COMMENTARY

Toward the reconstitution of synthetic cell motility

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ABSTRACT

Cellular motility is a fundamental process essential for embryonic development, wound healing, immune responses, and tissues development. Cells are mostly moving by crawling on external, or inside, substrates which can differ in their surface composition, geometry, and dimensionality. Cells can adopt different migration phenotypes, e.g., bleb-based and protrusion-based, depending on myosin contractility, surface adhesion, and cell confinement. In the few past decades, research on cell motility has focused on uncovering the major molecular players and their order of events. Despite major progresses, our ability to infer on the collective behavior from the molecular properties remains a major challenge, especially because cell migration integrates numerous chemical and mechanical processes that are coupled via feedbacks that span over large range of time and length scales. For this reason, reconstituted model systems were developed. These systems allow for full control of the molecular constituents and various system parameters, thereby providing insight into their individual roles and functions. In this review we describe the various reconstituted model systems that were developed in the past decades. Because of the multiple steps involved in cell motility and the complexity of the overall process, most of the model systems focus on very specific aspects of the individual steps of cell motility. Here we describe the main advancement in cell motility reconstitution and discuss the main challenges toward the realization of a synthetic motile cell.

KEYWORDS

active matter; cell motility; cytoskeleton; myosin contractility; synthetic reconstituted systems

Introduction

Cellular motility is a fundamental process essential for embryonic development, wound healing, immune responses, and development of tissues. Aberrant regulation of cell migration drives progression of many diseases, including cancer invasion and metastasis, which in the latter case, utilize their intrinsic migratory ability to invade adjacent tissues and vasculature, and ultimately metastasize. During motility, eukaryotic cells interact with their environment, i.e., nearby cells and surrounding substrates through cell-cell and cell-substrate contacts. In response to those external forces and signals,1,2 the cells actively change their shapes and internal structure to generate traction forces required for their movement. These features are attributed mainly to the mechanical and dynamic properties of the cytoskeleton, an active gel of filamentous proteins, including filamentous actin (F-actin) and microtubules, that is part of all biological cells.3,4 By “active,” we refer to those cytoskeletal processes that are driven by the hydrolysis of ATP. The activity combined with the filaments’ polarity produces a host of dynamic phenomena that do not exist in physical gels, e.g., filament treadmilling,5 and the generation of movement and contractile stresses by molecular motor aggregates.3 The cytoskeleton remolds itself through interactions of the filaments with various associated proteins, some of which affect the assembly and disassembly rates of the cytoskeletal filaments,6 others like molecular motors, crosslink filaments and actively generate mechanical and contractile stresses in the network. In this way the cytoskeleton can self-organize into a variety of subcellular structures such as protrusions,7 contractile networks such as stress fibers8 and the actin cortex.9 These different structures are directly implicated in the various stages of cell motility.

Eukaryotic cells are mostly moving by crawling on an external, or inside, substrate which can differ in their dimensionality, elastic properties, and surface composition.10 Despite its complexity (see recent reviews11,12 several main features of motile cells are rather universal: (i) retrograde F-actin flows from the leading edge of the cells toward its back, (i) polarization of different cellular components localized differentially between the front and the back of the cell.13

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Single cell motility has been extensively studied on 2D flat open surfaces and has been described as a multi-step process which includes: (i) formation of membrane protrusions at the cell leading edge, (ii) adhesion to the substrate, and (iii) myosin-dependent contraction of the cell body combined with detachment of adhesion points at the back, overall resulting in forward cell propulsion (Fig. 1). The different steps are by no means independent of each other and they also reflect the tight coupling between cell motility, contractility, and adhesion. The first step of extension of cellular protrusions is driven by the polymerization of actin filaments at the cell leading edge and gives rise to a retrograde flow of F-actin, enhanced by myosin contractility. The transformation of the (protrusive) polymerization forces into directed motion requires that the growing filaments are positioned next to the surface. This is achieved by localizing actin nucleators at the membrane surface which link the protruding actin network to the membrane. Extension of cellular protrusions is followed by the formation of new adhesions at the cell front. Adhesion to the substrate is required to balance the propulsive force generated by actin polymerization with the contractile forces generated by myosin motors at the rear. The balance between adhesion strength and contractility affects the cell speed and cell persistence (which quantifies the ability of a cell to maintain its direction of motion) and cell shape. If the overall adhesion strength is too low, contraction may even stall or retract the leading edge. Cell motility is also affected by the geometry of the surrounding substrate. Cells moving on open flat surfaces move differently than cells confined within narrow channels or squeezed in between 2 flat surfaces. Low adhesion and confinement was shown to give rise to bleb-based amoeboid motility, which is usually adopted by cells moving in a 3-dimensional (3D) environment. Bled-

**Figure 1.** Schematic diagram of the various steps of cell crawling on a 2D substrate (protrusion-based migration). The motion is powered by different traction forces produced by the cytoskeleton and is characterized by the following steps: 1. extension of cellular protrusions at the leading edge by the polymerization of a branched actin network through the localization of actin nucleators at the membrane surface (yellow dots - inset). 2. Formation of new adhesions at the cell front which connect the protruding network to the underlying substrate. 3. Myosin-dependent contraction of the cell body (inset) combined with detachment of adhesion points at the rear. 4. Retraction of the trailing edge and forward translocation of the cell.
based migration was shown to be driven by actin cortex contractility.9

Research on cell motility and adhesion in the last few decades has focused on uncovering their biochemical basis.6 Numerous studies are now converging into an overall picture of the identities of the major molecular players involved and their order of events.11,18,22 Nevertheless, despite the incredible progress, our ability to infer on the collective behavior from the molecular properties remains a major challenge.

For this reason reconstituted systems were developed.23,24 These systems are controllable and well-characterized, facilitating the characterization of specific processes and provide a complementary approach to live-cell studies. In principle, they allow for full control of the constituents and for studying the effects of specific changes in molecular composition and the impact of the system’s geometry, thereby providing insight into the roles of the various system parameters. This type of detailed investigation is practically unachievable in the in-vivo cellular environment where the changes in one parameter can entail simultaneous modifications on different levels rendering the interpretation of the direct effects of this parameter almost impossible. Also, such model systems provide quantitative information that can be used for physical modeling. The data extracted from such experiments provide useful input for the models as well as values of the system’s parameters. Eventually, such an approach balances the mutually conflicting demands for simplicity, which is required for systematic and quantitative studies, and for a sufficient degree of complexity that allows a faithful representation of biological functions.24

In this review we describe the various in vitro reconstituted systems that were developed in the past 2 decade. Because of the multiple steps involved in cell motility and the complexity of the overall process, most of the model systems focus on very specific aspects of the individual steps of cell motility. In the following we describe the main advancement in cellular reconstitution based on the 3 main steps of cell motility. We conclude with an outlook to open questions and future directions and discuss the main challenges toward the realization of a synthetic motile cell.

**Cellular reconstitution**

Cell motility and adhesion involve the participation of the cell cytoskeleton, membrane proteins (e.g., actin nucleators, adhesion proteins), and the plasma membrane. The membrane functions as a fluid boundary that links the intracellular cytoskeleton to the external world and enables transmission of signals and forces across the membrane. The membrane also serves as a substrate for enzymatic reaction. The physical linkage between the membrane and the cytoskeleton is established via membrane proteins or by direct physical interactions between filaments and lipids. Through this coupling, the cytoskeleton applies 2 kinds of forces: (i) protrusive forces that push the membrane outwards, mainly through directed actin polymerization, and (ii) contractile forces that pull the membrane inwards, mainly through the activity of molecular motors. Reciprocally, the membrane shape and elasticity may have an impact on cytoskeletal self-organization, e.g., through the distribution of membrane proteins.25,26

**Step 1 - Extension of cellular protrusions**

Among the different steps of cell motility, cell protrusion is probably the best understood. Initiation of cellular motility involves the extension of cellular protrusions, e.g., lamellipodia, at the cell leading edge. The lamellipodium (100-160 nm thick) is composed of a dense array of short-branched filaments that begins at the leading edge and extends several microns back, and then the lamella takes over, extending from the lamellipodium to the cell body.27 The driving force for lamellipodial membrane protrusion is the polymerization of a branched actin network at the membrane surface. Several regulatory proteins are involved in this process and are used to control the density, the length and the number of pushing filaments, and regulate the rate of actin turnover.6 The branched nucleation process is initiated by activation of the Arp2/3 complex by surface-bound Nucleation Promoting Factors (NPFs) (e.g., WASP) that are activated at the plasma membrane (e.g., Cdc42, PIP2). Cortactin stimulate the release of the surface-bound NPFs from the new branch, thereby increasing the turnover rate of branching per NPFs molecule and decreasing the network-membrane binding time, required for fast protrusion rates.28-30 The branched nucleation process is followed by filament elongation that pushes the membrane forward. In response to this deformation, the membrane applies resistive elastic forces on the growing filaments. Each filament grows only transiently, since barbed-end capping by capping proteins (CPs) terminates the growth. This limitation of growth produces short (few tens of nm) stiff filaments that are more effective than long filaments at pushing on the membrane without buckling. In addition, CPs control the location of the pushing force, and prevent nonproductive consumption of actin monomers elsewhere in the cell.6 At the rear, the actin network undergoes disassembly in order to refill the pool of actin monomers available for polymerization. The actin monomers diffuse back to the front and
assemble onto uncapped filaments of the protruding network. Cofilin was shown to participate in actin network disassembly by inducing filament severing which can then quickly depolymerize. Much more significant is the role of myosin II motors that are responsible for the majority of the depolymerized actin at the cell back. Myosin motors promote filament severing and polymerization with an efficiency that was shown to depend on their concentration and activity as well as on network architecture. Yet, the molecular mechanisms of myosin-dependent actin depolymerization remain unknown.

Overall, the branched actin networks that are continuously generated at the leading edge display features characteristic of a steady-state treadmill process. The actin filaments are oriented with their barbed ends toward the plasma membrane: their growth pushes the plasma membrane forward, while the pointed ends of the filaments are severed and depolymerized at the rear of the lamellipodium in order to refill the pool of actin monomer available for polymerization. The release of surface-bound NPFs from the newly formed branches after branch nucleation is initiated is required for enhanced cell motility and persistent motion. Actin polymerization forces are not restricted just to drive cellular protrusions of crawling cells but they also underlie a variety of biological processes such as the propulsion of endosomes, vesicles, and various pathogens. They are also important for processes such as endocytosis, phagocytosis and macropinocytosis.

Reconstituting actin-based cellular protrusions

Various in vitro systems consisting of cell-free extracts and purified proteins were developed to study the mechanism of force generation by actin-polymerization (see recent review ref.39). Since biological activities and reactions are confined to surfaces, e.g., soft (plasma membrane and vesicles) or stiff (pathogens) surfaces, reconstitution of cell motility was done by restricting the biochemical activities to surfaces, such as beads or lipid bilayers (Fig. 2).

Reconstitution began with studies of the pathogenic bacterium Listeria monocytogenes. Listeria constitutes a simple model system for studying movement induced by branched actin polymerization; it expresses an actin NPF, ActA, at its surface which exploits the cellular machinery of the host (infected) cell to generate its own movement. Listeria induces the assembly of a polarized branched actin comet tail at the bacterium rear by recruiting actin monomers and associated proteins from the pool of the infected cell. The propulsion forces generated by the elastic stresses developed in the growing actin network enable the Listeria to propel within the cell cytoplasm and spread from cell to cell. Listeria has been instrumental in determining the biochemistry and biophysics of the lamellipodial actin machinery of motile cells. Initially, Listeria motility was reconstituted in Xenopus eggs cell, these studies demonstrated that the recruitment of eukaryotic proteins is necessary for the motility of the Listeria. A major breakthrough was obtained in 1999, with the discovery of the essential set of purified proteins necessary and sufficient to reconstitute the motility of Listeria in vitro (Fig. 2A(a)). These experiments provided strong evidence that polymerization forces suffice to push a load in the absence of motor proteins.

Nevertheless, the use of Listeria suffered from the drawback that geometrical parameters such as the size and shape of the bacterium are predetermined and uncontrollable, and that the localization and surface density of ActA are unknown. Listeria was thus replaced with rigid microspheres which were coated with various NPFs. The first successful bead system was performed with ActA-coated particles in cell-free extracts (Fig. 2A(b)). The internal organization of actin filaments within the comet tail was shown to have a similar dendritic organization to that of lamellipodia, which validated the use of beads as a model system for lamellipodia protrusions. There is however an inherent problem associated with the growth of an actin network around a spherical bead. This is because it results in the formation of an actin shell which has to undergo symmetry breaking to form a polarized actin network (comet tail) that is capable to generate directional movement. Symmetry breaking was proposed to result from network fracture or depolymerization and it occurs when the stresses developed in the actin shell reach a critical value. Both processes are initiated at the outer surface of the spherical actin layer where the lateral stresses developed in the gel are maximal. These two scenarios recover the experimentally observed linear dependence of the time of symmetry breaking with the size of the beads.

Symmetry breaking is of course not unique to actin networks polymerizing on spherical objects. Cells have also to break their spherical symmetry to initiate motility. In the case of cells, fluctuations in myosin contractility and cell adhesion can trigger cell motility, and a positive feedback between actin retrograde flows and gradients in contractility maintains stable cell polarization.

The use of cell extracts limited the possibilities of changing the physico-chemical parameters of the medium. Cell extracts were thus replaced with a medium of purified proteins. Initial studies investigated the role of the density of surface-bound NPFs (WASP-VCA
fragment) and bead size on symmetry breaking, comet structure, and bead motility. With the full control of the composition of purified proteins mix it was now possible to study the effect of the individual proteins constituting the motility medium on system behavior. An inherent feature of actin-based motility is the need for network-surface binding. The binding is mediated by the surface-bound NPFs that initiate the

Figure 2. Protrusion based cellular reconstitution. (A) Actin based-motility of rigid particles. (a) Phase-contrast image of the movement of the *L. monocytogenes* in a solution of purified proteins. Scale bar is 10 μm. Reprinted by permission from Macmillan Publishers Ltd: Nature (43), © 1999. (b) Fluorescence image of a moving 0.5 μm carboxylated microsphere coated with ActA in Xenopus egg extract. Scale bar is 5 μm. From ref. 44 © 1999 National Academy of Sciences, USA. (c-d) Cortactin’s function in actin-driven motility. (c) Fluorescence images of the movement of WASP-VCA-coated beads in purified motility medium supplemented with cortactin. Cortactin (red) distributes over the entire actin comet in proportion to the density of actin (green). Scale bar is 10 μm. Reprinted from ref. 28 © 2011 with permission from Elsevier. (d) Schematic diagram showing a bead coated with WASP-VCA molecules polymerizing a branch actin network at its surface through the activation of Arp2/3 complex. At low concentrations, cortactin enhance the release of WASP-VCA molecules from the newly-formed branches thereby decreasing network-surface binding time, required for fast and persistent movement. Reprinted from ref. 30 © 2012 with permission from Landes Bioscience. (B) Actin based-motility of soft particles. (a) Fluorescence image of the movement of an oil droplet coated on its outer surface with WASP-VCA moving in Hela cell-free extracts. The forces generated by the growing network at the rear results in a pear-drop shape. (Top) the actin comet is shown (scale bar is 4 μm) and (bottom) shows the asymmetric distribution of WASP-VCA, which colocalizes with the actin network. Scale bar is 3 μm. Reprinted from ref. 63 © 2004 by the American Physical Society. (b) Fluorescence image of the movement of oil droplets in a purified proteins medium, coated with WASP-VCA on their outer surface. Initially, a symmetric actin shell grows on the droplet surface (inset - actin is fluorescently labeled). After symmetry is broken the droplets are propelled by an actin comet tail. No deformation of the droplet is observed. Scale bar is 5 μm. (Bernheim-Groswasser A., personal communication). (c) Fluorescence image of a vesicle coated with ActA on its outer surface moving in Xenopus egg extract. The forces generated by the growing network results in a pear-drop shape. Scale bar is 3 μm. From ref. 59 © 2003 National Academy of Sciences, USA (d) Fluorescence image of a cortical actin network polymerized at the inner surface of a vesicle. Scale bar is 10 μm. Reprinted from ref. 71 © 2009, with permission from Elsevier.
nucleation of new actin branches. Yet the same surface-bound NPFs also inherently prevent the translation of the polymerization forces into motion, essentially because the NPF molecule has to be in contact with the network during the formation of the new branch. In a recent study it was shown that cortactin relaxes this internal inhibition by enhancing the release of the NPFs from the new branch, thereby decreasing the network-surface binding time, required for fast and persistent movement\(^{28}\) (Fig. 2A(c,d)).

Other studies explored the effect of external applied forces on system behavior. For instance, micropipette manipulation was used to determine the impact of pulling and pushing forces on the propulsion velocity.\(^ {58}\) This assay provided values for the network elastic modulus, network-bead friction coefficient, and force required to stall the movement. The relevance of this study to cellular protrusion is evident, as the growing filaments in the lamellipodia can experience both pushing (due to membrane resistance) and pulling force (generated at the contractile forces by myosin motors) during cell motility.

Despite the ease of manipulation, proteins that are directly attached to rigid surfaces may adapt non-physiological conformation and are not free to diffuse along the surface as is the situation in cells; therefore their own dynamics cannot be studied.

Replacing the rigid beads by lipid vesicles\(^ {59-61}\) or oil droplets\(^ {62,63}\) offered the possibility to couple 2D diffusion with network reorganization and to study force generation by growing actin networks on deformable surfaces both in cell extracts (Fig. 2B(a,c))\(^ {62,63}\) and purified protein solution (Fig. 2B(b)). The use of fluid surfaces facilitates symmetry breaking. After symmetry was broken the surface-bound nucleators concentrated at the back of the droplet/vesicle, colocalizing with the actin comet (Fig. 2B(c)). These results provided evidence that the surface-bound actin nucleators are linked to the actin network,\(^ {62,63}\) supporting previous optical tweezers measurement on actin comet-Listeria binding strength.\(^ {64}\)

With the aim to reconstitute synthetic cells the next big step was to encapsulate the actin machinery inside cell-like compartments and promote actin polymerization at the surface. Incorporation of actin monomers and triggering their polymerization inside giant unilamellar vesicles (GUVs) or liposome, alone or in admixture with different actin binding proteins has been achieved long ago\(^ {65-70}\) and was shown to induce the deformation of the vesicles. The attachment of the actin filaments to the membrane was established by using specific anchoring proteins or relied on non-specific electrostatic interactions. Nevertheless, none of these studies included physiologically relevant actin nucleation and polymerization machinery. Using the reverse emulsion technique Pontani et al. succeeded to reconstitute a cortical actin layer inside a liposome, which polymerized at the membrane surface (Fig. 2B(d)).\(^ {71}\) The characterization of the physical and geometrical constraints that control the thickness of the actin layer that grew on the surface was established. Regardless of the actin network thickness and mechanical properties, the cortical layer remained stable and did not undergo symmetry breaking.

**Step 2 - adhesion to the substrate**

The second step of cell crawling on 2D surfaces (referred to as protrusion-based migration) is associated with adhesion to the underlying substrate which is mediated by specific surface receptors that firmly bind the actin network to the extracellular environment. These adhesions are required to balance the polymerization forces at the leading edge with the contractile forces generate by myosin motors at the back. Surface adhesion involves the formation of focal adhesions (FAs) which are dynamic structures consisting of hundreds of molecular components.\(^ {72}\) FAs are organized into 3D ‘nondomains’ that dynamically connect the actin network to the substrate\(^ {18}\) mediated by transmembrane proteins that localize to the cell surface.\(^ {73}\) The dynamic coupling of the actin network with the underlying substrate, via populations of adhesion molecules, generates a frictional interface that applies traction forces on the surface\(^ {74,75}\) (Fig. 1). Feedback between myosin contractility and adhesion strength was shown to maintain a front-to-back gradient in traction force, such that it is high at the front and low at the back. The high adhesion at the front generates high traction forces enabling the transformation of actin retrograde flow into net protrusion whereas the low adhesion at the back enables the detachment and retraction of the cell rear, overall resulting in directional cell movement. Cytoskeleton-substrate adhesion is mediated by cell adhesion molecules (CAMs), which include the transmembrane receptors integrin family that bind to the extracellular matrix (ECM).\(^ {76}\) The integrins interact with various EMC ligands such as fibronectin, collagen, and laminin that contain a RGD motif specifically recognized by most integrins.\(^ {77}\) The intracellular domain of integrin binds to proteins like talin and FAK.\(^ {78,79}\) Talin directly interact with filamentous actin and is important for the activation of integrins and for creating stable linkage with the actin cytoskeleton. In contrast, FAK form an indirect linkage to the actin cytoskeleton with other intracellular proteins such as vinculin and α-actinin helping to regulate and reinforce the actin network-integrin linkage.

The essential function of cell adhesion has created remarkable interests in developing experimental set-ups
and methods for measuring and studying cell adhesion properties. Initial studies focused on studying attachment and detachment events of single cells. These studies were used to extract the interaction forces between cells and their underlying substrate and study the impact of different physiological factors as well as surface chemical and mechanical properties on cell adhesion. In the following, the development of nanostructured substrate allowed studying the effect of the lateral organization of integrin receptors on cell adhesion with nanometer resolution. Using highly ordered gold nanoparticles functionalized with RGD peptides it was found that adhesion strength was essentially controlled by the distance between integrin molecules (defined by the interparticle spacing) rather than by their density. A universal distance of 58 to 73 nm was shown to optimize the adhesion efficiency of a variety of cells.

Reconstitution of integrin into lipid membranes

The complexity of native cell membranes and their interaction with the cellular environment prevent to study the direct impact of specific membrane proteins on cell adhesion. For that, various reconstituted model systems were developed to isolate processes involved in cell adhesion with reduced molecular complexity. Functional integrins were isolated from cells and incorporated into planar lipid bilayers and small unilamellar vesicles (SUVs). The binding energy of integrin-ligand pairs was determined by measuring the adhesion force of integrin reconstituted in planar bilayers against RGD peptide carrying GUVs. Experiments done with various ECM ligands showed that integrin-ligand binding was specific, which proved that integrin-functionalized membranes are well qualified as sensing devices for the detection of sensible ligand-receptor interactions.

A model system that is much closer to cells than planar lipid membranes and SUVs are giant unilamellar vesicles. The only work of integrins reconstitution in GUVs was done by Streicher et al., who had the goal to reconstitute the first steps of integrin-mediated cell spreading (Fig. 3A). Binding experiments of integrin-GUVs on surfaces and quantum dots coated with RGD ligands revealed that the incorporated integrins were biologically active. The authors found that integrins-GUVs adhered to the surface in a 2-step spreading process though they could not detect enrichment of integrins in the adhesion patches. This led them to conclude that the actin cytoskeleton has an important role in recruiting cytoplasmic proteins that stabilize more integrins in the adhesion zone to eventually form focal adhesion spots.

One study explored the role of the actin cytoskeleton on cell adhesion. This was done by polymerizing an actin layer on the inner surface of a GUV. The spreading dynamics of the GUV on a polyhistidine-coated glass surface was reminiscent of a natural cell, and turned out to be governed by the density and mechanics of the cortical actin layer (Fig. 3B).

After the successful reconstitution of functional integrins in GUVs, the reconstitution of the actin cortex inside GUVs, the next big step would be to introduce actin and the major proteins involved in the signalization between integrin and the actin cytoskeleton into these integrin-reconstituted GUVs.

Step 3 - myosin-dependent actin networks contractility

The last steps of protrusion-based motility involves myosin-dependent contraction of disordered actin networks at the cell rear combined with the detachment of FAs overall leading to the retraction of the cell rear. A negative feedback between myosin contractility and FAs adhesion, combined with positive feedbacks between myosin contractility, F-actin retrograde flow and network disassembly, was proposed to maintain cell polarity and promote fast and persistent movement. Myosin contractility also plays a major role in bleb-based amoeboid migration. In that case cell movement is driven by actin cortex contractility where positive feedback between rearward F-actin cortical flows and gradients in myosin contractility maintains stable-bleb cell polarization and motion. Transition between these migratory phenotypes can be triggered by varying the adhesion strength, myosin contractility, and confinement. In a recent work it was shown that reducing the adhesion strength and placing the cells under confinement promote the transition from protrusion-based to bleb-based migration.

Reconstitution of myosin-dependent actin networks contractility

The multiple functions of myosin motors in cell motility renders the investigation of their direct impact on network contractility, F-actin retrograde flow, actin turnover, and adhesion, practically impossible. For that, different model systems were developed with the aim to recreate some of the functions of myosin motors in cells.

Myosin-dependent contractility of disordered actomyosin networks

Initial studies on actomyosin systems revealed that myosin motors have the ability to reorganize actin into different mesoscopic structures, such as bundles, asters, and rings when mixed with an inert polymer or polymerized in the presence of passive crosslinkers. Actomyosin networks were successfully reconstituted for the first
Figure 3. (A,B) Reconstitution of cell adhesion and spreading. (A) GUVs containing integrins interacting with a fibrinogen-coated substrate. (a) Integrin-containing vesicle adhered and spread on the fibrinogen-covered surfaces, as shown by the static dark patch in RICM (91). Scale bar is 10 μm. (b) Scheme of the system (green-fibrinogen, blue-integrin). Reprinted from ref. 91 © 2009, with permission from Elsevier. (B) Spreading of liposomes carrying a cortex. (a) Area of contact as a function of time observed by reflection interference contrast microscopy (RICM) for high χ conditions (the higher the χ-value, the denser and more continuous the cortex is). The inset shows the spreading area with time (scale bars is 5 μm) (92). (b) Confocal microscopy image showing the adhered liposome at steady-state forming a contact angle θ between the liposome and the coated surface (92). Unit square is 1 μm. Reprinted from ref. 92 © 2011, with permission from Elsevier. (C,D) Reconstitution of an actin cortex and myosin-dependent cortical flow. (C) Reconstitution of actinomyosin cortex on the outer ('Outside' geometry) and the inner ('Inside' geometry) surface of lipid vesicles in a purified proteins medium. (a) 'Outside geometry': spinning disk confocal images of cortical network that ruptures and flow after addition of myosin motor.104 Reprinted from ref. 104 © 2013, by permission of the Royal Society. (b) 'Inside geometry': confocal images of a cortical actin layer at strong cortex-membrane attachment conditions in the absence (left), and in the presence of myosin motors (right). The addition of myosin motor results in symmetry breaking and cortical peeling.103 Scale bars are 5 μm. (D) Spinning disk confocal images of an actomyosin cortex polymerized at the inner surface ('inside' geometry) of water-in-oil (W/O) droplet in cell extracts. Actin (magenta) and the actin nucleator ActA (green). At 30°C a uniform cortex is formed; reducing the temperature to 20°C results in symmetry breaking and cortical flow. Scale bars are 10 μm. Reprinted from ref. 106 © 2014 eLife Sciences Publications Ltd.
time by Backouche et al.,96 who polymerized actin monomers in the presence of myosin motor aggregates and strong passive crosslinkers. Actomyosin networks formed above a certain myosin concentration and required the addition of strong passive crosslinkers. This work also provided the first direct evidence for the ability of myosin motors to promote network disassembly at high concentrations. Cryo-electron microscopy measurements reveal that at the local level myosin motors cause severing and depolymerization of the actin filaments.32

The next step was achieved with the reconstitution of disordered contractile networks using purified proteins107-110 and cell-free extracts.109 Myosin motors triggered network contraction only above a certain concentration. Network contractility was shown to depend on actin concentration and myosin aggregation state,109 and on the type and concentration of passive crosslinkers.98-100 The concentration of passive crosslinkers was proposed to regulate the level of connectivity within the network.98 Despite the fact that contractility seems to be a generic feature of actomyosin systems the exact mechanism that promote global network contraction is not fully understood. Especially, it is not clear why such a system contract rather than expand or even stall. Recent evidences from studies on reconstituted actomyosin bundles and cortical networks raised the possibility that contraction arise, among others, from filament buckling.101,102

**Reconstitution of cortical networks and myosin-dependent cortical flow**

With the aim to reconstitute the actin cortex and study the role of myosin-dependent contractility on cell shape changes and cortical actin flow, actomyosin networks were anchored to the outer or to the inner surface of cell-sized GUVs (Fig. 3C).103-105 The cortical actomyosin network was localized to the membrane surface either by immobilizing actin nucleators to the membrane which induced the polymerization of a cortical layer or by attaching biotinylated actin filaments to the membrane. The tension generated by the motors resulted in symmetry breaking and cortical flow though it did not promote GUV movement. Upon symmetry breaking, the cortical layer either spontaneously ruptured or crushed, depending on the amount of myosin, cortex connectivity, and cortex-membrane attachment.103,104 These dynamic instabilities were explained to result from the balance between active tension by myosin and mechanical resistance to rupture.103 Cortical actomyosin networks were also reconstituted in water-in-oil (W/O) droplets by encapsulating cell-free extracts supplemented with myosin motors and passive crosslinkers (Fig. 3D).106 Actin polymerization was triggered by localizing actin nucleators at the inner surface of the droplet. Although the use of cell extracts prevents the possibility to known and control the concentrations and activities of the various components, cell-free extracts can be useful to study cortical network dynamics because they naturally contain all the components involved in actin cortex assembly, such as the actin polymerization machinery, accessory proteins regulating actin turnover, and myosin motors. The cortical layer undergoes spontaneous symmetry breaking, which was driven by myosin contractility, resulting in cortex rupture and flow. Symmetry breaking was triggered by increasing the concentration of passive crosslinkers or by decreasing the temperature. Nevertheless, it never resulted in droplet movement.

**Discussion**

This paper reviews the various models systems that have been developed in the past decades to reconstitute specific features and processes of cell motility. Cellular protrusions have been successfully recreated as well as integrin-RGD adhesion. Another big step was achieved with the reconstitution of actin cortices and myosin-dependent symmetry breaking and actin flows. Despite all these achievements, there are still many barriers to overcome toward the realization of synthetic cell motility (Fig. 4).

One big barrier for realization of reconstituted systems for cell motility is the difficulty to recreate the conditions for actin turnover (or treadmilling) which seem to be an essential component of cell polarization and cell motility. The main reason for this is that it is difficult to find the right combination of severing agents and depolymerization factors, combined with nucleator of actin polymerization which optimizes network turnover. Despite the fact that Listeria motility has been recreated in vitro, turnover of the comet tail is usually not observed. Also, the treadmilling of individual actin filaments have never been reproduced in vitro.

Another big obstacle to overcome is associated with cell polarization. Symmetry breaking conditions were indeed recreated in vitro nevertheless the system usually reaches final non-dynamic state. If cell polarization is maintained by a positive feedback between the actin retrograde flow and front-back markers, such as myosin II gradient, it means that one should also recreate the right conditions for establishing this positive feedback keeping the system in dynamic steady state.

Adhesion also affects the coupling of actin retrograde flow and traction forces, and without confinement is
essential. Without confinement, in order to recreate motility on a flat substrate adhesion will have to be included to couple the actin network to integrin-mediated adhesion sites. In addition, if one aims to reconstitute the coupling of adhesion strength to myosin contractility then it may be necessary to use the entire adhesion complex which acts as a mechanosensor in the cell into reconstituted vesicles. This would make the reconstitution of cell adhesion much more complex, and at this point recreating this advanced synthetic adhesion machine is beyond our capabilities. This is reason why it would be simpler to start with systems where adhesion does not play an essential role in cell motility, which is the case when the cells are put under confinement conditions. Overall, the long-term goal would be to reconstitute the transition from bleb-based motility to protrusion-based migration, and find the conditions that trigger the transition, for instance, by including the effect of surface adhesion (Fig. 4).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

A.B.-G. thanks the Israel Science Foundation (grant #1618/15) for financial support.

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