A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

Rayan Awais, Department of Mathematics
Mykhailo Potomkin, Ph.D., Department of Mathematics

ABSTRACT

The dynamics of epithelial cells during wound healing exhibit significant complexity, notably in their size-dependent behavior. This work aims to depict a fundamental mechanism underlying this size dependence in cellular dynamics by developing a computational model Our research question investigates how the physical size of epithelial cells influences their motility and behavior patterns, specifically during the epithelial-to-mesenchymal transition critical for wound healing. Thus, we propose a model where the key mechanism involves a field of spatially coupled forces acting on the cell membrane, driven by the dynamics of actin monomers. These monomers, randomly distributed within the cell, become focal points for membrane protrusions, thus influencing cell behavior. Our model succinctly captures the essence of size-dependent cellular dynamics without resorting to changes in gene expression patterns, offering new insights into the variations in cell behavior. Through this computational framework, we demonstrate that the diversity in cellular responses during wound healing can be fundamentally attributed to differences in cell size. The model's insights into the correlation between cell size and motility highlight how the physical properties of cells influence wound healing.

KEYWORDS: epithelial-to-mesenchymal transition, size-dependent dynamics, computational model, protrusions, computational software, wound-closure

FACULTY MENTOR - Dr. Mykhailo Potomkin, Department of Mathematics

Dr. Potomkin is an applied mathematician. His principal area of research is modeling, analysis and numerical simulation of problems involving differential equations (ordinary, stochastic and partial differential equations). His primary interest is on problems arising in Mathematical Biology and Soft Matter Physics.

RAYAN AWAIS

Rayan Awais is a second-year Mathematics major at the University of Riverside. He conducts mathematical computational research under Dr. Potomkin. Rayan has presented at the Undergraduate Research Symposium and was one of the youngest presenters at the American Mathematical Society conference in Washington, D.C. As a Mathematics Ambassador, he is deeply involved in academic and outreach activities. He aims to explore his full potential in mathematics.

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

INTRODUCTION

Biological background

Epithelial cells, commonly recognized as the building units of the body, play a role in establishing protective barriers [1]. They contribute significantly to maintaining tissue integrity and overall balance within the body. Epithelial cells serve as the building blocks for linings in organs and structures. Not only do they act as barriers against environmental hazards, but they also actively participate in numerous physiological processes. These processes include absorbing nutrients in the tract, facilitating gas exchange, and filtering substances in the renal system. Another role is their involvement in tissue and organ regeneration. In cases of injury or damage, these cells play a part in the body's response to restore tissue integrity.

This natural healing process is supported by a mechanism known as epithelial to mesenchymal transition (EMT), which signifies a shift from their usual stationary state to motile. Epithelial cells undergo EMT, enabling them to close wounds, including minor cuts on the skin's outer layer and more severe injuries. The intricate dance of cells during the healing process regulated by EMT remains an area of great scientific interest and exploration [1,2].

Wound healing requires epithelial cells to fill the gap caused by the wound as fast as possible. Besides simply proliferating, epithelial cells start exhibiting different behaviors depending on how close they are to the wound edge [3,4]. The cells on the edge experience dramatic stresses due to the difference between the wound interior and the opposite side filled by neighboring cells. Such cells tend to extend long protrusions (so-called lamellipodia) towards the wound center and become motile (through EMT) in the direction of their protrusions. These cells are regarded as "leaders." Cells, which are adhered to the leader, follow it and thus are called "followers." Leaders have a pancake-like shape; they are flat and occupy a much larger area than followers. It is unclear if leaders generate force and pull followers toward the wound center or if leaders and followers have the same level of motility and the leader just guides followers [5]. The entire cluster of leaders and followers migrates persistently in an appropriate direction to close the wound. The coexistence of leaders and followers is an example of when epithelial cells exhibit diversity in sizes, and the cell's dynamics strongly depend on its size: leaders, when not adhered to other cells, are almost stationary without significant variations in shapes, whereas individual followers oscillate and extend fast thin protrusions (so-called filopodia). It is worth noting that the proliferation rate of leader cells is much lower than follower cells [4].

The emergence of leaders and followers is associated with the specific setting of the tissue's front propagation during wound closure. However, other types of cellular configurations are of great importance. For example, when cells are at low confluency, they form small clusters or migrate individually at the early stages of tissue formation. In these cases, besides leaders and followers, individual cells of the intermediate area can be observed; that is, they are larger than followers but smaller than leaders [2]. Extensions for these cells are smaller than for followers, and shape variations are smooth. For example, when retracted, an extension turns into a new extension nearby, or an extension does not retract fully. Still, it starts moving along the cell boundary, thus showing a boundary traveling wave. Note that, on the one hand, all these cells—small followers, large leaders, and intermediate cells—originated from the same tissue, where all cells are densely packed and look the same [4].

On the other hand, in this hierarchy of cells, each "class" behaves differently and has a specific function in collective cell migration. Similar to how leaders' behavior acquires them a function of navigating the

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

repairing tissue, mechanical properties of intermediate cells may elucidate their role in collective cell migration.

Purpose of the Study

In this work, we aim to develop a theoretical framework via a computational model, providing a mechanical description of cells exhibiting different behaviors so that these behaviors depend on a single geometric parameter, such as the cell size. Conversely, all other parameters, including microscopic ones, remain across all cells. This uniformity ensures that any observed differences are due to the variable under investigation.

Typically, cell shape and motility dynamics are described as a subtle interplay between cell constituents, such as actin filaments, myosin motors, and adhesion proteins, as well as the elasticity of the cell membrane [6]. Motility models assume that actin filaments tend to grow (polymerize) at the leading edge by attaching actin monomers on their front, thus pushing the elastic membrane forward. We focus on monomers and polymers' actin dynamics to elucidate the size-dependent qualitative behavior. More precisely, we consider randomly distributed monomers along the cell body and assume that they tend to cause intensive actin polymerization and growth of an extension when the number of actin monomers near a boundary point exceeds a certain threshold. Finally, we note that epithelial cells transitioning to a motile state are located on the edge of a tissue or crawl individually. This work will focus on the dynamics of an individual cell that does not adhere to any other cell.

Relevance to Current Research

Our work is situated within the broader context of cell biology research, where understanding the mechanisms of cell movement is essential. By focusing on size dependence, this study addresses a gap in research that predominantly assumes that cell differentiation or external chemical cues can cause differences in the behavioral patterns of cells. Our computational model provides a complementary perspective, offering insights into the physical and mechanical aspects of cell motility that are influenced by size. This unique angle broadens the scope of current research and sets the wave for future investigations into how size and chemotaxis interact to govern cell behavior in complex biological processes.

METHODS

We employed a methodology encompassing Physics, Mathematics, and Computational Modeling principles to comprehend size-dependent dynamics for motile epithelial cells. In modeling the cell, we followed the concept of the Subcellular Element Method [7]. We consider a two-dimensional domain representing the apical view of a cell crawling on a substrate. The cell membrane is described by a finite sequence of nodes connected by elastic springs. These nodes with springs form a closed curve determining the cell's shape. Springs connect the neighboring nodes only; see Figure 1A. As time progresses, the cell maintains its initial area with minor fluctuations. This aspect of the model enables us to simulate cells of a specified area and investigate the impact of this area on the dynamics of cell shape. Further details on how we model cell shape and the mechanisms of cell motility through the polymerization of actin monomers are provided in the Methods section below.

Model of Cell Shape

For each node, we impose the force balance. Namely, the viscous drag force is balanced by the spring force, surface tension, area preservation force, and protrusion force. The balance equation results in an ordinary differential equation for each node, and the solution of this equation, if protrusion force is neglected, tends to minimize the elastic energy of each spring, surface tension energy, and the discrepancy between the current

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

area of the cell and the initial one:

$$r_i'(t) = -\nabla_{r_i}(\mathcal{E}_{spring} + \mathcal{E}_{s.tension} + \mathcal{E}_{area}) + F_{protr}. \quad (1)$$

Here, $\mathcal{E}_{spring} = k_{spring}(|r_i(t) - r_{i1}(t)| - L_0)^2 + k_{spring}(|r_i(t) - r_{i2}(t)| - L_0)^2$ is the energy of two springs from both sides of the node r_i (indexes for two neighboring nodes are denoted by $i1$ and $i2$, see Figure 1A) and L_0 is the equilibrium spring length. The number of nodes is denoted by N_{mem}. The surface energy is defined as $\mathcal{E}_{s.tension} = k_{tension}\sum_{i=1}^{Nmem}\cos(\varphi_i)$ and φ_i is the angle formed by the two springs at the node r_i, and the area preservation energy is given by $\mathcal{E}_{area} = k_{area}(A(t) - A_0)^2$, where $A(t)$ is the cell area at time t and A_0 is the initial area of the cell. Model parameters k_{spring}, $k_{tension}$, k_{area}, L_0, and A_0, are calibrated so that numerical simulations are stable and observable dynamics is biologically reasonable. The force F_{protr} comes from protrusion, as described below.

When protrusive forces are disregarded, $F_{protr} = 0$, the minimization of total cellular energy leads to the convergence of the cell shape toward its equilibrium state, which is a circle. Specifically, the energy term $\mathcal{E}_{spring}$ penalizes deviations in the cell shape when the distance between adjacent nodes differs from the equilibrium spring length L_0. By minimizing the energy, $\mathcal{E}_{s.tension}$, the cell membrane tends to rectify at each node. Given that the membrane is a closed curve, a circular shape minimizes the energy term $\mathcal{E}_{s.tension}$. Additionally, the third energy term in equation (1), $\mathcal{E}_{area}$, acts to maintain the cell's initial area.

The dynamics described by equation (1) emerge from the interplay among these three energy minimization processes. For instance, if there is a disparity between the radii of the circle with the perimeter $N_{mem} \cdot L_0$, favored by the minimization of $\mathcal{E}_{spring}$, and the circle with the area A_0, favored by $\mathcal{E}_{area}$, the actual radius is determined by the coefficients k_{spring} and k_{area}. For example, when $k_{spring} >> k_{area}$, the minimization of $\mathcal{E}_{spring}$ dominates the minimization of $\mathcal{E}_{area}$, and the equilibrium radius is close to $(2\pi)^{-1}N_{mem}L_0$.

Model of Protrusions

We model cell protrusions extended due to an internal activity as follows. We track individual free actin monomers inside the cell (see green dots in Figure 1B). These monomers must be understood as coarse-grained since the number of monomers exceeds the one we use in the model by several orders of magnitude. A protrusion is formed if monomers are randomly concentrated at a membrane node (see Figure 1B). Namely, denote the number of monomers about r_i by $N_{m,i}$. We call corresponding monomers adjacent to the membrane node r_i (depicted as black dots in Figures 1B and 1C). Then, the condition for the formation of a protrusion r_i is $N_{m,i} > N_{threshold}$, where $N_{threshold}$ is the threshold value for the number of adjacent monomers. The formation of protrusion is incorporated in the modeling equations (1) via the term F_{protr}, which is non-zero when the condition $N_{m,i} > N_{threshold}$ is satisfied. The force F_{protr} is directed along the outward normal, and its magnitude and duration time values are chosen to resemble biologically relevant behavior. In addition, we model that adjacent monomers are attracted by the corresponding membrane node, preventing them from participating in protrusions elsewhere. When the protrusion is retracted, all monomers are pushed back inside the cell. All monomers not engaged in a protrusion exhibit a random walk confined by the polygon generated by membrane nodes. The formation of protrusions, represented by the term F_{protr} in Equation (1), serves as an active component of the system, actively maintaining the cell in a state away from equilibrium.

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

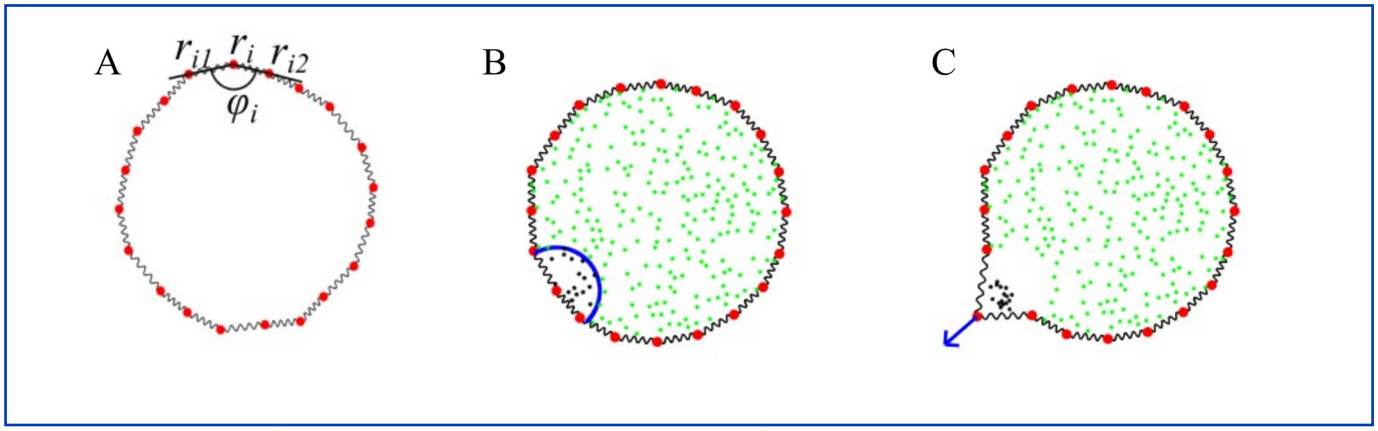

Figure 1. Illustration of the model setup. (A). Representation of cell shape as a polygon whose vertices are membrane nodes. Each side of the polygon is modeled as a spring and depicted as a zig-zag-shaped segment. If no Fprotr is exerted, Equations (1) result in convergence of the polygon to the regular one as time evolves, and, if the number of membrane nodes is very large, to a circle (B). Representation of cell with monomers (black and green isolated dots) and no protrusions. Each time step, the number of monomers at the vicinity of a membrane node is measured; the boundary of the vicinity is indicated as a blue arc and the corresponding monomers are black isolated dots (C).Illustration of protrusion formation, blue arrow depicts the force F_{protr}.

Implementation of the Computational Model

The model is developed using MATLAB (code is 367 lines). Ordinary differential equations in (1) are solved with the Forward Euler Method with a sufficiently small time step. The area of the cell $A(t)$ is calculated using the shoelace formula. The Brownian dynamics of the monomers are modeled as random jumps with standard deviation,$\sqrt{2D \cdot dt}$) for each time step dt, with the condition that a monomer does not exit the cell's boundary during these movements. As noted earlier, the values of the model parameters are carefully selected to ensure the stability of the computational code and to accurately reflect dynamics observed in various experimental and theoretical studies [2,6,7]. These values can be further calibrated using experimental data.

RESULTS

We numerically simulated cells of three different sizes. Specifically, we initialized cells as circles so that membrane nodes form a regular polygon, and we consider radii R=1.0, R=1.2, and R=1.6 corresponding to small, intermediate, and large cells, respectively. At the same time, we keep the number of membrane nodes N_{mem} and the number of free monomers N_{mon} the same for all radii, we adjust the spring equilibrium lengths according to the following equation:

$$L_0 = 2R \sin(2\pi/N_{mem}) . \quad (2)$$

Formula (2) is derived from representing the cell as a regular polygon with N_{mem} vertices so that R is the radius of the circumcircle of the polygon and L_0 is the side length. Monomers are generated randomly and uniformly inside the cell.

Representative cell dynamics obtained from the results of numerical simulations are presented in Figure 2. The small cell (R=1.0, see the first row in Figure 2) exhibited single or multiple protrusions at various distances from each other. When a protrusion is retracted, the next one may extend at a random point along the membrane. As we increase cell radius R and consider an intermediate cell (R=1.2, see the second row in Figure 2), correlations in protrusion locations become more evident. For example, the cell in the second row in Figure 2 extends three protrusions side-by-side in the

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

presented images. However, when we consider large cells (R=1.6, see the third row of Figure 2), protrusions are less frequent; they are usually single at each given frame and extend at random membrane locations. The decrease in correlations is because these correlations do not have time to preserve before the subsequent protrusion starts extending. Since the initial locations of monomers and their dynamics are random, we simulated the model with many realizations of initial conditions. The qualitative behavior was consistent throughout the realizations and the description of Figure 2 above.

Statistical properties of cell dynamics were also investigated. First, the probability of having a protrusion (or, equivalently, the protrusion frequency) decreases as we increase the cell size, see Figure 3A. It can be explained by that the number of monomers N_{mon} is the same for all cell sizes, implying that a larger cell will have a lower monomer density and, thus, a smaller number of protrusions. Similarly, the number of monomers protruding the membrane decreases with the cell size, see Figure 3B. Note that, though the protrusion frequency and the number of engaged monomers are strongly related, these two quantities have no one-to-one relation. To study the spatial distribution of protrusions, we computed the average cosine of the angle between two consecutive protrusions, $[\cos \Delta\varphi_{protr}]$. This quantity is close to 1 if protrusions tend to appear next to each other, and this quantity is close to -1 if protrusions appear on opposite sides of the cell. We see in Figure 3C that dependence on cell size is not monotonic: $[\cos(\Delta\varphi_{protr})]$ is positive for R=1.0, and then it becomes negative for R=1.2, slightly increasing for R=1.6. This observation supports the idea that even in a simple mechanical model, the behavioral patterns of a cell (and thus its role in cell migration) may be significantly altered by the change in a macroscopic parameter such as cell size. However, further research is necessary to uncover the correlations between successive protrusions and how these correlations depend on cell size. It is conceivable that there exists an optimal radius, R, with other parameters held constant, at which these correlations are maximized, leading to protrusions that emerge adjacent to each other, $[\cos(\Delta\varphi_{protr})] \approx 1$. Indeed, small cells with $R \ll 1$ tend to exhibit numerous protrusions, resulting in weaker correlations between protrusion locations. Conversely, in large cells with $R \gg 1$, the coupling between protrusions is diminished. This weakening of coupling is due to the constant number of monomers spread over a larger area, thereby reducing their concentration and, consequently, the correlations among protrusions.

Finally, the mean square displacement of the cell center was computed, see Figure 4. It was confirmed that a smaller cell moves faster than a larger one, similar to how smaller followers move faster than larger individual leaders [4].

In summary, the model demonstrates that smaller cells are more dynamic, exhibiting numerous protrusions and tending to displace their centers to a greater extent compared to larger cells. In contrast, larger cells remain closer to their equilibrium state, displaying minimal protrusion dynamics. This observed behavior aligns with the dynamics of isolated leader and follower cells in Madin-Darby Canine Kidney cells [3,4,5] which inspired this research.

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

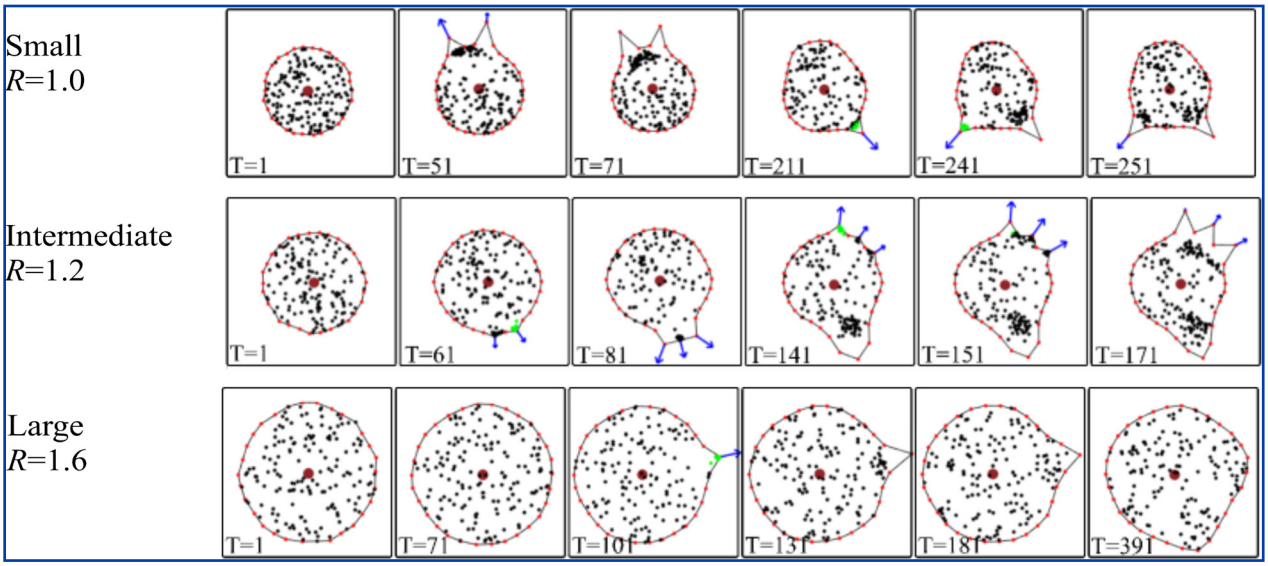

Figure 2. Cell dynamics obtained from numerical simulations for various values of radius *R*. Six subfigures in each row present the cell at different time instances (indicated in subtitles). Black and green dots represent free and engaged monomers, respectively. Red dots depict membrane nodes, and they are linked by black segments representing the cell boundary. Purple arrows are the protrusion active force. The large red dot represents the geometric center of the cell.

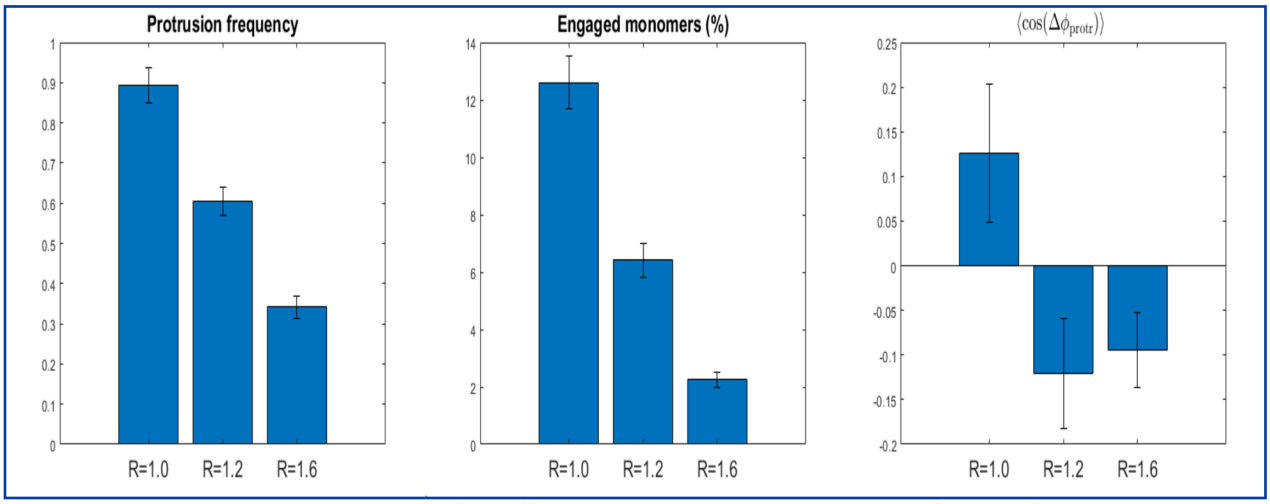

Figure 3. CBar graphs on cell activity for different sizes. Simulations were performed for 500 cells. (A). Protrusion frequency is defined as the number of existing protrusions per a time step. (B). Number of monomers engaged in protruding membrane (depicted in Figures 2, 3, and 4 as green dots). (C). The average cosine of the angle between two consecutive protrusions. The angle is computed with respect to the cell center.

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

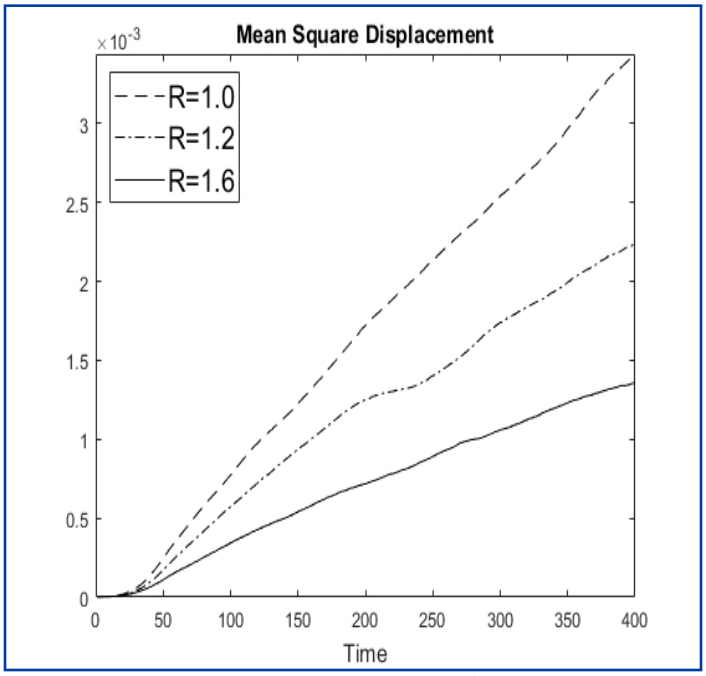

Figure 4. Mean square displacement of the cell center as a function of time. Three graphs correspond to various values of the cell radius, as indicated in the legend.

DISCUSSION

Cell motility results from very complex processes, including actin network formation, myosin dynamics, microtubule reorganization, and binding of adhesion bonds with the extracellular environment [8]. Additionally, a cell may alter its behavioral patterns in response to external chemical or mechanical cues [9,10]. However, cells may exhibit various behaviors even when no external perturbation is present, like leader and follower epithelial cells where isolated leaders are large (compared to follower cells) and passive and followers are active with a higher division rate [4]. It is known that epithelial cells, even within a single cluster, may be significantly different in size [11]. In this work, we hypothesized that the cell size may impact the geometric cell behavior. We confirmed this hypothesis by employing computational modeling. Our model utilizes actin network polymerization with freely floating monomers as the primary mechanism of protrusion formation in the spirit of a well-established modeling approach for actomyosin-driven cell motility [6,12]. Monomers engaged in actin network formation generate random forces on the cell boundary in our model. In this project, we assumed that these forces come from the dynamics of free actin monomers. Other intracellular processes may contribute to generating similar force fields, either related to the dynamics of myosin motors or cell-substrate adhesion. However, regardless of the biological origin of the force field, we believe that the size-dependent behavior is observed under two necessary conditions on the force field: the size-independent magnitude and spatial coupling. Here, the former condition is implemented by imposing that the number of monomers is constant throughout all sizes, and the latter one, spatial coupling, is through redistribution of monomer density, which deviates from the initial uniform configuration as time evolves.

Our model does not capture the rotation or traveling waves of intermediate-size cells observed in experiments and detailed computational models [13]. This is due to the symmetry of protrusions in the current model. As a future direction, we plan to incorporate a symmetry-breaking mechanism in the dynamics of an extension, which will allow for a preferred direction, clockwise or counterclockwise, and propagation of traveling waves along the cell's boundary. Finally, we plan to simulate the dynamics of heterogeneous cell clusters, where cells have different behaviors, and this difference originates from different cell sizes. Heterogeneity may benefit cell migration (thus wound closure) since different cells may acquire different functions useful for cell cluster navigation, chemotaxis, signaling, and cell activation [4].

The implications of our research are twofold. Firstly, it underscores the potential of intracellular processes beyond actin monomer dynamics to describe cell motility through similar force fields. It opens avenues for exploring the roles of myosin motors and cell-

substrate adhesion in future models. Secondly, our work highlights the necessity of incorporating mechanisms to break symmetry in extension dynamics, enabling the simulation of more complex behaviors like cell rotation and traveling wave propagation.

ACKNOWLEDGMENTS

I extend my heartfelt gratitude to Professor Mykhailo Potomkin from the Department of Mathematics at the University of Riverside for his invaluable support and guidance throughout this research. His expertise and encouragement have been instrumental to my work. Thank you, Professor Potomkin, for your unwavering dedication.

REFERENCES

[1] Freshney, R.I. and Freshney, M.G., eds. (2002). *Culture of Epithelial Cells* (Vol. 195). New York: Wiley-Liss.

[2] Vu, R., Dragan, M., Sun, P., Werner, S., and Dai, X. (2023). Epithelial-Mesenchymal Plasticity and Endothelial–Mesenchymal Transition in Cutaneous Wound Healing. *Cold Spring Harbor Perspectives in Biology*, 15(8), a041237.

[3] Omelchenko, T., Vasiliev, J.M., Gelfand, I.M., Feder, H.H., and Bonder, E.M. (2003). Rho-dependent formation of epithelial "leader" cells during wound healing. *Proceedings of the National Academy of Sciences*, 100(19), pp.10788-10793.

[4] Kozyrska, K., Pilia, G., Vishwakarma, M., Wagstaff, L., Goschorska, M., Cirillo, S., Mohamad, S., Gallacher, K., Carazo Salas, R.E., and Piddini, E. (2022). p53 directs leader cell behavior, migration, and clearance during epithelial repair. *Science*, 375(6581), p.eabl8876.

[5] Campanale, J.P. and Montell, D.J. (2023). Who's really in charge: Diverse follower cell behaviors in collective cell migration. *Current Opinion in Cell Biology,* 81, p.102160.

[6] Barnhart, E., Lee, K.C., Allen, G.M., Theriot, J.A., and Mogilner, A. (2015). Balance between cell-substrate adhesion and myosin contraction determines the frequency of motility initiation in fish keratocytes. *Proceedings of the National Academy of Sciences*, 112(16), pp.5045-5050.

[7] Nematbakhsh, A., Sun, W., Brodskiy, P.A., Amiri, A., Narciso, C., Xu, Z., Zartman, J.J., and Alber, M. (2017). Multi-scale computational study of the mechanical regulation of cell mitotic rounding in epithelia. *PLoS Computational Biology*, 13(5), p.e1005533.

[8] Okeyo, K.O., Adachi, T., and Hojo, M. (2010). Mechanical regulation of actin network dynamics in migrating cells. *Journal of Biomechanical Science and Engineering*, 5(3), pp.186-207.

[9] Kim, M.C., Neal, D.M., Kamm, R.D., and Asada, H.H. (2013). Dynamic modeling of cell migration and spreading behaviors on fibronectin coated planar substrates and micropatterned geometries. *PLoS Computational Biology*, 9(2), p.e1002926.

[10] Rangarajan, R. and Zaman, M.H. (2008). Modeling cell migration in 3D: Status and challenges. *Cell Adhesion & Migration*, 2(2), pp.106-109.

[11] Bermudez, A., Negrete Muñoz, S., Blaik, R., Rowat, A.C., Hu, J., and Lin, N.Y. (2023). Using histologic image analysis to understand biophysical regulations of epithelial cell morphology. *The Biophysicist*.

[12] Ziebert, F. and Aranson, I.S. (2014). Modular approach for modeling cell motility. *The European Physical Journal Special Topics*, 223(7), pp.1265-1277.

[13] Nickaeen, M., Novak, I.L., Pulford, S., Rumack, A., Brandon, J., Slepchenko, B.M., and Mogilner, A. (2017). A free-boundary model of a motile cell explains turning behavior. *PLoS Computational Biology,* 13(11), p.e1005862.