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PRINCIPAL INVESTIGATOR: Bo Sun

CONTRACTING ORGANIZATION: Oregon State University

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**14. ABSTRACT**

The vast majority of breast cancer deaths are related to metastasis, during which cell migrate and invade surrounding tissue. Attempts to design effective drug treatments for metastasis have largely failed. A major reason for this failure is the plasticity of migrating cancer cells: they are able to rapidly switch between different modes of migration when faced with different extracellular environment. As a consequence, drugs that target a single migration mode will not be effective in stopping metastasis. This plasticity is poorly understood but depends strongly on the mechanical properties of the extracellular matrix (rigidity, fiber alignment, pore size, etc.). In this project, we will carry out quantitative experiments which determine the modes of migration as a function of the extracellular matrix properties, quantify the transitions between migration modes, and determine how the remodeling of the extracellular matrix couples back to the migration mode and mode transitions.

**15. SUBJECT TERMS**

NONE LISTED

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
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1. **Introduction**

We aim to address the overarching challenge to understand why some breast cancers become metastatic. Metastasis is enabled by cell migration during which cancer cells navigate through and negotiate space within the extracellular matrix (ECM). During metastasis, cancer cells can dynamically switch migration modes and these transitions between modes may significantly contribute to the invasive properties of tumors. To directly address the overarching challenge, we hypothesize that bidirectional and mechanical interactions in the cell-ECM system regulate the migration mode switching of breast cancer cells, which ultimately determines the metastatic potential of breast tumors. We will employ a combination of quantitative experiments, automated algorithmic data analysis, and computational modeling. Our project has two specific aims:

Aim 1: To quantify how breast cancer cell migration mode transitions are determined by extracellular matrix properties and mechanotransduction pathways.

Aim 2: To determine how the invasiveness and migration mode transitions of disseminating breast cancer cells depend on collective extracellular matrix remodeling and tumor geometry.

2. **Keywords**

Cancer, metastasis, migration, morphology, migration modes, modeling

3. **Accomplishments**

**What were the major goals of the project?**

The major goals of this project are to quantify how breast cancer cell migration mode transitions are determined by extracellular matrix properties and mechanotransduction pathways and to determine how the invasiveness and migration mode transitions of disseminating breast cancer cells depend on collective extracellular matrix remodeling and tumor geometry.

Aim 1: To quantify how breast cancer cell migration mode transitions are determined by extracellular matrix properties and mechano-transduction pathways

Major Task 1: To quantify the ECM micromechanical control of migrational mode transitions
Milestone of Major Task 1: establish how ECM micromechanical rigidity and anisotropy modulate the migration mode transition rates of breast cancer cells of different subtypes.
Major Task 1 is 100% accomplished.

Major Task 2 To identify main molecular pathways that regulate cell migrational mode transitions
Milestone of Major Task 2: establish how mechanosensing pathways modulate the migration mode transition rates of breast cancer cells. Examine the pathways with different subtypes of breast cancer cells
Major Task 2 is 90% accomplished

Major Task 3: Development of a comprehensive cell motility model
Milestone of Major Task 3: develop a validated cell motility model that can be validated using experimental data and that can generate experimentally testable predictions.
Major Task 3 is 80% accomplished

Aim 2: To determine how the invasiveness and migration mode transitions of disseminating breast cancer cells depend on collective extracellular matrix remodeling and tumor geometry

Major Task 4: To determine individual cell migrational mode transitions in disseminating tumor organoids
Milestone of Major Task 4: establish the spatial-temporal pattern of cancer cell migration mode transitions disseminating from tumor organoids. Test the effects of ECM micromechanics remodeling in modulating cell migration mode transitions in these dissemination processes.
Major Task 4 is 80% accomplished

Major Task 5: Development of a computational model for collective ECM remodeling and tumor organoid invasion

Milestone of Major Task 5:  validate an efficient computational model for collective ECM remodeling and tumor organoid invasion

Major Task 5 is 90% accomplished.

**What was accomplished under these goals?**

Major Task 2: To identify main molecular pathways that regulate cell migrational mode transitions

Milestone of Major Task 2: establish how mechanosensing pathways modulate the migration mode transition rates of breast cancer cells.

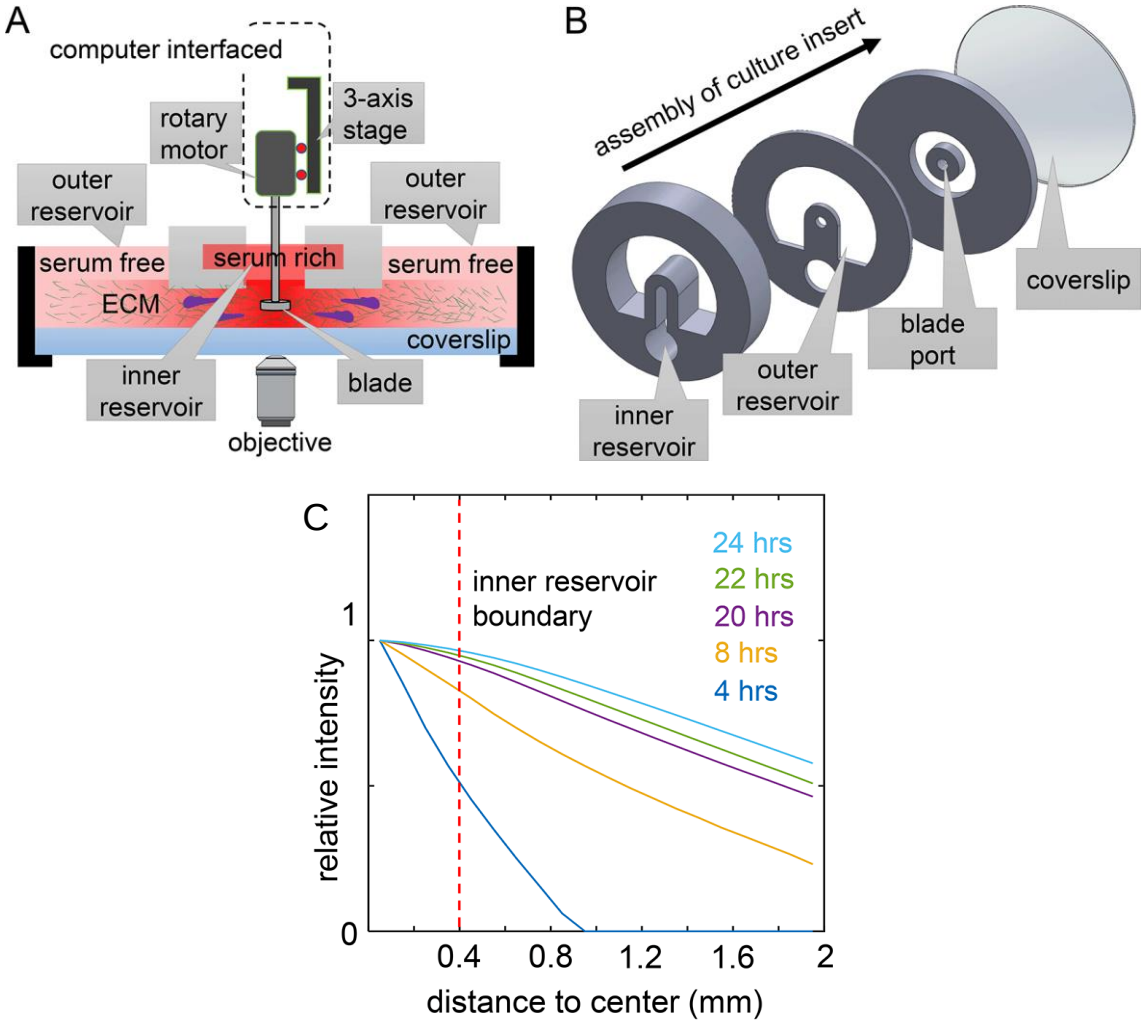


***Fig. 1*** *This figure shows the development of a 3D cell culture system which allows simultaneous control of ECM alignment and chemotaxis gradient to study the cancer cell motility. (A-B) show the explosive view of the device. To evaluate the chemoattractant profile, we fill the inner reservoir with fluorescent dye solution and measure the fluorescent intensity. As shown in (C), stable chemotaxis gradient can be maintained for more than 16 hours without the need for perfusion.*

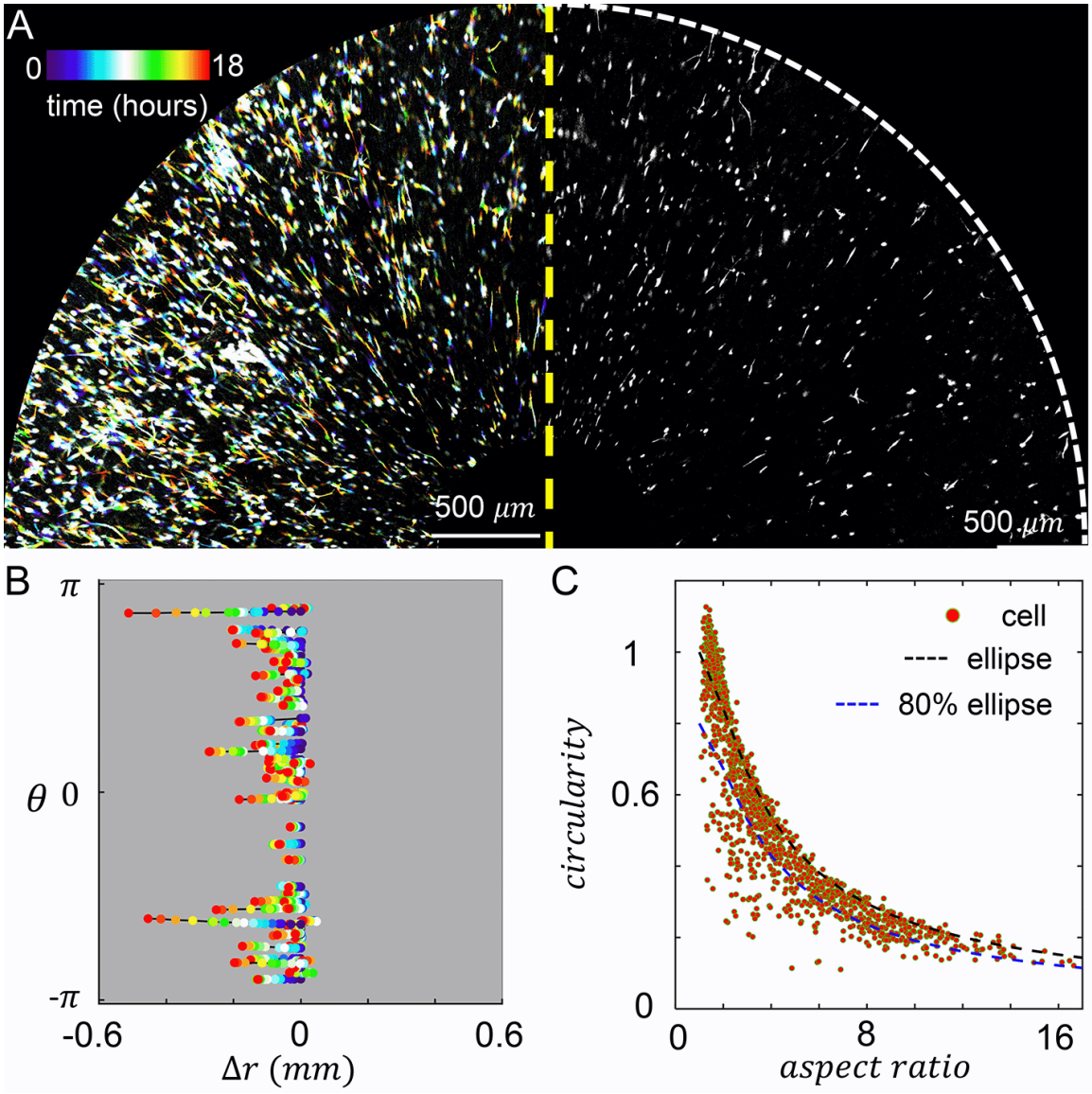


***Fig. 2*** *This figure shows the combined effects of chemotaxis and contact guidance on breast cancer cell morphology and motility. Here the chemical gradient and collagen fibers are aligned in parallel. (A) Left: temporal projected time-lapse imaging of breast cancer cells showing the cell motility. Right: a snapshot at five hours showing the cell morphology. (B) Cell trajectories in the radial (Δr) and azimuthal (θ) coordinates. (C) A scattered plot showing cell aspect ratio and circularity. The black dashed line indicates the circularity of an ellipse at a given aspect ratio. The blue dashed line indicates 80% of circularity corresponding to an ellipse at a given aspect ratio. We empirically consider data points below the blue dashed line as strongly protrusive cells. In (B-C) 70 cells are tracked.*

During the past reporting period, we made progress towards major task 2. In one published report, Esfahani et al, **Patterning ECM microstructure to investigate 3D cellular dynamics under multiplexed mechanochemical guidance**, F1000Research 2022, 11:1071, we develop a technology to combine

chemotaxis and contact guidance cues to study 3D breast cancer cell motility **(Fig. 1**). The device allows generation of well-maintained chemotactic gradient in the radial direction, and collagen fiber alignment in the radial or circumferential direction. The dual guidance therefore will control breast cancer cell migration through cross-talking chemical sensing and mechanical sensing pathways. As shown in **Fig. 2,** when ECM fibers are aligned in parallel with the chemical gradient, cell migration is strongly biased to the radial direction, indicating strengthened chemical and mechanical guidance. Cell morphology, which is indicative of migration phenotype, shows a broad distribution with most cells following the ellipsoidal shape with varying aspect ratios. In particular, a cell with large aspect ratio and low circularity is likely in the lamellipodial (LA) state; a cell with large aspect ratio and large circularity cells is likely in the filopodial (FP) state; a cell with small aspect ratio is likely in the blebbing state (BB).

Major Task 3: Development of a comprehensive cell motility model

We developed a modeling framework, which is able to simulate how cell deformations, cell polarization, cell-cell adhesion, and matrix remodeling can contribute to collective migration in confining ECM conditions (see **Fig. 3**). This framework is based on the cellular Potts Model that relies on minimization of the overall energy of the system using a Hamiltonian. In this Hamiltonian, we incorporated terms for cell motility, cell-ECM interactions, cell proliferation, and cell polarization. In addition, we defined a matrix-remodeling agent that is active either locally in the direction of the polarization vector or globally over the whole cell body. Our simulations showed that in the presence of strong local degradation, migration displays invasive behavior while in the presence of primarily global degradation, cells formed rotating acinar structures.

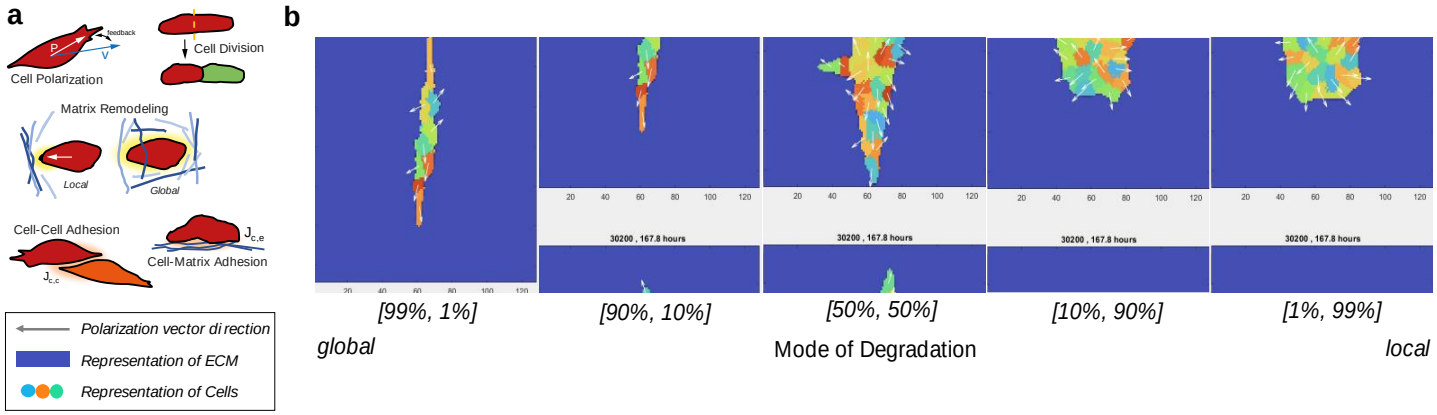


***Fig. 3*** *(a) Schematic representation of the cellular Potts model, which includes cell-cell and cell-ECM interaction terms. (b) Simulated phenotypes of increasing global (left) or local (right) matrix remodeling. Local matrix remodeling results in invasive structures, while global remodeling leas to rotating acinar structures.*

In addition we developed a stochastic model in which discrete particles, corresponding to the four experimentally observed phenotypes, are introduced into the computational domain at fixed rates (see **Fig. 4**). Using experimentally observed transition rates and cell speeds, we computed the fraction of different phenotypes as a function of the distance from the point of particle flux. We found that this fraction rapidly achieved equilibrium values, independent of the distance from this point. Furthermore, we found that the fraction of FP cells is larger at the leading edge than in the bulk, but at smaller values than found in the experiments. This suggests that different transition or speed rules apply at this leading edge.

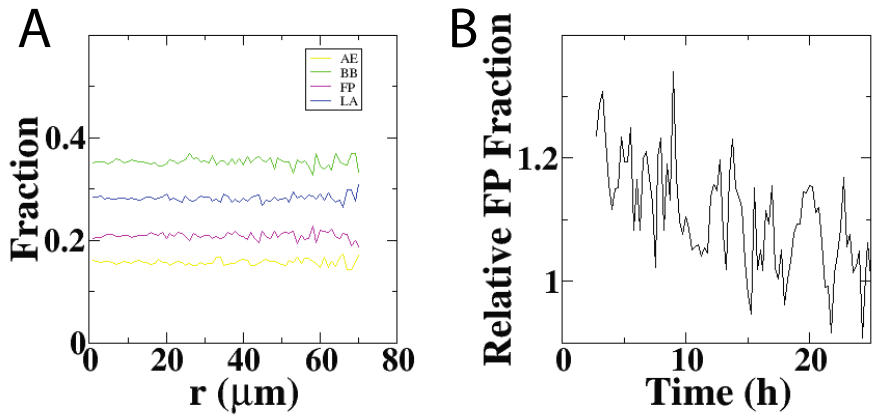


***Fig. 4*** *Results of a stochastic particle simulation. (A) Fraction of the 4 different phenotypes as a function of distance from the particle release point after 24 hours. (B) Relative fraction of FP cells at the leading edge of the invasive cell population.*

Major Task 4: To determine individual cell migrational mode transitions in disseminating tumor organoids

During the past reporting period, we made progress towards major task 4. In a manuscript under review (Eddy et al, **Facilitating cell segmentation with the Projection-Enhancement Network, Fig. 5**), we have developed a deep-learning based method to facilitate segmentation of individual cells at high cell density. The tool allows us to study migration phenotypes of breast cancer cells disseminating from tumor spheroids.

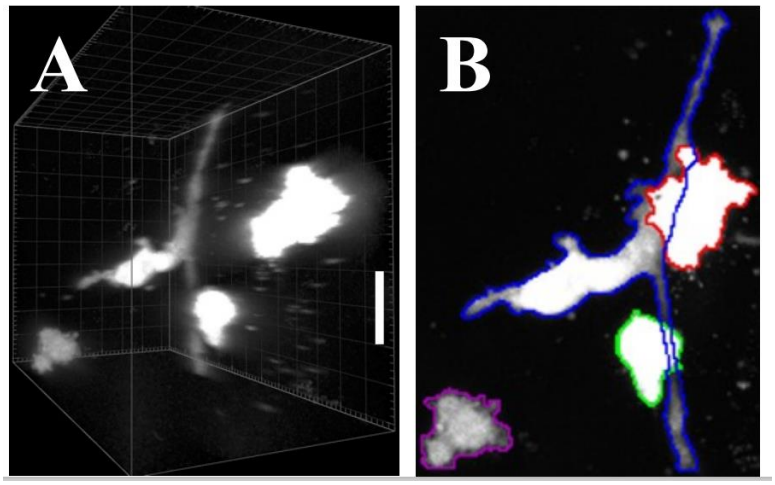


***Fig. 5*** *This figure shows closely packed breast cancer cells in 3D ECM (A) can be segmented using our deep-learning based algorithm Projection Enhancement Network (PEN), as shown in (B).*

Applying the PEN algorithms, we have studied the motility and migration phenotypes of breast cancer cells disseminating from tumor spheroids. As shown in **Fig. 6** We find that breast cancer cells rapidly leave the tumor spheroid and continuously invade in the 3D ECM. During the invasion process, cells make transitions between migration phenotypes. We have quantified the phenotype dynamics by computing the transition rates, which indicates typical transition happens once every two to four hours.

We also find the breast cancer cell motility depends on the phenotype. In particular, the radial step sizes follow logistic distributions, with the filopodial phenotype being the most invasive.

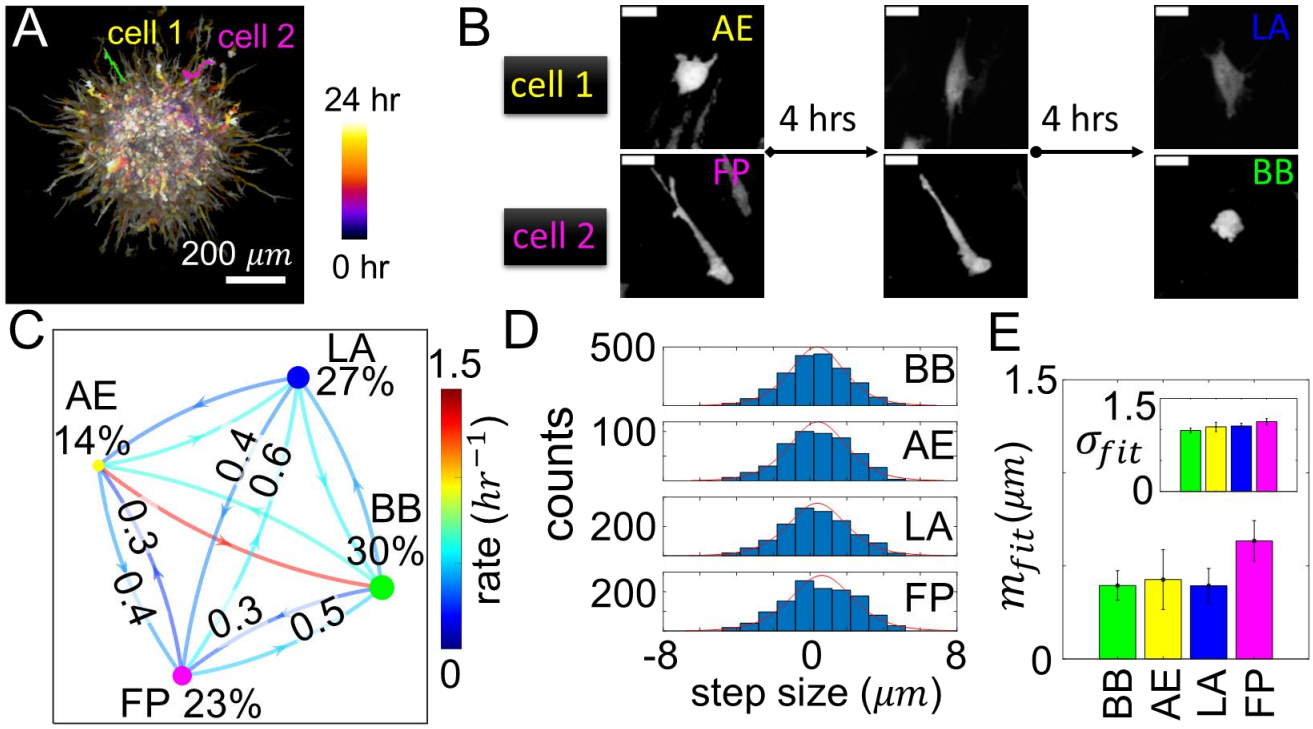


***Fig. 6*** *This figure shows breast cancer cells disseminating from tumor spheroids exhibit transitions between different migration phenotypes and phenotype-dependent motility. (A) Temporal projection showing the trajectories of disseminating breast cancer cells. (B) Two representative cells showing phenotype transitions as they invade into the surrounding collagen-based ECM. (C) The transition rates of single breast cancer cells between different phenotypes. (D) Histogram showing the radial step size distribution for each migration phenotype. (E) The histograms can be fitted by Logistic models with means $m$ and variances $\sigma$ that parameterize the motility.*

The spatial temporal map of phenotype probability of an invading tumor spheroid show that filopodial cells (FP) are enriched at the invasion front (**Fig. 7**). This implies that FP phenotype emerge as leader cells during the metastasis. Our results underscored the importance of fully characterizing the cancer cell migration phenotype in order to better understand invasion and metastasis.

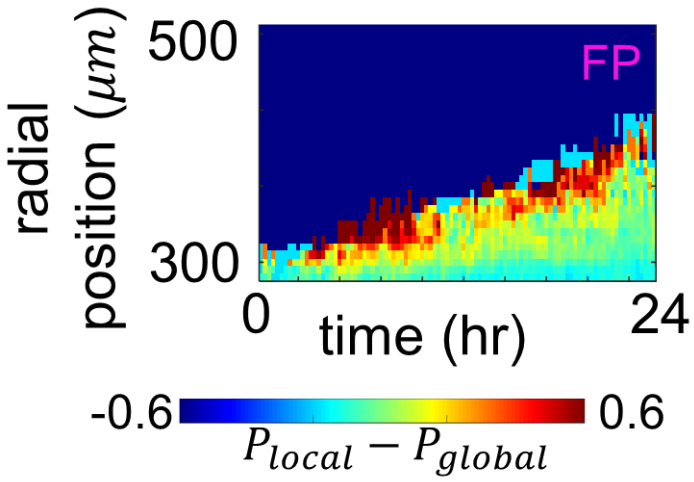


***Fig. 7*** *This figure shows the spatial-temporal evolution of breast cancer migration phenotype when disseminating from a tumor spheroid. The heatmap represents the elevated filopodial phenotype at the invasion front.*

Major Task 5: Development of a computational model for collective ECM remodeling and tumor organoid invasion

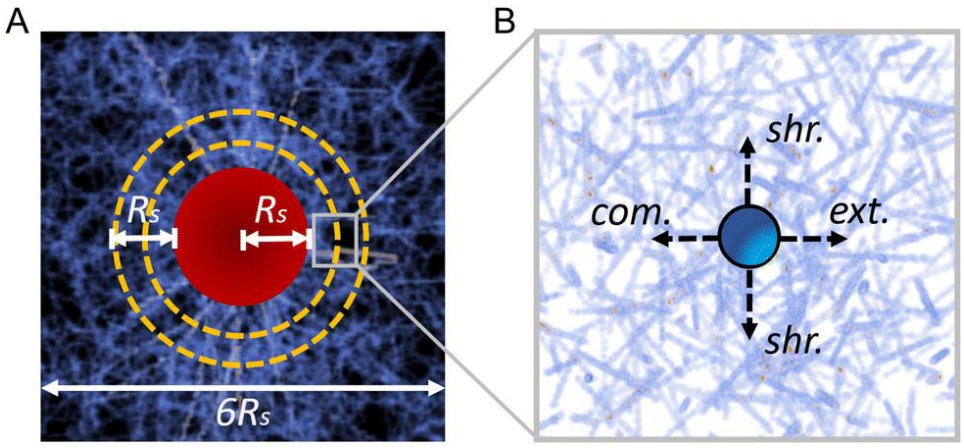


***Fig. 8*** *A computational model that incorporates a fibrous ECM network to examine the ECM micromechanical remodeling by an invading tumor. (A) A schematic showing the simulation representative volume element (RVE). (B) Micromechanics is quantified by measuring the directional compliance as in the experiments. In particular,* $J_{com}$, $J_{ext}$, *and* $J_{shr}$ *measure the compliances of the ECM in the directions toward the tumor, away from the tumor and sideways of the tumor.*

During the past reporting period, we have made progress on Major Task 5. In a published manuscript, (Naylor et al, **Micromechanical Remodeling of the Extracellular Matrix by Invading Tumors: Anisotropy and Heterogeneity**), we report an experiment-verified computational model of ECM remodeling by an invading tumor. We study the invasion of cancer cells in 3D fibrous ECM (**Fig. 8**), and quantify the local rigidity in compression (towards the tumor), extension (away from the tumor), and shear (sideway to the tumor) directions. We find that there are three independent processes, namely collective contraction, ECM degradation, and active pulling, all contributing to the ECM remodeling. In particular, ECM degradation by cells secreting matrix proteinase moderately softens the ECM moderately, but the pulling force from migrating cells may significantly stiffen the ECM. In all cases, the ECM is most stiff in the extension direction, and most soft in the compression direction, which elastic moduli decreasing by as much as 30% (**Fig. 9**). The results highlight the complex nature of ECM micromechanics and its remodeling by metastatic breast tumors.

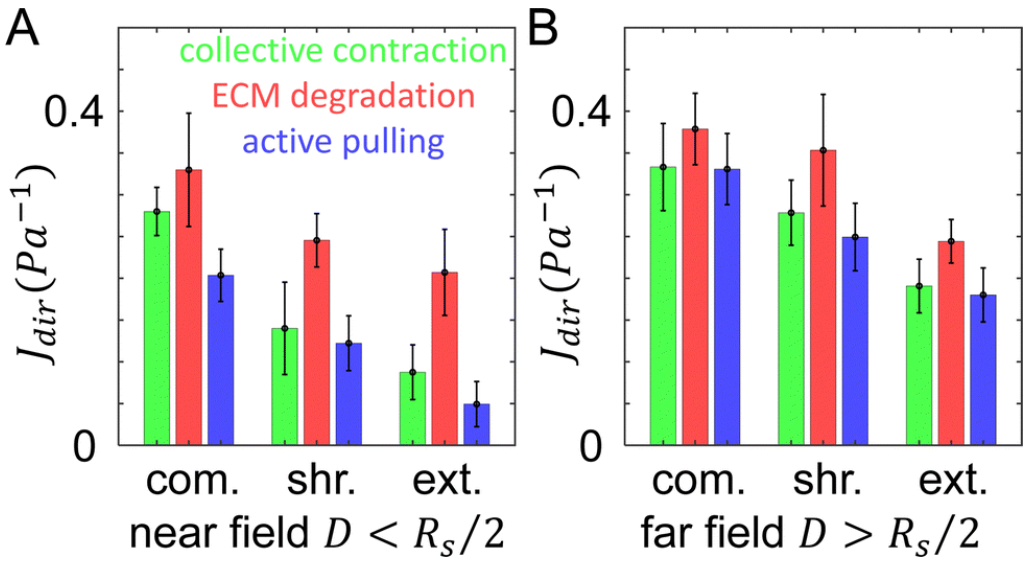


***Fig. 9*** *The directional compliance of the ECM from computational model simulations. (A) Directional compliance $J_{dir}$ along different directions in the near field with D < Rs/2, where D is the distance to the tumor boundary, and Rs is the radius of the tumor. (B) Directional compliance $J_{com}$ in the far field with D > Rs/2. Green: results by taking into account the collective contraction of the tumor (same as Fig. 5). Red: results when the tumor's collective contraction and ECM degradation are both considered. Blue: results when the tumor's collective contraction, ECM degradation and a nearby cell's active pulling are all considered.*

**What opportunities for training and professional development has the project provided?**

As part of this project, Dr. Pedram Esfahani, a graduate student turned to postodoc at Oregon State University was involved in the project. He was trained in conducting live cell experiments, extracellular matrix engineering and data analysis. He was trained in writing and presenting his scientific communication skills that helped him to defend his PhD thesis. Mr. Austin Naylor, currently a graduate student, is also involved in the project. He was trained in mechanical characterization of soft materials, data processing, and scientific presentation.

In addition, Dr. Elmi, a post-doctoral researcher at UC San Diego, was supported by this grant and was able to further develop her modeling and analysis skills. Finally, all participants were given the opportunity to improve their presentation skills during our group meetings.

**How were the results disseminated to communities of interest?**

Our study was reported in two publications: **Patterning ECM microstructure to investigate 3D cellular dynamics under multiplexed mechanochemical guidance**, Pedram Esfahani and Bo Sun, F1000Research 2022, 11:1071. and **Micromechanical Remodeling of the Extracellular Matrix by Invading Tumors: Anisotropy and Heterogeneity**, Austin Naylor, Yu Zheng, Yang Jiao and Bo Sun, Soft Matter, 2023, 19, 9 – 16 (also chosen as a cover story) In addition, one additional manuscript is currently under review: **Facilitating cell segmentation with the Projection-Enhancement Network**, Christopher z Eddy, Austin Naylor, Christian Cummingham and Bo Sun. Preprint of the manuscript is available at https://arxiv.org/abs/2301.10877 . In addition, Dr. Pedram Esfahani presented the research at invited seminars at the University of Chicago and Loyola University. Austin Naylor presented the research at Annual Meeting of American Physical Society in 2023. The title of his presentation was “Mechanosensing directs invasion and morphodynamics of spheroids”.

**Impact**

**What was the impact on the development of the principal discipline(s) of the project?**

Our results, reported in Esfahani et al, F1000Research 2022, provide insights into breast cancer cell metastasis in 3D extracellular matrices (ECMs) under physiological conditions where multiple external cues often exist simultaneously. Our results show a method to efficiently combine chemical gradient and ECM fiber alignment in 3D cultures. Our results demonstrate how cells integrate mechanical and chemical cues together to decide on the direction of motility. These results offer a generalizable experimental platform to understand and predict the metastasis of breast cancer cells in complex physiological environments.

Our result, reported in Naylor et al, Soft Matter 2023, provide insights into the ECM remodeling by metastatic breast tumors. Solid tumors, such as breast tumors, often significantly remodel the surrounding ECM. This is an important process that modulate the tumor growth, metastasis, and their interactions with immune cells. Our results show that the various biological functions of breast cancer cells, including active pulling, ECM degradation, and collective contraction each have distinct roles in remodeling the micromechanics of ECM.

Breast cancer cells and are known to exhibit multiple mode of migration, which poses significantly challenges to the treatment of metastasis disease. To facilitate studying the processing during multicellular tumor spheroid invasion, we have developed deep-learning based algorithm to segment the cells from confocal imaging. The segmented cells can then be classified into their corresponding migration phenotypes. The work is currently under review at Physical Biology.

In addition, we used a cellular Potts model to investigate the interactions necessary to produce emergent behaviors of individually seeded cancer cells. These cells are observed to form either cylindrical ductal tissues by invasive collective migration (ICM) or spherical acinar tissues by rotational collective migration (RCM). Our model simulations suggest that in 3D confinement, RCM and ICM emerge based on how cells localize their matrix remodeling activity, which can drive differences in cell polarization and protrusion dynamics. Quantitative microscopy experiments confirm that initial differences in cell protrusion length, lifetime, and rate are coupled to distinct matrix remodeling localization patterns that dictate cell shape, which propagates across cell division cycles and gives rise to RCM and ICM behavior. This study has been submitted to Nature Physics.

**What was the impact on other disciplines?**

Our project is highly muldisciplinary and involves, aside from cancer biology, the field of cell biology, computer vision and mathematical modeling. It will thus have an impact on these disciplines. For cell biology, for example, our experimental studies provide deeper insights how cell morphologies and cell migration are coupled to lead phenotype-dependent invasion. The insights can be applied to other motile cells such as fibroblasts and immune cells. For computer vision, our result provides a practical tool to detect individual cells from 3D image stacks with limited axial resolution. This tool can be applied to essentially any types of image stacks. In addition, our mathematical models are able to address morphological changes in migrating cells as well as the effect of matrix degradation factors. These models should be applicable to a wide variety of cell biology problems.

**What was the impact on technology transfer?**

Nothing to report

**What was the impact on society beyond science and technology?**

Nothing to report

## 4. Changes/Problems

**Changes in approach and reasons for change**

Nothing to report

**Actual or anticipated problems or delays and actions or plans to resolve them**

Due to the COVID-19 pandemic, the lab operation still encounters frequent disruption such as due to lab member being infected or exposed. In addition, many research supporting functionality, such as shipping of reagents, staff hiring, facility access, and equipment maintenance were occasionally impacted. As such, even with our best effort to address the challenges, we expect delays in the research output.
We are making our best efforts to prioritize the health of lab members while at the same time keeping research on pace. For instance, we have been working with multiple vendors to reduce delays of key experimental reagents. We have also been utilizing non-regular business hours to access key facilities.

**Changes that had a significant impact on expenditures**

Nothing to report

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

**Significant changes in use or care of human subjects**

Nothing to report

**Significant changes in use or care of vertebrate animals.**

Nothing to report

**Significant changes in use of biohazards and/or select agents**

Nothing to report

## 5. Products

**Publications, conference papers, and presentations**.

**Journal publications.**

Micromechanical Remodeling of the Extracellular Matrix by Invading Tumors: Anisotropy and Heterogeneity, Austin Naylor, Yu Zheng, Yang Jiao* and Bo Sun*, Soft Matter, 2023, 19, 9 – 16 *acknowledgement of federal support: yes*

Patterning ECM microstructure to investigate 3D cellular dynamics under multiplexed mechanochemical guidance, Pedram Esfahani and Bo Sun, F1000Research 2022, 11:1071*acknowledgement of federal support: yes*

## 6. Participants & Other Collaborating Organizations

**What individuals have worked on the project?**

**Participants at Oregon State University**

| | |
|---|---|
| **Name:** | Bo Sun |
| **Project Role:** | *PI* |
| **Researcher Identifier (e.g. ORCID ID):** | 0000-0001-7001-8781 |

| | |
|---|---|
| **Nearest person month worked:** | 1 |
| **Contribution to Project:** | Oversee overall project progress, analyze data, write manuscript, coordinate with collaborating labs |
| **Funding Support:** | DOD, NSF, NIH |

| | |
|---|---|
| **Name:** | Pedram Esfahani |
| **Project Role:** | Postdoc Scholar |
| **Researcher Identifier (e.g. ORCID ID):** | |
| **Nearest person month worked:** | 6 |
| **Contribution to Project:** | conduct experiment, analyze data, write manuscript |
| **Funding Support:** | DOD, NIH |

| | |
|---|---|
| **Name:** | Austin Naylor |
| **Project Role:** | Graduate student |
| **Researcher Identifier (e.g. ORCID ID):** | |
| **Nearest person month worked:** | 6 |
| **Contribution to Project:** | conduct experiment, analyze data, write manuscript |
| **Funding Support:** | DOD, NIH |

**Rappel personnel**

| | |
|---|---|
| **Name:** | Wouter-Jan Rappel |
| **Project Role:** | Collaborating PI |
| **Researcher Identifier (e.g. ORCID ID):** | 0000-0003-3833-7197 |
| **Nearest person month worked:** | 1 |
| **Contribution to Project:** | Dr. Rappel is the collaborating PI on the project and responsible for the modeling efforts |
| **Funding Support:** | DOD, NSF, NIH |

| | |
|---|---|
| **Name:** | Dorsa Elmi |
| **Project Role:** | Postdoctoral Scholar |
| **Researcher Identifier (e.g. ORCID ID):** | 0000-0002-9741-6816 |
| **Nearest person month worked:** | 11d |
| **Contribution to Project:** | Dr. Elmi is responsible for model development for cells in ECMs with varying composition |
| **Funding Support:** | DOD, NSF, NIH |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

**What other organizations were involved as partners?**

We collaborated the project with Dr. Herbert Levine from Northeastern University, Dr. Joe Gray from Oregon Health and Science University, and Dr. Stephanie Fraley from UC San Diego.

## 7. Special Reporting Requirements

## 8. Appendices