Enhanced mechanical heterogeneity of cell collectives due to temporal fluctuations in cell elasticity

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Changes in cell biophysical properties play fundamental roles in cancer progression [1, 2]. Biophysical techniques such as atomic force microscopy (AFM), optical trapping and micropipette aspiration used to probe individual cell mechanical properties [3] show that cell stiffness grades the ability of tumor cells to metastasize, with softer cancer cells exhibiting the highest migratory and invasive potential [4]. Interestingly, while cancer tissues are generally stiffer than normal tissues, individual cancer cells themselves are softer than normal cells [4, 5]. Given the importance of cell mechanics in cancer progression, insights into how mechanical heterogeneity - i.e. the idea that individual cells within a tumor can be characterized by different stiffnesses - emerge and consequently impact cell dynamics is important. To address these questions, we used a minimal 3D computational model of cell aggregates to show that time dependent change in single cell stiffness controls cell dynamics and mechanical heterogeneity of cell collectives.

Cell division, where a single cell divides into two, is a crucial process in the cell cycle marked by substantial changes in cell morphology, biochemistry and mechanics [6, 7]. Cell morphological change during division is driven by drastic remodeling of the cytoskeleton - a complex and dynamic network of proteins present in most animal cells [6, 8–10]. The cell cortex is composed of a thin actin protein network bound to the cell membrane with a dense crosslinked meshwork architecture [11] that determines cell deformation in response to intercellular and extracellular forces [12]. Actin protein filaments that make up the cortex can dynamically polymerize and depolymerize leading to time dependent variations in cell stiffness [13, 14]. Recently, high temporal resolution AFM measurements of dividing embryonic cells showed that cell stiffness remarkably increased immediately prior to cell division and softened after cell division, exhibiting a periodic stiffening and softening [15]. Cell stiffness increased ~3-fold from ~ 0.1 KiloPascal(KPa) to ~ 0.3 KPa prior to division and softened after division over a time scale of ~ 20 minutes, which is very short compared to typical cell division times of 15 hours [15]. Such periodic stiffening and softening is directly driven by the accumulation of actin filaments in the cortical regions immediately prior to cell division and then redistribution into the cytoplasmic regions after division respectively [15]. A recent study showed that tumor cells exhibit a similar mechanoadaptation by softening to facilitate invasion in confined channels [16].

Using a 3D computational model, we study the effect of rapid single cell level stiffness change on the overall growth and dynamics of multicellular collectives. By varying the probability for cells to soften after division, we discover that mechanical heterogeneity, 3D cell dynamics and tumor growth are all enhanced due to time dependent cell stiffness change. We reveal that cell division associated stiffening and softening determines the spatial structure and dynamics of three-dimensional (3D) multicellular aggregates. Our results provide an explanation why softer cells which are directly correlated with heightened cancer progression and metastasis are preferentially located at the periphery of multicellular tumor spheroids as observed in experiments [2, 4, 17].

This article is organized as follows. In Sec. II we present the model and the method. In Sec. III we characterize the mechanical heterogeneity of tumor cell collectives and its impact on tumor cell dynamics. Finally, in Sec. V we present our conclusions.
FIG. 1. 3D tumor growth model with time-varying single cell stiffness. (a) Schematic illustrating time-varying single cell stiffness change implemented in the simulation. The cell stiffness increases prior to division due to the accumulation of actin filaments (red lines) at the cell cortex and soften immediately after division due to the release of cortical actin into the cell cytoplasm, as controlled by the parameter for cell softening probability $\chi$. $\chi = 0$ implies no softening of the parent cell while $\chi = 1$ leads to both parent and daughter cell softening after division. The daughter cell stiffness after division is set from a fixed initial condition. (b) (Top panel) Time dependent individual cell stiffness change at $\chi = 0.0$. Lines with different colors are for selected individual cells from the simulation. (Middle panel) Cell stiffness vs time at $\chi = 0.5$ and (Bottom panel) at $\chi = 1$. (c) Average cell stiffness in the 3D cell collective as a function of time at three different values of $\chi = 0, 0.5, 1$.

II. MODEL AND METHOD

We utilized an agent based simulation scheme for three-dimensional (3D) tumor growth to quantify how time-varying single cell stiffness determines the spatial mechanical heterogeneity and dynamics of cells within a growing multicellular collective. Such off-lattice simulations are widely used in modelling tumor growth and recapitulate experimentally observed features of individual cell dynamics within cell collectives [18–24]. Agent based models can simulate biophysical interactions between individual cells and provide insight into bridging the gap between single cell and tissue scale behaviors while capturing emergent cell collective behaviors [20, 25, 26]. Cell-cell interactions are typically modelled with short-ranged forces consisting of two terms - (i) elastic (repulsion) and (ii) adhesive (attraction) forces. The magnitude of the elastic force ($F_{ij}^{el}$) between two cells $i$ and $j$ of radii $R_i$ and $R_j$ is given by [19, 20],

$$F_{ij}^{el} = \frac{h_{ij}^{3/2}}{3} \left( \frac{1-v_i^2}{E_i(t)} + \frac{1-v_j^2}{E_j(t)} \right) \sqrt{\frac{1}{R_i(t)} + \frac{1}{R_j(t)}}. \quad (1)$$
where \(v_i\) and \(E_i\) are the Poisson ratio and elastic modulus of the \(i\)th cell. Here, \(h_{ij}\) is the overlap (virtual) distance between the two cells. The time-varying cell elastic modulus, \(E_i(t)\), which we refer to as the cell stiffness is the key parameter that we focus on in this study. Prior works have considered the cell stiffness to be time independent [18–20, 22–24]. The adhesive force \(F_{ad}^{ij}\) between cells is,

\[
 F_{ad}^{ij} = A_{ij} f_{ad}^{ij} \frac{1}{2} \left( c_{rec}^{ij} + c_{lig}^{ij} c_{re}^{ij} \right),
\]

where \(A_{ij}\) is the overlap area between the two cells in contact and \(f_{ad}^{ij}\) determines the strength of adhesive bond [19, 20]. The receptor (rec) and ligand (lig) concentrations are normalized to satisfy \(c_{rec}^{ij} = c_{lig}^{ij} = 0.9\).

Starting with 100 cells randomly placed in a 3D cubic volume, we simulate tumor cell collective growth over \(\sim 7.5\) days, sufficient to account for multiple cell division cycles. As cells grow, divide and move the multicellular collective grows into a large spheroid with cells in the core and periphery, mimicking the growth of tumor spheroids [27] and organoids [17] as observed in experiments. The effect of forces that cells experience from its micro-environment on growth is accounted through the pressure, \(p_i\), that cells feel due to neighboring cells using the minimal definition [18–20],

\[
 p_i = \frac{1}{\sum_{j=1}^{NN(i)} |F_{ij}| A_{ij}}.
\]

Here, the sum is over the nearest-neighbors (\(NN\)) of the \(i\)th cell and \([\ldots]\) denotes the absolute value. If \(p_i\) is smaller than a predetermined threshold value, \(p_c\), cells grow in size and divide. However, if \(p_i > p_c\), the cell becomes dormant which stalls size growth and division. A cell can switch between dormancy and growth depending on whether the ratio of \(\frac{p_i(t)}{p_c}\) is greater than or less than 1 [21]. The volume of an individual cell grows in time at a mean rate,

\[
 r_V = \frac{2\pi \left( R_m \right)^3}{3 \tau},
\]

and divides into two cells upon reaching a critical radius \(R_m = 5 \mu m\). On division, the parent cell and the newly created daughter cell take on radii \(R_d = \frac{R_m}{2}\) to ensure volume conservation. Hence, a key time scale in the simulation is \(\tau\) - the average time it takes for a particle to divide, set to be \(\sim 15\) hours, comparable to typical cell division times [19, 28]. We incorporate cell death in the simulations by randomly removing particles at a rate \(k_d = 10^{-6} s^{-1}\). Owing to the death rate being much smaller than the birth rate \((k_d \ll \frac{1}{\tau})\), we are simulating a rapidly expanding collection of cells.

### A. Time variation in single cell stiffness

To investigate whether temporal variation in single cell stiffness affects 3D cell collective spatial organization and dynamics, we coupled cell division to cell stiffness change according to two simple rules: (i) First, as the size of a cell approaches the mitotic radius, at \(R_i(t = t')/R_m = 0.98\), its stiffness is increased to \(E_i(t > t') = \min[2.5 \times E_i(t'), 3\text{KPa}]\) i.e. minimum of the value between 2.5 times the cell stiffness at time \(t'\) and a threshold stiffness value of 3 KPa. This ensures that the maximum cell stiffness is 3KPa and prevents it from increasing to unphysical values. Tumor cells tend to be stiffer than embryonic cells and the stiffness range we consider have been experimentally measured [16]. The condition \(R_i(t = t') = 0.98 \times R_m\) is set to ensure that stiffness change occurs immediately before cell division at \(R = R_m\). (ii) Second, to mimic experimentally observed cell softening after division, we implement a probabilistic protocol for cell softening:

- draw a uniformly distributed random number, \(u\), in the interval (0,1)
- if \(u\) is less than or equal to the softening probability parameter \(\chi\), reduce cell stiffness to \(E_i(t > t') = \max[0.2 \times E_i(t'), 0.5\text{ KPa}]\). If \(u > \chi\), the parent cell does not soften. \(\chi\) is an input parameter that we vary in the model.

To prevent cell stiffness from approaching zero, we implement a lower bound of cell stiffness at 0.5 KPa. The initial condition for daughter cell mean stiffness is set to 1KPa and characterized by a Gaussian distribution with standard deviation of 0.1 KPa (see Table I). The spectrum of cell stiffness between 0.5 – 3KPa we consider is in the physiological range for cell stiffnesses [29] with the lower and upper end corresponding to embryo cell stiffness and lung cell stiffness respectively [30]. The schematic of single cell stiffness change is visualized in Fig. 1a. The time dependent single cell stiffness change obtained in the simulation for selected cells at three different \(\chi\) values are shown in Fig. 1b. At \(\chi = 1\) intermittent cell stiffening and softening events are visible (bottom panel, Fig. 1b) compared to \(\chi = 0\) (top panel, Fig. 1b).

We now describe the molecular underpinnings of the cell stiffness change implemented in the computational model. As cells progress through the cell cycle and approach division, actin filaments accumulate at the boundary of the cell, increasing cell stiffness. After division, the acto-myosin filaments are distributed into the cytoplasmic regions of the cell until the cell is ready for the next division event [15, 31, 32]. The probability for the cortical protein filaments to be re-distributed into the cytoplasm is modeled in our simulation via the parameter \(\chi\), which we vary from 0 to 1, at intervals of 0.1. Hence, \(\chi = 0.1\) implies a very low probability for cells to soften, while \(\chi = 1\) implies a high probability for cell softening after division. We note that the parent cell stiffness is dynamically increased before division. After division, the daughter cell stiffness is set from the initial condition as noted in Table I while the parent cell undergoes softening as determined by the parameter \(\chi\). When the daughter cell grows in size and approaches the mitotic radius as noted above \((R_i(t = t')/R_m = 0.98)\), they can undergo stiffening followed by softening. Furthermore, some cells may become dormant after stiffening and not progress to division depending on the pressure parameter. This would lead to the arrest of single cell stiffnesses at heightened values irrespective of \(\chi\). The overall
change in average cell stiffness of the cell collective as a function of time is shown in Fig. 1c. At $\chi = 1$, the cell collective is on average the softer as opposed to a stiffer cell collective at $\chi = 0$. Based on varying the parameter $\chi$ we can now study the impact of cell division associated cell softening and stiffening on cell dynamics within the 3D cell collective.

### B. Cell dynamics

In addition to the active forces due to cell growth and division, the passive forces experienced by a cell due to interaction with its neighbors contributes to cell dynamics. The net force $F_i$ on the $i^{th}$ cell is the vectorial sum of elastic and adhesive forces that the neighboring cells exert on it, $F_i = \sum_{j=1}^{NN(i)} F_{ij}$. Here, $j$ is summed over the number of nearest neighbors $NN(i)$. We performed over damped (low Reynolds number [33]) dynamics without thermal noise because the viscosity of the medium surrounding cells is assumed to be $10^{-6}$ kg/s. At least 12 simulations each for 10 different parameters utilized in the computational model are summed over the number of nearest neighbors $NN(i)$. We performed over damped (low Reynolds number [33]) dynamics without thermal noise because the viscosity of the medium surrounding cells is assumed to be $10^{-6}$ kg/s. As expected, the overall cell subpopulation is stiffer at low $\chi$ which corresponds to low probability for cells to soften post division. We discover that the stiffness heterogeneity between cell subpopulations in the core and periphery increases with $\chi$. To quantify this further, we grouped cells according to their positions with respect to the tumor center of mass, $R_{CM} = \frac{1}{N} \sum_{i=1}^{N} r_i$, where $N$ is the total number of cells. By calculating the cell distances from the tumor center of mass, $d_i = |\vec{r}_i - \vec{R}_{CM}|$, where $[...]$ indicates vector magnitude we group cells into 8 cell subpopulations. Cells closest to the center of mass compose the core of the spheroid and we refer to the outermost subpopulation as the periphery. The thickness of each layer composing the cell subpopulation is set to 15 $\mu$m. The statistical average of single cell stiffness within each subpopulation is computed at time $t = 12\tau$ using,

$$\langle E(r_d) \rangle = \frac{\sum_i E_i \delta(r_d - d_i)}{\sum_i \delta(r_d - d_i)},$$

where $r_d$ is the binning distance from the tumor center of width 15 $\mu$m.

Notably, cells located near the core of the tumor spheroid are stiffer as compared to cells near the periphery (Fig. 2c), irrespective of the value of $\chi$. As expected, the overall cell subpopulation is stiffer at low $\chi$ which corresponds to low probability for cells to soften post division. We discover that the stiffness heterogeneity between cell subpopulations in the core and periphery increases with $\chi$. To quantify the spatial mechanical heterogeneity between cell subpopulations, we calculated the difference in average stiffness between the core and periphery,

$$\Delta E = \langle E \rangle_{core} - \langle E \rangle_{periphery}$$

(see Fig. 2d). At $\chi = 0$, the spatial mechanical heterogeneity is low with a mean $\Delta E \sim 0.35$ KPa as compared to $\Delta E \sim 0.8$ KPa at $\chi = 1$. The spatial mechanical heterogeneity is therefore enhanced at $\chi = 1$, indicating that time-varying cell stiffness change during cell division is an important determinant.

### III. RESULTS

While the molecular factors that determine tumor growth is better understood, much remains to be known about the impact of time dependent changes in cell physical properties on the spatial mechanical heterogeneity of 3D cell collective. Given that individual cells that make up a tumor can be characterized by broadly varying stiffnesses, are cell subpopulations i.e. clusters of cells with differing levels of stiffness spatially organized within cell collectives? To answer this question we visualized the multicellular spheroids generated from our simulations at $t = 12\tau$ and $\chi = 1$ (Fig. 2a). A mixture of soft (lighter color) and stiff (darker color) are visible on the surface of the spheroid. As we are interested in understanding the spatial variation in cell stiffness, a cross-section view with respect to a 2D plane cutting through the center of the 3D cell collective is shown in Fig. 2b. Remarkably, a clear trend in spatial mechanical heterogeneity with stiffer cells in the core and softer cells at the periphery is visible. To quantify this further, we grouped cells according to their positions with respect to the tumor center of mass, $R_{CM} = \frac{1}{N} \sum_{i=1}^{N} r_i$, where $N$ is the total number of cells. By calculating the cell distances from the tumor center of mass, $d_i = |\vec{r}_i - \vec{R}_{CM}|$, where $[...]$ indicates vector magnitude we group cells into 8 cell subpopulations. Cells closest to the center of mass compose the core of the spheroid and we refer to the outermost subpopulation as the periphery. The thickness of each layer composing the cell subpopulation is set to 15 $\mu$m. The statistical average of single cell stiffness within each subpopulation is computed at time $t = 12\tau$ using,

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### TABLE I. The parameters used in the simulation. The parameters where we indicate the mean and standard deviation are sampled from a normal distribution. For details, see [20].

| Parameters                             | Values                  |
|----------------------------------------|-------------------------|
| Timestep ($\Delta t$)                  | 10s                     |
| Critical Radius for Division ($R_{cr}$) | 5 $\mu$m                |
| Environment Viscosity ($\eta$)         | 0.005 kg/($\mu$m s)     |
| Average Division Time ($\tau$)         | 54000 s                 |
| Adhesive Coefficient ($f_{ad}$)        | $1 \times 10^{-3}$ $\mu$N/$\mu$m$^2$ |
| Initial Mean Elastic Modulus ($E_{f}$) | 1KPa (0.1KPa)           |
| Mean Poisson Ratio ($\nu$)             | 0.5 (0.02)              |
| Death Rate ($k_d$)                     | $10^{-6}$s$^{-1}$        |
| Mean Receptor Concentration ($c^{R}$)  | 0.9 (0.02) [Normalized] |
| Mean Ligand Concentration ($c^{L}$)    | 0.9 (0.02) [Normalized] |
| Threshold Pressure ($p_t$)             | $10^{-4}$KPa            |

The spatial mechanical heterogeneity is therefore enhanced at $\chi = 1$, indicating that time-varying cell stiffness change during cell division is an important determinant.
FIG. 2. Spatial heterogeneity in cell subpopulation stiffness between core and periphery in growing 3D cell collectives. (a) Snapshot of the 3D collection of ∼6,000 cells at t = 7.5 days for χ = 1. Each small sphere is a single cell of maximum diameter 10 μm with the color visualizing cell stiffness (see color bar). (b) Cross section through one plane of the 3D cell collective showing the core and periphery. Stiffer cells (darker color) are visible at the core with softer cells at the periphery. (c) Average stiffness of cell subpopulations as a function of distance from the core. Cell subpopulations are categorized according to their distances from the center of mass of the 3D cell collective. Circles indicate mean values and the error bar is the standard deviation. A marked difference between cell subpopulation stiffness at the core vs periphery is noted at χ = 1, as quantified by ∆E. (d) Mechanical heterogeneity of the cell subpopulation stiffness is quantified using ∆E. Differences in the average cell stiffness between the core and periphery is most pronounced at χ = 1.

of mechanical heterogeneity. Indeed, mechanical heterogeneity during disease progression is thought to facilitate metastasis [2, 29, 35]. Moreover, spatial heterogeneity of tumor organoids with a stiffer core and softer periphery of cells may be a general feature of 3D tumor cell collectives [17].

Next, we investigated whether the dynamics of individual cells that make up the spheroid could be affected by the spatial mechanical heterogeneity. Prior studies report that metastatic tumor cells are softer compared to non-metastatic tumor cells [17, 36–39]. As cell division events fluidize cell collectives and lead to superdiffusive dynamics [20, 22], division dependent cell softening could affect the non-equilibrium active forces that cells experience and thus affect individual 3D cell dynamics. By tracking single cell trajectories, we calculate both single cell mean-squared displacement (scMSD) and ensemble averaged MSD,

\[ \Delta(t) = \left( \frac{1}{N} \sum_{i=1}^{N} \left| \mathbf{r}_i(t) - \mathbf{r}_i(0) \right|^2 \right)^{1/2}, \]

where \( N \) denotes the total number of tracked cells from the beginning to the end of the simulation. The ensemble average \( \langle ... \rangle \) is over 12 different simulation runs at each value of \( \chi \) for different initial conditions (see Appendix A). scMSD (without averaging over \( N \) or multiple simulation runs) shown in Fig. 3a reveal that distances traversed by cells are highly heterogenous. While majority of the cells traverse distances less than \( \sqrt{500 \mu m^2} = 22.4 \mu m \), a population of highly dynamic cells exist that traverse distances on the order of \( \sqrt{3000 \mu m^2} = 54.7 \mu m \) (Fig. 3a) at \( \chi = 1 \). Another interesting feature is the intermittent change in scMSD clearly visible in the highly dynamic group of cells where there are steep increases in scMSD
followed by time regimes where scMSD does not change much.

As cell collectives exhibit glass to fluid-like transition due to cell division events [20, 40, 41], we surmise that cell displacement could be linked to cell division events and the associated time dependent change in stiffness. Hence, we investigate the effect of cell softening probability on ensemble averaged MSD (Fig. 3b). At short times, \( t < 2 \) days, probability of cell softening (\( \chi \)) has no visible effect on the cell dynamics as observed from the MSD plots. In contrast, at longer times \( t > 2 \) days, cell dynamics is significantly restricted at low \( \chi \). As the MSD is significantly enhanced at \( \chi = 1 \), we confirm that higher \( \chi \) values resulting in enhanced spatial mechanical heterogeneity with larger stiffness asymmetry between cells in the core and the periphery (see Fig. 2d) is more conducive to 3D cell dynamics. To confirm that the space explored by cells increases with \( \chi \), we analyze the maximum (max) MSD at \( t = 12 \tau \) during each individual simulation run (see stars in the inset of Fig. 3b). The max MSD at multiple \( \chi \) values are summarized in Fig. 3c. Therefore, on the basis of the spatial mechanical heterogeneity we report in Fig. 2, a stiffer core and softer peripheral cells is conducive to heightened cell dynamics as indicated by the larger MSD values.

MSD depends on the mechanical resistance of the surrounding medium [42], but, the influence of individual particle level change in mechanical properties such as stiffness on MSD is unclear. Time dependent scaling of MSD based on a fit to power law \( \Delta(t) \sim t^\beta \) reveals important features of cell dynamics (see black lines in the inset of Fig. 3b for details). When \( \alpha = 1 \), cells exhibit diffusive random walk. For cells undergoing directed motion, the power law exponent is greater than one (\( \alpha > 1 \)) in contrast to restricted cell motion which leads to a sublinear rise in MSD with \( \alpha < 1 \). Interestingly, median \( \alpha \) values show that cells exhibit subdiffusive motion due to time varying stiffness change except at \( \chi = 1 \). The median MSD exponent (white circles in Fig. 3d) are all below 1, except at \( \chi = 1 \). Additionally, heightened mechanical heterogeneity leads to enhanced super-diffusive dynamics as there is a marked increase in median MSD exponent at \( \chi = 1 \) (\( \alpha > 1 \)). For \( \chi < 0.5 \), no clear trend in MSD exponent is visible in Fig. 3d even though the max MSD increases in the same range. Despite the fact that all the MSD exponents are characterized by a wide scatter, we observe that enhanced spatial mechanical heterogeneity led to heightened MSD and MSD exponent.

IV. INDIVIDUAL CELL SOFTENING REGULATES CELL COLLECTIVE GROWTH RATE

The cell softening probability clearly determines the cell dynamics as evident from the MSD dependence on \( \chi \) (discussed above). We next sought to evaluate whether cell softening impacts the volumetric growth of tumor cell collectives. Finding the biophysical underpinnings of tumor growth is of much interest. This is an important problem because accurate tumor growth modeling can be crucial in evaluating patient screening strategies [43], establishing radiation treatment protocols [44] as well as assisting treatment decisions [45]. To answer this question, we quantified the 3D spatial spread of the cell collective using radius of gyration squared,

\[
R^2_g(t) = \left\{ \frac{1}{N} \sum_{i=1}^{N} (|\mathbf{r}_i(t) - \mathbf{R}_{CM}(t)|)^2 \right\}.
\]

(9)

The bracket \(...\) denotes ensemble average over 12 different simulation runs at each value of \( \chi \) for different initial conditions (see Appendix A). The average squared distance of all the cells from the center of mass gives a sense of the size of the 3D cell collective. Small \( R^2_g \) values indicate cell positions that are localized in close proximity to the center of mass. In contrast, cells spatially distributed farther away from the center of mass leads to significantly larger \( R^2_g \) values [46, 47]. As a result, \( R^2_g \) as a function of time is a readout of the 3D cell collective volumetric growth. The time varying \( R^2_g \) in Fig. 4 shows slow change at \( t < \sim 3 \) days followed by faster growth at \( t > 4 \) days. The \( R^2_g \) values are indistinguishable between \( \chi \) values at time below 4 days as compared to later times when \( R^2_g \) is significantly larger for \( \chi = 1 \). Time dependent scaling of \( R^2_g \) based on a fit to power law \( R^2_g(t) \sim t^\beta \) reveals important features of cell spatial distribution dynamics in 3D [48, 49] (see Inset of Fig. 4). When \( \beta = 1 \), cells exhibit diffusive random spread compared to when cells undergo directed spreading at \( \beta > 1 \). By contrast restricted cell spreading leads to sublinear rise in \( R^2_g \) with \( \beta < 1 \). The maximum (max) \( R^2_g \) values (marked as stars in Inset of Fig. 4) show a clear linear trend with \( \chi \) (see Fig. 5a). This implies that enhanced mechanical heterogeneity leads to significantly more spread out morphology of the 3D cell collective. The median value of max \( R^2_g \) at \( \chi = 0.1 \) is \( \sim 5800 \mu m^2 \) as compared to \( \sim 7100 \mu m^2 \) at \( \chi = 1 \) as shown in Fig. 5a.

Our results therefore indicate that heightened spatial mechanical heterogeneity leads to enhanced volumetric growth of the 3D cell collective with time dependent spatial expansion of the cell collective being restricted when individual cells are stiffer. In contrast, cell softening favored faster expansion of the cell collective into the surrounding viscous medium with a median value of \( \beta \sim 1.2 \) (Fig. 5b). Our results provide evidence into how spatial mechanical heterogeneity determines the spatial spread of 3D cell collectives. Hence, spatial mechanical heterogeneity consisting of a stiffer core cells and softer peripheral cells aid in more efficient volumetric growth of cell collectives.

V. CONCLUSION

Understanding how individual cell level mechanical changes impact cell dynamics and tumor growth is critical to understanding cancer progression. In this respect, we studied how time varying individual cell stiffness drives spatial mechanical heterogeneity in multicellular collectives by incorporating stiffening of cells immediately prior to division and softening post division into our minimal 3D tumor growth...
FIG. 3. Cell softening after division leads to distinct cell dynamic behaviors. (a) Single cell MSD (scMSD) versus time, at \(\chi = 0\) (blue), \(\chi = 0.5\) (red) and \(\chi = 1\) (yellow). \(\sim 60\) scMSDs per \(\chi\) value show highly heterogeneous dynamics with some cells traversing large distances while other cells move less in comparison to the typical cell diameter of 10\(\mu\)m. (b) Ensemble averaged MSD of cells versus time at three different values of \(\chi\). The data is averaged over 12 independent simulation runs by tracking \(\sim 800\) cells over the complete simulation at each value of \(\chi\). Inset: MSD from averaging over cells from individual simulation runs at \(\chi = 1\). The maximum (max) MSD value for 2 individual simulation run is marked with stars. The time regime where MSD is fit to power law in order to extract the MSD exponent is shown. (c) Max MSD increases as a function of \(\chi\). Colored dots represent each of the Max MSD values from individual simulation runs. White dots are the median values and the thick line within the violin distribution represent the interquartile range between the first and third quartiles. The bottom and top edge of thinner gray lines mark the lower and upper adjacent values respectively. (d) By fitting cell averaged MSD in each of the 12 simulation runs to a power law, we extracted the MSD exponent (\(\alpha\)) as a function of \(\chi\). Cell dynamics is significantly enhanced at \(\chi = 1\) as compared to \(\chi = 0\).

Our simulations show that cell division associated softening drives the emergence of spatial mechanical heterogeneity between the core and periphery of multicellular spheroids. The resulting spatial stiffness pattern consisting of a core made up of stiffer cells and peripheral softer cells enhances the 3D collective cell dynamics and volumetric growth of multicellular spheroids. Broadly, our computational results are consistent with experimental observations of spatial mechanical heterogeneity in 3D tumor organoids [17], and the heightened ability of softer tumor cells to metastasize [4, 50]. As polymerization and depolymerization of the actomyosin network in the cell cortex leads to time varying stiffening and softening of the cell, we show that such temporal stiffness variation at the single cell level is essential in the emergence of mechanical heterogeneity. In addition to the increased space that cells explore in 3D cell collectives due to periodic stiffening and softening, our study shows that increased spatial mechanical heterogeneity is correlated with enhanced 3D spheroid growth. Our results therefore have important implications into understanding how time variations in single cell mechanical properties determine the spatial organization and dynamics at the cell collective scale.
FIG. 4. Cell softening after division control cell collective growth. (a) Quantification of the ensemble averaged (over 12 simulation runs) radius of gyration squared ($R^2_g$) of the 3D tumor cell collective over 7.5 days at $\chi = 0$, 0.5 and 1. Inset: $R^2_g$ from averaging over cells from individual simulation runs at $\chi = 1$. Maximum (max) $R^2_g$ values for 2 individual simulation runs are marked with stars. The time regime where $R^2_g$ is fit to power law in order to extract the exponent is shown.

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FIG. 5. Cell softening after division control cell collective growth. (a) Max value of $R^2_g$ at the end of simulation run at $t = 7.5$ days indicate significantly enhanced growth of tumor cell collective with increased probability of individual cells to soften (compare $\chi = 1$ to $\chi = 0$). Max $R^2_g$ value from each of the 12 simulations after averaging over individual cell $R^2_g$ is shown as colored dots. White dots are the median values and the thick line within the violin distribution represent the interquartile range between the first and third quartiles. The bottom and top edge of thinner gray lines mark the lower and upper adjacent values respectively. (b) By fitting the average $R^2_g$ in each of the 12 simulation runs, we extracted the $R^2_g$ exponent $\beta$ as a function of $\chi$. Volumetric growth of the 3D cell collective is significantly enhanced at $\chi = 1$ as compared to $\chi = 0$.

Appendix A: Initial Conditions

We initiated the simulations by placing 100 cells whose $x$, $y$, $z$ coordinates are chosen from a normal distribution with zero mean and standard deviation 40 $\mu m$. In the present study, all the individual cell parameters are fixed except single cell stiffness $E_i$ which is varied within a physiological cell stiffness range. The simulated dense 3D cell aggregate was evolved for 650,000 sec or 12$\tau$. At each $\chi$ value, 12 different simulation runs allow for random initial positions of cells. Hence, our reported results account for varying initial conditions. Rele-
vant simulation parameters are shown in Table 1. The time-dependent coordinates of particles were recorded to calculate the dynamical observables relevant to this study.

Appendix B: Simulation movies

Movies generated from the simulated 3D cell collective are shown. The total duration of the movie is 650,000 sec or ≈ 12τ. The time interval between consecutive frames is 1000 sec.

Movie 1: 3D cell collective simulated at χ = 1. Color bar indicates the stiffness of cells with dark blue indicating stiffer cells at 3KPa. Softer cells are shown in yellow color at 0.5KPa. The observation frame is rotated to allow for a full 3D view of the tumor cell collective. The box is for 3D visualization purposes only. (Link)

Movie 2: Cross-section view of a 3D cell collective simulated at χ = 1. Color bar indicates the stiffness of cells with dark blue indicating stiffer cells at 3KPa. Softer cells are shown in yellow color at 0.5KPa. A view of a fixed 2D plane cutting through the 3D cell collective is shown. (Link)

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