Heterogeneous force network in 3D cellularized collagen networks

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Abstract

Collagen networks play an important role in coordinating and regulating collective cellular dynamics via a number of signaling pathways. Here, we investigate the transmission of forces generated by contractile cells in 3D collagen-I networks. Specifically, the graph (bond-node) representations of collagen networks with collagen concentrations of 1, 2 and 4 mg ml\textsuperscript{-1} are derived from confocal microscopy data and used to model the network microstructure. Cell contraction is modeled by applying correlated displacements at specific nodes of the network, representing the focal adhesion sites. A nonlinear elastic model is employed to characterize the mechanical behavior of individual fiber bundles including strain hardening during stretching and buckling under compression. A force-based relaxation method is employed to obtain equilibrium network configurations under cell contraction. We find that for all collagen concentrations, the majority of the forces are carried by a small number of heterogeneous force chains emitted from the contracting cells, which is qualitatively consistent with our experimental observations. The force chains consist of fiber segments that either possess a high degree of alignment before cell contraction or are aligned due to fiber reorientation induced by cell contraction. The decay of the forces along the force chains is significantly slower than the decay of radially averaged forces in the system, suggesting that the fibrous nature of biopolymer network structure can support long-range force transmission. The force chains emerge even at very small cell contractions, and the number of force chains increases with increasing cell contraction. At large cell contractions, the fibers close to the cell surface are in the nonlinear regime, and the nonlinear region is localized in a small neighborhood of the cell. In addition, the number of force chains increases with increasing collagen concentration, due to the larger number of focal adhesion sites in collagen networks with high concentrations.

1. Introduction

The extracellular matrix (ECM) is an interconnected network of biopolymers that provides structural support for cells and allows the diffusion of biochemicals within tissues. The most abundant component of ECM is type I collagen (COL-I), a fibrous protein responsible for giving the ECM its material stiffness [1]. Cells attach and move through the ECM using protein complexes that link the ECM to the force-generating cell cytoskeleton [2]. However, these cell-ECM adhesions also act as sensors, sending information to the cell about the structure and mechanical properties of the surrounding matrix [3, 4] and helping to regulate cell behavior such as motility, morphology, and differentiation [5–7]. The stiffness and the relative alignment of fibers in the network are particularly important to cell function. For example, dense and rigid collagen gel can promote growth and progression of cancer cells and tumors [8, 9]. Other important examples are durotaxis in which cells tend move in the direction of increasing matrix stiffness [10], and contact guidance in which cells tend to align and move in the direction of fiber alignment [11, 12].

Cell-ECM interaction is a dynamic process in which the cell actively remodels the network [4, 13] and these effects can propagate over long distances, even affecting the bulk properties of the network.
Specifically, tension exerted by the cells can align the fibers in the network leading to long range force transmission [14, 16, 17]. Fiber mediated stresses can trigger mechano-sensitive pathways of distant cells affecting behaviors such as force generation [18, 19] and cell-ECM adhesions [20] and leading to diverse collective behaviors [21]. This coupling of cells provides a means for mechanical communication and plays an important role in regulating and coordinating collective cellular dynamics in a wide range of biological processes, such as morphogenesis, tissue regeneration, and immune response, as well as diseases such as muscular dystrophy, fibrosis, and cancer [4, 13, 22–26].

Due to their effects on cell behavior and communication, a significant amount of work has been carried out to characterize the structural and physical properties of biopolymer networks. Traditional morphological descriptors for such networks include the distribution of fiber length [27], porosity [28, 29], pore-size distribution [30–33] and turbidity [34, 35], which are mainly bulk averaged properties. Recently, local topological and geometrical statistics such as distribution of number of fibers at a cross-link node (i.e., valency number) and relative fiber orientations (i.e., direction cosine) are employed to successfully reconstruct COL-I network computationally [36]. Higher order spatial fluctuations has also been utilized to characterize the evolution of COL-I network during gelation process [37]. In addition, the transport properties (e.g., macromolecule diffusivity) [33, 38–47] and mechanical properties (e.g., elastic moduli, bulk rheology, stress distribution, etc) [14, 16, 18, 36, 48–54] of biopolymer networks, which are respectively crucial to the chemical and mechanical signalling between the cells, strongly depend on the network microstructure. In addition, stress heterogeneity and strain localization of collagen networks especially when interacting with cells have been systematically investigated [55–57].

In the past decade, a variety of discrete fiber-based models have been devised to investigate the mechanical behavior and force transmission in polymer networks [14, 54, 58–63]. It is found that under both global and local perturbations, a 2D random network can exhibit both non-affine and affine deformation regimes, which are respectively bending and stretching dominant [58, 59]. The distribution of forces in the network is highly heterogeneous, with the majority of forces carried by a small fraction of fibers [58]. Very recently, force transmission in cellularized network has been studied using model random networks and cells in 2D [14, 54, 62]. It has been shown that a contractile elliptical cell can lead to highly heterogeneous force networks in a random network and result in significant fiber orientation [14]. Importantly, the remodeled network supports transmission of cell generated forces over a distance that is one order of magnitude larger than the linear cell size, suggesting fiber-mediated long-range mechanical signaling between distant cells [54, 62]. In addition, the relation between the range of force transmission and cell morphology has been elucidated [54].

Although many useful insights have been obtained, the preponderance of previous studies employed simple beam elements to model the mechanical behavior of individual fibers, and thus, did not explicitly incorporate the strain-hardening behavior upon stretching nor buckling upon compression. It has been recently shown that such nonlinear behaviors of individual fibers may have significant effects on the mechanical properties of collagen networks [60, 62, 64]. In addition, 2D model random networks were typically used to represent the cell-ECM system. However, the actual 3D collagen networks may possess more complex microstructure and topology. Indeed, unique bulk rheology properties have been observed in networks directly reconstructed based on confocal microscopy data [36, 63].

In this paper, we systematically investigated the mechanical behavior of 3D cellularized ECM, in particular, fiber-mediated transmission of forces generated by active contraction of embedded cells. The biopolymer networks are represent by a 3D graph (i.e., bond-node) model derived from confocal microscopy data, in contrast to the 2D random network models used in previous studies [14, 54, 58–60, 62]. Collagen networks with different collagen concentrations, i.e., 1, 2 and 4 mg ml$^{-1}$ are investigated. Spherical contractile cells are embedded in the network and cell contraction is modeled by applying correlated displacements at specific nodes, representing the focal adhesion sites. A nonlinear elastic model is employed to characterize the mechanical behavior of individual fiber bundles including strain hardening during stretching and buckling under compression. A force-based relaxation method is employed to obtain equilibrium network under cell contraction.

In particular, we find that for all collagen concentrations, the majority of the forces are carried by a small number of heterogeneous force chains emitted from the contracting cells, which is qualitatively consistent with our experimental observations. The force chains consist of fiber segments that either possess a high degree of alignment before cell contraction or are aligned due to the fiber reorientation induced by cell contraction. The decay of the forces along the force chains is significantly slower than the decay of radially averaged forces in the system, suggesting that the fibrous nature of biopolymer network structure can support long-range force transmission, which is consistent with the recent investigation using 2D model networks [54, 62]. Moreover, the force chains emerge even at very small cell contractions, and the number of force chains increases with increasing cell contraction. At large cell contractions, the fibers close to the cell surface are in the nonlinear regime, and the nonlinear region is localized in a small neighborhood...
of the cell. In addition, the number of force chains increases with increasing collagen concentration, due to the larger number of focal adhesion sites in collagen networks with high concentrations.

2. Materials and methods

2.1. Preparing and imaging collagen gel

Gels were prepared from high-concentration rat tail collagen I in acetic acid (Corning, ≈ 10 mg ml⁻¹). The collagen was diluted with dH₂O, 5, 10 and 20 × PBS (phosphate buffered saline), and 0.1 N NaOH to respective final concentrations of 1 mg ml⁻¹, 2 mg ml⁻¹ and 4 mg ml⁻¹ and a pH of 7.4. The gelation temperature is 35 °C, which was regulated using a stage top incubator (ibidi Heating System, universal Fit) equipped with an external temperature sensor (thermo-couple type K).

Confocal reflection microscopy images of the collagen gels were taken using an inverted laser scanning confocal microscope (LSCM, Leica TCS SPE) with a 20× oil immersion objective. Samples were illuminated with a 532 nm laser and reflected light passed through a 30/70 RT filter and confocal pinhole before being collected by a photomultiplier tube detector (PMT). The scan size was 1024 × 1024 pixels (367 μm × 367 μm) and reflected light intensity was collected as 8 bit gray scale images. One confocal z-stack was taken for the collagen sample with the first scan ≈10 μm above the glass to avoid reflection interference. 100 scans were taken per well with a 1.7 μm z-spacing between each scan.

2.2. 3D reconstruction and skeletonization

The individual scans (i.e., 2D slices) were then stacked along the z-direction using ImageJ, to render a digitized volume of the collagen gel. In the 3D volume, the grayscale value of a voxel (i.e., the 3D analogy of a pixel) indicates the local reflection intensity and thus, whether that voxel belongs to a collagen fiber or the interstitial liquid. A dilation–erosion method [65–67] is then employed to extract the skeleton of the collagen network. Specifically, the 3D volume is thresholded to generate a binary field with black voxels indicating the ‘collagen phase’. The collagen phase is then dilated by adding another layer of black voxels on top of fiber surface voxels to preserve the connectivity of the network. Then the collagen phase is eroded by iterative removing layers of black voxels, while maintaining the connectivity of the network. The skeletonized network is then converted to the graph (bond-node) model [36], in which a node represents a crosslink and a bond connecting a pair of nodes represents a segment of a collagen fiber between two crosslinks.

We emphasize that resolution limit of our confocal microscopy is about 300 nm and that collagen fibrils with thickness less than this value cannot be well resolved and reconstructed. In our subsequent modeling, we only explicitly consider the thick fiber bundles that can be well resolved using our confocal microscopy. One way to take into account the effects of the finer background fibers is to employ an effective-medium approach, and treat the fibers as a continuum with effective elastic properties that coupled of the discrete thick fiber bundles explicitly considered in our current model.

2.3. Nonlinear elastic collagen model

In order to study the mechanical behavior of the network, we need to specify the properties of individual fibers. We note that recent studies have shown that single fiber bundles exhibit linear elastic behavior even upon large elongation, which is sufficient to induce an overall strain hardening behavior of the bulk ECM [63, 68, 69]. However, recently there has been an increasing interest to understand the mechanical behavior of cellularized ECM, for which it is hypothesized that fibers sufficiently close to cell surface are under very large deformation and exhibit nonlinear behavior [64]. Continuum models based on this hypothesis have been developed to accurately resolve the traction forces on cells [64]. Thus, we are motivated to employ similar nonlinear fiber model with both strain hardening upon stretching and buckling upon compression in our discrete cellularized ECM network model to understand the effects of nonlinearity, which has not been systematically investigated in discrete network models of cellularized ECM.

Here, we employed a nonlinear elastic model recently developed by Steinwachs et al to characterize the stretching/compression of a collagen fiber [64]. In particular, upon stretching, a fiber first enters a linear elastic regime, which is followed by a strong strain-hardening regime once the elongation is larger than a prescribed threshold. Upon compression, we consider the fiber immediately buckles and thus, possesses a much smaller compression modulus. The elongation stiffness k of the fiber is thus given by

\[
k = \begin{cases} 
\rho E A, & \lambda < 0 \\
E A, & 0 < \lambda < \lambda_0 \\
E A \exp[(\lambda - \lambda_0)/\lambda_0], & \lambda > \lambda_0,
\end{cases}
\]

where E and A are respectively the Young’s modulus and cross-sectional area of the fiber bundle, and we use \(EA = 8 \times 10^{-7} \text{N} [36]\); \(\lambda = \delta \varepsilon / \varepsilon\) is elongation strain, and \(\lambda_0 = 0.02\) and \(\lambda_0 = 0.05\) are parameters for the strain-hardening model [64]; \(\rho = 0.1\) describes the effects of buckling [62].

In addition, we consider the fiber segments as well as the cross links (nodes) can resist bending and employ a first-order bending approximation [58], for which the bending energy \(E_b\) is a function of transverse displacement \(u\) of the two nodes of a fiber, i.e., \(E_b = \alpha E I u^2 / \ell_0^3\), where the bending modulus \(EI = 5 \times 10^{-22} \text{N m}^2 [36]\), I is the second moment of area, \(\ell_0\) is the original length of the fiber segment, and
\[ \alpha = 1.8. \] We find that in our collagen gels, fiber bending does not play an important role in determining the response of the network to local perturbations due to contractile cells \[ \text{[62, 64]} \]. We also note that the effects of interstitial fluid, which quickly dissipates the kinetic energy generated due to cell contraction, are not explicitly considered. The mechanically equilibrated network possesses the minimal strain energy, which can be obtained by the force-based relaxation method described in section 2.5.

2.4. Contractile cell model

We model an embedded cell as a sphere with radius \( R \) centered at a prescribed location in the network. This simple shape is consistent with the ‘rounded’ cell morphology (i.e., those with an aspect ratio very close to unity) seen in our experiments (see figure 4). We note that in actual in vivo cellularized ECM systems, round-shaped cells are rarely found. Nonetheless, we believe that this very simple model with ideal geometry can still provide useful insights. For example, the spherical symmetry allows us to carry out radial average of force/deformation field generated by the contracting cell and compare them to the continuum mechanics predictions. The effects of elongation of cell shape on the resulting force network has been systematically investigated using 2D random network models \[ \text{[54]} \]. The cell shown in figure 4 is a NIH 3T3 cell cultured in 3D collagen I gel \((1.5 \text{ mg ml}^{-1})\) at 30 °C for 48 h, which is particularly selected due to its rounded shape and its contraction state, in order to qualitatively compare with our simulation using spherical cell models. The majority of the cells in the experiments are nonspherical.

The nodes of the network within a certain distance \( \delta R \) to the cell surface (i.e., \( R + \delta R \) to the cell center) are considered as ‘coarse-grained’ focal adhesion sites, through which the cell is mechanically coupled with the network \[ \text{[75]} \]. Cell contraction is then modeled by displacing the focal adhesion nodes towards the cell center with a prescribed magnitude (see figure 1). In particular, with the boundary nodes of the simulation box kept fixed (a node is a boundary node if its distance to the box boundary is smaller than 3% of the box length), the set of adhesion nodes are displaced towards the cell center and then kept fixed. This local perturbation leads to a stressed network and the resulting equilibrium network configuration is obtained via a force-based relaxation method (described in detail below): all of the other nodes (free nodes) are allowed to be displaced until the entire network settles down in a new configuration with minimal strain energy such that all free nodes are in a force balanced state. In the supporting information, we show that varying the boundary layer thickness and cell-ECM interaction \( \delta R \) do not significantly affect the simulation results.

2.5. Force-based relaxation method

We employ a stochastic optimization scheme to find the mechanical equilibrium network configuration under the prescribed local perturbations that mimic cell contraction. As indicated above, in our system the equilibrium network is associated with the minimal elastic energy \( E_G \), including contributions from both stretching and bending.

In order to find the minimal energy states, a randomly selected node of the network is given a small displacement (i.e., a trial move), which leads to stretching/compression/bending of fibers that connected to this node and thus, causes a change of total elastic energy \( E_G \) of the network. If \( E_G \) decreases, the displacement is accepted, otherwise, it is accepted with a probability given by the Metropolis rule (see details below). A local energy update method \[ \text{[76–78]} \] is employed: before displacing the selected node, the energy associated with this node, i.e., the ‘local energy’ \( E_L \), which is defined as the elastic energy of all the bonds (fibers) connected to this specific node, is calculated. Because the displacement of a node only affect the bonds connected to this node, the change of the total energy \( \delta E_G \) is exactly equal to the change of this local energy \( \delta E_L \), i.e., \( \delta E_G = \delta E_L \). The acceptance probability of the trial move \( p_{\text{acc}} \) is simply given by

\[
p_{\text{acc}} = \begin{cases} 
1, & \delta E_L \leq 0 \\
\exp(-\delta E_L/T), & \delta E_L > 0,
\end{cases}
\]

where \( T \) is an effective ‘temperature’ initially chosen to be high and gradually decreases and simulated annealing \[ \text{[70]} \] is used to drive the energy to a (local) minimum.
Furthermore, force-based node displacements are used for the trial moves. Specifically, a randomly selected node $i$ is displaced along the direction of the net force on this node, with the magnitude of the displacement proportional to the magnitude of the force $F_i$, i.e.,

$$\delta_i = \gamma |F_i|,$$

where $\delta_i$ is magnitude of the displacement and $\gamma$ is a random multiplier between $[0, \gamma_{\text{max}}]$. The upper bound is chosen such that the maximal individual displacement is $\sim 1/500$ average fiber length, which leads to fast convergence of the optimization. About 15 000 trial moves are performed for each node for each temperature stage. The simulation is terminated when the total energy is smaller than a prescribed tolerance. The convergence analysis of the force-based relaxation method is provided in the supporting information.

### 3. Results

#### 3.1. Structural characteristics of 3D collagen networks

Figure 2 shows the distributions of pore sizes for the 3D collagen networks with different concentrations. Specifically, a pore is defined as the largest spherical void that can be inserted into a randomly selected location in the system. The average pore sizes for collagen gels with concentrations of 1 mg ml$^{-1}$, 2 mg ml$^{-1}$ and 4 mg ml$^{-1}$ are respectively 1.22 $\mu$m, 0.988 $\mu$m and 0.684 $\mu$m. Moreover, the average fiber-segment lengths are respectively 1.96 $\mu$m, 1.81 $\mu$m and 1.28 $\mu$m; and the average coordination numbers are respectively 3.19, 3.24 and 3.26 for these collagen networks. These structural statistics are consistent with those reported in literature [36, 71, 72] for collagen gels at corresponding concentrations. We also note that due to the low coordination number (i.e., $Z < 6$), the 3D networks would exhibit floppy modes if one only considers the stretching resistance of the fibers [58, 73]. However, it has been shown that heterogeneous force network can emerge in 2D random network with only stretching resistance fibers in response to local perturbation of contractile cells [62].

#### 3.2. Emergence of heterogeneous force network

The force-based relaxation method is applied to study the mechanical response of a collagen network due to the contraction of a spherical virtual cell with radius $R_c = 7.5 \mu$m (and $\delta R = 0.5 \mu$m) embedded in the network. In particular, the virtual cell is placed in the center of cubic simulation boxes with edge lengths 65 $\mu$m, 55 $\mu$m and 40 $\mu$m for collagen concentrations 1 mg ml$^{-1}$, 2 mg ml$^{-1}$ and 4 mg ml$^{-1}$, respectively. The maximum contraction ratio $\Gamma_c$ (defined as the ratio of the cell radius after and before the contraction) is chosen to be $\Gamma_c = 0.85$ and 15 contraction steps are successively applied to deform the collagen gel. We consider the virtual cell is initially embedded in a stress-free network. Therefore it is easy for the cell to perturb the network via contraction, leading to relatively large $\Gamma_c$ used here. The force-based relaxation method is then employed to find force-balanced network configuration.

Figure 3 shows the emergence of heterogeneous force networks in collagen networks with different collagen concentrations due to continuous cell contraction. The upper, middle and lower panels respectively show collagen networks with a concentration of 1, 2 and 4 mg ml$^{-1}$. For each row, the images from left to right show the force networks associated with cell contraction ratio $\Gamma_c = 0.95$, 0.9 and 0.85. In particular, the average tensile force on each fiber is computed (see figure 5) and a fiber with force $f^*$ satisfying the following condition is considered to participate in the force network:

$$\frac{f^* - f_{\text{min}}}{f_{\text{max}} - f_{\text{min}}} > \gamma_f,$$

where $f_{\text{min}}$ and $f_{\text{max}}$ are respectively the smallest and largest forces on the fibers, and $\gamma_f$ is a prescribed ratio which is chosen to be 0.2 here. In other words, we only consider the fibers that carry a force that is roughly greater than 20% of the largest force in the system to contribute to the force network, and such fibers are shown in red. We note that the resulting force network is not very sensitive to the value of $\gamma_f$, e.g., varying $\gamma_f$ by 50% does not significantly change the force network structure (see supporting information).

A more closer examination of the force networks reveals that the forces are mainly carried by linear chain structures composed of the fiber segments, which are emitted from the cell and extend to the boundary of the simulation domain. Branching only occurs in regions relatively far away from the cell.

![Figure 2](image-url)
surface. These observations are qualitatively consistent with our experimental results. For example, figure 4 shows a confocal microscopy image of a rounded contracting cell (NIH 3T3). It can be clearly seen that linear chain structures are emitted from the cell surface. Most of such linear chain structures surrounding the cell possess lengths of 20–30 μm, which are much larger than the average fiber segment length (a few microns). This suggests that such linear chain-like structures consisting of aligned fiber segments arise due to cell contraction as well. We emphasize that the fluorescence image does not show the actual force chains as the fluorescent intensity does not correspond to force amplitude. The force chains emerged from our simulations are characterized in detail in the following sections.

It can be seen from figure 3 that at each collagen concentration, the force chains emerge even at very small cell contractions, and the number of force chains increases with increasing cell contraction. This suggests that even at very small contraction, the resulting forces can propagate to a distant location with the magnitude remains to be the same order of magnitude of the largest force in the system. The observed heterogeneous force chain structures support the hypothesis that the fibrous nature of the collagen network supports long-range mechanical signaling between the cells [54, 62]. In addition, the number of force chains increases with increasing collagen concentration. Recall that in our model the nodes of the network within δR = 0.5 μm to the cell surface are considered as ‘coarse-grained’ focal adhesion sites, through which the cell is mechanically coupled with the network.

Figure 3. Emergence of heterogeneous force networks in collagen networks with different concentrations due to continuous cell contraction. The fibers carrying large forces (>20% of the largest force in the system) are shown in red, and the remaining fibers are shown in light blue. Upper panels: force networks in 1 mg ml⁻¹ collagen with cell contraction ratio G = 0.95, 0.9 and 0.85 from left to right. Middle panels: force networks in 2 mg ml⁻¹ collagen with cell contraction ratio G = 0.95, 0.9 and 0.85 from left to right. Lower panels: force networks in 4 mg ml⁻¹ collagen with cell contraction ratio G = 0.95, 0.9 and 0.85 from left to right.
Thus, the average number of adhesion sites is proportional to the cell surface area and collagen concentration and is found to be 37, 52 and 119 respectively for 1 mg ml$^{-1}$, 2 mg ml$^{-1}$ and 4 mg ml$^{-1}$ networks. Thus, the increasing number of force chains is due to the larger number of focal adhesion sites in collagen networks with high concentrations.

### 3.3. Heterogeneous distribution of forces

Figure 5 shows the distribution of forces on the fibers in the collagen networks with different concentrations at different cell contraction ratios. It can be seen that as the contraction ratio $\Gamma_c$ increases, the tail of the force distribution moves up, indicating an increasing number of larger forces. This is also consistent with the observed increasing number of force chains as contraction ratio increases. In addition, the majority of forces (i.e., with magnitude larger than that given in equation (4)) are carried by a small fraction of fibers. At the largest contraction ratio (i.e., $\Gamma_c = 0.85$), the fraction of large-force-bearing fibers is 0.056, 0.061, and 0.064 respectively for 1 mg ml$^{-1}$, 2 mg ml$^{-1}$ and 4 mg ml$^{-1}$ collagen networks. Again, the increase is due to the higher focal adhesion density at higher collagen concentration.

### 3.4. Identifying force chains

To understand how the forces due to cell contraction are propagated throughout the system, we investigate the spatial correlations among large-force-bearing fibers and identify well-defined structures formed by these fibers. In particular, starting from a given large-force-bearing fiber segment directly connected to the cell surface, we identify the next large-force-bearing fiber segment directly connected to original segment and require that the mid-point of new fiber segment is further away from the contracting cell. When there are multiple large-force-bearing segments connecting to the original segment, each branch will be tracked separately. This process is repeated until the boundary of the simulation domain is reached or there are no successive large-force-bearing fiber segments. Consistent with our visualization observation, we identify well-defined linear chain-like structures formed by successive large-force-bearing fiber segments, and the branching only occurs in regions far away from the contracting cell. We refer to these chain-like structures as ‘force chains’ in the collagen network. We note that although the aforementioned procedure is not the only method to identify the force chains in a stressed disordered network, it enables us to investigate the largest linear extent of the region affected by the contractile cell, and thus, the range of fiber-mediated mechanical signaling between cells.

How does such chain-like structures arise in response to the perturbation due to cell contraction? To investigate the mechanism for the formation of such linear structure, we compare the angles $\alpha$ between two successive fiber segments along the force chains, before and after the cell contraction (with $\Gamma_c = 0.85$). Figure 6 shows the comparison. It can be clearly seen that for all three collagen concentrations, most of the angles $\alpha_f$ after cell contraction are relatively small (i.e., less than $0.15\pi$), which is consistent with observed linear chain-like structures. However, a significant fraction of $\alpha_0$ ($\sim$70%) before contraction possesses large values (e.g., $>0.25\pi$). This suggests that roughly 70% fibers undergo significant re-orientation due to cell contraction in order to support the propagation of forces through the linear force chains. We note this result is consistent with those reported in [14]. The remaining angles, whose values before the contraction are relatively small, do not significant change after the perturbation. This indicates that certain pre-existing chain-like structures in the non-stressed network can also be selected to carry large forces and contribute to the force chains. The relative contributions of the aforementioned two mechanisms for force-chain formation, i.e., fiber orientation and selection of pre-aligned fiber segments, generally depends on the network of microstructure and mechanical properties.

### 3.5. Force decay along force chains

To quantify how the averaged tensile force propagates along the force chains, the fiber segments along each force chain have been identified and the magnitude of the forces on the fibers along each force chain are obtained. Figure 7 shows the comparison of the decay of forces along the force chains $f_{\Gamma_c}(r)$ and the decay of radially averaged force $\bar{f}(r)$ as the distance $r$ form the contractile cell increases. We note that $\bar{f}(r)$ is computed by averaging the magnitude of forces carried by the fiber segments whose centers are in a
concentric thin spherical shell with radius \( r \) and thickness \( d_r \).

It can be clearly seen in figure 7 that the decay behavior of \( f_{C}(r) \) is distinctly different than \( \bar{f}(r) \). In particular, \( f_{C}(r) \) decays much slower than \( \bar{f}(r) \), although both are characterized by a power law decay as can be clearly seen in the logarithmic plot. The figure also shows the linear fit of \( \ln \bar{f} \) versus \( \ln r \), i.e.,

\[
\ln \bar{f}(r) = A + B \ln r, \tag{5}
\]

where the slope \( B = -0.963, -0.958, -0.931 \) respectively for collagen concentration 1 mg ml\(^{-1}\), 2 mg ml\(^{-1}\) and 4 mg ml\(^{-1}\). This is to be compared to the decay of \( \bar{f}(r) \) at large \( r \) values for the Eshelby’s inclusion problem for linear elastic medium [79], i.e.,

\[
\bar{f}(r) \sim 1/r. \tag{6}
\]

We believe the deviation of \( B \) value from \(-1\) is due the strain-hardening of collagen fibers close to cell surface.
at large cell contractions (see next section for detail). Following the same procedure, the decay behavior of $f_c(r)$ along the force chains can be approximated via

$$f_c(r) \sim 1/r^n,$$

(7)

where $\eta = 0.384, 0.361$ and $0.328$ respectively for collagen concentration $1 \text{ mg ml}^{-1}$, $2 \text{ mg ml}^{-1}$ and $4 \text{ mg ml}^{-1}$. In addition, the magnitude of the forces along the force chain is much higher than the radially averaged forces. This is consistent with our observation from the distribution of forces shown in figure 5, i.e., the majority of forces are carried by a small number of fibers constituting the force chains, while the remaining fibers carry very small forces. Additional simulations based on large random network models suggest that the finite-size effects are negligible for the system sizes considered here. These results indicate that although the average mechanical behavior of celluarized ECM can be obtained via an effective-medium approach, discrete-fiber-based models are clearly more appropriate to capture the heterogeneous distribution and long-range propagation of forces generated by contracting cells.

3.6. Localized nonlinear deformation

For large cell contractions (e.g., $\Gamma_c = 0.85$), it can be expected that the large-force-bearing fiber segments close to the contracting cell are in the nonlinear regime, i.e., the axial strain is larger than $\lambda_i = 0.02$. To quantify the extent of such nonlinear deformation region, we compute the radially averaged nonlinear tensile strain $\bar{\lambda}$ on fiber segments as a function of the distance of the fiber center to the cell center. As shown in figure 5, for all three collagen concentrations, the nonlinear regions are localized in the immediate neighborhood of the contracting cell, i.e., within 4–6 $\mu$m from the cell surface. In addition, no fibers are found in nonlinear regime for networks with cell contraction ratio $\Gamma_c < 0.88$ in our simulations.

However, the threshold deformation for strain hardening behavior for individual fiber segment (i.e., $\lambda_i = 0.02$) is much smaller than the linear shrinkage of the cell for even intermediate $\Gamma_c$. This indicates that the linear extent cell contraction does not directly translate to the deformation of individual fibers. In general, we expect the size of the nonlinear region depends on both $\lambda_i$ and the linear extent of the force chains. For the collagen networks studied in this work, strain hardening of collagen networks only occurs at very large cell contractions (i.e., larger than 12%) and is localized in the immediate neighborhood of the cell.

4. Conclusions and discussion

We have numerically investigated the mechanical response of collagen networks with different collagen concentrations due to the active contraction of embedded cells. The networks are reconstructed from confocal microscopy images and a nonlinear elastic model is employed to characterize mechanical behavior of individual fiber segments. Cell contractions are modeled by applying correlated displacements at specific nodes, representing the focal adhesion sites. A force-based stochastic relaxation method is employed to obtain force-balanced network under cell contraction. We find that the majority of the forces are carried by a small number of heterogeneous force chains emitted from the contracting cells. The force chains consist of fiber segments that either possess a high degree of alignment before cell contraction or are aligned due to the reorientation induced by cell contraction. In addition, the decay of the forces along the force chains are significantly slower than the decay of radially averaged forces in the system. These results suggest that the fibrous nature of collagen network structure can support long-range force transmission and thus, long-range mechanical signaling between cells [80]. One of the advantages of mechano-signal transduction is that it could be ~40 times faster than the diffusion-based chemical signaling, and it is operational in systems whether normal chemical signaling pathways are blocked due to abnormal cellular structure (e.g., in cancer cells), as reviewed in [81]. Our study also suggests that although the average mechanical behavior of celluarized ECM can be obtained via an effective-medium approach, discrete-fiber-based models are more appropriate to capture the heterogeneous distribution and long-range propagation of forces generated by contracting cells.

We note that the collagen networks studied in this work are statistically isotropic and the fiber segments do not have any preferred global orientation. In future work, the effects of fiber alignment in the original non-stressed network work will also be studied. Such collagen networked can be experimentally prepared under lower gelation temperature [37]. It is expected that the pre-orientated fiber chains will dominant the...
force-chain formations, and thus, significantly biases the force propagation as well as the cell migration. In addition, realistic cell morphology obtained from 3D reconstruction based on confocal microscopy images will be incorporated into the model. Multi-cell interactions will also be investigated. Lastly, experimental visualization and quantification of the force chains in cellularized ECM is crucial to quantitatively validating our model and further deepening our understanding of the force transmission in such systems. We will explore different labeling proteins that are sensitive to the elongation of fibers in order to directly observe and quantify the force networks.

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Abbreviations list

- ECM: extracellular matrix
- COL-I: type I collagen
- NMR: nuclear magnetic resonance
- PBS: phosphate buffered saline
- PMT: photomultiplier tube detector

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