Multiscale model predicts increasing focal adhesion size with decreasing stiffness in fibrous matrices

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We describe a multiscale model that incorporates force-dependent mechanical plasticity induced by interfiber cross-link breakage and stiffness-dependent cellular contractility to predict focal adhesion (FA) growth and mechanosensing in fibrous extracellular matrices (ECMs). The model predicts that FA size depends on both the stiffness of ECM and the density of ligands available to form adhesions. Although these two quantities are independent in commonly used hydrogels, contractile cells break cross-links in soft fibrous matrices leading to recruitment of fibers, which increases the ligand density in the vicinity of cells. Consequently, although the size of focal adhesions increases with ECM stiffness in nonfibrous and elastic hydrogels, plasticity of fibrous networks leads to a departure from the well-described positive correlation between stiffness and FA size. We predict a phase diagram that describes nonmonotonic behavior of FA in the space spanned by ECM stiffness and recruitment index, which describes the ability of cells to break cross-links and recruit fibers. The predicted decrease in FA size with increasing ECM stiffness is in excellent agreement with recent observations of cell spreading on electrospun fiber networks with tunable cross-link strengths and mechanics. Our model provides a framework to analyze cell mechanosensing in nonlinear and inelastic ECMs.

Focal adhesions (FAs) are large macromolecular assemblies through which mechanical force and regulatory signals are transmitted between the extracellular matrix (ECM) and cells. FAs play important roles in many cellular behaviors, including proliferation, differentiation, and locomotion, and pathological processes like tumorigenesis and wound healing (1–4). For this reason, intense efforts have been devoted to understanding how key signaling molecules and ECM characteristics influence the formation and growth of FAs. In particular, in vitro studies using elastic hydrogels have shown that forces generated by actomyosin contraction are essential for the stabilization of FAs (5, 6). Numerous observations have convincingly demonstrated that cells form larger FAs as well as develop higher intracellular traction forces on stiffer ECMs (7, 8), evidencing the mechanosensitive nature of FAs which has been quantitatively modeled using different (continuum, coarse-grain, and molecular) approaches (9, 10).

It must be pointed out that in all of the aforementioned investigations, the substrates considered were flat (2D) and linear elastic. However, in vivo, many cells reside within 3D fibrous scaffolds where the density and diameter of fibers can vary depending on the nature of the tissue (11–13). The local architecture of these fibrous networks may change significantly when cells exert forces on them, leading to phenomena such as nonlinear stiffening, reorientation, and physical remodeling of the ECM (14, 15). Our recent study on cells in synthetic fibrous matrices with tunable mechanics and user-defined architecture showed that increasing fiber stiffness suppresses spreading, in contrast to hydrogels, where increased stiffness always promotes cell spreading (16). Other recent studies have found that the spreading of cells cultured on soft viscoelastic substrates that exhibit stress relaxation is greater than those on elastic substrates of the same modulus but similar to that of cells spreading on stiffer elastic substrates (17). Although these studies demonstrate a clear departure from the well-described relationship between material stiffness and spreading established with elastic hydrogel surfaces, a quantitative description of how cells are able to physically remodel matrices to mature FAs, which in turn can lead to greater spreading, is currently lacking. In particular, models that connect ECM structure (i.e., fiber properties such as size and stiffness and the strength of cross-links) with cell adhesion formation and spreading can guide the development of materials to engineer the cellular responses, as well as to better understand the cell–matrix interactions in physiologically relevant states.

Here we propose a multiscale chemomechanical model to describe the evolution of FAs in cross-linked fibrous networks that resemble native ECMs. Specifically, possible breakage of cross-links in the fibrous network is considered, which allows contractile cells to recruit fibers and increase the density of ligands available for the formation of adhesions. By combining the mechanics of fiber recruitment with stress-dependent growth kinetics of FA plaques, we predict a phase diagram for the stable size of focal adhesions as a function of the ECM stiffness and a parameter we introduce, namely, the recruitment index of the ECM that characterizes how easily fibers can be recruited by the contractile cells. Our model explains how cell-driven fiber recruitment can lead to a departure from the monotonic stiffness versus cell spreading relationship observed in hydrogels.

Materials and Methods

To understand the influence of cell-driven fiber recruitment on the formation of FAs, we developed a multiscale chemomechanical model. Specifically, the

Significance

Focal adhesions play crucial roles in mechanotransduction and regulate processes such as spreading, proliferation, differentiation, and motility. It is well known that cells develop larger adhesions when cultured on stiffer elastic hydrogels, but the native extracellular matrix (ECM) is fibrous, nonlinear, and dissipative. We developed a multiscale model showing that adhesion size decreases with increasing stiffness in fibrous matrices, in excellent agreement with our experiments on engineered fibrous matrices. Our model shows that this is due to the feedback between cell contractility and the physical remodeling of ECM, which does not exist in elastic substrates. The basic stiffness–adhesion size principle uncovered can be applied to understand tumor progression fundamentally or to better design biomaterial scaffolds to control cell behavior.


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correlation between fiber density (which then determines the density of ligand/integrin bonds composing FAs) and cell contractility is first obtained using discrete fiber network (DFN) simulations. The mechanical response of the FA–ECM complex to actomyosin contractile forces is then determined by developing a coarse-grained model, where discrete FAs are homogenized and treated as an adhesion band along the rim of the cell. By coupling the stiffness dependence of the actin contractile force and the stress-dependent kinetics of adding new adhesion plaque units, the growth dynamics of the FA band and its equilibrium size are evaluated. The details of each of the elements of the model are described in the following sections.

**Discrete Fiber Network Model for the ECM.** Following our earlier work on active biopolymer networks (14, 15), 2D fiber networks representing electrospun matrices were created with randomly organized linear elastic fibers and breakable cross-links. The fiber properties used in our DFN simulations were based on recent experiments on electrospun methacrylated xerogel (16) scaffolds. Specifically, individual fibers were modeled as beams having circular cross-sections with Young’s moduli, Poisson’s ratios, and radii of 140 MPa, 0.3, and 1.8 μm, respectively. The initial configuration was created by randomly placing discrete fibers in a 2D plane and cross-linking the fibers that are closer than a threshold value. New fibers were added until the experimentally observed network pore size was reached. A circular void was introduced in the middle to represent the contractile cell which applies a uniformly distributed and radiating contractile force to the network near the periphery of the hole (Fig. S1). During the simulation, the force applied at the cell periphery was raised incrementally, whereas the network displacement was fixed at the outer boundaries, far from the hole. The cross-links were checked at each loading increment and removed if the transmitted strain energy exceeded a threshold value. This allowed for the detachment of fibers from their initial positions and densification toward the cell area. Fiber recruitment was quantified as the number of fibers that were pulled into the cell area.

**Coarse-Grained Model for the Mechanical Response of the FAs.** It is well known that FAs, which consist of clusters of integrins that bind to the ECM and to an intracellular plaque of reinforcing actin binding proteins, are connected to the cell nucleus via actomyosin stress fibers as shown in Fig. S2. Furthermore, such assemblies are mostly distributed at the cell periphery (18, 19), as shown in Fig. 1A. Based on these observations, we proceed by adopting an axially symmetric coarse-grained computational model where we represent the cell nucleus via actomyosin stress fibers as shown in Fig. 1C. The FA bands are not considered here, but we adopt a homogenized description where the total area of the adhesions is predicted based on the effective width of the band. The FA band is treated as an elastic plaque representing the stiffness of the constituent molecules (with Young’s modulus $E_D$) connected to the ECM through an array of integrins (modeled as springs with stiffness $k$) whose density $\phi$ is assumed to be proportional to the fiber density $\phi_f$ underneath the cell. Although more complicated models with strain-dependent detachment rates can be used for integrins, recent experiments have shown that a simple description (i.e., treating the integrin as a linear spring) can capture the response of integrins sufficiently because the timescale for integrin binding dynamics (i.e., a few seconds (9, 20)) is much shorter than that for FA growth (i.e., a few minutes (18)). In addition, the proximal end of the band is connected to the cell nucleus through stress fibers (Fig. 1B) that generate contractile forces.

Increases in the density of ECM fibers $\phi_f$ underneath a cell can occur as the contractile forces break the cross-links in the ECM and recruit fibers, which will further influence the integrin–ECM bond density $\phi_i$; for simplicity, we assume that $\phi_i = \phi_f$. Specifically, our DFN simulations show that the fiber density $\phi_f$ increases with the applied force (once it exceeds a threshold value) based on large levels of force. To capture this behavior, $\phi_f$ is phenomenologically related to the contractile stress $\sigma$ as

$$\frac{\phi_f}{\phi_i} = \left(1 + 5\epsilon \left[1 - (\sigma/\sigma_c)^{1/n}\right]\right)^{1/n}, \quad \sigma < \sigma_c,$$

$$\frac{\phi_f}{\phi_i} = \left(1 + 5\epsilon \left[1 - (\sigma_c/\sigma)^{1/n}\right]\right)^{1/n}, \quad \sigma \geq \sigma_c.$$ 

[1]

Here $\phi_f$ is the initial fiber density, $\sigma_c$ corresponds to the threshold stress for fiber recruitment, $\epsilon$ stands for the error function, and $n$ (recruitment index) is a measure of the ease with which fibers can be recruited. Physically, large values of $n$ correspond to the cases where cross-links are weak (i.e., can be broken easily), and therefore, more fibers will be recruited by the cell. As we show below (Cross-Link Breakage Enables Ligand Recruitment in Fibrous Networks), Eq. 1 captures the essential features of cross-link failure and fiber recruitment observed in our DFN simulations. With this description in hand, we can then use our coarse-grained model to address the outstanding issue of how cellular contracture influences the formation of FAs (via remodeling the ECM) within a continuum framework. The limitations of applying Eq. 1 to the 1D model for FA growth are discussed in Supporting Information.

When the actomyosin system applies a stress $\sigma$ at the proximal edge of the FA band/plaque (as shown in Fig. 1C), the FA–ECM system deforms in response, leading to spatially varying elastic fields. To determine the stress and strain distributions, we implemented the coarse-grained model shown in Fig. 1B (governing equations and the boundary conditions are given in Supporting Information), together with the phenomenological description for fiber recruitment (Eq. 1), in the finite element method package (COMSOL 5.1). The effective modulus of the FA–ECM complex (i.e., the modulus sensed by the cell through active contraction) can be expressed as

$$E_{FA} = E_{ECM} \left(1 + 5\epsilon \left[1 - (\sigma_c/\sigma)^{1/n}\right]\right),$$

[2]

where $E_{ECM}$ is the radial strain of the plaque at the proximal edge, which depends on the stiffness of the ECM, the size of the adhesion plaque as well as the degree of fiber recruitment. Note that because the contractile stress depends on the effective stiffness $E_{FA}$, the mechanical deformation of the FA–ECM complex has to be obtained in a self-consistent manner due to chemomechanical feedback (Fig. 2C; see Supporting Information for details).

**Model for Stress-Dependent Growth of the FA Band.** Given that integrin binding/unbinding occurs within seconds (9, 20), whereas the assembly of proteins in the FA takes several minutes (18), the growth of FA should primarily depend on how fast adhesion proteins are added/removed from the plaque. Furthermore, as suggested by experiments, we proceed by assuming that protein recruitment/disassembly can only take place at the edge of the FA plaque (21). Finally, the driving force for growth of the plaque is assumed to be the chemical potential difference between plaque units recruited to the plaque and those in the cytosol. In particular, the work done by the contractile stress as the new units are recruited is expected to facilitate their incorporation in the plaque (22). Following this line of reasoning, we express...
ECM bond density could vary the effective stiffness of FAs. 

The free energy difference for a segment of the plaque (with size \( \Delta r \) and radial angle \( \Delta \theta \)) as shown in Fig. 2D and Fig. 3C as 

\[
\Delta E = -\sigma h \Delta r (r_c - n \Delta r) d\theta + 2\pi h (r_c - n \Delta r) d\theta,
\]

where \( h \) is the thickness of the FA plaque and \( \Delta \theta \) (with unit \( 1/m^2 \)) represents the free energy gained per unit area for growing the plaque. The first term corresponds to the mechanical work performed by the actomyosin fibers. 

Putting it all together: Prediction of the stable FA size based on mechanical response. The rate-limiting step in the growth of the FAs is the incorporation of new plaque units, a process influenced by the stress level in the plaque exerted by the actomyosin network. The contractility of the network, in turn, depends on the effective stiffness of the adhesion complex determined by the size of the plaque, the stiffness of the ECM, and the density of integrin links between the ECM and the plaque. For ECMs that can be remodeled by cells, the integrin density is expected to be proportional to the density of fibers that can be recruited by the cells as they break the cross-links, which is controlled by the contractile force. 

Results 

Cross-link Breakage Enables Ligand Recruitment in Fibrous Networks. Our DFN simulations showed that the fiber strains decay gradually away from the periphery of the cell where contractile forces are applied (Fig. 3A). Furthermore, fibers oriented in the radial direction are stretched, whereas strains in the fibers aligned circumferentially were predominantly compressive (Fig. 3B). As the level of contractile force increases, the compressed fibers buckle, whereas the cross-links between the radial fibers could undergo higher stretching. 

Tensile forces generated by cellular contraction leads to the breakage of the fiber cross-links, which allows the cell to recruit more fibers. The fiber recruitment index increases when the cell contractile force \( \sigma \) is above the critical level (Fig. 3C) and gives its equilibrium size.
fewer fibers on stiffer ECMs, as shown in Fig. 3 and D, a phenomenon that is well captured by our model (Fig. S4).

Nonuniform Stress Distribution in the FA Leads to Biphasic Stiffness Sensing by the Actomyosin System. A quantity of key interest is the effective stiffness ($E_{FA}$) of the FA–ECM complex, which physically represents the apparent mechanical modulus of the extra-cellular environment that a cell senses through the FA. This quantity is determined using the coarse-grained model and plotted as a function of the FA size in Fig. 4A and C for different values of the ECM moduli ($E_{ECM}$) and fiber recruitment indexes ($n$), respectively. In both cases, $E_{FA}$ is small when the FA band size is either very large or very small but reaches a maximum at a certain intermediate FA band size. To understand this biphasic behavior, the force distribution in the integrin layer is first examined in the absence of fiber recruitment. As shown in Fig. 4B, the force transmitted to the ECM is distributed almost uniformly over all of the integrins for small FAs (solid blue curve), whereas the load distribution becomes highly nonuniform for large FAs, with the proximal edge carrying the majority of the transmitted force (solid magenta curve). This is known as the shear lag effect (23), where the stresses get unevenly distributed in the connecting layer (integrin layer) due to the difference in the deformation of the connected elements (the FA plaque and the ECM). The non-uniform distribution of force in the integrin layer becomes significant above a critical size of the FA, namely, the shear lag length (23). This length is determined by the stiffnesses of the FA plaque, integrins, and ECM, as well as the density of integrins (see the detailed form in Supporting Information and Fig. SSB). Hence, for FAs that are smaller than this characteristic size, the load is almost evenly shared by the integrins, and their growth results in bringing more active (i.e., load bearing) integrins and leads to a monotonic increase of $E_{FA}$. In contrast, for larger FAs (much larger than the shear lag length), the load is concentrated in a limited region at the proximal edge, and only inactive (i.e., those carrying no load) integrins are introduced as they grow. The effective stiffness of this inactive part correlates negatively to its size, whereas the response of the active part is not sensitive to how large the adhesion plaque is (refer to Fig. SSB and related discussion for details). Hence, $E_{FA}$ decreases for large FAs as they grow.

When recruitment of fibers due to breaking of the cross-links is considered, the effective stiffness of the FA–ECM complex ($E_{FA}$) increases significantly compared with the case where fiber recruitment is not possible (Fig. 4C). For ECMs of the same modulus, higher fiber recruitment index (larger $n$) means more cross-links can break for the same level of applied load (as shown in Fig. 4D, Top). As a result, the cell can recruit more fibers and form more integrin–ECM bonds (as shown in Fig. 4D, Bottom), which contributes to additional stiffness and ultimately leads to the overall increase of effective stiffness ($E_{FA}$).

![Fig. 4](image-url)

Fig. 4. Mechanosensing of the FA shows biphasic behavior with respect to the FA size. (A) Effective stiffness of a FA as a function of its size and the ECM modulus. (B) Normalized integrin force distribution for FAs of different sizes with $E_{ECM} = 7.5$ kPa. For small FAs, integrin force is almost uniformly distributed. The force is concentrated at the proximal edge as the FA becomes larger. (C) Influence of fiber recruitment index on the effective stiffness FA size profile with $E_{ECM} = 7.5$ kPa. Fiber recruitment significantly increases the effective stiffness of the FA–ECM complex. (D) Schematics for the influence of fiber recruitment: higher fiber recruitment index indicates more cross-link breakage, leading to more fibers and ligands, providing more integrins within the FA. Therefore, fiber recruitment significantly increases the effective stiffness. (E) Generic shape of the FA growth rate as a function of FA size, from which two quantities of central interest, i.e., the nucleation size for the nascent adhesions to develop into mature ones and the stable size for a fully developed FA, can be identified. The value of FA growth rate is only positive when FA size is between these two sizes. $E$ reprinted with permission from ref. 24, with permission from Elsevier (www.sciencedirect.com/science/journal/00063495).
Fiber recruitment promotes FA formation. We first examined the correlation between FA size and ECM modulus without fiber recruitment, a scenario relevant to most elastic hydrogels and cross-linked ECMs that cannot be physically remodeled by the cells. As shown in Fig. 5A, we found that cells cannot form stable FAs on very soft ECMs. Furthermore, FA size increases monotonically with the ECM modulus. This can be explained by the fact that higher contractile stress will be developed on stiffer ECMs, which eventually leads to larger FAs.

When cells can recruit fibers [as in the case of fibrous ECMs with breakable cross-links (16)], the stable FA size increases compared with the cases without fiber recruitment (solid red curve in Fig. 5A) for a given level of ECM stiffness. This is because more integrins are available with the recruitment of fibers, leading to a stiffer FA–ECM complex as shown in Fig. 4C and therefore to higher levels of contractility. The FA size will reach its maximum at a certain intermediate ECM modulus when fiber recruitment is possible (Fig. 5A), in direct contrast to the monotonic trend observed on substrates that cannot be remodeled. The reason is that cross-links are ruptured more easily in a softer ECM due to the large deformation caused by cell contraction. Consequently, more integrin–ECM bonds will be formed in the FA, which will result in a stiffer FA–ECM complex (and hence a larger FA) even though the ECM modulus is smaller. This competition between increases in the fiber/integrin density and the ECM modulus (both promoting the formation of larger FAs) leads to a peak in the FA size at intermediate levels of the ECM modulus (Fig. 5A). These findings are consistent with our recent experimental observations where the FA size was found to increase with the stiffness of the hydrogel substrate that cannot be remodeled (i.e., n = 0), whereas larger FAs can be formed on softer remodelable fibrous scaffolds (refer to Fig. 5 C and D).

When the recruitment index is at an intermediate level (n = 2), the solid green curve in Fig. 5A), the FA size–ECM modulus relation still shows a monotonic variation. These results predict that a critical level of fiber recruitment is essential for the presence of a nonmonotonic FA size–ECM modulus relation. Above this critical level, the nonmonotonicity becomes less significant by reducing the fiber recruitment index (n = 4; solid magenta curve in Fig. 5A), which has been validated by our experiments (16).

By varying the values of n and L_ECM, the stable FA band size as a function of the ECM modulus and the fiber recruitment index is shown in Fig. 5B. The heat map predicts how the FA size varies with the ECM modulus and the fiber recruitment index. Similar to previous studies (24), our model suggests that the cell cannot form stable FAs on very soft ECMs. The threshold modulus for stable FA formation decreases with increasing fiber recruitment index because FA formation is favored at higher ligand densities. The cells can form stable FAs in ECMs with weak cross-links. In that case, FA growth may be favorable by an increase in ligand density resulting from the recruitment of fibers. Another key prediction of the model is the increase of FA size with stiffness in an intermediate range of stiffness and decrease of FA size at larger matrix stiffness (e.g., the red and magenta curve in Fig. 5A); we have not been able to engineer our ECMs to span the entire phase space to validate the predictions of the model. We hope these predictions can provide guidelines to design matrices to engineer the cell response.

Discussion

In summary, we developed a multiscale coarse-grained chemo-mechanical model to describe the evolution of FAs in cross-linked fibrous networks that resemble native ECMs as well as widely used hydrogel ECM systems. In particular, by considering the elastic deformation and fiber recruitment within the ECM along with the stress-dependent growth kinetics of the FA, we predict the stable FA band size as a function of ECM modulus and fiber recruitment index. Our results show that, FA size is positively correlated with ECM modulus for ECMs that can be remodeled (i.e., hydrogels), but the relation departs if the ECM is remodelable for cells (i.e., fibrous network), as shown in Fig. 5A. The reported FA size–ECM modulus relation is consistent with recent experiments (Fig. 5 C and D) (16).

To further understand the nonmonotonic behavior of the FA size as a function of the level of fiber recruitment, we study how FA adhesion size varies when the ligand density and the ECM modulus are independently altered based on our recent published 1D FA model. This analysis was motivated by the experimental work of Engler et al. (25), who controlled the density of collagen on the surface of hydrogels (of fixed stiffness) and hence effectively designed a method to decouple the effects of ligand density and ECM stiffness. They found a nonmonotonic dependence of cell area on ligand density; the cell area shows a peak at an intermediate density of ligands on the surface. They also suggested that other cellular responses (focal adhesion growth, cell shape, and cytoskeletal organization) should follow similar trends (25). However, an explanation for these phenomena is still lacking (26). Specifically, we found that L_c (the shear lag length that determines the size over which contractile stresses are transmitted to the ECM) decreases with increasing ligand density (Fig. S5E), which results in a larger value of the ratio L_c/L_f, even if the FA size (L) remains unchanged. As we have shown earlier, this ratio determines the integrin force distribution profile: at small L_c/L_f, integrin force distributes uniformly, whereas the force becomes highly localized at the proximal end.
when \( L/L_c \) is large (Fig. S5A). This change in the integrin force distribution (induced by either increasing \( L \) or decreasing \( L_c \)) eventually leads to the biphasic response of the effective stiffness of FA. Therefore, increasing ligand density (\( \phi_i \)) has a similar effect on \( E_{FA} \) as that of increasing the FA size (\( L \)); that is, the effective stiffness of FA increases with the growing \( \phi_i \) initially, reaches its maximum, and then decreases gradually as the ligand density further increases (refer to Fig. SSF and related discussions). By coupling the effective stiffness of FAs with the stiffness-dependent generation of actin contractile stress and the force-dependent kinetics of adding new adhesion plaque units, the stable FA size as a function of ECM stiffness and ligand density can be obtained. As shown in Fig. S6, cells respond positively to ECM stiffness (i.e., forming larger FAs) but non-monotonically to ligand density, which is consistent with the observations on cellular contractility and spreading (1, 25). Specifically, the effective stiffness of FA–ECM complex (with fixed ECM stiffness) will be small if the ligand density is either too low or too high, leading to low intracellular contractility. Because cells form larger FAs at higher contractility levels, the stable size of FAs will reach its maximum at intermediate ligand density. In comparison, under fixed ligand density, stiffer ECM always results in higher contractility and consequently a monotonic increase in the FA size. We carried out steps b–d (Fig. 2) by treating ligand density and ECM modulus as two independent parameters and obtained the FA size profile as shown in Fig. S6F, which shows trends similar to the 1D model (Fig. S6F). In summary, we predicted the non-monotonic FA size–ECM stiffness–ligand density map (Fig. S6) that was pointed out previously (1, 25) but thus far has not been explained from a theoretical perspective.

The behavior of cells on different ECM systems is best summarized by the nonmonotonic response map (Fig. S6). In particular, for ECMs that cannot be remodeled (i.e., hydrogels), the ECM modulus and ligand density are decoupled from each other (this is applicable for most artificial ECM systems, as shown in Fig. 6A). Therefore, as ECM becomes stiffer, the response of cells follows a linear path with no variation in ligand density (Fig. 6C), resulting in positive correlation between FA size and ECM modulus (Fig. 6E). From a comparison, for fibrous ECMs that can be remodeled (such as DexMA fiber network), cells are able to recruit more fibers from their soft microenvironment and hence form more integrin bonds (Fig. 6B), as demonstrated by recent experiments (16) and our simulations (Fig. S4). Because the ligand/ligand density and ECM modulus are coupled in this case, the cells will react to changes in the properties of their surroundings along a much more complicated path where ligand density decreases with increasing ECM modulus as shown in Fig. 6D, leading to the nonmonotonic FA size–ECM modulus relationship (Fig. 6F). Of course, the actual shape of the path has to be determined by the cross-talk among cell contraction, fiber recruitment, and ECM stiffness.

We must point out that local fiber recruitment is closely related to the inelastic (history-dependent) bulk response of biopolymer networks (27–29). In particular, mechanical straining accelerates the dissociation of weak cross-links (30), leading to macroscopic plastic deformation of the matrix. It had been shown that such an effect is more prominent at long timescales (27) and large strains (28, 29), whereas it diminishes with the addition of permanent covalent cross-links (28, 29). In contrast, networks that only have weak cross-links are more dissipative and undergo larger stress relaxations (29).

In this regard, it is expected that the fiber recruitment index, \( n \), introduced here is a quantitative measure of the plastic response of the ECM, a parameter that has not been considered and appreciated in previous theoretical investigations. By introducing this parameter, we are able to characterize the coupled relation between ligand density and ECM stiffness for fibrous ECMs. Recently, the influences of time-dependent matrix properties [such as viscoelasticity (17) and viscoplasticity (31)] on cell behaviors have drawn lots of attention. By applying the corresponding theoretical models, we may be able to characterize the corresponding recruitment index for these matrices and therefore apply the proposed multiscale model to probe the cellular mechanosensing in these matrices. Experiments have shown that matrix degradation by the action of enzymes such as MMPs decreases with increasing tensile forces (32), but this newly described phenomenon of force-mediated changes in active, cell-induced matrix degradation is not considered here, because cells cannot degrade the synthetic DexMA fibers considered in our experimental study. Our model generically applies to fibrous ECMs with interfiber bonds that follow a Bell-like breaking behavior, where cross-links dissociate more readily with increasing levels of force. Recent experiments (29) on the nonlinear viscoelastic response of collagen suggest that cross-link breaking in collagen networks is facilitated by tensile forces, in agreement with the assumptions of our model. Because cross-link breakage occurs within 1–10 s (29), which is separable from the timescale for FA growth and evolution (5–10 min) (18), our model can be applied to address viscoelastic effects of the ECM. Incorporating properties such as cell-mediated matrix degradation and synthesis into the current model will be critical in the future to capture the long-term evolution of cell-matrix interactions in natural matrices.

To summarize, our results from the multiscale chemomechanical model and recent reports (16) show that as studies move from smooth and flat hydrogel surfaces to more complicated 2D or 3D fibrous scaffolds (mimicking in vitro ECMs), the ability of cells to remodel their microenvironment needs to be taken into consideration when modeling the growth of focal adhesions. In addition to providing explanation for a variety of experimental observations, this study can serve as a theoretical framework for assessing the role of FAs in cell functions such as cell spreading, migration, and differentiation in nonlinear extracellular environments.

![Fig. 6. Ligand recruitment leads to nonmonotonic behavior of FA size with stiffness.](Image 0x1 to 19x816)
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