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Review

Implications of a poroelastic cytoplasm for the dynamics of animal cell shape

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Abstract

Two views have dominated recent discussions of the physical basis of cell shape change during migration and division of animal cells: the cytoplasm can be modeled as a viscoelastic continuum, and the forces that change its shape are generated only by actin polymerization and actomyosin contractility in the cell cortex. Here, we question both views: we suggest that the cytoplasm is better described as poroelastic, and that hydrodynamic forces may be generally important for its shape dynamics. In the poroelastic view, the cytoplasm consists of a porous, elastic solid (cytoskeleton, organelles, ribosomes) penetrated by an interstitial fluid (cytosol) that moves through the pores in response to pressure gradients. If the pore size is small (30–60 nm), as has been observed in some cells, pressure does not globally equilibrate on time and length scales relevant to cell motility. Pressure differences across the plasma membrane drive blebbing, and potentially other type of protrusive motility. In the poroelastic view, these pressures can be higher in one part of a cell than another, and can thus cause local shape change. Local pressure transients could be generated by actomyosin contractility, or by local activation of osmogenic ion transporters in the plasma membrane. We propose that local activation of Na^+/H^+ antiporters (NHE1) at the front of migrating cells promotes local swelling there to help drive protrusive motility, acting in combination with actin polymerization. Local shrinking at the equator of dividing cells may similarly help drive invagination during cytokinesis, acting in combination with actomyosin contractility. Testing these hypotheses is not easy, as water is a difficult analyte to track, and will require a joint effort of the cytoskeleton and ion physiology communities.

Keywords: Cytoskeleton; Cell mechanics; Poroelasticity; Cytoplasm; Cytokinesis; Viscoelasticity

Contents

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1. Introduction

The forces that control shape and drive motility in animal cells have long been considered different from those in plant and fungal cells. Plant and fungal cells (Fig. 1, [1]) normally have an internal pressure, known as turgor, that can be as large as a few atmospheres (∼1 MPa) [2,3], that is sustained by a combination of active osmotic mechanisms and resisted by a relatively stiff polysaccharide cell wall [4]. Shape and size change in these cells occurs by softening or remodeling of the wall, either locally as in tip-growing cells, or globally as in diffusely growing cells (Acetabularia, Nitella). Shape change is coupled to volume change [5] and occurs over a range of time scales, from a few seconds (in rapidly growing pollen tubes) to minutes (in growing root hairs). Animal cells, on the other hand, have a much lower internal pressure, at most a small fraction of an atmosphere, and a much softer boundary layer. The shape of a typical animal cell shape (in the absence of forces from other cells and the substrate) is governed largely by the spatio-temporal organization of the actomyosin cortex that underlies the plasma membrane. Shape change is driven by polymerization and contraction of this cortex, and is usually assumed to occur without local or global changes in cytoplasmic volume. In this predominantly biochemical view, hydrostatics with an osmotic origin are thought to be all-important in shaping plant cells, but not relevant in determining the shape or dynamics of animal cells. Here, we will argue that this view captures only part of the picture, and that hydrodynamic forces may play an important role in shaping animal cells.

2. A minimal microstructural model for cytoplasm: poroelasticity

The simplest physical models for shape, and shape change, in animal cells have focused on the cortex, and assumed the cytoplasm to be a viscous material that adopts the shape defined by the cortex (Fig. 2A). More realistic, and more complicated, models view the cytoplasm as a medium that has both viscous, elastic and plastic properties (Fig. 2B), whose dynamics

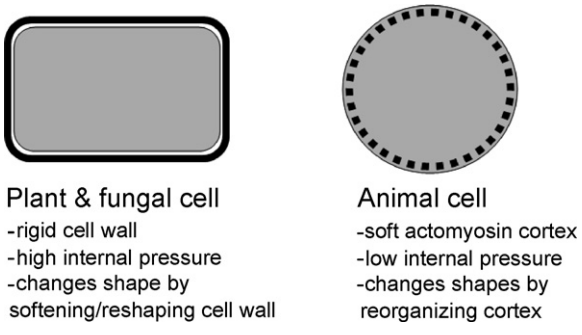

Fig. 1. Plant and animal cell shape. In the classic view, plant and fungal cell shape is determined by a rigid cell wall (heavy line). The cytoplasm (grey) is under high turgor pressure, and conforms to the shape of the cell wall. Animal cell shape (in the absence of forces from other cells or a rigid substrate) is determined by a soft, elastic cortex made of actin and myosin (dashed line), and the cytoplasmic pressure is assumed to be so small as to be irrelevant in shape determination.

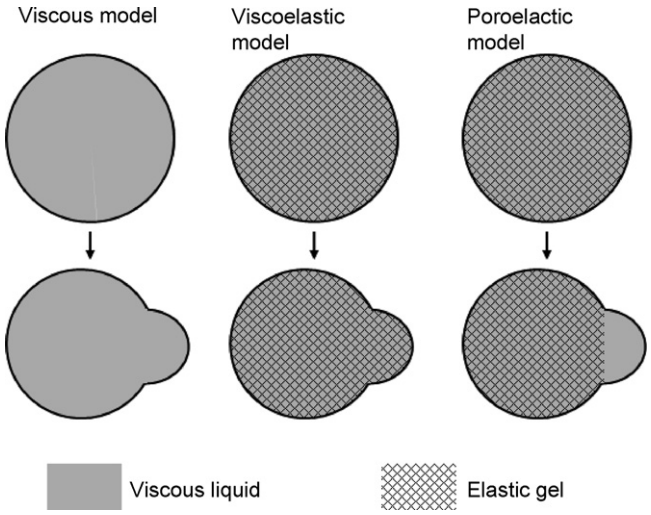

Fig. 2. Towards a microstructural model for cytoplasm. In the viscous model the cytoplasm is viewed simply as a homogeneous viscous liquid. In the viscoelastic model it is viewed as a homogeneous network immersed in a fluid with no ability to discern the underlying microstructure. The poroelastic model is qualitatively different in that now there is a distinct microstructure that allows us to describe how the liquid can move relative to the network during shape change.

can be described in terms of the theory of viscoelasticity and viscoplasticity [6,7]. This formalism is based on the assumption that the material is a single homogeneous phase, i.e. it ignores the microscopic structure and dynamics of the constituents. This is of course not true: the cytoplasm is made up of water, ions, metabolites, soluble proteins, large protein aggregates and organelles, such as the cytoskeletal and membrane network, all of which are dynamically changing. Thus, even at the simplest level, one must account for the fact that the cytoplasm has two distinct phases, a solid phase consisting of a network, membranes and particulates, and a fluid phase consisting of water, ions, metabolites and soluble proteins, that interpenetrate each other. Viscoelasticity and viscoplasticity formalisms implicitly assume that there is no movement of these phases relative to each other. We consider it extremely unlikely that the solid and fluid phases always move together, and that relative motion will naturally occur when a cell sticks, moves, blebs, or divides, since all these phenomena have spatially non-uniform time courses. To account for relative movement between the fluid and solid components of the cytoplasm (Fig. 2C), we need a new formalism. Perhaps the simplest is poroelasticity [8], and its generalizations. This formalism accounts for the dynamics of cytoplasm by including the movement of liquid through a soft porous mesh. It has been previously applied in various forms to understand aspects of the mechanics of whole cells [9,10], and extracellular matrix gels such as collagen [11], and cartilage [12,13]. Here, we review its implications for motility of single cells that involves shape change, such as leading edge protrusion or cleavage furrow invagination. Poroelasticity is potentially of particular importance in these situation for two reasons: change in shape of the plasma membrane implies the possibility that water enters or exits the cell across the membrane, and local actomyosin contraction may squeeze liquid towards a different region of the cell. In both cases cytoplasmic

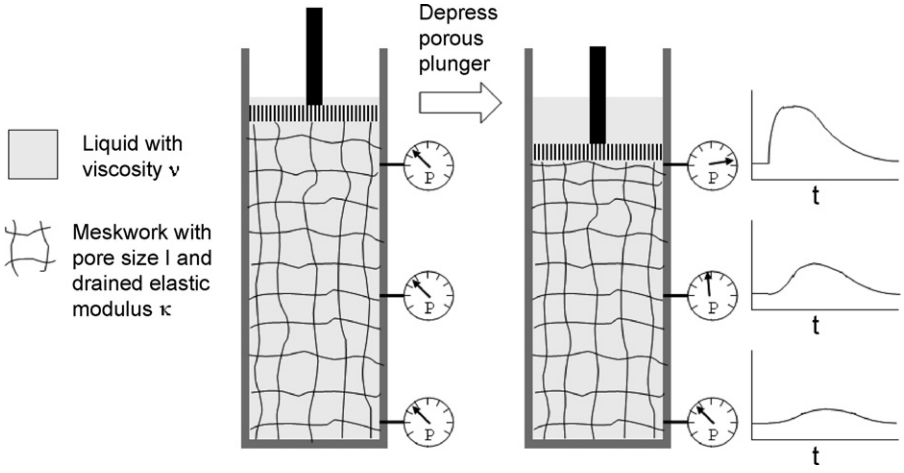

Fig. 3. Thought experiment demonstrating slow equilibration of pressure in the poroelastic regime. A rigid cylinder is provided with pressure gauges at different depths, and filled with a medium consisting of an elastic gel permeated with a viscous liquid. When a porous plunger is depressed by a constant load, initially the liquid flows though the holes in the plunger. With time, the liquid must flow through pores in the gel over longer and longer distances, and the rate of liquid flow limits the rate at which the system equilibrates. The displacement which is step-like and localized near the plunger, with time behaves diffusively as does the pressure [4,10,13], and eventually is uniform over the entire system, as liquid flow slows down and elastic stresses balance the external load. Initially, pressure is high near the plunger, but low elsewhere. Over time, pressure equilibrates, and eventually vanishes.

fluid moves relative to the gel phase, implying poroelasticity may be important.

To understand the theory in a minimal setting, we consider a thought experiment in which a load is suddenly applied onto a very long, confined, hydrated, soft, porous gel via a porous plunger (Fig. 3), first introduced in Ref. [12] to explain and interpret some observations of cell blebbing. In a cellular situation, the porous plunger could, for example, represent the plasma membrane, and it could be depressed by a hypertonic shock, or contractile force. Contrary to naïve intuition, the gel does not equilibrate at the speed of sound, but instead relaxes slowly over time. After the plunger is depressed rapidly, the pressure rises rapidly to a high value at the closest gauge but is barely felt further away. Eventually, as water exits the system and progressively more of the load is born by the gel, the pressure at all positions reaches the same value, and the gel relaxes until it reaches an equilibrium where the applied load is everywhere balanced by the elastic stresses in the network [14]. This thought experiment and its variants have been well studied in simple physical gels [15,16], as well as in biogels such as cartilage and collagen networks [11,17]. It demonstrates that pressure, as well as the stress and the relative displacement (or strain) in the network, are all functions of space and time in a fluid-infiltrated soft network. A brief description of the underlying theory and scaling ideas are outlined below; details may be found, for example, in Ref. [14].

Assuming that the displacement field of the gel swelling (assumed to be made of incompressible constituent materials) is given by $u(x,t)$, the stress in it at a given cross-section is $K\,\partial_x u$, where K is the drained bulk (compressibility) modulus that characterizes the resistance of the porous network to volume changes in the absence of a fluid. Balancing the change in stress from one cross-section to another with the fluid pressure gradients yields $K\,\partial_{xx}u = \partial_x p$. Since the relative swelling or shrinkage of the gel is driven by fluid flow, which in turn is proportional to the local pressure gradient, we also have the following relation for the rate of deformation $\partial_t\,\partial_x u = \partial_x k\,\partial_x p$ where k is the hydraulic permeability of the gel which characterizes the ease with which fluid can flow through it (and is proportional to viscosity of the infiltrating fluid and inversely proportional to the square of the mesh size of the gel). Combining the two equations, we find that the strain $\partial_x u$ satisfies the diffusion equation with diffusion constant $D = Kk$. For a suddenly applied load that is held constant, at a boundary, say $x = 0$, of a gel of finite length L, we find that the displacement itself satisfies the diffusion equation $\partial_t u = D\,\partial_{xx}u$ as does the pressure $p(x,t)$. Thus the solution of the above partial differential equation may be written as:

$$u(x,t) = \sum_n \exp\left[-\lambda_n^2 t\right]\left(A_n \sin\left[\frac{n\pi x}{L}\right] + B_n \cos\left[\frac{n\pi x}{L}\right]\right)$$

where $\lambda_n^2 = nD/L^2$ are the inverse time constants associated with different modes of relaxation in the gel, and the constants A_n, B_n are determined by the initial conditions. We see that the slowest characteristic time scale for relaxation $\tau \sim L^2/D$, i.e. the larger the system, the longer it takes for the displacements and pressure to equilibrate, for a given gel. Similarly, the smaller the diffusion constant (corresponding to a small very stiff mesh infiltrated by a very viscous fluid), the longer it takes for the pressure to equilibrate.

3. Parametrizing poroelasticity: fluid viscosity, network hydraulic permeability and network elasticity

The framework outlined above will be relevant to cell shape and movement if there is inter-phase movement, i.e. the liquid must move relative to the meshwork. We believe this occurs in a variety of situations such as cell blebbing, cell division, cell motility and indeed in any situation where there are spatially heterogeneous mechanical events in a cell. However, it is also necessary that the distances and times over which displacements and pressures equilibrate are relevant to some aspect of

cell behavior—i.e. there are windows in space-time where these concepts are relevant. Since the diffusion constant for the equilibration of stresses, displacements and pressure depends on the liquid viscosity, the bulk compressional modulus of the gel, and its structure (pore/mesh size), this raises the question of what are typical values of these parameters, which of them might be subject to active regulation by the cells, and what cellular phenomena fall in the relevant space-time window.

The cytoplasmic liquid is a composite of water and a variety of proteins and organelles. Changing the local concentration of these objects can change the shear and extensional viscosity by orders of magnitude, as is well-known from our knowledge of the physics of colloids, suspensions and polymers. The pore size needs to be relatively small for poroelastic effects to be significant over the 1–1000 s time window associated with most animal cell dynamics. We estimate pore diameters of 30–60 nm are required to account for data on blebbing and local sucrose perfusion using a poroelastic model ([18] and GC, TM and LM, manuscript in preparation). This value is consistent with that obtained from analyzing diffusion of different sized probes in some [19], but not all experiments [20]. 30–60 nm is smaller than the pore size we expect from cytoskeletal networks alone [21,22], with the possible exception of the extreme leading edge [23], where actin filaments are highly concentrated. Abundant membrane systems in the cytoplasm may make an important contribution to the meshwork, but since a typical vesicle or tubule has a diameter of 100 nm or more, these cannot account for the small pore size. The most abundant object in the cytoplasm of growing cells are ribosomes (∼30% volume occupancy, [24]). Ribosomes are ∼25 nm in diameter, close to the effective pore size, and they tend to link together, via the mRNA they are translating, into jointed chains called polysomes, with extended lengths in the hundreds of nm. We know from classic cell permeabilization experiments that most mRNA behaves as an insoluble molecule [25], suggesting polysomes may be part of the meshwork rather than the liquid. Thus the cytoplasmic meshwork may be comprised of elements with long distance organization but large pores, including the actin–microtubule–intermediate filamentous cytoskeleton as well as endomembranes, mixed with jointed chains of ribosomes and other medium-sized objects that tend to clog the pores. Even single ribosomes may add to effective viscosity as they are pushed through the meshwork. Cryotomography images tend to confirm this crowded, complex cytoplasm view [26]. In reality, the cytoplasm may contain components that move relative to each other at different rates when stress is applied to the meshwork, with cytoskeleton truly immobile, water, small molecules and small proteins truly mobile, and medium-sized components like ribosomes and polysomes moving at intermediate rates.

It is worth emphasizing here that by controlling the mesh/pore size alone, it is possible to change both the permeability and the compression modulus by orders of magnitude due to the strong dependence of both parameters on the pore size. Thus, one can control the rate of equilibration of pressure over a large range as well, from the sub-second time to tens of minutes. For example, the modulus $K \sim Ef(\varphi_s)$ where E is the bulk modulus of the material of which the cytoskeletal network is made, and

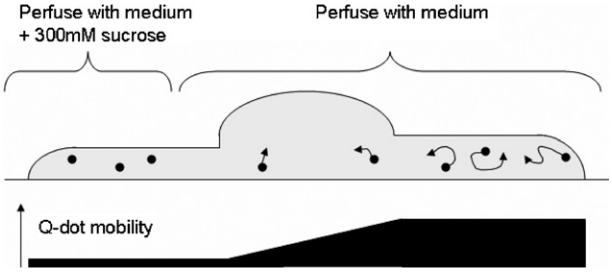

Fig. 4. Local perfusion of cells with osmolyte. HeLa cells were microinjected with 20 nm, passivated quantum dots. In control cells, Q-dots this size diffuse freely. The left side of the cell was perfused with medium + 300 mM sucrose (or another osmolyte) while the right side was perfused with medium alone. The cytoplasm on the left side rapidly dehydrates, causing the Q-dots to cease moving, as they are trapped in the shrunken pores of the cytoplasmic meshwork. On the right side, normal movement continues. In the middle, a gradient in mobility is observed over a zone of 5–10 μm. A non-equilibrium steady state of this kind can persist for many minutes. When the osmolyte flow is removed, the cell recovers rapidly and completely to its initial state. This experiment shows that water crosses the cell membrane faster than it moves within the cell, under conditions of osmotic forcing. It also shows that the cytoplasm can be non-equilibrated with respect to swelling state of the meshwork, consistent with a poroelastic description of the cytoplasm.

φ_s is the solid volume fraction, with $f(\varphi_s)$ varying over orders of magnitude [27]. Similarly, the permeability $k \sim l_p^2/\mu\, g(\varphi_s)$, with l_p the pore size, and $g(\varphi_s)$ a function of the fluid fraction varying over orders of magnitude [28], so that Kk can vary over a wide range indeed. These estimates would of course vary widely from one location to another in a cell. Indeed, even our simple picture of a two-phase continuum with a homogenous fluid infiltrating a uniform mesh is likely to be incorrect. Nevertheless, it provides a basis for understanding a number of phenomena in cytoplasmic dynamics that cannot be explained otherwise, and sets the stage for a further exploration.

A simple but direct indication of how stresses and pressures may be out-of-equilibrium in cells over distances smaller than a cell length can be seen in an experiment where liquid was deliberately forced through cells into which passivated quantum dots had been microinjected using an external osmotic gradient (Fig. 4, GC, TM and LM, manuscript in preparation) using the local perfusion apparatus described in Ref. [29]. We found that two parts of the cytoplasm, separated by as little as ∼5 μm, could be strongly out of hydrodynamic equilibrium. On the normal medium perfused side, the Q-dots diffused normally, since they were chosen to be smaller than the normal pore size, which we estimated as 30–60 nm in diameter, based on the size of the Q-dots (20 nm diameter) and the change in cell volume during hyperosmotic shock [18]. On the osmolyte perfused side, Q-dot probes were completely immobilized due to dehydration of the cytoplasmic meshwork, trapping the Q-dots in the shrunken pores. This experiment shows that water can cross the plasma membrane faster than it can move through the cytoplasm, in response to external osmotic forcing, and that the viscous retardation of water movement through the pores of the elastic cytoplasmic meshwork is sufficient to cause non-equilibration of pressure on a distance of tens of microns and a time scale of tens of seconds. Thus poroelastic effects might indeed be important on length and time scales relevant to cell

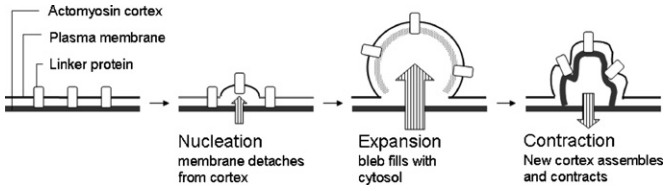

Fig. 5. A model for bleb mechanics based on current data [12,13,17]. Blebs are nucleated when a pressure difference across the plasma membrane of 100–500 Pa causes the membrane to detach from the underlying cortex. The bleb rapidly inflates as cytosol flows in (cytosol flow is indicated with arrows). Within ~30 s a new cortex starts to polymerize under the naked membrane. Over ~100–300 s, this cortex recruits a sufficient density of actin and myosin-II to initiate contraction, driving the cytosol back into the cell body.

motility, especially rapid protrusive events such as blebbing and pseudopod extension.

4. Exhibit 1: Blebbing as a window into cell hydraulics

We were drawn to consider poroelasticity by analysis of blebbing [12], a dramatic type of motility that many animal cells display when they divide [30,31] or undergo apoptosis [32]. Blebbing is typically a cyclic phenomenon where a bleb nucleates, expands, and contracts over 1–3 min without moving laterally (Fig. 5, [33,34]). Some cell types, such as dissociated embryonic blastomeres, exhibit traveling blebs that propagate circumferentially around the cell, a phenomenon called circus movement [35,36]. Blebs in most cells appear directionally uncontrolled, though blebbing of the leading edge thought to power directed motility in certain embryonic [37,38], amoeboid [39], and metastatic tumour cells [40–43]. Blebs, that protrude at rates of ~0.4 $\mu m\,s^{-1}$ [29], are more dramatic than standard, controlled protrusive events at the leading edge of motile cells. For example, lamellipodia protrude at ~0.15 $\mu m\,s^{-1}$ in rapidly moving fish keratocytes [44], and slower in most other cell types.

All data on blebs are consistent with the model shown in Fig. 5, in which a pressure difference across the plasma membrane causes the membrane to detach from the cortex, and inflate with cytosol. Using reasonable estimates for other parameters, we estimated that a pressure of ~100–500 Pa drives bleb expansion [18]. This is much smaller than estimated pressure differences across the plasma membranes of yeast and fungal cells ($6–8 \times 10^5$ Pa [45]), but it is sufficient to drive shape change in the absence of a rigid cell wall. Thus, the very existence of blebs implies the classic view that the cytoplasm of animal cells is not under pressure is incorrect. If contraction-induced pressure can drive the formation of blebs by tearing the bilayer from the cortex, it is reasonable to ask if pressure also plays a role in protrusion of lamellipodia and filopodia, an idea we explore below.

Blebbing per se does not require a poroelastic cytoplasm; contraction of the cortex could equally drive blebbing in the viscous and viscoelastic models in Fig. 1 (and this is the case in lipid vesicles coated with actin [46]), but with an important difference. In the viscous and viscoelastic models, the pressure that drives blebbing must be uniform throughout the cytoplasm, while in the poroelastic model it could be different at different locations. Using blebs as crude gauges for pressure difference across the plasma membrane at a particular location, we asked if pressure equilibrates globally, or can be locally different. Perfusion of one side of blebbing cells with drugs that relaxed the cortex (inhibitors of actin polymerization or myosin-II contractility) blocked blebbing on the drug-perfused side, while it continued on the non-perfused side (Fig. 6). We interpreted this observation as showing that the pressure that drives blebbing is generated, and used, locally, and does not equilibrate across the whole cell. Localized pressure transients require a poroelastic cytoplasm.

What is the source of the pressure that drives blebbing, and might it also play a role in other types of protrusive motility? Most work on this problem has focused on the role of actomyosin. Blebbing depends on myosin-II contraction, and is thought to occur when this force is unusually high, or when the cortex–membrane attachment is unusually weak [32,34]. Consistent with this model, blebbing can be induced in round animal cells by damaging the cortex, which allows the membrane to detach and bleb out [47–49]. For example, tissue culture cells rounded up in mitosis can be induced to bleb by treatment with latrunculin, which damages the actin cortex. Pretreatment of the cells with blockers of contractility (blebbistatin, an inhibitor of myosin-II, or Y27632, an inhibitor of Rho-kinase) prevented this drug-induced blebbing, showing that the pressure depends on myosin-II driven contractility [18]. Thus, cortical tension caused by contraction of the cortical actomyosin gel is one source of pressure for blebbing. However, we should also consider a second source of pressure due to osmotic effects. Indeed, recent experiments with cell fragments [47] show that a weakened cortex–bilayer link coupled with osmotic effects can also lead to blebbing, and even oscillations.

It would be convenient for students of the actin cytoskeleton if we could treat the plasma membrane as an impenetrable barrier, in which case we could assume that cell volume is constant, both globally and locally. Unfortunately, reality is more complex, since both water and ions can cross the plasma membrane rapidly. We know this from responses to changes in external osmolarity [50] (Fig. 4), and from direct observation of H_2O for D_2O exchange, which was followed using CARS microscopy, revealing a timescale of ~2 s [51]. The transporters that promote rapid movement of water and ions across the relatively impermeable lipid bilayer are discussed below. Because the plasma membrane is permeable to water, any pressure difference across it must be due to three contributions: osmotic effects, membrane tension and curvature, and cytoskeletal/cortical effects. When the cytoplasm is locally stressed by myosin-II driven contractility, e.g. at the site of a nascent bleb, water will tend to exit the cell at that site. This will dehydrate the cytoplasm, generating an osmotic potential that drives a backflow of water. If we assume that only water, and not ions, cross the membrane at a bleb, then a combination of the osmotic potential and membrane tension and curvature will locally balance the pressure from actomyosin, preventing large net efflux of water. In this case, we can neglect water crossing the membrane in our consideration of how actomyosin contraction generates the pressure that drives blebbing. If, however, the cell actively regulates osmotic potential, by importing or exporting ions across the membrane, the

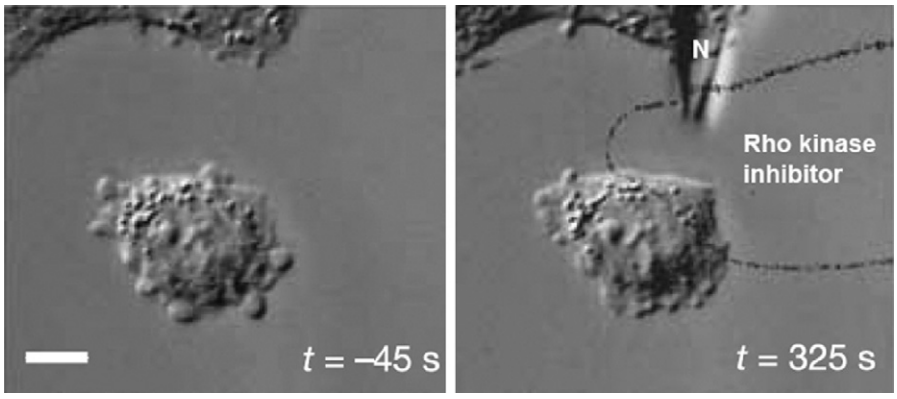

Fig. 6. Evidence that pressure does not equilibrate globally in blebbing cells. The left panel shows a constitutively blebbing human melanoma cell [16], imaged by DIC. The right panel shows what happened when one side of the cell was perfused with medium containing a drug that inhibits Rho-kinase, while other side was perfused with medium alone. Rho-kinase activates myosin-II, and inhibiting it relaxes the cortex. Blebbing stopped on the drug-perfused side of the cell, while it continued on the medium-perfused side. Similar results were found with other inhibitors of cortical contractility. We interpret this experiment as showing that pressure generated by actomyosin contraction on the non-drugged side does not equilibrate globally, because the cytoplasm is poroelastic. Scale bar = 10 μm. From Ref. [12].

consequent pressure differentials could be strong, fast-acting, and local.

5. Exhibit 2: Osmotic regulator localization and motility

Animal cells contain a complex, and energy-intensive, system of ion pumps and transporters to regulate their volume on short time scales, and this system is central to many aspects of normal and pathological physiology [52,53]. Although we do not know how cells sense their volume, physiology experiments have revealed volume regulating mechanisms in mammalian cells: short-term volume decrease is driven primarily by export of K^+, Cl^- and small organic osmolytes through various channels and transporters. Short-term volume increase is driven primarily by import of Na^+ through NHE family anti-porters (NHE1 being the most widely expressed) that exchange import of one Na^+ in exchange for export of one H^+. Because H^+ is strongly buffered in the cytoplasm, and Na^+ is not, Na^+ import coupled to H^+ export tends to increase the cell's osmotic potential. These volume-regulating transporters and channels are typically not ATPases. They catalyze transport in one direction because they utilize gradients in concentration and/or electrical potential generated by active systems, including metabolism (that generates small molecules and H^+) and the Na^+/K^+ ATPase (that generates gradients of Na^+, K^+, and electrical potential across the plasma membrane). Water crosses the plasma membrane in response to gradients in osmotic potential created by transport of ions and metabolites. Water movement across pure lipid bilayers is relatively slow, and in animal cells, the flow rate is greatly increased by proteins that act as selective water transporters, called aquaporins [54]. It is important to realize that short term regulation of cell volume occurs by processes that act on times scales similar to cytoskeleton biology. For example, a cell challenged with a step decrease in external osmolarity will typically swell over a few seconds, and then pump out the excess volume over a few tens of seconds (e.g. [50]). The existence of active volume regulating mechanisms implies that it is very important for the cytoplasm to maintain a particular degree of hydration. We believe this hydration set-point is important, at least in part, because it allows poroelastic control of motility.

Cell volume physiologists typically consider volume to be a global parameter of cell physiology, yet a considerable body of evidence points to local control of volume regulators. Key players, including K^+ and Cl^- exporters, Na^+/K^+ ATPase, NHE1, and aquaporins, are all subject to spatial as well as temporal regulation. In particular, NHE1 tends to concentrate at the leading edge of motile cells, where both its activity and localization are regulated by the same Rho family GTPase systems that regulate the actin cytoskeleton [52,55]. Some aquaporins also localize to the leading edge of motile cells [56,57]. These localization data suggest an involvement of NHE1 and aquaporins in cell migration. Consistent with this idea, active ion pumping, and activity of both NHE1 and aquaporins, are required for normal migration in some cell types [56–60]. While these localization and necessity data point to a role of osmotic regulators in cell migration, they are subject to multiple interpretations, because water, sodium and protons play many roles in cell physiology. For example, NHE1 may promote actin dynamics by alkalinizing the cytoplasm, and it also serves as a platform for attaching the cytoskeleton to the plasma membrane [52,55]. It is difficult to experimentally disentangle these coupled phenomena, and NHE1 probably plays multiple roles in cell migration.

6. Hypothesis: Cooperation between osmotic and cytoskeletal dynamics to generate hydrodynamic forces

Combining the poroelastic nature of cytoplasm, which allows the existence of local pressure transients, and local control of osmotic regulators discussed above, we hypothesize that a combination of osmotic, hydrodynamic and elastic forces contribute to dynamic shape change in animal cells. Fig. 7 shows a model in which local influx of Na^+ at the leading edge though NHE1 causes local influx of water, locally swelling the cytoplasm, and facilitating protrusion. Similar

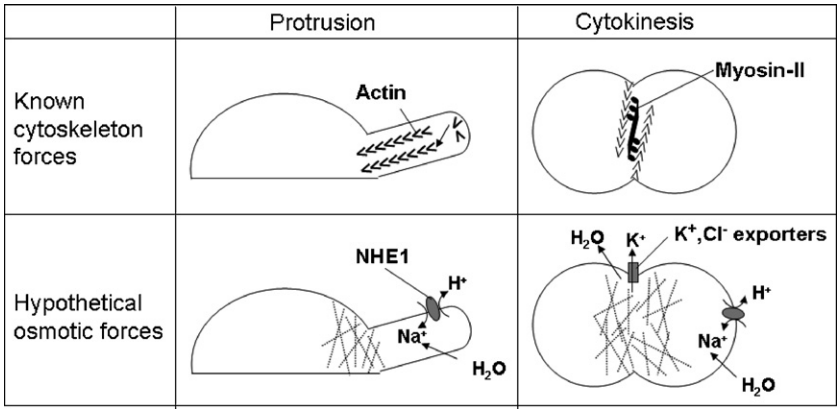

Fig. 7. Models for participation of osmotic forces in animal cell shape change. Protrusion of the leading edge (top row) is known to require actin polymerization. We and others [56,57,61], hypothesize it is also driven, in part, by local swelling of the leading edge. Local swelling is driven by influx of Na^+ ions through NHE1 antiporters. Furrowing during cytokinesis (bottom row) is known to require myosin-II driven contraction in most cells. We hypothesize it is also be driven, in part, by local shrinkage of the cytoplasm at the equator due to K^+ (and/or Cl^-) efflux at the equator. K^+ efflux has been detected in furrowing frog eggs [63]. In both cases, for water influx to cause swelling locally, rather than globally, requires that pressure not equilibrate globally on the time- and length-scales of motility. This requires a poroelastic barrier to fluid flow, indicated by the dotted lines.

model has been proposed by others [56,57,61], though they did not emphasize the requirement for a poroelastic cytoplasm to prevent instantaneous equilibration of pressure. Fig. 7 also shows a model in which local ion efflux at the equator, and influx at the poles, contributes to cytokinesis by shrinking the cytoplasm at the equator, and swelling it at the poles. Water influx at the poles might help account for the dramatic blebbing that occurs there during cytokinesis in some cell types.

How might we test the models in Fig. 7? The role of cytoskeletal forces has been confirmed by protein localization, pharmacology and genetics. Actin and myosin-II are clearly *necessary* for protrusive and furrowing shape change in most cells, but we do not know they are *sufficient* for physiological rates of shape change. Detecting the water flows predicted in Fig. 7 may be very difficult. Water is a challenging analyte, but it may be possible to detect water flows near cells by confining them in microfluidic chambers, and using optical measurements of flow [62]. Measuring water flow across the leading edge membrane would distinguish alternative models for a hydraulic contribution to protrusion. If myosin-II contractility drives fluid from the cell body into the leading edge, as it does in blebs (Fig. 5), we would expect to see water efflux at the front. If, instead, ion flows promote local swelling as proposed in Fig. 7, we would instead see water influx at the front. Detecting the predicted ion flows might be easier, indeed outflow of ions at the cleavage furrow in frog eggs, probably driven by K^+ efflux, was detected using vibrating electrodes [63]. Perturbation of both the osmotic and cytoskeletal players in Fig. 7 is possible using specific small molecule inhibitors. However, the complexity of ion homeostasis, multiple effects of changing ion concentrations in the cytoplasm, and a requirement for cytoskeleton to localize ion transporters and vice versa, will make it challenging to interpret such experiments. Local perfusion of inhibitors may be especially useful, since this will minimize systemic effects of changes in ion concentration and physical state in the bulk cytoplasm [29,64,65].

7. Afterword

We hope this article inspires others to question the orthodoxy that hydrodynamic forces can be ignored in animal cells. In recent years the fields of cytoskeleton and ion physiology have largely separated, and both have become so complicated by molecular details that they are difficult for outsiders to penetrate. The study of the forces that shape cells has been designated a mostly cytoskeletal question, with some input from adhesion systems, but little connection to the field of ionic physiology. While we do not question a primary role of cytoskeleton in cell shape change, we think it is necessary to take a broader, systems-level view of this problem, that includes the effects of ionic physiology, transport and hydrodynamics. In the pre-molecular era, students of shape change and motility considered many possible models, concomitant with the diverse range of mesoscopic observable solutions to how these are in fact played out in different cell types [66,67]. These early studies, for reasons of simplicity and lack of molecular knowledge, focused on very coarse-grained models for the cell and the cytoplasm, treating them as homogenous materials. Our ever-increasing molecular knowledge, and ability to probe cytoplasmic dynamics on finer length and time scales, poses the risk of drowning in a morass of parameters that would lead to little more than an exercise in curve fitting. We believe it will be better to instead refine physical models gradually, incorporating only relevant molecular details as they are discovered. Our simple poroelastic picture of the cytoplasm is a step in this direction. It takes as inputs measurable geometric and physical parameters and leads to falsifiable predictions. However, as our brief discussion of the molecular basis of pore size indicates, the physical picture of cytoplasm is still incomplete and somewhat mysterious, and in need of novel research approaches to understand how it is maintained and how it is changed. Maintaining an effective pore size large enough for diffusion of proteins, but small enough that poroelastic effects are important on time and length scales relevant to motility, may be important for many aspects of cell physiology.

References

[1] Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J. Molecular biology of the cell. New York: Garland; 2004.

[2] Harold FM. To shape a cell: an inquiry into the causes of morphogenesis of microorganisms. Microbiol Rev 1990;54:381–431.

[3] Bartnicki-Garcia S, Bracker CE, Gierz G, Lopez-Franco R, Lu H. Mapping the growth of fungal hyphae: orthogonal cell wall expansion during tip growth and the role of turgor. Biophys J 2000;79:2382–90.

[4] Preston RD. The physical biology of plant cell walls. London: Chapman and Hall; 1974.

[5] Cosgrove DJ. Water uptake by growing cells: an assessment of the controlling roles of wall relaxation, solute uptake, and hydraulic conductance. Int J Plant Sci; 154:10–21.

[6] Boal DH. Mechanics of the cell. Cambridge University Press; 2002.

[7] Bausch AR, Moller W, Sackmann E. Measurement of local viscoelasticity and forces in living cells by magnetic tweezers. Biophys J 1999;76:573–9.

[8] Biot M. General theory of three-dimensional consolidation. J Appl Phys 1941;12:155–64.

[9] Herant M, Marganski WA, Dembo M. The mechanics of neutrophils: synthetic modeling of three experiments. Biophys J 2003;84:3389–413.

[10] Dembo M, Harlow F. Cell motion, contractile networks, and the physics of interpenetrating reactive flow. Biophys J 1986;50:109–21.

[11] Chandran PL, Barocas VH. Microstructural mechanics of collagen gels in confined compression: poroelasticity, viscoelasticity, and collapse. J Biomech Eng 2004;126:152–66.

[12] Skotheim JM, Mahadevan L. Soft lubrication. Phys Rev Lett 2004;92:245509.

[13] Gu WY, Lai WM, Mow VC. A triphasic analysis of negative osmotic flows through charged hydrated soft tissues. J Biomech 1997;30:71–8.

[14] Wang H. Theory of linear poroelasticity with applications to geomechanics and hydrogeology. Princeton University Press; 2000.

[15] Manley S, Skotheim JM, Mahadevan L, Weitz DA. Gravitational collapse of colloidal gels. Phys Rev Lett 2005;94:218302.

[16] Kim C, Liu Y, Kuhnle A, Hess S, Viereck S, Danner T, et al. Gravitational stability of suspensions of attractive colloidal particles. Phys Rev Lett 2007;99:028303.

[17] Soltz MA, Ateshian GA. Experimental verification and theoretical prediction of cartilage interstitial fluid pressurization at an impermeable contact interface in confined compression. J Biomech 1998;31:927–34.

[18] Charras GT, Coughlin M, Mitchison TJ, Mahadevan L. Life and times of a cellular bleb. Biophys J 2008;94:1836–53.

[19] Luby-Phelps K, Castle PE, Taylor DL, Lanni F. Hindered diffusion of inert tracer particles in the cytoplasm of mouse 3T3 cells. Proc Natl Acad Sci USA 1987;84:4910–3.

[20] Seksek O, Biwersi J, Verkman AS. Translational diffusion of macromolecule-sized solutes in cytoplasm and nucleus. J Cell Biol 1997;138:131–42.

[21] Heuser JE, Kirschner MW. Filament organization revealed in platinum replicas of freeze-dried cytoskeletons. J Cell Biol 1980;86:212–34.

[22] Hartwig JH. Actin filament architecture and movements in macrophage cytoplasm. Ciba Found Symp 1986;118:42–53.

[23] Svitkina TM, Verkhovsky AB, McQuade KM, Borisy GG. Analysis of the actin–myosin II system in fish epidermal keratocytes: mechanism of cell body translocation. J Cell Biol 1997;139:397–415.

[24] Kempner ES, Miller JH. The molecular biology of Euglena gracilis. IV. Cellular stratification by centrifuging. Exp Cell Res 1968;51:141–9.

[25] Cervera M, Dreyfuss G, Penman S. Messenger RNA is translated when associated with the cytoskeletal framework in normal and VSV-infected HeLa cells. Cell 1981;23:113–20.

[26] Medalia O, Weber I, Frangakis AS, Nicastro D, Gerisch G, Baumeister W. Macromolecular architecture in eukaryotic cells visualized by cryoelectron tomography. Science 2002;298:1209–13.

[27] Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsudaira P, Weitz DA. Elastic behavior of cross-linked and bundled actin networks. Science 2004;304:1301–5.

[28] Bear J. Dynamics of fluids in porous media. New York: American Elsevier Publishing Company Inc.; 1972.

[29] Charras GT, Yarrow JC, Horton MA, Mahadevan L, Mitchison TJ. Non-equilibration of hydrostatic pressure in blebbing cells. Nature 2005;435:365–9.

[30] Burton K, Taylor DL. Traction forces of cytokinesis measured with optically modified elastic substrata. Nature 1997;385:450–4.

[31] Fishkind DJ, Cao LG, Wang YL. Microinjection of the catalytic fragment of myosin light chain kinase into dividing cells: effects on mitosis and cytokinesis. J Cell Biol 1991;114:967–75.

[32] Mills JC, Stone NL, Pittman RN. Extranuclear apoptosis. The role of the cytoplasm in the execution phase. J Cell Biol 1999;146:703–8.

[33] Cunningham CC, Gorlin JB, Kwiatkowski DJ, Hartwig JH, Janmey PA, Byers HR, et al. Actin-binding protein requirement for cortical stability and efficient locomotion. Science 1992;255:325–7.

[34] Cunningham CC. Actin polymerization and intracellular solvent flow in cell surface blebbing. J Cell Biol 1995;129:1589–99.

[35] Fujinami N, Kageyama T. Circus movement in dissociated embryonic cells of a teleost, Oryzias latipes. J Cell Sci 1975;19:169–82.

[36] Johnson KE. Circus movements and blebbing locomotion in dissociated embryonic cells of an amphibian, *Xenopus laevis*. J Cell Sci 1976;22:575–83.

[37] Trinkaus JP. Surface activity and locomotion of Fundulus deep cells during blastula and gastrula stages. Dev Biol 1973;30:69–103.

[38] Blaser H, Reichman-Fried M, Castanon I, Dumstrei K, Marlow FL, Kawakami K, et al. Migration of zebrafish primordial germ cells: a role for myosin contraction and cytoplasmic flow. Dev Cell 2006;11:613–27.

[39] Yoshida K, Soldati T. Dissection of amoeboid movement into two mechanically distinct modes. J Cell Sci 2006;119:3833–44.

[40] Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer 2003;3:362–74.

[41] Sahai E, Marshall CJ. Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. Nat Cell Biol 2003;5:711–9.

[42] Voura EB, Sandig M, Siu CH. Cell–cell interactions during transendothelial migration of tumor cells. Microsc Res Tech 1998;43:265–75.

[43] Keller HU. Redundancy of lamellipodia in locomoting Walker carcinosarcoma cells. Cell Motil Cytoskel 2000;46:247–56.

[44] Small JV, Herzog M, Anderson K. Actin filament organization in the fish keratocyte lamellipodium. J Cell Biol 1995;129:1275–86.

[45] Money NP, Harold FM. Extension growth of the water mold Achlya: interplay of turgor and wall strength. Proc Natl Acad Sci USA 1992;89:4245–9.

[46] Haeckl W, Barmann M, Sackmann E. Shape changes of self-assembled actin bilayer composite membranes. Phys Rev Lett 1998;80:1786–9.

[47] Paluch E, Piel M, Prost J, Bornens M, Sykes C. Cortical actomyosin breakage triggers shape oscillations in cells and cell fragments. Biophys J 2005;89:724–33.

[48] Paluch E, Sykes C, Prost J, Bornens M. Dynamic modes of the cortical actomyosin gel during cell locomotion and division. Trends Cell Biol 2006;16:5–10.

[49] Boulbitch A, Simson R, Simson DA, Merkel R, Hackl W, Barmann M, et al. Shape instability of a biomembrane driven by a local softening of the underlying actin cortex. Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics 2000;62:3974–85.

[50] Lucio AD, Santos RA, Mesquita ON. Measurements and modeling of water transport and osmoregulation in a single kidney cell using optical tweezers and videomicroscopy. Phys Rev E Stat Nonlin Soft Matter Phys 2003;68:041906.

[51] Potma E, de Boeij WP, van Haastert PJ, Wiersma DA. Real-time visualization of intracellular hydrodynamics in single living cells. Proc Natl Acad Sci USA 2001;98:1577–82.

[52] Alexander RT, Grinstein S. Na^+/H^+ exchangers and the regulation of volume. Acta Physiol (Oxf) 2006;187:159–67.

[53] Pasantes-Morales H, Lezama RA, Ramos-Mandujano G, Tuz KL. Mechanisms of cell volume regulation in hypo-osmolality. Am J Med 2006;119:S4–11.

[54] Borgnia M, Nielsen S, Engel A, Agre P. Cellular and molecular biology of the aquaporin water channels. Annu Rev Biochem 1999;68:425–58.

[55] Putney LK, Denker SP, Barber DL. The changing face of the Na^+/H^+ exchanger, NHE1: structure, regulation, and cellular actions. Annu Rev Pharmacol Toxicol 2002;42:527–52.
[56] Loitto VM, Forslund T, Sundqvist T, Magnusson KE, Gustafsson M. Neutrophil leukocyte motility requires directed water influx. J Leukoc Biol 2002;71:212–22.
[57] Saadoun S, Papadopoulos MC, Hara-Chikuma M, Verkman AS. Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. Nature 2005;434:786–92.
[58] Bereiter-Hahn J, Voth M. Ionic control of locomotion and shape of epithelial cells: II. Role of monovalent cations. Cell Motil Cytoskel 1988;10:528–36.
[59] Ritter M, Schratzberger P, Rossmann H, Woll E, Seiler K, Seidler U, et al. Effect of inhibitors of Na^+/H^+-exchange and gastric H^+/K^+ ATPase on cell volume, intracellular pH and migration of human polymorphonuclear leucocytes. Br J Pharmacol 1998;124:627–38.
[60] Denker SP, Barber DL. Cell migration requires both ion translocation and cytoskeletal anchoring by the Na–H exchanger NHE1. J Cell Biol 2002;159:1087–96.
[61] Loitto VM, Huang C, Sigal YJ, Jacobson K. Filopodia are induced by aquaporin-9 expression. Exp Cell Res 2007;313:1295–306.
[62] Irimia D, Charras G, Agrawal N, Mitchison T, Toner M. Polar stimulation and constrained cell migration in microfluidic channels. Lab Chip 2007;7:1783–90.
[63] Kline D, Robinson KR, Nuccitelli R. Ion currents and membrane domains in the cleaving Xenopus egg. J Cell Biol 1983;97:1753–61.
[64] Popov S, Brown A, Poo MM. Forward plasma membrane flow in growing nerve processes. Science 1993;259:244–6.
[65] O'Connell CB, Wang YL. Mammalian spindle orientation and position respond to changes in cell shape in a dynein-dependent fashion. Mol Biol Cell 2000;11:1765–74.
[66] Kamiya N, Kuroda K. Bot Mag (Tokyo) 1956;69:544–54.
[67] Allen RD, Roslansky JD. The consistency of ameba cytoplasm and its bearing on the mechanism of ameboid movement. I. An analysis of endoplasmic velocity profiles of Chaos (L.). J Biophys Biochem Cytol 1959;6:437–46.