Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Mechanics and dynamics of reconstituted cytoskeletal systems☆

Mikkel H. Jensen [a,*], Eliza J. Morris [a], David A. Weitz [a,b]

[a] School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA
[b] Department of Physics, Harvard University, Cambridge, MA 02138, USA

ARTICLE INFO

Article history:
Received 2 April 2015
Received in revised form 22 June 2015
Accepted 23 June 2015
Available online xxxx

Keywords:
Biopolymer rheology
Actin
Cytoskeleton
Dynamic arrest
Composite networks
Active soft matter

ABSTRACT

The intracellular cytoskeleton is an active dynamic network of filaments and associated binding proteins that control key cellular properties, such as cell shape and mechanics. Due to the inherent complexity of the cell, reconstituted model systems have been successfully employed to gain an understanding of the fundamental physics governing cytoskeletal processes. Here, we review recent advances and key aspects of these reconstituted systems. We focus on the importance of assembly kinetics and dynamic arrest in determining network mechanics, and highlight novel emergent behavior occurring through interactions between cytoskeletal components in more complex networks incorporating multiple biopolymers and molecular motors.

Biological cells are dynamic mechanical objects able to crawl, change shape, divide, and remodel. They are robust enough to withstand substantial external strains, but unlike passive soft materials, they are able to actively tune their mechanics in response to their surrounding environment. These unique active material properties are essential for cellular life, and are owed in large part to the cytoskeleton, the active and richly heterogeneous network within the cellular interior. The three primary cytoskeletal determinants of intracellular mechanics in metazoan cells are filamentous actin (F-actin), microtubules (MTs), and intermediate filaments (IFs), each with their own distinct polymerization dynamics and mechanical properties [1–5]. Together, these polymers form a diverse set of structures in the cell, the mechanics of which are determined both by the properties of the filaments themselves and by the wealth of associated filament binding proteins. However, identifying specific physical properties or interactions between individual mechanical components is difficult, and underlying details or mechanisms are often obfuscated due to the inherent complexity of the cell. Reconstituted *in vitro* biopolymer networks serve as experimental systems in which the complexity can be controlled by adding only a subset of cellular proteins [6]. This bottom-up approach to cytoskeletal mechanics makes it possible to dissect details of protein interactions and filament mechanics, and thus yields insights complementary to whole cell studies. Understanding the physics and mechanics of these active soft materials using model systems is the focus of this review.

The simplest reconstituted system consists of a single species of cytoskeletal filament protein. Over the past two decades, the mechanics of single-species biopolymer networks have been extensively studied [7–14], serving as a basis for our current understanding of *in vitro* network mechanics. The most heavily studied system is the crosslinked actin network (for a review specifically of crosslinked actin networks, see Ref. [15]). A key feature of sufficiently crosslinked semiflexible biopolymer networks is their ability to stiffen dramatically under applied strain, in some cases increasing their network elasticity by several orders of magnitude [9]. Explaining this strain stiffening and relating the network mechanics to biochemical properties, such as polymer concentration and crosslinking density, have posed a challenge due to the semiflexible nature of F-actin and intermediate filaments. In the affine network model, semiflexible polymers are treated as thermal, entropic springs [12,16]. As individual filaments are stretched, the number of available entropic configurations of each filament is reduced, and the filament elasticity increases non-linearly. Assuming an affine network strain, strain stiffening of the bulk network is a product of the stretching of individual filaments. This model captures the experimentally observed scaling relations of elasticity with polymer concentration and the network strain stiffening in crosslinked semiflexible biopolymer networks [10,11], and was more recently used to compare the single filament stiffness to the network elasticity using intermediate filaments [17] and F-actin networks with varying filament flexibility [18]. However, discrete network models have yielded quantitatively similar strain stiffening behavior, and have highlighted non-affine filament bending and network rearrangements as alternative origins of network strain stiffening [19,20]. Thus, while network shear can induce the stretching of individual filaments, it also leads to network reorganization, as

☆ This article is part of a Special Issue entitled: Mechanobiology. Guest Editors: Andreas Janshoff and Sarah Köster.

* Corresponding author.
E-mail address: mjensen@seas.harvard.edu (M.H. Jensen).

http://dx.doi.org/10.1016/j.bbamcr.2015.06.013
0167-4889/

shown in Fig. 1. These recent efforts focusing on disordered, non-affine networks provide an alternative, non-thermal explanation for the strain stiffening behavior [21,22], and it is becoming increasingly clear that the mechanics of semiflexible biopolymer networks depend not only on the physical properties of the filaments themselves, but also on the exact network morphology [23,24].

Furthermore, the exact morphology of the final network, and therefore the network mechanics, is highly dependent on the kinetics of network formation and deformation history [25]. This becomes particularly apparent in crosslinked networks, as network crosslinking often occurs rapidly before the system reaches thermal equilibrium. Initially, filaments are polymerized and form bundles, but further evolution of the network is gradually halted through dynamic arrest, trapping the network in a metastable state not in thermal equilibrium, as illustrated in Fig. 2. Such behavior is observed for the intermediate filament protein keratin, an essential structural component of epithelial tissue, which forms filaments by the compaction and annealing of self-assembled unit-length filaments [26]. Even in the absence of any other cellular components or regulating factors, simply tuning the assembly kinetics to favor elongation or bundling allows keratin to polymerize into a range of different network structures [27]. The dynamics of network formation can also be altered by filament binding partners which change the rates of bundle formation, changing the relative rates of bundling and elongation and driving the network into different final structural states [28]. In networks of actin and the actin-bundling protein α-actinin, actin filament elongation and α-actinin-induced bundling occur concomitantly but are not independent of each other, and as filaments form and are crosslinked the dynamics of the network are dampened and inhibit further bundling. Thus, the final network state is highly dependent on the competing kinetics of filament elongation and α-actinin-induced bundling [29], resulting in a range of heterogeneous network structures [30]. Importantly, these networks do not reach thermal equilibrium as their evolution is halted by crosslinking, and a rich phase space of network morphologies is accessible across biochemically identical samples simply by altering the assembly kinetics. As a result, internal stress in the networks accumulates locally during network formation [31] and is not able to be released without crosslinker dissociation [32]. Thus, biopolymers assembled and crosslinked *in vitro* polymerize into diverse structures far from thermal equilibrium even in the absence of active components such as molecular motors.

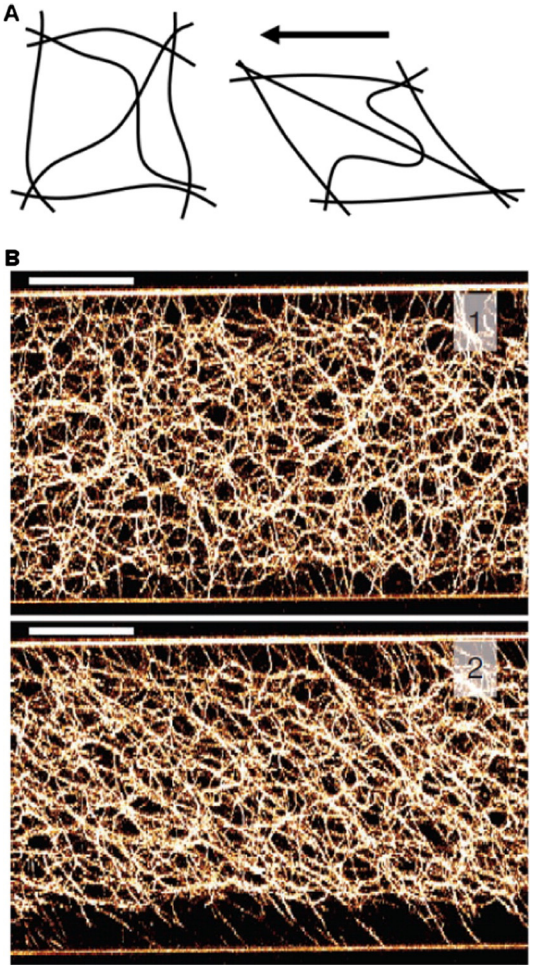

Fig. 1. Crosslinked actin networks subjected to external shear typically exhibit strain stiffening behavior. This is attributed to the rearrangement of filaments, as shown in panel A, which can involve both the stretching of individual network elements, as well as non-affine changes to network architecture. Confocal imaging of F-actin networks crosslinked with α-actinin before and after a 56% strain reveal structural reorganization within the sample under shear, as shown in panel B (reproduced from Ref. [25]). Scale bar is 50 μm.

The strong dependence of the final network structure on network formation kinetics leads to unexpected behavior in *in vitro* reconstituted networks consisting of multiple species of biopolymers. Emergent behavior in such composite biopolymer networks is not simply attributable to the properties of the individual polymers, but is strongly dependent on interactions between polymer species with very different mechanical and dynamical characteristics. For example, the addition of microtubules to sparsely crosslinked F-actin networks has been found to promote strain stiffening, as the stiff microtubules suppresses strain inhomogeneities in the network [33]. Passive microrheological studies of F-actin-microtubule composite networks showed that microtubules unexpectedly confer an increased local compressibility to F-actin networks [34]. The presence of a second polymer species in a semiflexible biopolymer network can also influence the final dynamically arrested network state. For example, since the IF protein vimentin forms networks significantly faster than actin under similar buffer conditions, rapidly forming IFs can sterically interfere with actin filaments before these have time to fully crosslink. Counterintuitively, the additional vimentin polymer can therefore result in a weaker overall composite network, as steric interactions lead to a loss of F-actin crosslinking, as shown schematically in Fig. 3 [35]. Notably, these emergent behaviors occur without direct biological or chemical crosslinkers between the different polymer species, supporting observations in cells suggesting that steric interactions between polymers greatly affect their mechanical behavior. For example, microtubules in cells can withstand large compressive loads with reduced buckling due to the lateral reinforcement of the surrounding elastic cytoskeleton [36]. Beyond such steric polymer interactions, cytoskeletal crosstalk in cells between the different polymer species also involves direct crosslinking by cytolinkers [37–41], an aspect not explored in reconstituted biopolymer systems. Overall, there are many unexplored aspects of composite biopolymer network mechanics and dynamics, and *in vitro* reconstituted systems offer a promising avenue for further investigating the physical interplays between cytoskeletal elements.

In addition to the passive mechanical properties of the filaments themselves, the cytoskeleton is rich in active processes, most notably filament polymerization and depolymerization, and motor activity. This activity makes the cytoskeleton greatly different from passive mechanical systems. The constant activity ensures that the cellular interior is always far from thermal equilibrium, and equilibrium thermodynamics principles do not apply to the cytoskeleton [42]. Additionally, the activity of F-actin- and microtubule-associated motors impact cytoskeletal mechanics by generating internal forces in the networks. Molecular motors are known to stir the cytoplasm and enhance intracellular dynamics [43], amplifying the motion of intracellular organelles even when these are not undergoing active transport. While this enhanced motion appears diffusive due to the stochastic nature of the motor activity in the network, the energy in the network far exceeds thermal energy, and can be traced back to motor activity by combining active and passive microrheology techniques to extract a force spectrum of the cellular

Please cite this article as: M.H. Jensen, et al., Mechanics and dynamics of reconstituted cytoskeletal systems, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamcr.2015.06.013

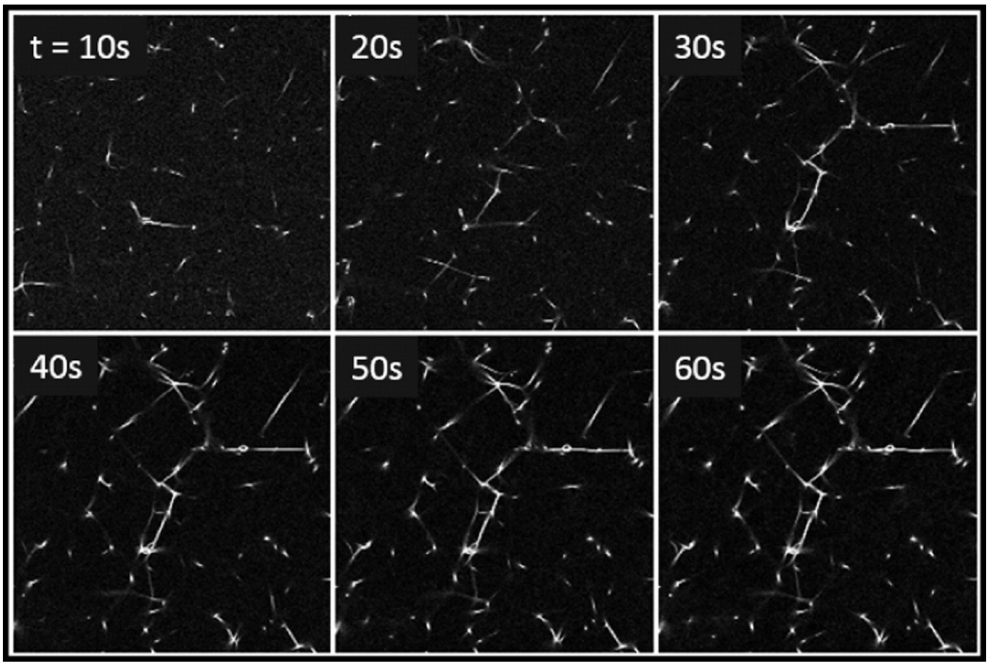

Fig. 2. Actin bundles form rapidly at concentrations typically used in reconstituted actin networks. Here, 12 μM actin is mixed with 1.2 μM crosslinker and immediately imaged by fluorescence confocal microscopy. Initially, actin bundles grow and change orientation as filaments polymerize and are crosslinked. The subsequent network formation is a dynamic process that gradually arrests, as crosslinking prevents the relaxation of internal stresses that develop during polymerization. Each frame is 100 μm.

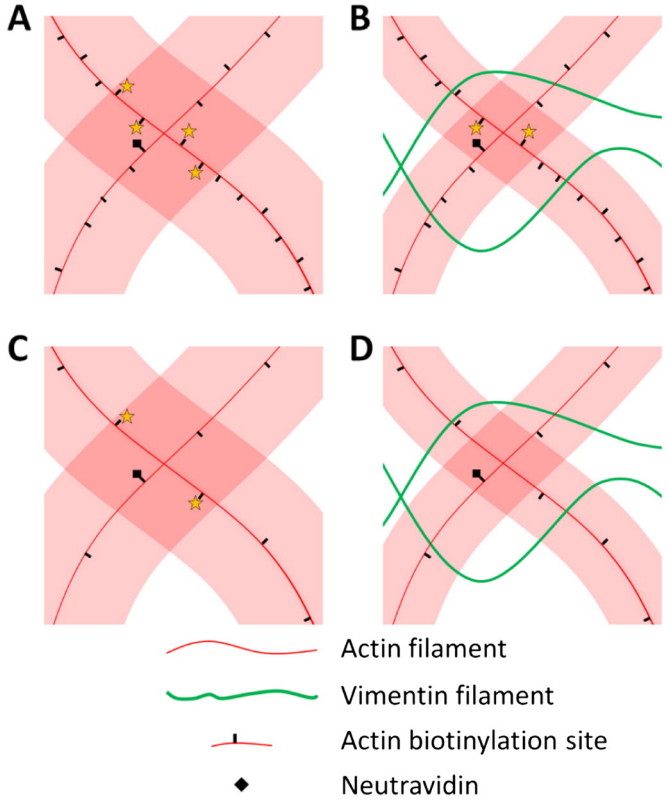

Fig. 3. Composite networks of crosslinked actin and vimentin IF demonstrate complicated emergent behavior. In some cases, the presence of vimentin can impede the actin crosslinking, thereby weakening the overall network structure. The addition of vimentin restricts F-actin fluctuations before dynamic arrest, as indicated by the shaded regions. When crosslinkers are abundant, potential crosslinking sites remain within reach, as illustrated in panels A and B. However, when crosslinkers are sparse, the additional constraint can lead to a loss of F-actin crosslinking, as shown in panels C and D. Figure is reproduced from Ref. [35].

interior [44]. Motors also add substantial tension to the stress fibers of the cell. Intracellular ablation using laser nano-scissors has revealed that stress fibers behave as viscoelastic tension-bearing cables tensed mostly by myosin motors [45]. The internal tension of the fibers not only changes the basic mechanical properties of the filaments [12,46], but is also crucial in maintaining the mechanical shape and integrity of the cell [47]. Furthermore, microfilament tension alters the affinities of binding proteins [48,49], and can directly serve as a mechanism of mechanotransduction by exposing previously inaccessible cryptic binding sites [50].

Because of the dominant role of active processes in the cell, the incorporation of motors into reconstituted *in vitro* systems has received considerable attention over the past decade. Two-dimensional reconstituted systems of myosin motors and F-actin on a lipid membrane have been used to mimic the actin cortex of the cell [51], and showed that motor activity induces both tension and compression in actin, which can cause contraction and induce filament buckling and breaking. In three-dimensional reconstituted networks of F-actin, motor activity causes temporal coarsening and the development of a variety of mesoscopic structures [52–54], and enhances the non-equilibrium fluctuations in the network [55], reminiscent of the motor-driven enhanced motion observed in cells [44]. Clustered *in vitro* acto-myosin structures can exhibit dynamic reorganization, and are able to either fuse into larger structures or rupture into smaller ones [56,57]; however, this dynamic behavior is dependent on the crosslinking and motor concentrations in the network. On the one hand, if the actin is highly crosslinked by a separate F-actin crosslinking protein or by inactive myosin at low ATP concentrations, myosin motor activity can serve to generate internal tension in the network. This internal tension in turn stiffens the network, analogous to strain stiffening in passive networks under an externally applied strain [9,58], and suggests that internal network tension generated by motor activity can serve as a mechanism for regulating network elasticity, as shown in Fig. 4. On the other hand, at low crosslinking densities, the lack of network connectivity causes the motors to fluidize the network, rather than contracting it into clusters. Robust network contraction is thus dependent on the interplay between crosslinking to maintain network connectivity, and motor-generated forces which tend to both tense and fluidize the

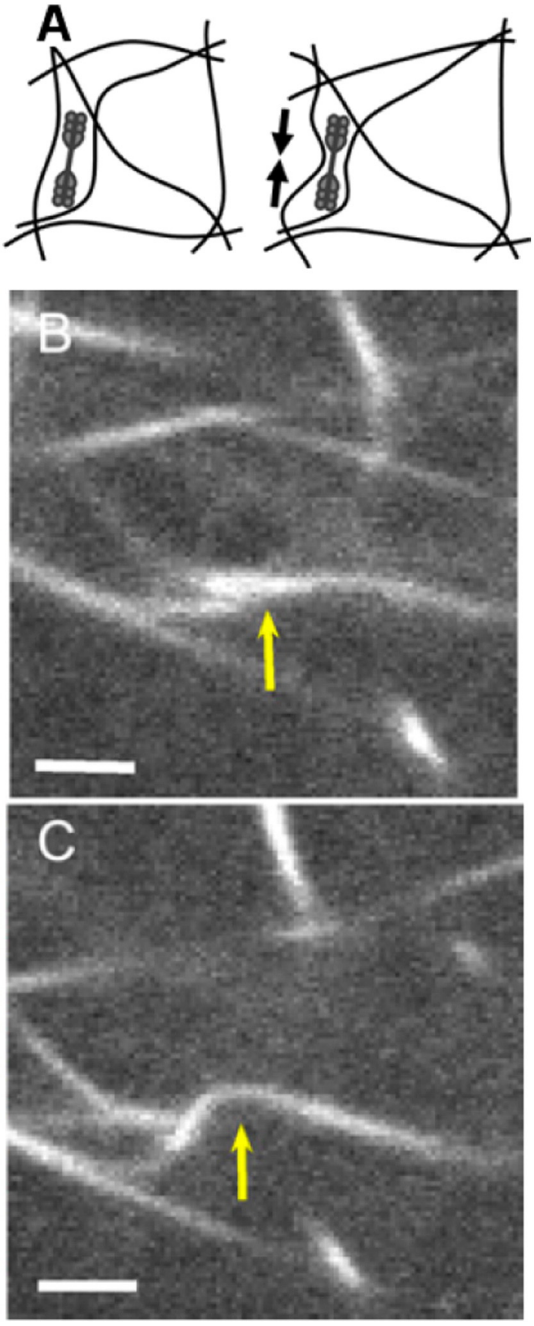

Fig. 4. Acto-myosin networks stiffen due to internally generated tension, reminiscent of the stiffening observed in networks under external shear, as discussed in Ref. [58]. The network tension is generated by myosin, as shown schematically in panel A, and can increase the network elasticity by several orders of magnitude as compared to an unstressed F-actin network. Myosin activity in an actin/α-actinin network can also cause both network contraction and local structural rearrangements, as shown by confocal fluorescence in panels B and C (reproduced from Ref. [59]). Scale bar is 2 μm.

network [57,59]. The right concentration range of crosslinkers is required to ensure that the network is fully connected, yet not so dynamically arrested that motor forces are insufficient to induce contraction [60]. In this regime, in which the network is balanced between full fluidity and static crosslinking, motor activity allows for a rich dynamic behavior and constant remodeling of heterogeneous structures around a steady-state equilibrium of cluster sizes [56,57]. The degree of crosslinking and motor activity also dictates whether contraction occurs globally or locally within the network, and since many crosslinkers respond to tension, the motor activity can dynamically affect the connectivity of the network, leading to different mesoscopic structures [61].

The bottom-up cytoskeletal model systems discussed here contain at most a few cytoskeletal elements, such as one or two biopolymer species, and a filament crosslinking protein. However, even with a relatively limited number of components, these soft biological materials display a wealth of unique behavior very different from more traditional, well-studied solids, and in many cases begin to capture qualitatively similar mechanics to that of the cytoskeleton. While the mechanics of the individual cytoskeletal polymers and single-species networks have been quite well studied, composite networks have shown additional surprising emergent properties, and the addition of motors to reconstituted biopolymer systems further introduces novel mechanics and dynamics, the details of which are still not fully explored. A hallmark of these active soft matter systems, and of living systems in general, is their highly non-equilibrium nature. While cytoskeletal polymers undergo turnover on the order of tens of seconds in cellular regions such as the leading edge of motile cells [62], the gradual onset of dynamic arrest occurs on a similar time scale as illustrated in Fig. 2, and regions of cells undergoing slower polymer turnover could experience arrested network evolution similar to observations in reconstituted networks. ATP-dependent processes contribute greatly to the dynamic behavior of the cytoskeleton, and reconstituted networks incorporating these elements, such as molecular motors, have already yielded insights into potential cellular mechanisms. The addition of motors to more complex reconstituted network systems is an exciting avenue of future research. Since the structure of biopolymer networks is dependent on the formation kinetics pathway, incorporating additional cytoskeletal elements into active networks is likely to alter both the network mechanics and active dynamical properties. For example, introducing a second biopolymer species to a contractile acto-myosin network would likely affect the contractile behavior and marginal stability of the composite structure. As future studies incorporate additional cytoskeletal components, these reconstituted systems will continue to provide additional insights into intracellular behavior, while also serving to broaden our knowledge of basic physical properties of active soft matter.

Transparency document

The Transparency document associated with this article can be found, in the online version.

Acknowledgements

This work was funded by the National Institutes of Health program project grant P01 GM096971, by the National Science Foundation (DMR-1310266), and by the Harvard Materials Research Science and Engineering Center (DMR-1420570).

References

[1] C.P. Brangwynne, G.H. Koenderink, E. Barry, Z. Dogic, F.C. MacKintosh, D.A. Weitz, Bending dynamics of fluctuating biopolymers probed by automated high-resolution filament tracking, Biophys. J. 93 (2007) 346–359.
[2] F. Gittes, B. Mickey, J. Nettleton, J. Howard, Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape, J. Cell Biol. 120 (1993) 923–934.
[3] S. Koster, D.A. Weitz, R.D. Goldman, U. Aebi, H. Herrmann, Intermediate filament mechanics in vitro and in the cell: from coiled coils to filaments, fibers and networks, Curr. Opin. Cell Biol. 32C (2015) 82–91.
[4] J. Howard, A.A. Hyman, Dynamics and mechanics of the microtubule plus end, Nature 422 (2003) 753–758.
[5] L.S. Tobacman, E.D. Korn, The kinetics of actin nucleation and polymerization, J. Biol. Chem. 258 (1983) 3207–3214.
[6] A. Bausch, K. Kroy, A bottom-up approach to cell mechanics, Nat. Phys. 2 (2006) 231–238.
[7] Y.-C. Lin, G.H. Koenderink, F.C. MacKintosh, D.A. Weitz, Viscoelastic properties of microtubule networks, Macromolecules 40 (2007) 7714–7720.
[8] Y. Yang, M. Bai, W.S. Klug, A.J. Levine, M.T. Valentine, Microrheology of highly crosslinked microtubule networks is dominated by force-induced crosslinker unbinding, Soft Matter 9 (2013) 383–393.

[9] M.L. Gardel, F. Nakamura, J.H. Hartwig, J.C. Crocker, T.P. Stossel, D.A. Weitz, Prestressed F-actin networks cross-linked by hinged filamins replicate mechanical properties of cells, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 1762–1767.

[10] M.L. Gardel, J.H. Shin, F.C. MacKintosh, L. Mahadevan, P. Matsudaira, D.A. Weitz, Elastic behavior of cross-linked and bundled actin networks, Science 304 (2004) 1301–1305.

[11] M.L. Gardel, J.H. Shin, F.C. MacKintosh, L. Mahadevan, P.A. Matsudaira, D.A. Weitz, Scaling of F-actin network rheology to probe single filament elasticity and dynamics, Phys. Rev. Lett. 93 (2004) 188102.

[12] F.C. Mackintosh, J. Kas, P.A. Janmey, Elasticity of semiflexible biopolymer networks, Phys. Rev. Lett. 75 (1995) 4425–4428.

[13] R. Tharmann, M.M. Claessens, A.R. Bausch, Viscoelasticity of isotropically cross-linked actin networks, Phys. Rev. Lett. 98 (2007) 088103.

[14] Y.C. Lin, N.Y. Yao, C.P. Broedersz, H. Herrmann, F.C. MacKintosh, D.A. Weitz, Origins of elasticity in intermediate filament networks, Phys. Rev. Lett. 104 (2010).

[15] O. Lieleg, M.M. Claessens, A.R. Bausch, Structure and dynamics of cross-linked actin networks, Soft Matter 6 (2010) 218–225.

[16] C. Storm, J.J. Pastore, F.C. MacKintosh, T.C. Lubensky, P.A. Janmey, Nonlinear elasticity in biological gels, Nature 435 (2005) 191–194.

[17] Y.C. Lin, N.Y. Yao, C.P. Broedersz, H. Herrmann, F.C. Mackintosh, D.A. Weitz, Origins of elasticity in intermediate filament networks, Phys. Rev. Lett. 104 (2010) 058101.

[18] M.H. Jensen, E.J. Morris, C.M. Gallant, K.G. Morgan, D.A. Weitz, J.R. Moore, Mechanism of calponin stabilization of cross-linked actin networks, Biophys. J. 106 (2014) 793–800.

[19] P.R. Onck, T. Koeman, T. van Dillen, E. van der Giessen, Alternative explanation of stiffening in cross-linked semiflexible networks, Phys. Rev. Lett. 95 (2005) 178102.

[20] T. Van Dillen, P. Onck, E. Van der Giessen, Models for stiffening in cross-linked biopolymer networks: a comparative study, J. Mech. Phys. Solids 56 (2008) 2240–2264.

[21] G. Zagar, P.R. Onck, E. Van der Giessen, Elasticity of rigidly cross-linked networks of athermal filaments, Macromolecules 44 (2011) 7026–7033.

[22] G. Zagar, P.R. Onck, E. van der Giessen, Two fundamental mechanisms govern the stiffening of cross-linked networks, Biophys. J. 108 (2015) 1470–1479.

[23] E. Huisman, T. Van Dillen, P. Onck, E. Van der Giessen, Three-dimensional cross-linked F-actin networks: relation between network architecture and mechanical behavior, Phys. Rev. Lett. 99 (2007) 208103.

[24] B. Wagner, R. Tharmann, I. Haase, M. Fischer, A.R. Bausch, Cytoskeletal polymer networks: the molecular structure of cross-linkers determines macroscopic properties, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 13974–13978.

[25] K.M. Schmoller, P. Fernandez, R.C. Arevalo, D.L. Blair, A.R. Bausch, Cyclic hardening in bundled actin networks, Nat. Commun. 1 (2010) 134.

[26] A. Kolsch, R. Windoffer, T. Wurflinger, T. Aach, R.E. Leube, The keratin-filament cycle of assembly and disassembly, J. Cell Sci. 123 (2010) 2266–2272.

[27] J. Kayser, H. Grabmayr, M. Harasim, H. Herrmann, A.R. Bausch, Assembly kinetics determine the structure of keratin networks, Soft Matter 8 (2012) 8873–8879.

[28] J. Kayser, M. Haslbeck, L. Dempfle, M. Krause, C. Grashoff, J. Buchner, H. Herrmann, A.R. Bausch, The small heat shock protein Hsp27 affects assembly dynamics and structure of keratin intermediate filament networks, Biophys. J. 105 (2013) 1778–1785.

[29] T.T. Falzone, M. Lenz, D.R. Kovar, M.L. Gardel, Assembly kinetics determine the architecture of alpha-actinin crosslinked F-actin networks, Nat. Commun. 3 (2012) 861.

[30] O. Lieleg, K.M. Schmoller, C.J. Cyron, Y. Luan, W.A. Wall, A.R. Bausch, Structural polymorphism in heterogeneous cytoskeletal networks, Soft Matter 5 (2009) 1796–1803.

[31] K.M. Schmoller, O. Lieleg, A.R. Bausch, Internal stress in kinetically trapped actin bundle networks, Soft Matter 4 (2008) 2365–2367.

[32] O. Lieleg, J. Kayser, G. Brambilla, L. Cipelletti, A.R. Bausch, Slow dynamics and internal stress relaxation in bundled cytoskeletal networks, Nat. Mater. 10 (2011) 236–242.

[33] Y.C. Lin, G.H. Koenderink, F.C. MacKintosh, D.A. Weitz, Control of non-linear elasticity in F-actin networks with microtubules, Soft Matter 7 (2011) 902–906.

[34] V. Pelletier, N. Gal, P. Fournier, M.L. Kilfoil, Microrheology of microtubule solutions and actin-microtubule composite networks, Phys. Rev. Lett. 102 (2009) 188303.

[35] M.H. Jensen, E.J. Morris, R.D. Goldman, D.A. Weitz, Emergent properties of composite semiflexible biopolymer networks, BioArchitecture 4 (2014) 138–143.

[36] C.P. Brangwynne, F.C. MacKintosh, S. Kumar, N.A. Geisse, J. Talbot, L. Mahadevan, K.K. Parker, D.E. Ingber, D.A. Weitz, Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement, J. Cell Biol. 173 (2006) 733–741.

[37] Y.M. Yang, J. Dowling, Q.C. Yu, P. Kouklis, D.W. Cleveland, E. Fuchs, An essential cytoskeletal linker protein connecting actin microfilaments to intermediate filaments, Cell 86 (1996) 655–665.

[38] V. Prahlad, M. Yoon, R.D. Moir, R.D. Vale, R.D. Goldman, Rapid movements of vimentin on microtubule tracks: kinesin-dependent assembly of intermediate filament networks, J. Cell Biol. 143 (1998) 159–170.

[39] B.T. Helfand, A. Mikami, R.B. Vallee, R.D. Goldman, A requirement for cytoplasmic dynein and dynactin in intermediate filament network assembly and organization, J. Cell Biol. 157 (2002) 795–806.

[40] C.L. Leung, K.J. Green, R.K. Liem, Plakins: a family of versatile cytolinker proteins, Trends Cell Biol. 12 (2002) 37–45.

[41] C.L. Leung, D. Sun, M. Zheng, D.R. Knowles, R.K. Liem, Microtubule actin cross-linking factor (MACF) a hybrid of dystonin and dystrophin that can interact with the actin and microtubule cytoskeletons, J. Cell Biol. 147 (1999) 1275–1286.

[42] D. Mizuno, C. Tardin, C.F. Schmidt, F.C. Mackintosh, Nonequilibrium mechanics of active cytoskeletal networks, Science 315 (2007) 370–373.

[43] C.P. Brangwynne, G.H. Koenderink, F.C. MacKintosh, D.A. Weitz, Cytoplasmic diffusion: molecular motors mix it up, J. Cell Biol. 183 (2008) 583–587.

[44] M. Guo, A.J. Ehrlicher, M.H. Jensen, M. Renz, J.R. Moore, R.D. Goldman, J. Lippincott-Schwartz, F.C. Mackintosh, D.A. Weitz, Probing the stochastic, motor-driven properties of the cytoplasm using force spectrum microscopy, Cell 158 (2014) 822–832.

[45] S. Kumar, I.Z. Maxwell, A. Heisterkamp, T.R. Polte, T.P. Lele, M. Salanga, E. Mazur, D.E. Ingber, Viscoelastic retraction of single living stress fibers and its impact on cell shape, cytoskeletal organization, and extracellular matrix mechanics, Biophys. J. 90 (2006) 3762–3773.

[46] X. Liu, G.H. Pollack, Mechanics of F-actin characterized with microfabricated cantilevers, Biophys. J. 83 (2002) 2705–2715.

[47] D.E. Ingber, Tensegrity I. Cell structure and hierarchical systems biology, J. Cell Sci. 116 (2003) 1157–1173.

[48] T.Q. Uyeda, Y. Iwadate, N. Umeki, A. Nagasaki, S. Yumura, Stretching actin filaments within cells enhances their affinity for the myosin II motor domain, Plos One 6 (2011) e26200.

[49] K. Hayakawa, H. Tatsumi, M. Sokabe, Actin filaments function as a tension sensor by tension-dependent binding of cofilin to the filament, J. Cell Biol. 195 (2011) 721–727.

[50] A.J. Ehrlicher, F. Nakamura, J.H. Hartwig, D.A. Weitz, T.P. Stossel, Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A, Nature 478 (2011) (260-U154).

[51] M.P. Murrell, M.L. Gardel, F-actin buckling coordinates contractility and severing in a biomimetic actomyosin cortex, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 20820–20825.

[52] M. Soares e Silva, M. Depken, B. Stuhrmann, M. Korsten, F.C. MacKintosh, G.H. Koenderink, Active multistage coarsening of actin networks driven by myosin motors, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 9408–9413.

[53] S. Kohler, A.R. Bausch, Contraction mechanisms in composite active actin networks, Plos One 7 (2012) e39869.

[54] F. Backouche, L. Haviv, D. Groswasser, A. Bernheim-Groswasser, Active gels: dynamics of patterning and self-organization, Phys. Biol. 3 (2006) 264–273.

[55] B. Stuhrmann, E.S.M. Soares, M. Depken, F.C. Mackintosh, G.H. Koenderink, Nonequilibrium fluctuations of a remodeling in vitro cytoskeleton, Phys. Rev. E Stat. Nonlinear Soft Matter Phys. 86 (2012) 020901.

[56] F.C. MacKintosh, Active gels: motors keep dynamics steady, Nat. Mater. 10 (2011) 414–415.

[57] S. Kohler, V. Schaller, A.R. Bausch, Structure formation in active networks, Nat. Mater. 10 (2011) 462–468.

[58] G.H. Koenderink, Z. Dogic, F. Nakamura, P.M. Bendix, F.C. MacKintosh, J.H. Hartwig, T.P. Stossel, D.A. Weitz, An active biopolymer network controlled by molecular motors, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 15192–15197.

[59] P.M. Bendix, G.H. Koenderink, D. Cuvelier, Z. Dogic, B.N. Koeleman, W.M. Brieher, C.M. Field, L. Mahadevan, D.A. Weitz, A quantitative analysis of contractility in active cytoskeletal protein networks, Biophys. J. 94 (2008) 3126–3136.

[60] S. Wang, P.G. Wolynes, Active contractility in actomyosin networks, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 6446–6451.

[61] J. Alvarado, M. Sheinman, A. Sharma, F.C. MacKintosh, G.H. Koenderink, Molecular motors robustly drive active gels to a critically connected state, Nat. Phys. 9 (2013) 591–597.

[62] J.A. Theriot, T.J. Mitchison, Actin microfilament dynamics in locomoting cells, Nature 352 (1991) 126–131.

Please cite this article as: M.H. Jensen, et al., Mechanics and dynamics of reconstituted cytoskeletal systems, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamcr.2015.06.013