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Probing cooperative force generation in collective cancer invasion

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Abstract

Collective cellular dynamics in the three-dimensional extracellular matrix (ECM) plays a crucial role in many physiological processes such as cancer invasion. Both chemical and mechanical signaling support cell–cell communications on a variety of length scales, leading to collective migratory behaviors. Here we conduct experiments using 3D in vitro tumor models and develop a phenomenological model in order to probe the cooperativity of force generation in the collective invasion of breast cancer cells. In our model, cell–cell communication is characterized by a single parameter that quantifies the correlation length of cellular migration cycles. We devise a stochastic reconstruction method to generate realizations of cell colonies with specific contraction phase correlation functions and correlation length \( a \). We find that as \( a \) increases, the characteristic size of regions containing cells with similar contraction phases grows. For small \( a \) values, the large fluctuations in individual cell contraction phases smooth out the temporal fluctuations in the time-dependent deformation field in the ECM. For large \( a \) values, the periodicity of an individual cell contraction cycle is clearly manifested in the temporal variation of the overall deformation field in the ECM. Through quantitative comparisons of the simulated and experimentally measured deformation fields, we find that the correlation length for collective force generation in the breast cancer diskoid in geometrically micropatterned ECM (DIGME) system is \( a \approx 25 \mu m \), which is roughly twice the linear size of a single cell. One possible mechanism for this intermediate cell correlation length is the fiber-mediated stress propagation in the 3D ECM network in the DIGME system.

1. Introduction

Collective cellular dynamics in three-dimensional extracellular matrix (ECM) plays a central role in many physiological processes including morphogenesis, wound healing and particularly tumor metastasis.
These processes, such as the formation of tubular structures during the development of branched organs [1–3], the repair of epithelial wounds [5,6] and the growth of vessels during tumor angiogenesis [7], involve highly orchestrated multicellular dynamics over large spatial and temporal scales. Quantitative measurement of these length and time scales provides key information that allows us to distinguish individual from emergent multicellular cellular behaviors.

Coordination between cells requires efficient cell–cell communications in the forms of chemical [8], electrical [9] or mechanical signals [10, 11]. The need to overcome physical barriers during 3D migration [12] and the excellent force transmission within the ECM [13, 14] strongly suggest a role for mechanical cues in orchestrating collective cancer invasion.

The mechanical interaction between cells involves force generation, propagation and sensation. The three steps do not occur in sequence but rather are coupled dynamically to maintain tissue homeostasis [15]. In particular, traction force from one cell will remodel the local geometry and elasticity of the ECM [16] therefore modulating the force generation of nearby cells [17]. The presence of direct or ECM-mediated intercellular feedback mechanisms has suggested the notion of collective force generation, especially in the context of tumor metastasis [18]. Indeed, it has been observed that tumor organoids exert highly organized traction forces [19], while isolated individual cells generate fluctuating forces that vary significantly from one cell to another [20].

To further investigate collective force generation during cancer cell migration, here we present a phenomenological model based on the recently developed tumor diskoid in geometrically micropatterned ECM (DIGME) system [21]. In our model, cell–cell communication is characterized by a single parameter that quantifies the correlation length of cellular migration cycles. Specifically, we consider that each tumor cell undergoes a series of periodic contraction cycles from its migration machinery. For simplicity, the contraction cycles of all of the cells are considered to possess the same periodicity. Each cell is also characterized by a unique contraction phase, which is spatially correlated with that of other cells via an inverse exponential function with the correlation length \( a \) (i.e. \( \sim \exp(-r/\alpha) \)). We devise a stochastic reconstruction method to generate realizations of contracting cell colonies with specific phase correlation functions and correlation length \( a \).

The deformation field resulting from collective cell contraction with different \( a \) values is then computed and quantitatively compared with the experimentally measured deformation field in the breast cancer DIGME system in order to identify the correlation length \( a \) for the system.

Our study shows that as the correlation length increases from 0 (i.e. the case where the cell contractions are completely random), the size of the region of cells with similar contraction phases also increases. In the limit \( a \to \infty \), the entire cell colony is completely synchronized and all cells contract simultaneously. For small \( a \) values, the large fluctuations in individual cell contraction phases smooth out the temporal fluctuations in the time-dependent deformation field in the ECM. On the other hand, for large \( a \) values, the periodicity of individual cell contraction cycles is clearly manifested in the temporal variation of the overall deformation field in the ECM. This generic dependence of temporal variation of the deformation field on \( a \) is utilized to identify the correlation length in DIGME. Through quantitative comparisons of the simulation results and experimental data, we find that the correlation length for collective force generation in the breast cancer DIGME system is \( a \approx 25 \mu m \), which is roughly twice the linear size of a single cell. One possible mechanism for this intermediate cell correlation length is fiber-mediated stress propagation in the 3D ECM network in DIGME [14].

2. Methods

2.1. Cell culture and 3D patterning

High-concentration type I rat tail collagen (9.95 mg ml\(^{-1}\), Corning) was diluted with Dulbecco’s modified Eagle’s medium, phosphate-buffered saline (PBS, 10×) and sodium hydroxide (NaOH, 0.1 M) to a concentration of 2 mg ml\(^{-1}\) with pH 7.4. All solutions were held and mixed at 37 °C, prior to the gelation process at 37 °C. To create 3D patterned cell disks, we employed the DIGME method described previously [21]. Briefly, a stainless steel cylinder was used to mold a well in the collagen gel. Then high-density human breast carcinoma cells (MDA-MB-231/GFP, Cell Biolabs) were mixed with neutralized collagen solution and used to fill the molded well. The sample was placed in a tissue culture incubator at 37 °C with 5% CO\(_2\) for 3 h then moved to an on-stage incubator for imaging.

2.2. Micromechanical model for tumor DIGME

Here we develop a 2D micromechanical model for the tumor DIGME. Specifically, the ECM is modeled as a nonlinear elastic continuum with strain hardening behavior and elastic moduli \( K = 150 \) Pa (bulk modulus) and \( \mu = 100 \) Pa (shear modulus) [22], which has been shown to correctly capture the mechanical behavior of ECM. A detailed description of the micromechanical model for the collagen network is provided in the supporting information (stacks.iop.org/PhysBiol/14/045005/mmedia). As we will show below, the exact values of the elastic moduli do not affect the determination of the cell correlation length in the system. In addition, we note although a collagen network generally exhibits viscous behavior we believe it is legitimate to model it as a nonlinear elastic medium in the current work. This is because the characteristic periodicity for cellular contraction (\( \sim 220 \) min) in our DIGME system is generally much larger than
the relaxation time scale for the viscous behavior of collagen gels (~30 s).

The cells, which are embedded in the ECM, are modeled as equal-sized 2D circular disks. Initially, a fixed number of non-overlapping disks are randomly placed in a square simulation domain with periodic boundary conditions. The disk packing is subsequently densified by repeatedly shrinking the size of the simulation domain while randomly displacing the disks via the Monte Carlo method [23]. Once a desirable cell packing density has been achieved, a circular region representing the tumor diskoid is ‘cut out’ from the resulting packing configuration and embedded in the middle region of the ECM. In our simulations, the diskoid contains ~1000 cells with a covering fraction of ~0.55, consistent with the experimentally measured cell density.

Once the geometrical model for DIGME has been established, the volume-compensated lattice-particle method (VCLP) [24] is employed to study the mechanical behavior of DIGME due to cell contraction. In the VCLP, the continuum is treated as an ordered network of interacting material points, which obeys a cohesive law directly obtained from basic constitutive relationships with analytical solutions. The interactions among the material points include both the local pair-wise potential between two particles $U_{\text{pair}}$ and the multi-body potential among non-local particles $U_{\text{VCLP}}$, i.e. $U = U_{\text{pair}} + U_{\text{VCLP}}$. Accordingly, the force field between two neighboring particles only depends on their relative displacement (i.e. pair-wise potential), but it also includes a contribution from all the neighboring particles surrounding them (i.e. non-local multi-body potential). The VCLP has been successfully applied to study nonlinear failure properties of heterogeneous materials such as particle-reinforced composites [25], which possesses a similar microstructure to the DIGME.

In our DIGME model we consider each cell to be perfectly bonded with the ECM, and when a cell contracts periodically it pulls the ECM bonded to its perimeter inwardly. This is to mimic the effect of the pulling forces generated via the contraction of actin filaments and transmitted to ECM fibers through focal adhesion during cell migration. During the initial phase of the invasion (the focus of this paper) most cells possess a rounded morphology and the slightly elongated cells do not appear to show any patterns of alignment. Therefore we believe that isotropic contraction is a good approximation for studying collective force generation in our DIGME system. In addition, the contraction process occurs much faster than the focal motion of the cells, so that the cell positions are considered stationary for a few contraction cycles. We consider the cell contraction to be characterized by a sinusoidal function, i.e.

$$\delta(t) = \delta_0 \sin(2\pi \omega t + \phi),$$

where $\delta(t)$ is shrinkage of the cell diameter due to contraction with module $\delta_0$ chosen to be 15% of the cell diameter, $\omega = 1/T$ is the individual cell contraction frequency ($T$ is the corresponding period) and $\phi$ is the phase for cell contraction with respect to a global reference time. It is important to note that in our current model possible fluctuations of the individual cell contraction frequency $\omega$ are not explicitly considered, i.e. we assume that all cells possess identical $\omega$. In general there are other possible sources for the fluctuations, such as variation of $\omega$ among different cells and variation of $\omega$ in a single cell over time. To quantify the effects of these possible dephasing mechanisms we carry out a systematic sensitivity analysis by considering a random variation in the single cell contraction frequency across different cells or in a single cell over time and report the results in the supporting information. The implications of the analysis are provided in section 4.

As we will discuss in the following section, the correlation of $\phi$ between different cells quantifies the degree of collectivization of cell contraction (migration) in the system. For the subsequent micromechanical analysis the contraction of each cell is applied as a time-dependent displacement boundary condition for the ECM; and the fixed zero displacement boundary conditions are applied to the external boundaries of the square simulation domain for the ECM. Due to the symmetry of the tumor diskoid, the overall effect of cell migration leads to isotropic growth of the diskoid, which can be mimicked in the model by isotropically expanding the circular cell packing region (i.e. re-scaling the intercellular distances).

### 2.3. Stochastic phase construction for modeling collective cell contraction

In our system, the tumor cells in the diskoid can migrate and invade the surrounding ECM. During the migration, a cell pulls the collagen fibers via contraction of actin filaments to generate locomotion. In our model we consider the collective nature of the cell migration to be manifested as the coordinated contraction of cells. As described in section 1, we consider that each tumor cell undergoes a series of periodic contraction cycles with the same periodicity. In particular, each cell $i$ at position $\mathbf{r}_i$ possesses an intrinsic contraction phase $\phi_i(\mathbf{r}_i)$ (see equation (1)). The degree of collectivization is then quantified via the following contraction phase correlation function:

$$S(r) = \langle \phi(\mathbf{r}) \cdot \phi(\mathbf{r} + \mathbf{a}) \rangle,$$

where $\mathbf{r} = |\mathbf{r}_i - \mathbf{r}_j|$ and $\langle \cdot \rangle$ denotes the ensemble average. Without loss of generality, we use an exponential correlation function, i.e.

$$S(r) = (\phi_1 - \phi_2) \exp(-r/a) + \phi_2,$$

where $\phi_1 = \frac{1}{T} \int_0^T \phi \, d\phi = \pi^2/3$ and $\phi_2 = (\frac{1}{T} \int_0^T \phi \, d\phi)^2 = \pi^2/4$. The parameter $a$ is the correlation length that characterizes the degree of collectivization in the system. For example, for $a = 0$, $S(r) = \pi^2/4$, indicating that the contraction (migration) of the cells in the system is completely uncorrelated (asynchronized).
On the other hand, for $a \rightarrow \infty$, $S(r) = \pi^2/3$, indicating that cell contraction (migration) is fully synchronized (i.e. all cells contract simultaneously). The major task of our modeling effort is to estimate the $a$ value in the DIGME from the experimentally measured ECM displacement field profile in order to assess the degree of collectivization in such system.

Collective cell contraction characterized by the phase correlation function equation (3) with a specific correlation length $a$ can be realized using inverse reconstruction using stochastic optimization [26]. Initially, each cell is assigned a phase $\phi$ that is randomly selected from $[0, \pi]$. The phase correlation $S(r)$ is then computed from this initial configuration. We define an ‘energy’ as the squared difference between the computed and target correlation function $S'(r)$, i.e.

$$E = \sum_r (S(r) - S'(r))^2. \tag{4}$$

Then simulated annealing is employed to evolve the phase distribution in a tumor diskoid. Specifically, at each step the phase $\phi_i$ of a randomly selected cell $i$ is varied by a random amount $\delta \phi_i$ leading to a change in the computed $S(r)$ and thus an energy change $\Delta E$. This variation of $\delta \phi_i$ is accepted with a probability $p = \min[1, \exp(-\Delta E/T)]$. The parameter $T$ is a fake temperature which is chosen to be high initially and gradually decreases during the simulation, in order to improve the convergence of the algorithm to the global optimum.

3. Results

3.1. Experimental results

In order to study collective cancer invasion and force generation in three dimensions, we have previously developed a mechanical-based strategy for micropatterning 3D in vitro tumor models, namely DIGME [21]. DIGME allows one to control the micro- to mesoscale geometry of the ECM surrounding cell aggregates. In this study we take advantage of DIGME to create circular diskoids consisting of MDA-MB-231 cells, a highly invasive breast cancer cell line. We note that although tumor spheroids have been widely used as invasion models in previous studies, the diskoid geometry employed here leads to a plane strain deformation of the ECM and allows us to more accurately capture the cell contraction-induced deformation field in both experiments and subsequent 2D simulations.

As shown in figure 1, a circular diskoid is a cylindrical cell aggregate confined in a 3D collagen matrix. The diskoid has a high aspect ratio, with a diameter of approximately 920 \(\mu m\) and thickness of 150 \(\mu m\). Within 3 d of seeding the cells, collective invasion only slightly changes the initial shape of the diskoid. Over the longer term, the invasion is more pronounced in the radial than the vertical direction [21]. The morphological distribution and density evolution of the cells resemble the middle plane of tumor spheroids [18].

In order to probe the effects of the mechanical forces generated by the cells, we embedded 1 \(\mu m\) red fluorescent particles in the collagen matrix so that the GFP-labeled cells and the matrix deformation could be monitored at the same time. In particular, we recorded images at the middle plane of the diskoid where such deformation is most significant. For each experiment, we imaged continuously for 3 d at 5 min intervals, employing particle image velocimetry (PIV) to obtain the velocity field of the ECM by comparing images taken 5 h apart.

We find that there are three distinct phases in collective force generation (figure 2(A)). Immediately following seeding into the diskoid mold, the tumor diskoid pushes the matrix outward. This is due to the initial spreading of the cells and because the cells are closely packed in the diskoid. The pushing phase is followed by a period of almost zero velocity, after which the diskoid starts to generate a contractive force that pulls the matrix radially inward.

To better visualize the three different phases, we have calculated the average radial velocity $(v_r(t, r))$ over the angle $2\pi$, as shown in figure 2(B). The spatial–temporal map shows that the matrix velocity field decays at a larger distance, and that the initial stable pushing force is gradually replaced by a fluctuating pulling force generated by the diskoid.

With the time–dependent velocity field highlighting three different phases in the collective force generation of the cells, we have also computed the strain field in order to further elucidate the stress distribution in the ECM. In particular, we obtain the infinitesimal strain tensor $\varepsilon(t, r)$, which is a $2 \times 2$ matrix that varies in space and time. Equivalently, the strain tensor can be characterized by its two orthogonal principal directions—eigenvectors $\mathbf{v}_1(t, r)$ and $\mathbf{v}_2(t, r)$ and the corresponding eigenvalues $\lambda_1(t, r)$ and $\lambda_2(t, r)$.

As shown in figure 3(A), in the pushing phase the two principal directions are strongly aligned with the radial and tangential directions. The matrix is compressed radially, and extended in the tangential direction. On the other hand, during the pulling phase the strain decomposition has much larger fluctuations, but overall the matrix is extended in the radial direction and compressed in the tangential direction. To further illustrate the quantitative variations of the strain field, we have calculated the angular averaged trace of the strain field $(Tr(\varepsilon))(t, r) = \langle \lambda_1(t, r) + \lambda_2(t, r) \rangle_{r=0 \ldots \infty}$ at fixed radial distances from the center of the diskoid. As shown in figure 3(B), near the boundary of the tumor diskoid the ECM is expanded during the pushing phase and compressed during the pulling phase. The angular averaged trace of the strain field decays rapidly away from the diskoid center. Also consistent with the velocity field, we find that in regions where significant strain is observed, the principal axis of the strain tensor aligns with the radial direction. To further decipher the patterns in the spatial–temporal evolution of the deformation field in figure 3(B), we perform a Fourier analy-
sis (see supporting information) which clearly shows periodicity in the temporal domain.

After characterizing the collective traction field of a tumor diskoid, we take advantage of computational modeling to address the central question of the report: to what extent do the cells coordinately apply traction force to the extracellular matrix.

3.2. Simulation results
In this section, we present our simulation results including the reconstruction of contraction phase distributions with specific correlations in the tumor diskoid as well as the subsequent micromechanical analysis.

Figure 4 shows the distribution of the contraction phase in tumor cell aggregates (modeled as packings...
of non-overlapping congruent circles) associated with different phase correlation length $a$ obtained via the stochastic reconstruction method described in section 2.3. In the figure, red indicates large $\phi$ values ($\sim\pi$) and blue indicates small $\phi$ values ($\sim0$). Figure 5 shows the scaled target and reconstructed phase correlation functions (see equation (3)), i.e.

$$f(r) = (S(r) - \phi_2)\cos(\phi_1 - \phi_2) = \exp(-r/a),$$

associated with different correlation lengths $a$. The final squared error (i.e. the energy; see equation (4)) between the target and reconstruction is smaller than $10^{-6}$ for all cases. It can be seen from figure 5 that the reconstructed correlation functions are virtually identical to the corresponding target functions.

It can be clearly seen in figure 4 that as the correlation length $a$ increases, the regions with similar colors are growing in size, i.e. regions in which the cells possess similar $\phi$ values. This indicates that regions containing collectively contracting cells are also growing, which is consistent with the imposed stronger spatial correlations. In addition, as the correlation length increases, the distribution of the $\phi$ values also shifts from a uniform distribution (as in the case of $a = 0$ m) to a bimodal distribution (i.e. for $a = 50$ $\mu$m). The reconstructed cell packings with specific phase correlations
are then mechanically coupled with a continuum ECM model to study collective force generation in circular diskoid tumor invasion.

We now investigate the deformation of the ECM surrounding the tumor diskoid due to collective cell contraction. In particular, each cell is allowed to contract and deform the ECM according to equation (1) with its specific phase \( \phi_i \) with respect to a global reference time \( t_0 = 0 \). The contraction of the cells imposes time-dependent displacement boundary conditions at the cell–ECM interfaces, while the outer surface of the ECM is considered to be fixed (i.e. with zero displacement). The VCLP method is subsequently employed to solve the displacement field in the ECM with the aforementioned boundary conditions.

Figure 6 shows snapshots of the displacement field, i.e. the distribution of the magnitude of the radial component of the local displacement vector, for different phase correlation lengths. It can be clearly seen that as the correlation length increases, the magnitude of displacement at fixed distance \( r \) from the center of the tumor diskoid increases. This is due to the larger pulling forces generated by more collective cell contraction (i.e. with larger phase correlations). In addition, the displacement fields for all cases are almost circularly symmetric, which is due to the isotropy of the tumor diskoid.

Figure 7 shows the temporal evolution of the circularly averaged trace of the strain tensor field \( \langle \text{Tr}(\varepsilon) \rangle(t, r) = \langle \lambda_1(t, r) + \lambda_2(t, r) \rangle_{\varepsilon_{rr}} \) normalized with respect to \( \langle \text{Tr}(\varepsilon) \rangle_{\text{max}} \) in the system for different phase correlation lengths, where \( \lambda_1 \) and \( \lambda_2 \) are the eigenvalues of the local strain tensor. The quantity \( \langle \text{Tr}(\varepsilon) \rangle \) characterizes the local volumetric deformation (i.e. the volume strain) of the ECM due to the contraction of cells in the tumor diskoid, which results in a state of bi-axial tension in the ECM. Consistent with the experimental observation, the principal axis of the local strain tensor is aligned with the radial direction.

In addition, we note that increasing the correlation length, i.e. the degree of collectivization in cell contraction (force generation), also directly affects the temporal evolution of the circularly averaged volume strain profile. Specifically, for the totally uncorrelated case (i.e. with \( a = 0 \) indicating a random distribution of the contraction phase among the cells), the random fluctuations in the contraction phase distribution significantly smooth out the temporal variation in the strain profile. For example, the maximum

![Figure 5](image_url)  
**Figure 5.** Scaled phase correlation functions equation (3) associated with different correlation lengths \( a \). The reconstructed functions (solid symbols) are virtually identical to the corresponding target functions (solid lines).

![Figure 6](image_url)  
**Figure 6.** Spatial maps showing the magnitude of the radial displacement from simulated tumor diskoids. One typical snapshot is shown for each phase correlation length. The color scheme linearly scales with the radial displacement magnitude (0 to 1) normalized with respect to the peak displacement value.
fluctuation of the strain field at the same location but different time points is about 50% and the deformation of ECM never vanishes. On the other hand, for the fully synchronized case (i.e. with \( a = \infty \)), indicating the ideal case that all cells contract simultaneously), a significant temporal variation of the volume strain profile that is consistent with the cell contraction period can be identified. It can be clearly seen that the deformation field in ECM almost completely vanishes at \( t \approx 250 \text{ min} \) (where \( n = 1, 2, 3, \ldots \)) and reaches peak values at \( t \approx 125 + 250 \text{ min} \) (where \( n = 0, 1, 2, \ldots \)). For intermediate correlations (i.e. intermediate \( a \) values), as the correlation length increases, the temporal variation in the strain profile is more significant. We note that this intrinsic dependence of the temporal variation of the deformation field on the spatial correlation length \( a \) is sensitive to neither the individual cell contraction magnitude nor the exact values of ECM mechanical properties, and thus this property is sufficiently robust to be utilized to identify the correlation length in DIGME.

### 3.3. Probing the collectivization in DIGME

We now utilize the correlation length-specific time-dependency feature of the volume strain profile to identify the correlation length, i.e. to quantify the degree of collectivization in force generation from the experimental data for the DIGME system. We first note that the experimentally obtained volume strain profile contains a bi-axial compression state during the initial stage of tumor diskoid evolution, which is mainly due to the volumetric expansion of the entire tumor cell colony when seeded in the ECM. Since this strain state is not associated with the forces generated via cell contraction during collective migration, we exclude this part when comparing with our simulation results.

For quantitative comparison of the experimental and simulation results, we first normalize the volume strain profile with respect to the corresponding peak value and the large-distance asymptotic value, i.e.

\[
\frac{\langle \text{Tr}(e) \rangle(t, r)}{\langle \text{Tr}(e) \rangle_{\text{max}}} = \frac{\langle \text{Tr}(e) \rangle(t, r) - \langle \text{Tr}(e) \rangle(t, r \to \infty)}{\langle \text{Tr}(e) \rangle_{\text{max}}}.
\]

(6)

For each \( a \) value, we shift the phase \( \Phi \) and re-scale the time with factor \( \gamma \) for the entire volume strain profile to minimize the squared error between the simulated and smoothed experimental data, i.e.

\[
E = \min_{\{\Phi, \gamma\}} \left[ \sum_{t, r} \left( \langle \text{Tr}(e) \rangle_{\text{sim}}^*(t, r) - \langle \text{Tr}(e) \rangle_{\text{exp}}^*(t, r) \right)^2 \right].
\]

(7)

Then the \( a \) value associated with the smallest \( E \) will be identified, and considered as the phase correlation length for the experimental DIGME system.

Through the aforementioned analysis, we find that the strain profile associated with a correlation length \( a \approx 25 \mu m \) minimizes the squared error (see equation (7)) among all \( a \) values examined (see supporting information). In our model, the cell size (i.e. the diameter of the circles) is set to be 10 \( \mu m \). Thus, the identified correlation length corresponds to the size of about two to three cells. We note that our analysis mainly emphasizes the intrinsic dependence of the
temporal variation of the deformation field (e.g. strain profile) on the correlation length, instead of trying to match the exact values of the simulated and experimental deformation fields. Therefore, the obtained correlation length is not affected by the different reference states that are used to define the strain field in the simulation and experiment (e.g. a global stress/strain-free state for the simulation and local reference states taken every 5 h for the experiment). It is also important to note that the scaling parameter $\gamma$ in equation (7) is related to the individual cell contraction frequency, i.e. $\gamma = 2\pi \omega = 2\pi T/\gamma$. The obtained $\gamma$ value that minimizes the error (7) corresponds to $T = 1/\omega \approx 220$ min. We also verify our estimated individual cell oscillation periodicity (\sim 220 min) by comparing the experimental value ($\sim 230 \pm 30$ min) obtained by observing individual cells randomly dispersed in an ECM with lower cell density. However, it is important to note the oscillation periodicity could also depend on the cellular microenvironment, which could lead to the observed discrepancy. The experimental data are reported in the supporting information.

4. Discussion and conclusions

In this paper we have presented a phenomenological model to probe coordinated force generation during collective migration of breast cancer cells. In our model, cell–cell communication is characterized by a single parameter that quantifies the correlation length of cellular migration cycles, without explicitly considering the detailed mechanisms of the communication. Realizations of contracting cell colonies with different correlation lengths are generated using a stochastic reconstruction method. We find that the time-dependent ECM strain profile possesses an intrinsic dependence on the cell correlation length $a$. This feature is subsequently utilized to identify the correlation length in experiments on cancer diskoid invasion, which leads to $a \approx 25 \mu m$. This intermediate correlation length is roughly twice the linear size of the cells in the system. Interestingly, such intermediate correlation lengths have also been observed for 2D cell monolayers where the cells are either communicating chemically [27] through gap junctions or mechanically through direct contacts [28].

It is not surprising that an intermediate correlation length arises from our analysis. The tumor diskoid possesses a fairly high cellular density. Thus, it can be expected that the biochemical and biomechanical mechanisms giving rise to long-range cell–cell communications might be suppressed by the strong nearest-neighbor screening effects. In 3D collective cell migration, one of the possible mechanisms for coordinating collective force generation is collagen fiber-mediated stress transmission. In particular, it has been shown recently that the actomyosin-generated contraction force of a migrating cell can propagate to the ECM fibers via focal adhesion sites, leading to a heterogeneous quasi-long-range stress network in the ECM [14]. This implies that the local stress state near a particular cell may result from the pulling of a distant cell, which in turn biases focal adhesion formation for the current cell and its migratory behavior. We expect this mechanism to be important in the tumor diskoid system where cells are embedded in a 3D, albeit thin, block of ECM. Another important mechanism for collective force generation involves the mechanosensitivity of individual cells [29]. For example, it was shown in [29] that cells can adjust their contractile forces according to the forces they experience from other cells, which in turn could lead to collective contraction of the entire colony.

An important question is whether the identified correlation length is intrinsic to breast cancer cells or is dependent on the specific system. Based on the aforementioned analysis, we believe the near-neighbor screening effects could play an important role in the densely packed DIGME system and effectively reduce the correlation length among cells. In a more spread-out cell configuration with a much lower cellular density we expect to observe a longer correlation length for the tumor cells. Nonetheless, this also illustrates the importance of the heterogeneous microenvironment in determining collective tumor cell behavior.

Finally, we note that in our model we have assumed that all cells possess the same contraction periodicity (or equivalently frequency) and we ignored any possible fluctuations in these quantities. As shown in the supporting information, increasing fluctuations in the contraction periodicity or frequency of individual cells will result in less coherent cell contraction and force generation, which would further smooth out the temporal variation in the deformation field. This in turn would result in an effectively smaller correlation length in the tumor diskoid. This analysis suggests that ignoring the possible fluctuations in individual cell contraction frequency and periodicity would lead to an underestimate of the correlation length in the system. Therefore, the obtained $a = 25 \mu m$ from the current study should be considered as a lower bound on the correlation length in a rigorous sense. In addition, the possible plastic behavior of the collagen network is also not explicitly considered in our current model. The plastic behavior of the ECM due to the permanent remodeling of the collagen network by cellular forces will result in accumulated plastic strain in the ECM. However, we expect that the temporal variation of the overall strain profile still strongly depends on the contraction correlation length, which can be utilized to determine the correlation length. We will investigate these issues in future work using a similar procedure to that introduced here.

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