Supplementary material for

Multi-compartment cell-based modeling of confined migration: regulation by cell intrinsic and extrinsic factors

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1 Spreading and stiffness of MDA-MB-231 cells

1.1 Experimental methods

Cell culture: MDA-MB-231 cancer cell lines were obtained from National Center for Cell Science (NCCS) (Pune, India) and cultured in high glucose Dulbecco’s Modified Eagle Medium DMEM (Invitrogen) containing 10% fetal bovine serum (FBS, Hi-media). Cells were maintained at 37°C under 5% CO₂ humidified atmosphere. Cells were maintained in 60 mm culture dishes (Tarsons) and passaged when 80-90% confluent using 0.25% trypsin-EDTA (Hi-media).

Cell spreading: Cells were cultured on collagen type I coated substrate (1 µg/cm²) at a seeding density of 2 × 10⁴ cells/cm² for 12-15 hrs. Cells were labeled with live cell imaging dye calcein AM (10 µg/ml) (Invitrogen) for 20 mins and then imaged using an inverted microscope (Olympus IX71) at 20x magnification. Quantification of spreading analysis was done using Fiji-Image J software. Briefly, after background subtraction, images were thresholded to similar extent, and cell spread area was obtained using the ImageJ-Analyze Particle tool.

AFM experiments: For measuring cell and nuclear stiffness, samples were probed with a MFP3D Asylum AFM mounted on Zeiss inverted microscope. Pyramidal cantilevers of nominal spring constant 30 pN/nm (10 kHz, TR400PB, Asylum research) were used for probing cell cortical stiffness. Exact values of cantilever stiffness were obtained using thermal calibration method. For measuring cell cortical stiffness, cells were probed slightly off their center to get the best estimate of cortical stiffness, and the first 500-800 nm of indentation data was fit using Hertz model [2]. For nuclear stiffness measurement, cells were first treated with 5µM cytochalasin-D (Calbiochem) for 30 mins to depolymerized cytoplasmic F-actin and then indented at the center with a stiff 200 pN/nm tip (22 kHz, TR800PB, Asylum research) [3]. Indentation data between 500–2000 nm was fitted using Hertz model to obtain estimates of nuclear stiffness.

Cell Motility: For 2D motility experiments, 2 × 10³ cells/cm² were cultured for 8-12 hrs, and then time lapse imaging was performed for every 10 mins for 3 hrs duration using a temperature and CO₂ control stage. (Nikon Eclipse Ti, 20x objective). Cell speed was obtained by using the manual tracking plugin of Image J.
1.2 Spreading and stiffness of MDA-MB-231 cells

Figure S1: Spreading, cell stiffness and nuclear stiffness of MDA-MB-231 cells. A. Representative calcein stained fluorescent images (green) of MDA-MB-231 cancer cells cultured on col I-coated substrates. B. Histogram of 2D cell spreading area of cells cultured on Col I coated substrates (n = 3, 149 cells). C. Experimental setup for probing nuclear stiffness. Cells were treated with 5 M CytochalasinD (Cyto D) for 30 mins for depolymerizing the actin cytoskeleton, and then probed with a stiff pyramidal AFM probe. D. Histogram of cell cortical stiffness probed with soft AFM pyramidal probe. First 500 nm of the force curves were fit with Hertz equation to obtain estimates of cortical stiffness (n = 2, 119 cells). E. Histogram of nuclear stiffness of cells cultured on col I coated substrate. 500-2000 nm of the force curves were fit with Hertz equation to obtain estimates of nuclear stiffness. (n = 3, 94 nuclei).
2 Simulation implementation

Figure S2: Copy-index process and simulation algorithm. A. Schematic shows copy-index process for a system with two type of pixels represented by white and brown colors. Each block represents a single pixel. During copy-pixel attempt, a pixel is randomly selected and designated as target pixel. Another pixel adjacent to the target pixel is then selected and designated as source pixel. Finally, type of target pixel (i.e., color of pixel) is then changed to that of source pixel. B. Flowchart showing the procedure of the system state transition thereby regulating spatio-temporal dynamics of the system.

The complete simulation framework was implemented using already available open source software package CompuCell3D (CC3D) [1]. The framework is based on GGH algorithm in which systems evolves through random copy-pixel attempt (Figure S2). In CC3D, energy terms (e.g., listed in Equation (1)) are encoded using plugins and Steppables are used to perform custom actions (e.g., storing simulation data to file) after every simulation step (MCS). The brief description of all the plugins used in our study are as follows:

1. **CellType**: Represents different type of pixels. We have 4 type of pixels in our simulation lattice viz. Cytoplasm (C), Nucleus (N), Matrix (Mat) and Medium (free space).
2. **Contact**: It is used to define the interface energy between pixel of different entities.
3. **ContactInternal**: Since our single cell is a multi-compartment entities, this plugin is used to define interface energy between different compartments of same cell.
4. **FocalPointPlasticity**: It is used to defined a constraint on distance between centre of masses of two entities. We used this plugin to make sure that different compartments of a cell remain attached for avoiding cell fragmentation.
5. **Volume**: This term is used to define the area constraint ($\lambda_p$, refer main text for further details). In 3D simulations, the same plugin is used to model the volume constraints.

6. **SurfaceArea**: This term is used to define the perimeter constraint ($\lambda_p$, refer main text for further details). In 3D simulations, the same plugin is used to model the surface area constraints.

7. **CenterOfMass**: This is a tracking plugin and is used to monitor the centroid of all cytoplasmic and nuclear compartment. The cell centroid was then determined by taking vector average of centroid of all 9 compartments.

8. **ExternalPotential**: This plugin is used to model a directed force (in positive-x direction).

Detailed description of these plugins can be found in the CC3D documentation [1]. Next, to implement additional functionalities (e.g., storing of simulation data), we used following steppables in our model:

1. **PIFInitializer**: This steppable allowed us to input the cell description with details about the relative positioning of different compartments.

2. **UniformInitializer**: This is used to define the geometry of confinement.

3. **IdFieldVisualizationSteppable**: We wrote this custom steppable in python and integrated it with other modules. This steppable is used for visualization.

4. **Cytoplasm_NucleusSteppables**: We wrote this custom steppable in python and integrated it with other modules. This steppable is used for determining the cell centroid from centroid of individual compartment at every MCS. Writing simulation data (e.g., cell centroid) to file is also done in this steppable.
3 Effect of confinement on cell length

Figure S3: Cell length \((L)\) during migration though channels of varying width. Represented images showing cell position and size (left) and enlarged images (right) for three different values of confinement. \(\lambda_{a,N} = \lambda_{a,C} = 2E/L^4\), \(\lambda_{p,N} = \lambda_{p,C} = 2E/L^2\).
4 Influence of cell and nuclear area constraints on invasion efficiency

Figure S4: Cell/nuclear area constraint influence invasion efficiency. A. Statistics of $t_{entry}$ for varying combinations of cell/nuclear area constraints and different channel widths. $(\lambda_{p,C}, \lambda_{p,N}) = (2, 2) \frac{E}{L^2}$. B. Statistics of $t_{transit}$ for varying combinations of cell/nuclear area constraints and different channel widths. $(\lambda_{p,C}, \lambda_{p,N}) = (2, 2) \frac{E}{L^2}$. Errorbars: ± Standard Error of Mean (SEM).
5 Effect of confinement-dependent stiffness tuning on invasion efficiency

Figure S5: Influence of confinement-dependent stiffness tuning on invasion efficiency. (A-Upper) Cell migration was simulated through a multi-step channel with four 70 µm long regions of different widths ($\phi = (17, 11, 7, 5)$ µm). (A-Lower) Cell/nuclear stiffness was tuned depending upon the extent of confinement. (B) Transit time ($t_{\text{transit}}$) defined as the time required to pass through the central 50 µm segments (schematic) inside different regions was quantified for case of dynamic tuning and for $(\lambda_{p,C}, \lambda_{a,N}) = (10,10)$ E/L. $(\lambda_{p,C}, \lambda_{a,N}) = (2,2)$ E/L was used in these simulations. 20-30 simulations per conditions were performed. Errorbars: ± Standard Error of Mean (SEM).
6 Influence of channel shape on transit time

Figure S6: Influence of channel shape on transition time. Transit times ($t_{transit}$) for tapered and flat channels for $(\lambda_{p,C}, \lambda_{p,N}) = (2,2)$ and $(5,5) \frac{E}{L^4}$. $(\lambda_{a,C}, \lambda_{a,N}) = (2,2) \frac{E}{L^4}$ was used in these simulations. Errorbars: ± Standard Error of Mean (SEM).
Effect of perimeter and area constraints on cell and nuclear deformability.

Figure S7: Effect of perimeter and area constraints on cell and nuclear deformability. A. Temporal fluctuations in cell area for different combinations of cell perimeter constraint ($\lambda_{p,C}$) and nuclear perimeter constraint ($\lambda_{p,N}$). B. Temporal fluctuations in nuclear area for different combinations of cell perimeter constraint ($\lambda_{p,C}$) and nuclear perimeter constraint ($\lambda_{p,N}$). C. Temporal fluctuations in cell area for different combinations of cell area constraint ($\lambda_{a,C}$) and nuclear area constraint ($\lambda_{a,N}$). D. Temporal fluctuations in nuclear area for different combinations of cell area constraint ($\lambda_{a,C}$) and nuclear area constraint ($\lambda_{a,N}$).
Figure S8: Motility of MDA-MB-231 cells. A. Representative trajectories of MDA-MB-231 cells. B. Histogram of 2D motility of cells cultured on Col I coated substrates. (n = 2, 50 cells).
9 Supplementary Videos

Supplementary Videos V1 - V3. Perimeter constraints $\lambda_{p,C}$ and $\lambda_{p,N}$ used to model cells with different extent of cytoplasmic and nuclear deformability, respectively. Videos show cell shape for $(\lambda_{p,C}, \lambda_{p,N}) = (2, 2) \frac{E_L}{\ell^2}$ (Video V1), $(\lambda_{p,C}, \lambda_{p,N}) = (2, 10) \frac{E_L}{\ell^2}$ (Video V2) and $(\lambda_{p,C}, \lambda_{p,N}) = (10, 20) \frac{E_L}{\ell^2}$ (Video V3). $(\lambda_{a,C}, \lambda_{a,N}) = (2, 2) \frac{E}{\ell^2}$ was used in these simulations. Other parameters were kept same as listed in Table 1.

Supplementary Videos V4 - V6. End position of the cell was determined at the end of the simulation to determine the entry efficiency. Video V4. Cell did not enter into the channel. $(\lambda_{p,C}, \lambda_{p,N}) = (10, 20) \frac{E_L}{\ell^2}$. $\phi = 3 \mu m$. Video V5. Cell entered into the channel but trapped inside the channel. $(\lambda_{p,C}, \lambda_{p,N}) = (10, 20) \frac{E_L}{\ell^2}$. $\phi = 5 \mu m$. Video V6. Cell entered into the channel and existed from the channel $(\lambda_{p,C}, \lambda_{p,N}) = (2, 2) \frac{E_L}{\ell^2}$. $\phi = 3 \mu m$. $(\lambda_{a,C}, \lambda_{a,N}) = (2, 2) \frac{E}{\ell^2}$ was used in these simulations.

Supplementary Video V7: Video shows cell migration through channel of size $\phi = 17 \mu m$. $(\lambda_{p,C}, \lambda_{p,N}) = (2, 2) \frac{E_L}{\ell^2}$. $(\lambda_{a,C}, \lambda_{a,N}) = (2, 2) \frac{E}{\ell^2}$. Video shows that cells prefer to move along one side of the wall when wall is wider than cell size.

Supplementary Video V8: Video shows cell migration through two confined regions separated by an unconfined region of size 35 $\mu m$. $(\lambda_{p,C}, \lambda_{p,N}) = (2, 2) \frac{E_L}{\ell^2}$. $(\lambda_{a,C}, \lambda_{a,N}) = (5, 5) \frac{E}{\ell^2}$.

Supplementary Video V9: Video shows cell migration through two confined regions separated by an unconfined region of size 75 $\mu m$. $(\lambda_{p,C}, \lambda_{p,N}) = (2, 2) \frac{E_L}{\ell^2}$. $(\lambda_{a,C}, \lambda_{a,N}) = (5, 5) \frac{E}{\ell^2}$.

References

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