ACTIVE STIFFENING OF F-ACTIN NETWORK DOMINATED BY STRUCTURAL TRANSITION OF ACTIN FILAMENTS INTO BUNDLES

Shengmao Lin ^{a*}, Xinwei Han ^{b*}, Gary C.P. Tsui ^c, David Hui ^d, Linxia Gu ^{a, b, e #}

^a Department of Mechanical and Materials Engineering, University of Nebraska-Lincoln, NE 68588-0656

^b Department of Interventional Radiology, The First Affiliated Hospital of Zhengzhou University, Henan Province, P. R. China

^c Department of Industrial and Systems Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, P. R. China
LA 70148

^d Department of Mechanical Engineering, The University of New Orleans, LA 70148

^e Nebraska Center for Materials and Nanoscience, Lincoln, NE 68588-0656

* both authors contributed equally to this paper

Corresponding author. Tel.: 1-402-4727680; Fax: 1-402-4721465;

E-mail: lgu2@unl.edu

This work is submitted as an Original Paper.

ABSTRACT

Molecular motor regulated active contractile force is key for cells sensing and responding to their mechanical environment, which leads to characteristic structures and functions of cells. The F-actin network demonstrates a two-order of magnitude increase in its modulus due to contractility; however, the mechanism for this active stiffening remains unclear. Two widely acknowledged hypotheses are that active stiffening of F-actin network is caused by (1) the nonlinear force-extension behavior of cross-linkers, and (2) the loading mode being switched from bending to stretching dominated regime. Direct evidence supporting either theory is lacking. Here we examined these hypotheses and showed that a reorganization of F-actin network from cross-linked filament state to bundled stress fiber state plays a key role on active stiffening of actin network. We demonstrated through computational models that the stretching of cross-linkers and molecular motors has less impact on the active stiffening, while it is more sensitive to cytoskeleton reorganization during the elasticity sensing. The proposed new mechanism involving the cytoskeletal remodeling was able to integrate discrete experimental observations and has the potential to advance our understanding of active sensing and responding of cells.

Keywords: F-actin network; molecular motor; active stiffening; cross-linker; bundle; computational biomechanics

1. INTRODUCTION

Mechanical properties of extracellular matrix (ECM) play an important role in mediating cellular form and function. Cells respond to the ECM stiffness by adjusting adhesion, cytoskeleton structure,

and contractile force [1]. For example, mesenchymal stem cells were differentiated into neurogenic, myogenic, or osteogenic cell types by only varying the ECM stiffness [2]. It is well acknowledged that cells tune up their own stiffness in response to a stiffer ECM [3]. In particular, cells on a soft substrate demonstrate a diffuse cytoskeleton with random arrangement of actin filaments. In contrast, cells on a stiff substrate contain more aligned stress fibers, aggregations of actin, and increased cell contractile force [4]. Indirect data support that the active response of cytoskeleton, i.e., the actin fiber network, was regulated by myosin II motors, actin filament, and cross-linker proteins. Specifically the experimental study by Koenderink et al. [5] has demonstrated that myosin II motors were able to stiffen the F-actin network by two orders of magnitude by switching nonlinear filament A (FLNa) cross-linkers to rigid scruiin. However, the mechanism that drives active stiffening of the cytoskeleton remains poorly understood [1].

There are two hypotheses for the active stiffening of the actin fiber network. The first hypothesis is that active stiffening of the network is attributed to the nonlinear force-extension behavior of cross-linkers, corresponding directly to the pre-mentioned experimental protocol [5]. It was illustrated in the theoretical work by Chen et al. [6] where the nonlinear spring cross-linkers were used to capture the two orders of magnitude stiffening in a two-dimensional (2D) fiber network model. The second hypothesis is the fiber loading mode being switched from softer bending to stiffer stretching dominated regime [7]. The behaviors of cross-linkers in existing 2D models are usually simply represented as intersection points constrained with rotating pin joints or springs, which might overestimate the role of the cross-linker as well as the molecular motor contraction on the active stiffening [8]. Moreover, current models generally ignore the remodeling of actin filaments into stress fibers at higher level of contraction [9], which was captured by Walcoot et al. [10].

In the present study, we developed a three dimensional (3D) computational model, which considered the spatial configuration of both cross-linkers and molecular motors, as well as the structural reorganization from actin filaments to stress fibers during the elasticity sensing, to further test these hypotheses. The contractile forces are designated to molecular motor proteins which induced pre-stress in the F-actin network. The role of nonlinear cross-linkers and bending/stretching ratio were delineated. Results showed that cytoskeleton reorganization plays an essential role in active stiffening of the cell, supporting a new mechanism that molecular motors induced active stiffening is sensitive to cytoskeleton reorganization.

2. MATERIALS AND METHODS

The remodeling of F-actin networks is illustrated in Fig.1. Its cross-linked state was constructed by three components: F-actin filaments (black line), cross-linker (green line) and myosin-II motors (red line). After remodeling, these three components were assumed bundled together to form stress fibers, which is referred to as bundled state, corresponding to a higher contraction level.

Both states were modeled within the same size of representative volume element (RVE), i.e., 40 μm in side length, which is the same size used in another study [6]. For cross-linked state, 3D F-actin filaments (1600 in total) were randomly distributed inside the RVE, representing F-actin concentration of 1mg/ml [5]. A total of 860 cross-linkers and 1634 myosin-II motors were then generated between the F-actin filaments based on a distance threshold of 800 nm and 1200 nm respectively. The detailed modeling technique was described in our previous work [8]. For bundled state, a Voronoi-based network was adopted, which has demonstrated its efficiency [11-13]. It was assumed that four F-actin filaments form into one stress fiber in average [14, 15]. The volume fraction of the F-actin filaments or fibers were kept the same for these two states while the diameters

of F-actin filaments and bundled fibers are 7 nm [16] and 14 nm respectively. The 10% contraction was applied on each myosin-II motor in cross-linked state, which was reverse fitted based on the estimated 1-pN-force per myosin head in one experimental study [5]. In bundled state, a 20% contraction was adopted and applied on each stress fiber, per the measurement reported in the stress fiber contraction test [17]. The material properties F-actin fiber were assumed as 1.6 GPa in Young's modulus and 0.3 in Poisson's ratio [18]. The Young's modulus of cross-linkers varied as 0.016 GPa, 0.16 GPa, 1.6 GPa and 16 GPa. In addition to the contraction, a 10% shear strain was also applied on the upper surface of RVE to obtain the stiffness of F-actin networks. The developed computational models were solved using ABAQUS 6.12 (Simulia, Providence, RI).

Figure 1: F-actin network in (a) cross-linked state and (b) bundled state.

3. RESULTS

The active contractility of the F-actin network without external loading are demonstrated in Fig.2. Even without external loadings, the network deformed itself to sense its environment, i.e., the fixed top and bottom surfaces. It is clear that no distinguishable contraction was observed in cross-linked state. The peak reduction in cross-sectional area is merely 4%. In contrast, stress fiber formation led to approximately 43.8% reduction in cross-sectional area for bundled state at the

middle plane. The larger contraction also corresponded to increased internal stresses in larger percentage of fibers. The average axial force for each myosin motor is approximately 0.65 pN in cross-linked state. The average axial force for each stress fiber is 68.63 pN per the bundled network model.

Figure 2: Active contraction of F-actin network induced von-Mises stress (GPa) distributions for (a) cross-linked state, and (b) bundled state.

The effect of molecular motors on active stiffening of F-actin network was delineated through mechanical characterization of four configurations, i.e, cross-linked passive, cross-linked active, bundled passive and bundled active. These models represented the F-actin network at two different states (cross-linked or bundled) with and without considering the motor contraction (active or passive). The resulting mechanical behaviors of these networks in terms of stress-strain relationship as well as their shear modulus are depicted in Fig.3. Two orders of increasing shear modulus were clearly observed. In addition, F-actin network in bundled state has a higher shear modulus compared to the cross-linked state. Also, the molecular motor contraction induced a relatively higher stiffness in networks, and its role is more profound at the bundled state. Specifically, the active contraction of

myosin motor induced approximately 26 times increase in the shear modulus of network, while it is not even double the network stiffness in cross-linked state.

Figure 3: F-actin network: (a) shear stress-strain relationship and (b) shear modulus.

The stiffness of cross-linkers was altered for up to four orders difference in magnitude to test the hypothesis that active stiffening of the F-actin network can be attributed to the mechanical properties of the cross-linker. The Young's modulus of cross-linkers varied between 0.016 GPa and 16 GPa with an increment of 10 times. Results in Fig.4 have shown that the shear modulus of F-actin network only increased 2.5 times from $5.00E-05$ Pa to $1.25E-04$ Pa, even with 10000 times increase in the cross-linkers' stiffness. This indicated that cross-linker stiffening alone is not sufficient enough to capture the reported active stiffening of F-actin network by nearly two orders of magnitude [5].

Figure 4: The role of cross-linker stiffness on shear modulus of F-actin network.

The filament stretching or bending stiffness was also varied to test the other hypothesis regarding the transition from bending to stretching dominated mode [7], where the stretching stiffness μ was defined as EA and bending stiffness κ as EI , with A as the cross-section area of the filament and I as the moment of inertia. Three cases were then considered by changing the filament radius R and Young's modulus E , as listed in Table 1. Case 1 and 2 have the same stretching stiffness but the bending stiffness of case 1 is four times case 2. Both cases 2 and 3 have the same bending stiffness but the ratio of stretching stiffness is four. The resulting shear modulus of F-actin network has the same ratio between cases as the bending stiffness. This indicates that the deformation of the cross-linked network is dominated by the bending mode.

Table 1: The role of filament stretching or bending stiffness on the shear modulus of F-actin network

	R (nm)	E (GPa)	μ (EA)	κ (EI)	Shear modulus (Pa)
Case 1	7	0.4	61.56	754.4	4.59 E-04
Case 2	3.5	1.6	61.56	188.6	1.15 E-4
Case 3	7	0.1	15.4	188.6	1.15 E-4

4. DISCUSSION

In this work, the active stiffening of F-actin network was examined through computational models. The cytoskeleton reorganization of F-actin network, i.e., cross-linked vs. bundled state, was considered by integrating discrete experimental evidence from actin filaments and stress fibers. The contractile forces correspond to the stretching of molecular motor proteins. A 10% contraction strain was applied on the molecular motors in a cross-linked network resulting in an average axial filament force of 0.65 pN, which is in consistent with the experimental study by Koenderink et al. [5]. At the

bundled state, an observed 20% contraction on each stress fiber [17] was adopted, implying the effect of reinforced molecular motors.

Results have demonstrated that molecular motors induced active stiffening of up to two orders is sensitive to the F-actin network reorganization. This has been accredited to the increased cross-linker concentration [5, 19]. Experimental evidence supports that F-actin networks are weakly cross-linked and largely unbundled at low concentrations of cross-linkers [5]. As the concentrations of cross-linker increases, F-actin filaments were then formed into stress fibers, which were comprised of better aligned actin filaments bundled by cross-linkers and myosin II motors [20-22]. We also captured the contraction induced pre-stress in the network as well as bulk volume changes when actin filaments were formed into stress fiber, i.e., the bundled state, which is consistent with the experimental observations [5, 23]. Specifically, we have demonstrated that the average axial force for each myosin motor is approximately 0.65 pN in cross-linked state. This is within the range of values reported by Koenderink et al. [5]. In addition, Bendix et al. observed the bulk volume reduction during contraction of a simplified gel composed of purified F-actin, myosin motors and cross-linkers [23]. This contractility occurs only with sufficient motor concentrations and appropriate cross-linker concentrations, i.e., bundled state in this work.

We also tested two competing hypothesis for active stiffening of F-actin network. One is that active stiffening of the network is attributed to the nonlinear force-extension behavior of cross-linkers, inspired by the observations that the biopolymer network constituted with nonlinear FLNa cross-linker proteins causes a two-order increase in network stiffness and the network reconstituted with rigid scruin cross-linker didn't show much active stiffening effect [5]. The 2D fiber network model by Chen et al. [6] was able to capture the two orders of magnitude stiffening

using nonlinear spring cross-linkers, which was not explicitly defined in terms of magnitude. The active stiffening was implemented by randomly distributed molecular motors, i.e., force dipoles pulling out the F-actin fibers, which could stretch cross-linkers up to a strain of 100%. This strain level seems excessive, which might need additional evidence to support. Using our 3D cross-linked network model, we examined the non-linear force-extension behavior of cross-linkers with a modulus increase over a range of four orders of magnitude. The observed results showed the shear modulus of the network was only doubled. This implied that the nonlinearity of cross-linkers might not play a major role in active stiffening.

We also tested the other hypothesis regarding fiber loading mode switching from softer bending to stiffer stretching dominated regime [7]. The motors, again modeled as force dipoles in a 2D network, might also lead to excessive strain in filaments due to the enforcement of fiber stretching. The characterization of bending (EI) /stretching (EA) might be over simplified considering the 3D network topology. Per their definition, we observed the bending dominated behavior in our 3D networking models. This was expected considering large non-affine deformation at low stretch ratio [8]. The discrepancy between our results and the existing hypothesis could be explained by the different configuration of molecular motor, cross-linkers and dimensionality. The associated question could be: does a stretching dominated mode exist for a highly dynamic fiber network? This question merits further investigation and was beyond the scope of this work.

It is worth noting that stress fiber was composed of four F-actin filaments, which is at the lower end of the range [14]. This configuration is sufficient for illustrating the mechanism of active stiffening of F-actin network. For stress fibers with more actin filaments, we expect that the

cytoskeletal reorganization plays a more important role than the cross-linking in the active stiffening of F-actin network.

In summary, this work demonstrated that cytoskeleton reorganization plays an essential role in active stiffening of the cell, supporting a new mechanism that molecular motors induced active stiffening is sensitive to cytoskeleton reorganization. The correlation between the active contractility and stress fiber formation could be further studied for better regulating cell sensing. This work would improve the understanding of active cellular mechanics and provide a platform for designing active biometric materials [24]. This work could also be extended to study the cell-ECM interaction.

Conflict of interest statement

None of the authors has any conflict of interest.

Acknowledgments

This work was supported by the National Science Foundation CAREER award (CBET-1254095).

REFERENCE

1. Discher, D.E., P. Janmey, and Y.-l. Wang, *Tissue cells feel and respond to the stiffness of their substrate*. Science, 2005. **310**(5751): p. 1139-1143.
2. Engler, A.J., et al., *Matrix elasticity directs stem cell lineage specification*. Cell, 2006. **126**(4): p. 677-689.
3. Solon, J., et al., *Fibroblast adaptation and stiffness matching to soft elastic substrates*. Biophysical journal, 2007. **93**(12): p. 4453-4461.
4. Janmey, P.A., T. Yeung, and L.A. Flanagan, *Effect of substrate stiffness on cell morphology*. ASME-PUBLICATIONS-BED, 2001. **50**: p. 709-710.
5. Koenderink, G.H., et al., *An active biopolymer network controlled by molecular motors*. Proceedings of the National Academy of Sciences, 2009. **106**(36): p. 15192-15197.
6. Chen, P. and V.B. Shenoy, *Strain stiffening induced by molecular motors in active crosslinked biopolymer networks*. Soft Matter, 2011. **7**(2): p. 355-358.
7. Broedersz, C. and F. MacKintosh, *Molecular motors stiffen non-affine semiflexible polymer networks*. Soft Matter, 2011. **7**(7): p. 3186-3191.
8. Lin, S. and L. Gu, *Influence of Crosslink Density and Stiffness on Mechanical Properties of Type I Collagen Gel*. Materials, 2015. **8**(2): p. 551-560.
9. Burridge, K. and E.S. Wittchen, *The tension mounts: stress fibers as force-generating mechanotransducers*. The Journal of cell biology, 2013. **200**(1): p. 9-19.
10. Walcott, S. and S.X. Sun, *A mechanical model of actin stress fiber formation and substrate elasticity sensing in*

adherent cells. Proceedings of the National Academy of Sciences, 2010. **107**(17): p. 7757-7762.

11. Lake, S.P., et al., *Mechanics of a fiber network within a non-fibrillar matrix: model and comparison with collagen-agarose co-gels*. Annals of biomedical engineering, 2012. **40**(10): p. 2111-2121.
12. Zhang, L., et al., *A coupled fiber-matrix model demonstrates highly inhomogeneous microstructural interactions in soft tissues under tensile load*. Journal of biomechanical engineering, 2013. **135**(1): p. 011008.
13. Lin, S., et al., *Towards Tuning the Mechanical Properties of Three-Dimensional Collagen Scaffolds Using a Coupled Fiber-Matrix Model*. Materials, 2015. **8**(8): p. 5254.
14. Tojkander, S., G. Gateva, and P. Lappalainen, *Actin stress fibers—assembly, dynamics and biological roles*. J Cell Sci, 2012. **125**(8): p. 1855-1864.
15. Pellegrin, S. and H. Mellor, *Actin stress fibres*. Journal of cell science, 2007. **120**(20): p. 3491-3499.
16. Cooper, G.M., *Structure and organization of actin filaments*. 2000.
17. Katoh, K., et al., *Isolation and contraction of the stress fiber*. Molecular Biology of the Cell, 1998. **9**(7): p. 1919-1938.
18. Gardel, M.L., et al., *Mechanical response of cytoskeletal networks*. Methods in cell biology, 2008. **89**: p. 487-519.
19. Mizuno, D., et al., *Nonequilibrium mechanics of active cytoskeletal networks*. Science, 2007. **315**(5810): p. 370-373.
20. Kaunas, R., H.J. Hsu, and S. Deguchi, *Sarcomeric model of stretch-induced stress fiber reorganization*. Cell Health Cytoskeleton, 2011. **3**: p. 13-22.
21. Gardel, M., et al., *Elastic behavior of cross-linked and bundled actin networks*. Science, 2004. **304**(5675): p. 1301-1305.
22. Lieleg, O., M.M. Claessens, and A.R. Bausch, *Structure and dynamics of cross-linked actin networks*. Soft Matter, 2010. **6**(2): p. 218-225.
23. Bendix, P.M., et al., *A quantitative analysis of contractility in active cytoskeletal protein networks*. Biophysical journal, 2008. **94**(8): p. 3126-3136.
24. Cheung, H.-Y., et al., *A critical review on polymer-based bio-engineered materials for scaffold development*. Composites Part B: Engineering, 2007. **38**(3): p. 291-300.