[73]

At the level of the local cell–cell interaction, the conventional reductionist view is that the collective migration is regulated by the cooperation of a variety of physical factors including cell-generated forces, polarization, selective affinity, and differential adhesion together with gradients of morphogens and phase-gradient encoding of gene oscillations.[74–79] Cell motility then provides the required mechanical forces to overcome cohesive energy barrier. Therefore, the system can explore various configurational possibilities before ultimately stabilizing into a favorable final state.[79]

The motion of any object cannot be fully understood except in the context of forces. In the case of collective cellular migration, the physical forces in question include intracellular forces supported by the cytoskeleton, local tractions exerted beneath an advancing monolayer, and intercellular forces exerted across boundaries between a cell and its immediate neighbors.[80] Although, the intercellular forces have been recognized as playing a fundamental role in biological processes,[81] for almost as long they had remained virtually hidden because they were difficult to measure.

Through monolayer stress microscopy, these hidden forces are now measurable and can be resolved into normal (tensile) versus shear components.[5,7,8,62,82–84] Surprisingly, maps of intercellular stresses in a structurally homogeneous monolayer are severely rugged (Figure 3). These stress landscapes remain strongly heterogeneous and fluctuate rather dramatically in space and in time[5,8,9] and as such reveal a physical picture that is dominated by dynamic heterogeneity.[8,9,62] The observed heterogeneity is dynamic in nature not structural.

Collective Dynamics and Plithotaxis

From a map of the complete stress field, the local stress anisotropy within the monolayer plane can be signified by ellipses whose major and minor axes correspond to the maximal and minimal principal stresses (Figure 3).[5] Either the ratio of major and
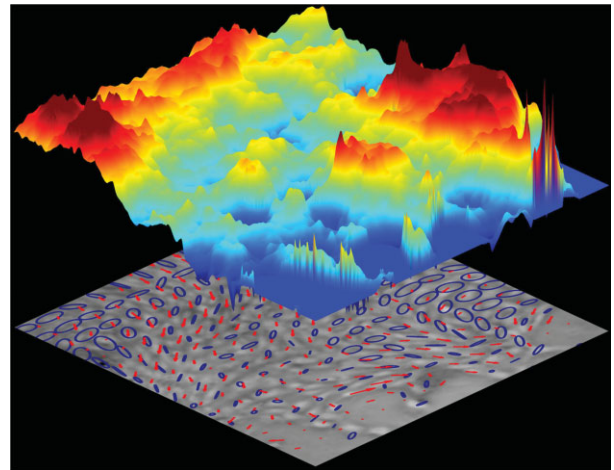

FIGURE 3 | Intercellular stress forms a rugged landscape (colored topography denotes local tensile stress) and cell migrations (red arrows) follow stress orientation (blue ellipses). (Reprinted with permission from Ref 5. Copyright 2011 Nature Publishing Group)

minor radii (the eigenvalues of the local stress tensor) or the eccentricity of the ellipse can be used as a quantitative measure for the deviation from circularity, i.e., isotropic local stress. Local tension, which is the sum of the two principal radii, is then the trace of local stress tensor. Furthermore, the orientation of each ellipse defines the orientations of the local principal stress. Within this rugged stress landscape there is a strong and systematic tendency for the velocity vectors (red arrows, Figure 3) of a broad range of epithelial or endothelial cell types to correlate preferentially with the local orientation of the maximal principal stress, i.e., the orientation of stress ellipse.[5] By definition, along principal orientations the shear stress is zero, implying that cell–cell junctions do not tolerate intercellular shear stress, and that the cell preferentially tends to migrate in a direction that minimizes shear stress across mutual cell–cell junctions. This collective tendency, called plithotaxis, represent a potent physical explanation for collective cellular migration and is mediated through the agency of local intercellular stresses exerted between neighboring cells.[5,83] The stronger is the stress anisotropy, the greater is the plithotaxis. However, the plithotaxis was found to be attenuated when cell–cell junctions were disrupted using calcium chelation or anticadherin antibodies. Under this condition, the cells no longer migrate along the orientation of the stress ellipse. Similarly, in epithelial cell lines expressing weak or nonexistent levels of cell–cell junctional proteins, plithotaxis is inhibited. Therefore, collective migration is an emergent property of the cell group, and requires the cooperativity of mechanical forces across many cell–cell contacts. In agreement, recent

molecular dynamics simulation shows an efficient spreading of a monolayer where cells tend to have an alignment between their motile force and velocity.[85]

Kinetic Arrest

Dynamic heterogeneity is ascribed to the formation of cooperative clusters of cellular units, in which cluster size increases with increasing cellular density.[8,62] At lower densities, the orientation of cell motion is less constrained by intercellular attractions and the cells have enough freedom to rotate or free space to diffuse. With proliferation, however, cellular density increases and cells become increasingly crowded. As cellular crowding approaches some critical threshold, the number of cells that must rearrange for any single cell to change its neighbors expands dramatically, and therefore cooperative clusters become progressively bigger and slower, and the motions become more intermittent.[8] In inert soft condensed matter, similar behavior is called kinetic arrest. Upon crowding, spontaneous local motion of the cells, represented by velocity fields, becomes progressively more heterogeneous. The observations of force chains, intermittent dynamics, cooperativity, dynamic heterogeneity, and kinetic arrest, when taken together, are again reminiscent of a jamming transition from a liquid- to a solid-like state.[9,18,56,86−88]

Although the jamming transition remains controversial and poorly understood, it promises to unify the dynamics and rheology of a remarkably wide range of SGMs. As in the dynamics of inert soft glasses and the cytoskeleton, those of a living cellular monolayer depend upon volume exclusion, volume (size),[24] adhesive interactions,[56] imposed mechanical deformation (stretch or shear),[6,84,89,90] and deformability[37] of the unitary particle. Nevertheless, unlike inert soft glasses, of course, the unitary particles comprising the cellular monolayer are active and self-propelled.

The strength of cohesive forces influences orientational degrees of freedom and size of force chains across many cell–cell junctions. In a monolayer of epithelial breast-cancer MCF10A cells, upon over-expressing the oncogene ErbB2/HER-2/neu, which promotes proliferation and cellular crowding, plithotaxis is strong[5] and the system might be imagined to approach a fully jammed state.[57] In contrast, by over-expressing the oncogene 14-3-3ζ, which decreases cellular cohesion, plithotaxis is attenuated and the system becomes fluidized.[5,57] Remarkably, the dynamics within a confluent epithelial cell sheet can be quantified by the so-called Avramov–Milchev equation describing the rate of structural rearrangements,[8,11] as well as by using the more rigorous 4-point

susceptibility[5,91] demonstrating growing scales of length and time. Furthermore, using model monolayers of crawling keratocytes, Szabo et al.[61] reported a kinetic phase transition with much the same features of the glass transition. Glass-like features is also reported during a wound-healing like essay where two separate confluent layers meet and starting to jam as soon as the directed motion is hampered.[10] The correlation lengths were observed to increase with time and followed the Kohlrausch–William–Watts stretched exponential function, a characteristic of aging glassy systems.[10] In addition, numerical studies of jamming phase diagram by molecular dynamics simulation reveal that for a confined dense collection of two-dimensional self-propelling polar repulsive soft disks, at low densities or high propulsive speed, the collection tends to behave liquid-like.[92] The motors undergo super-diffusion and the orientation of their motion scrambles over time. At intermediate densities the direction of propulsive velocity (polar axis) for a cell tends to align with the polar axis of neighboring cells. This leads to a linear displacement of particle with time, a characteristic of ballistic motion. At higher densities or lower propulsive speed, however, the particles are jammed and their displacements oscillate about their mean position. [92]

All together, these theoretical and experimental results suggest that as cells become less crowded, or less mutually adhesive, or more forceful, they will move more freely. If the situation is reversed, structural rearrangements in the monolayer will become gradually slowed, cooperativity will increase, and, eventually, the monolayer will become frozen.[7−9,62,82,83] As such, it is reasonable to ask if the jamming hypothesis might unify within one mechanistic framework the effects of diverse biological factors previously considered to be acting more or less separately and independently.

Jamming Phase Diagram

The existence of the liquid-like and jammed solid-like states leads to a hypothetical jamming phase diagram.[14,57] Such a diagram can be imagined in a multidimensional parameter space where the jammed state is at or near the origin. The physical parameters satisfying this criterion may include reciprocal of cellular density,[8] reciprocal of cell–cell adhesion,[5] motility, cellular stiffness,[37] substrate stiffness,[62,93] stretch or shear loading and cellular volume. Figure 4 demonstrates a hypothetical jamming phase diagram in three-dimensions based on the first three of the aforementioned parameters. Over-expressing the oncogene 14-3-3ζ leads to decrease in cell–cell
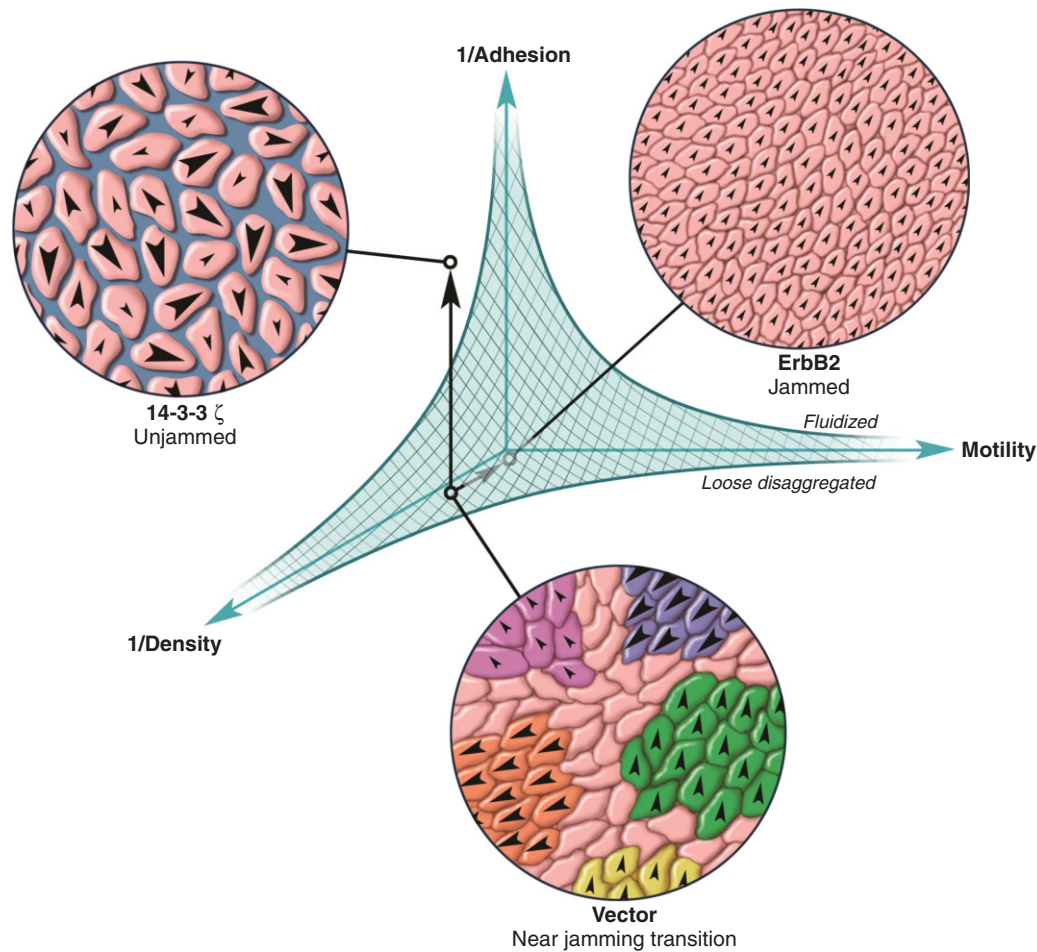
FIGURE 4 | Hypothetical jamming phase diagram for the cellular monolayer in the inverse density, adhesion, and external stress space. The jammed region is the one close to the origin, bounded by the hypothetical jamming surface. Arrowheads depict the migration speed and migration direction of individual cells. Colors depict cell clusters (packs) that move collectively. As cell density increases, MCF10A cells in the controlled state move from outside the jamming transition towards the jammed region while their motion becomes more cooperative and slower. Cell migration follows the local orientation of maximal principal stress, the phenomenon called plithotaxis. As cells proliferate and crowd more due to overexpression of oncogene ErbB2, cell packs become progressively larger and slower, and plithotaxis becomes amplified. However, overexpression of oncogene 14-3-3ζ disrupts cell-cell junctions, therefore the monolayer becomes unjammed and fluidized, collectivity is lost and plithotaxis is ablated. (Reprinted with permission from Ref 57. Copyright 2013 Elsevier)

adhesion and a fluid-like phase. On the other hand, overexpressing the oncogene ErbB2 leads to proliferation, crowding and resulting solid-like behavior. As we approach from a liquid-like state to the origin of the phase diagram, motions of cells become increasingly cooperative; cell clusters become progressively bigger and slower, and ultimately span the entire monolayer.

CONCLUDING REMARKS

In this review, we provided an overview of recent developments in understanding of intracellular and intercellular dynamics within the framework of glassy behavior. This perspective neither minimizes specific signaling events nor ignores them, but rather seeks to set them into a unifying framework. Furthermore, the perspective of the glassy dynamics and cell jamming in which cells and cellular collectives are considered as a phase of soft condensed matter might suggest insights into physiological and pathophysiological mechanisms. For example in asthma, a corollary hypothesis is that asthmatic patients cannot dilate their airways with a deep inspiration because the cytoskeleton of airway smooth muscle cells fails to fluidize and remains jammed in a solid-like glassy phase.[20,21,28,94,95] In a more general sense, the glass hypothesis is likely to be fundamental to a wide variety of higher integrative cell functions in which cell types of different function for instance the smooth muscle cells that line the iris, the gut, the

bladder, and the blood vessels are subjected to mechanical stresses. Moreover, in the context of monolayer biology, this perspective leads logically to new biological questions. In normal physiology, does the epithelial monolayer tend to form a solid-like aggregated sheet—with little possibility of cell invasion or escape—because constituent cells are kinetically arrested?[96] In pathophysiology, can transformation, paracellular leak, escape, or invasion of certain cell populations be attributed to their unjamming? In wound-healing assays, does the wounded cell layer become unjammed?[97] If so, what is the nature of the critical physical threshold? What are the signaling events that control cell jamming? In what ways cell jamming affects gene expression

and cell signaling? In cancer research, the attempts to reduce tumor progression have proven to be unsuccessful, and this ineptness has been inferred as reflecting that, to maintain invasiveness, migration events are biochemically reprogrammed.[64,72,98,99] Might jamming hypothesis offer the alternative but not exclusive possibilities that certain tumor cell subpopulations may unjam, awake from quiescence and thus evolve so as to maintain invasiveness by selection for tradeoffs among adhesive interaction, compressive stress, and cyclic deformation? These questions can bridge biology and soft matter physics and, with the existing experimental and theoretical tools, they are indeed conceivable to be broached.

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