# Modeling Persistence in Mesenchymal Cell Motility Using Explicit Fibers

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ABSTRACT: Cell motility is central to a variety of fundamental processes ranging from cancer metastasis to immune responses, but it is still poorly understood in realistic native environments. Previous theoretical work has tended to focus on intracellular mechanisms or on small pieces of interaction with the environment. In this article, we present a simulation which accounts for mesenchymal movement in a 3D environment with explicit collagen fibers and show that this representation highlights the importance of both the concentration and alignment of fibers. We show good agreement with experimental results regarding cell motility and persistence in 3D environments and predict a specific effect on average instantaneous cell speed and persistence. Importantly, we show that a significant part of persistence in 3D is directly dependent on the physical environment, instead of indirectly dependent on the environment through the biochemical feedback that occurs in cell motility. Thus, new models of motility in three dimensions will need to account for the effects of explicit individual fibers on cells. This model can also be used to analyze cellular persistence in both mesenchymal and nonmesenchymal motility in complex three-dimensional environments to provide insights into mechanisms of cell motion seen in various cancer cell types in vivo.

Two-Dimensional

Three-Dimensional With Implicit Matrix

Three-Dimensional With Explicit Matrix
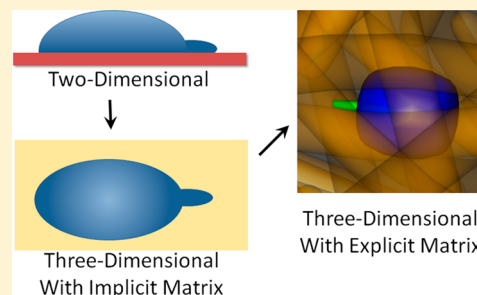
INTRODUCTION

Cell motility plays a vital role in many physiological systems, ranging from wound healing to tumor metastasis. The complexity of the processes underlying motility and the need for quantitative understanding necessitate computational modeling to understand the processes which can be difficult to study experimentally. Most modeling efforts of cell motility have unfortunately tended to focus on intracellular mechanisms[1,2] or on cells in artificial two-dimensional environments. These models generally focus on the environment's effects on internal cell signaling and tend to ignore the direct effects of the cell's environment on motility. Without such factors, we cannot see the entire picture of cell motility in vivo. Cells in vivo, instead of moving on a flat surface, move along, around, and through a three-dimensional mesh of extracellular matrix fibers. The role of these fibers in mediating and regulating cellular motion in vitro and in vivo has been underscored through a variety of recent experimental studies.[3−6]

Even with this knowledge, it is difficult to recognize which aspects of the three-dimensional matrix need to be incorporated into a model. The earliest models included an averaged mesh and the overall cellular behavior seen in experiment.[7−9] Later models included some matrix detail, e.g., a randomly arranged set of matrix fibers a cell could move around.[10] Few, however, included one of the most important details: cells do not simply move from one space adjacent to the matrix to another nearby space adjacent to the matrix. Needing to actually take the necessary steps to get from point A to point B, cells exert forces on the matrix, reaching out with lamellipodia and pulling themselves forward on a matrix fiber. This is modeled to some effect in recent work,[11] but in this case

another important detail of the fiber matrix is missing; even in cases with an overall average fiber alignment, some randomness to the alignment and spacing between fibers is natural. One especially important case is when the average size of empty spaces (pores) in the matrix is comparable to the smallest space a cell can easily squeeze through, and in this case, realism means one random direction will be easily traversable while another will require significant effort. Given the importance of directionality in cell movement, effective modeling cannot be performed without both randomness in matrix fiber alignment and a realistically moving cell.

Explicit modeling of these important aspects of the matrix allows for detailed models of cells to bridge the gap between the scale of intracellular mechanics and that of the cell–environment interaction properly. Indeed, even modeling these aspects of the matrix in a two-dimensional approximation of a three-dimensional system gives rise to interesting behavior.[12] Additionally, even without a detailed cellular model we can determine significant effects of the matrix environment on motile cells in general.

As has been seen in experimental work[13,14] on mesenchymal cell motility, cells pull themselves by exerting forces via lamellipodia, meaning that cells frequently move along the length of fibers they are already attached to. That cells move along the length of matrix fibers is an important consideration. A cell's ability to turn is limited in a realistic three-dimensional fiber matrix; it may go forward along a fiber, backward along

the same fiber, around the circumference of that fiber, or along one of a small number of other fibers the cell is in contact with. These options may not seem limiting, but depending on the number and alignment of other fibers, there could be severe restrictions on viable directions. Once multiple directions are allowed, the cell also must deal with collagen fibers being physically in its way, a phenomenon called steric hindrance. Second, when a cell passes through a matrix environment, it rearranges that environment: specifically, it removes some fibers and places aligned fibers in the direction it travels.[15] In this way, a single cell moving through an extracellular matrix leaves a path for other cells, since other cells will follow the collagen the first cell lays down. While these phenomena have been observed experimentally, even the basic effects of fiber alignment have not truly been considered in modeling. Our model, presented here, bridges this gap in our understanding and focuses on persistence in cell migration in three dimensions. Our model considers the effects of the cell's fiber environment on cell motility based on a simple but realistic general mesenchymal cell motility model.

■ MODEL

We modeled the three-dimensional extracellular matrix as a set of randomly placed elastic rods roughly as per previous work[16] but placed around a cell. These rods could not overlap each other or be deformed other than bending according to the elastic rod force—distance relationship. The environment was given repeating boundary conditions. Each fiber had a radius of 4 μm, corresponding to a rough average in 2 and 3 mg/mL collagen environments.[10] We modeled enough collagen fibers to fill 15% of the volume to represent 2 mg/mL gels and enough to fill 23% of the volume to represent 3 mg/mL gels.

Our cell model was intended to be as simple as possible while containing the most important details and was also intended to be generic for broad application. Therefore, quantities specific to cell type were tested for their effects on the eventual results. The cell was modeled as a short (6 μm length, 8 μm radius) elastic cylinder with hemispherical ends of the same radius, with a lamellipodium modeled as a thinner (1 μm radius, variable length) cylinder with a hemispherical end protruding from the center of the cell's front hemisphere. In agreement with previous work, cell adhesion to the matrix was modeled with slip-bond dynamics,[17,18] and any forces applied to the adhesions were assumed to be spread over a large number (100) of integrins. Several of these blocks of detachable integrins were modeled in both the cell rear and the lamellipodium. The cell's directional orientation was defined as the direction from the rear to the front of the cell. Lamellipodium movement was modeled as Gaussian, distributed in three dimensions but biased toward the cell's orientation vector on the millisecond scale, as per recent measurements of lamellipodium force and distance.[19] The retracting lamellipodium was modeled in the same way, but instead of being directed away from the cell, it was biased toward the cell front.

The precise number of integrins over which the force was spread, the number of blocks of integrins, and the numerical force values had an effect on the eventual absolute cell speed but no apparent effect on the relative speed or persistence length (data not shown). Unsurprisingly, removing the preference for free lamellipodium movement toward the direction of the cell's orientation resulted in no apparent persistence at all (data not shown). Because of the removal of

cell-specific numbers, the time units are semiarbitrary; for highly motile cells, a single time unit could reasonably be 15–30 min, while for much less motile cells it could be as large as 90 min or perhaps even higher.

In order to analyze only the effect of fiber concentration and alignment, the current model does not incorporate any explicit MMP activity, though effects of matrix deposition and decreases in fiber concentration over time by the cells can be analyzed. In order to keep the dependence on MMPs low, we compare our results to experimental scenarios where a cell can move without significantly degrading the matrix. These include experimental studies where MMPs have been explicitly blocked or the expression level is low[3,10] or environments where cells do not need to degrade the environment for motility. In these environments, migration is based largely on cells moving along, deforming, and aligning fibers rather than actively degrading them. Our model is able to quantify and reproduce cellular behavior in these environments with explicit fibers.

■ RESULTS AND DISCUSSION

First, we present average cell motility data in multiple environments: a low-concentration randomly aligned collagen gel, a high-concentration randomly aligned collagen gel, and a low-concentration highly aligned gel. Our results are shown in Figure 1. A straight line (d(MSD)/dt constant) implies random motion, an upward-curving line implies persistence, and a downward-curving line implies impeded motion.
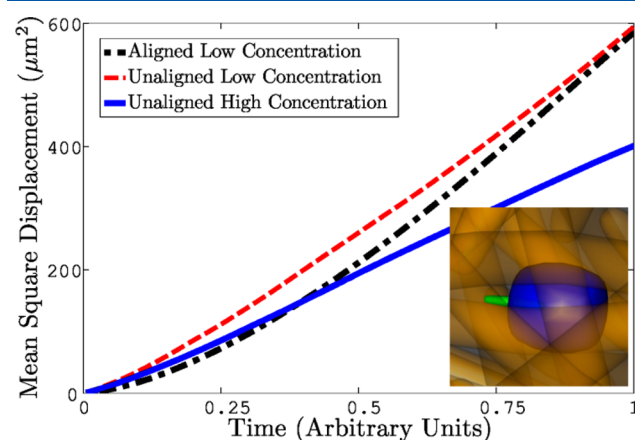

Figure 1. Mean square displacement over time lag of a simulated cell in aligned 2 mg/mL collagen, unaligned 2 mg/mL collagen, and unaligned 3 mg/mL collagen. MSD is averaged over at least seven simulations for each line, and each run lasted at least 8 time units. Inset: cell in a simulated unaligned collagen gel.

We have also used more traditional fitting to equations relating to ideal persistences,[20] but because none have any means of accounting for impeded motion, none fit the data as well as we feel necessary. We argue that the curve of the line is a reasonable persistence measure in this case. For reference, the most traditional persistence fit for speed S, time lag τ, and persistence time P, via the equation MSD = $3S^2P[\tau - P(1 - e^{-\tau/P})]$, would always curve upward for positive P, but the second derivative of MSD with respect to time would decay as $e^{-\tau/P}$. Fits to this ideal persistence time yield 0.60 ± 0.02 time units for the aligned case with an R^2 of 0.998, 0.11 ± 0.01 time units for the unaligned low-concentration case with an R^2 of

0.998, and 0 with an R^2 of 0.996 for the unaligned high-concentration case.

Fits to the ideal persistence length required using the fundamental equation for persistence length, $\langle \cos \theta \rangle = e^{-L/P}$, where the cosine in this case is averaged, not over time but over the spatial track of the cell for a set of lengths L, and then fit to the equation to yield persistence length P. These fits yield 334 ± 28 μm for the aligned case with R^2 of 0.66, 27.7 ± 2.2 μm for the unaligned low-concentration case with an R^2 of 0.73, and 7.6 ± 1.7 μm for the unaligned high-concentration case with an R^2 of 0.49. The poor fits (especially in the high-concentration case) are clearly related to persistence measures that lack the ability to deal with both persistence and impeded motion.

In Figure 1, the high-concentration gel partially impedes the cell, but in addition to impeding, it also provides more alternate pathways along which the cell can move than a low-concentration gel does. By both steric hindrance and the addition of choices of direction, the higher-concentration gel makes cell motility slower—not by decreasing the instantaneous speed $(((d(MSD))/(d\tau))^{1/2}$ at time lag 0), but by decreasing the persistence. In contrast, the aligned low-concentration matrix slightly decreases the relative speed but increases the persistence (both in time and in length) significantly. We suggest that the reduced instantaneous speed is due to the fact that the lamellipodium takes longer to search a mostly empty area directly in front of the cell in the aligned case. The increase in persistence corresponds well to experimental observations;[21] the difference in instantaneous cell speed has not yet been observed in experiments.

Modeling our cells for a longer time period (Figures 2 and 3) further highlights the difference between the unaligned and
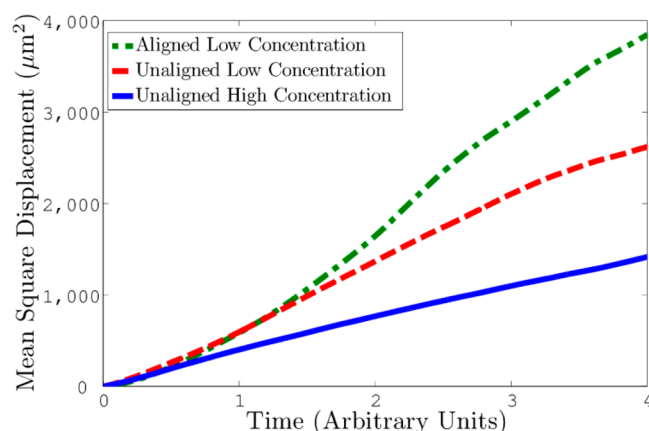

Figure 3. Second derivative of the mean square displacement with respect to time lag. High values imply persistence; zero values imply random motion; negative values imply impeded motion (also known as subdiffusive motion, due to steric hindrance). The second derivative of a straightforward fit of the cell in the aligned high-concentration matrix to a persistence-time equation is also displayed. This simplistic fit is incapable of properly accounting for steric hindrance.

Figure 2. Mean square displacement for longer time lag periods, highlighting the persistence difference in the aligned collagen. Again, MSD is averaged over at least seven simulations for each line, and each run lasted at least 8 time units (but provided poor enough statistics at high time lag that the inclusion of high time lag data points would be misleading).

aligned matrixes. The cell in the low-concentration unaligned matrix, after approximately a cell length, loses much of its persistence. In the aligned matrix, however, the cell continues being persistent for multiple cell lengths. Figure 3 shows the second derivative of MSD with respect to time lag, further demonstrating the persistence behavior.

It is important to note that the modeled cell is precisely the same in all environments. Thus, the effects seen are purely based on the physical environment of the cell, not on any
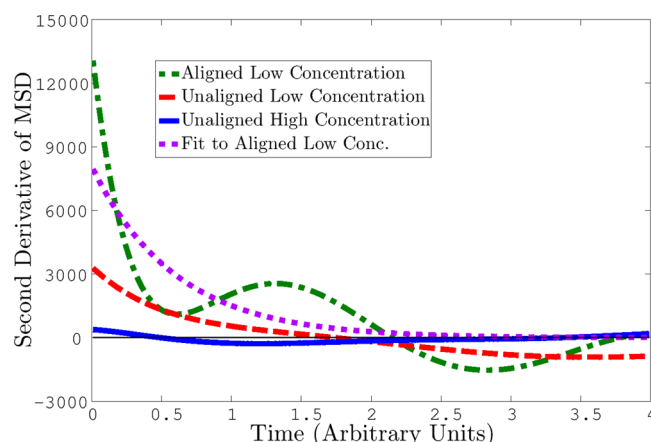
intracellular mechanisms that differ based on the physical environment of the cell. The distinction is important but almost impossible to see in a normal cell without modeling work. We find, then, that differences seen during experiments in apparent cell motility behavior are not necessarily due to the cell's internal signaling response to its environment; the difference in the environment can itself cause significantly different overall behavior without changing integrin concentrations, actin dynamics, or any such quantities which could be looked for via biochemical markers. Models of cell motility which include cell-type-specific intracellular details will therefore need to incorporate an explicit fiber matrix or the results of this model in order to produce reasonable persistence behavior.

CONCLUSIONS

Our simulation uses basic concepts of mesenchymal cell motility and the geometry of the cell environment to yield persistence data which corresponds well to experiment. This suggests that a large part of the persistence seen experimentally is directly dependent on steric hindrance and the availability of movement directions (instead of being indirectly dependent on them through biochemical signaling). This phenomenon is unique to 3D and cannot be observed in 2D environments. In addition to finding increased persistence, our results predict that a highly aligned matrix should yield a smaller instantaneous cell speed, which after a short time should be offset by that increased persistence.

To our knowledge, our work is the first to mathematically model the 3D environment in sufficient detail to have an effect on persistence. It demonstrates that the current paradigm of cell motility modeling, in which only rudimentary aspects of the surrounding materials such as the overall stiffness and ligand concentration are considered, is insufficient to capture central motility behaviors. Future work could involve a combination with quantitative data on cell remodeling to predict long-distance motility interactions and the incorporation of cell–cell interactions in nativelike 3D environments.

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Author Contributions
The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
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