Understanding Effects of Matrix Protease and Matrix Organization on Directional Persistence and Translational Speed in Three-Dimensional Cell Migration

MUHAMMAD H. ZAMAN,[1] PAUL MATSUDAIRA,[2,3] and DOUGLAS A. LAUFFENBURGER[3]

[1]Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX 78712, USA; [2]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA; and [3]Biological Engineering Division, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139, USA

Abstract—Recent studies have shown significant differences in migration mechanisms between two- and three-dimensional environments. While experiments have suggested a strong dependence of *in vivo* migration on both structure and proteolytic activity, the underlying biophysics of such dependence has not been studied adequately. In addition, the existing models of persistent random walk migration are primarily based on two-dimensional movement and do not account for the effect of proteolysis or matrix inhomogeneity. Using lattice Monte Carlo methods, we present a model to study the role of matrix metallo-proteases (MMPs) on directional persistence and speed. The simulations account for a given cell's ability to deform as well as to digest the matrix as the cell moves in three dimensions. Our results show a bimodal dependence of speed and persistence on matrix pore size and suggest high sensitivity on MMP activity, which is in very good agreement with experimental studies carried out in 3D matrices.

Keywords—Cell motility, Extra cellular matrix, Matrix metallo-protease, Monte Carlo simulations.

INTRODUCTION

Quantitative description of cell motility is desirable for a deeper understanding of migration in metastasis, development and wound healing. Recent attempts to quantify *in vivo* and *in vitro* migration trends have shed light on the underlying biophysical mechanisms by which cells respond to external gradients, integrate extra- and intracellular signals and show persistent or random motion.[1–3] Cellular motion is thus often described in terms of speed and some metric of random or persistent motility such as persistence or random

motility coefficients. While speed is often the metric of choice to quantify a cell's ability to migrate, it provides no information about the direction or randomness of this motion. Persistence in cell migration, on the other hand, describes significant changes in the direction of motion of a cell over time. Though quantitative experimental and theoretical studies on persistence have studied the origins and role of persistence in invasion and migration, almost all of these studies are based on cells moving on two-dimensional (2D) substrates.[4–6] Unlike migration on 2D surfaces, migration in three-dimensional (3D) environments requires a cell to steer its way through dense matrix fibers, actively change its morphology due to steric obstacles and use matrix metallo-proteases (MMPs) to degrade the matrix.[7–10] Individual or collective perturbation of these factors has been shown to affect a cell's ability to migrate in 3D matrices.[8,11,12]

A thorough and quantitative understanding of cell migration in 3D environments requires that we not only study migration and persistence by individually perturbing the biophysical and biochemical parameters, but also study the cellular response to simultaneous variations in these parameters. To date the simultaneous effects of these biochemical, structural and mechanical factors have not been quantitatively studied in the context of directional motion in 3D migration. In the present study, we report the results of a new computational approach that allows us to quantify the individual and collective effects of these factors on cell motility. We employ lattice Monte Carlo methods to simulate the interactions of a cell with its 3D environment and analyze our results using standard formalism for directional persistence time. While Monte Carlo methods have been used previously to study cell migration, our simulation is the first attempt to study the simultaneous role of matrix steric

Address correspondence to Muhammad H. Zaman, Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX 78712, USA. Electronic mail: mhzaman@mail.utexas.edu

and biochemical parameters on persistent motion in a degradable 3D environment. The results of our simulation are presented in terms of experimentally measurable variables and show good agreement with recent experimental findings.

SIMULATION METHODS

The simulation algorithm is depicted in Fig. 1. The 3D ECM is divided into cubic lattice spaces with dimensions larger than those of an "average" cell (for our simulations each lattice space $= 35 \times 35 \times 35$ μm, while the cell size is $20 \times 20 \times 20$ μm, i.e. the total cell volume is approximately 20% of the lattice volume). For simplicity, we assume that in each time step (Δt) a cell can move only one lattice space. The cell can move into any of its neighboring lattice spaces, however diagonal moves are not allowed as each time step allows for movement by a maximum distance of one lattice space (a diagonal move would mean movement by $\sqrt{2} \times$ lattice space).

The 3D lattice spaces (referred to as "allowed lattice spaces" or ALS from hereon) are filled with a known concentration of protein-ligands required for cell adhesion and migration. Extra cellular protein ligands such as fibronectin, collagen and laminin bind with integrins and provide necessary adhesion and traction for migration. As the migration of a cell in a 3D pore depends more on the cross-sectional area of the pore than its depth, we model the normalized pore size (in units of length^2) of each ALS by the following relation:

$$
\begin{aligned}
\text{Pore Size} = &(\text{ALS cross section}) \\
&\times \{1 - (\text{Number of ligands}) \\
&\times \frac{\text{Area per ligand}}{\text{ALS cross section}}\}
\end{aligned} \quad (1)
$$

In other words, if there is no ECM protein, the pore size is equal to ALS. The only variable in the above equation is the number of protein ligand molecules as all the other parameters are fixed for the purposes of our simulation. For our simulation, ALS cross section is 35×35 μm and the ligand area is on the nanometer scale (area of ligand $= 1 \times 1$ nm). The number of ligands is varied between get a pore size that varies from 90% to 10% of the ALS, i.e. 1.1×10^9 ligands would cover approximately 90% of the lattice and would result in a pore that is 10% of the lattice. On the other hand, a decrease in the total ligand number to 1.2×10^8 would result in net coverage of only 10% and the pore size would be 90% of the ALS. The choice of
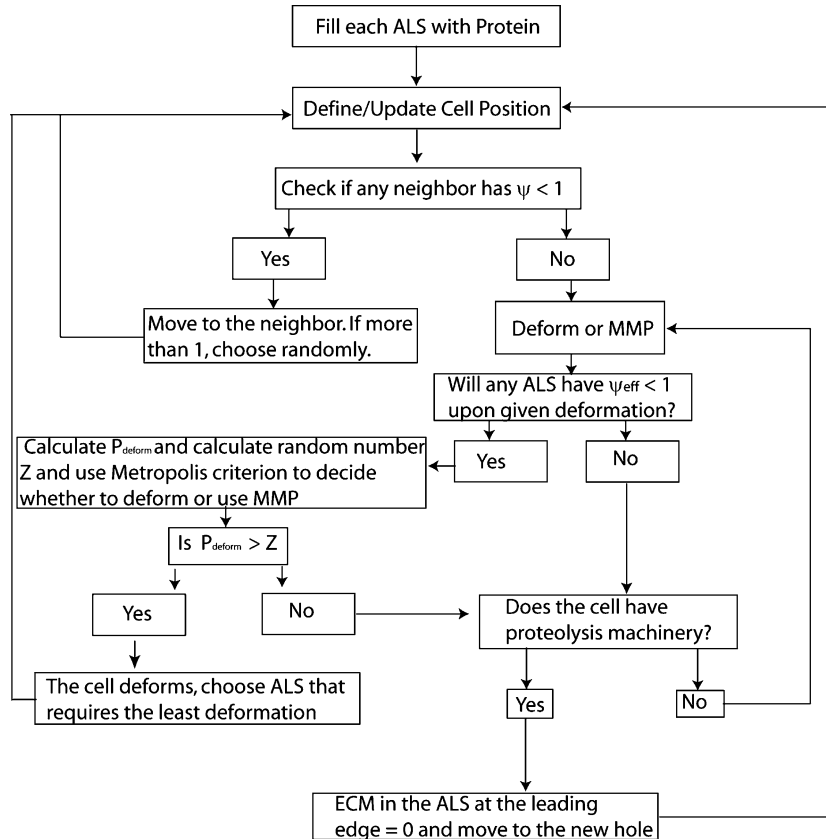

FIGURE 1.   A schematic of Monte Carlo routine used in our simulation.

the lower bound (i.e. coverage by 10% ECM ligand) corresponds to several computational and experimental studies suggesting that a minimum concentration of ECM ligands is required for motility and invasion.

The cell's initial ALS is chosen randomly, and the cell is allowed to move to any of its neighboring ALS. In other words at each time step, there are a maximum of six possible allowed neighboring sites. The model assumes that in the absence of an external chemical gradient, a cell's decision to move in a particular direction is influenced by steric factors. A cell migrates in a direction with the least amount of steric hindrance yet with a sufficient concentration of extracellular ligands for traction.

At each time step, the cell decides to move to its neighboring ALS based upon its ψ value for each of its neighboring ALS. The ψ value (a dimensionless quantity) for any given ALS is defined as:

$$\psi = \frac{\text{Cell cross section}}{\text{Pore size}} \qquad (2)$$

The cell moves into any of its neighboring ALS if $0.36 < \psi < 1$. The value of 0.36 arises by using the assumption that there is the minimum (normalized) concentration of protein required for traction and adhesion for the cell to migrate. In our simulations, we assume that the pore must be filled with 10% protein for the cell to move in ($\sim 1.2 \times 10^8$ ligand molecules). Since in the absence of any protein ligand, the minimum possible value of ψ is equal to 0.33 (cell cross section/ ALS cross section ~ 0.33), decrease in pore size by 10% would result in the ψ value to be ~ 0.36 (i.e. in the case when 10% of the pore is occupied by ligand, the resulting ψ value is 0.36). If there is more than one ALS with ψ value between 0.36 and one, the cell chooses the neighbor randomly. This move is defined as the "most probable move", as the cell can move into its neighboring ALS without any deformation or secretion of proteases.

If the ψ value for all the neighbors is greater than 1, the cell has two options: it can either deform and decrease the effective ψ value (by decreasing its cross-sectional area, i.e. going from spherical geometry to an elliptical geometry) or it can use its MMP machinery and deplete the ALS of its protein (hence increasing the pore size and the decreasing effective ψ value). As cell migration in 3D is often achieved by cell deformation that changes the cross-sectional area (while keeping constant volume)[7,8,12] we define effective ψ value as:

These moves, where the ψ value is greater than one and the effective ψ value is less than one are less probable than the moves with ψ value < 1, as both require some kind of work, either in the form of deformation, or release of MMPs.

In case of $\psi \geq 1$ and $\psi_{\text{eff}} < 1$, the cell's choice to move into a neighboring ALS depends upon deformation probability, P_{deform}, which depends upon the cell type, the number of ALS with pore size big enough that a given percent deformation would lead to $\psi_{\text{eff}} < 1$. Mathematically, for a given cell type P_{deform} can be written as :

$$P_{\text{deform}} = \delta \times \frac{m}{6} \qquad (4)$$

where δ has a value of one if the cell can deform at all and zero otherwise. "m" is the number of neighboring ALS that will result in $\psi_{\text{eff}} < 1$ at a given deformation The factor 6 appears because of the number of allowed neighbors around the cell.

Based upon Metropolis Monte Carlo criterion, the cell decides whether to deform or not by choosing a random number Z between 0 and 1. If the random number Z is greater than P_{deform}, the cell does not deform and decides to use its proteolysis machinery (if there is proteolysis machinery inside the cell), and if Z is less than P_{deform} the cell deforms and moves to the neighbor with that will result in $\psi_{\text{eff}} < 1$ at that given deformation. We can either pre-determine the maximum deformation possible in a given cell or choose it randomly within a certain range. If there are more than one neighboring ALS that fulfill this criterion, the cell moves to the neighboring ALS which requires least deformation to achieve $\psi_{\text{eff}} < 1$. In case the cell does not have an internal machinery to digest the matrix by proteolysis, and P_{deform} is less than Z, the cell does not move in that time step and repeats the process in the next time step. This process is continued until the random number Z is less than P_{deform} (Metropolis criterion).

For a rigid cell, or in situations where the pore size of all the neighboring ALS is so small that cell deformation will not lead to $\psi_{\text{eff}} < 1$, the cell can still move using its proteolysis machinery. Proteolysis depletes the ECM in the ALS chosen randomly from the six possible lattice sites. Our model assumes that ECM degradation is only at the leading edge, and upon degradation the cell moves to the lattice site by degrading ECM in that lattice site. We also assume

$$\psi_{\text{eff}} = \frac{\text{Cell cross section } - \text{ decrease in cross section due to deformation}}{\text{Pore size}} \qquad (3)$$

that ECM degradation is irreversible, and since the ALS is now depleted of any ECM, the cell cannot come back to an ECM-depleted ALS. (Fig. 1). As the time scale of proteolysis is much shorter than that of migration, we can safely assume that the proteolysis of matrix proteins at the leading edge is complete in the time scale of migration.

We calculate mean-squared displacement, speed and velocity from our lattice Monte Carlo simulations. The mean squared displacement is given by:

$$\langle d^2(t) \rangle = \langle x(t_0+t) - x(t_0) \rangle^2 + \langle y(t_0+t) - y(t_0) \rangle \\ + \langle z(t_0+t) - z(t_0) \rangle \quad (5)$$

$x(t_0 + t)$ is the x-coordinate of the cell at time t, $y(t_0 + t)$ is the y-coordinate of the cell at time t and $z(t_0 + t)$ is the z-coordinate of the cell at time t. The squared displacement is averaged over all previous time steps.

At long times, when the mean-squared displacement varies linearly with the number of time steps, the mean squared displacement is related to cell speed and persistence by the following relationship as has been used previously in experimental and computational studies[20]

$$\langle d^2(t) \rangle = 2S^2 p[t - p(1 - e^{\frac{t}{p}})] \quad (6a)$$

p is the directional persistence time, t is the total time and S is the cell speed. For our simulation, where $t \rightarrow \infty$, the relationship reduces to:

$$\langle d^2(t) \rangle \approx 2S^2 pt \quad (6b)$$

and hence persistence time p can be calculated by:

$$p = \frac{\langle d^2(t) \rangle}{2S^2 t} \quad (6c)$$

Over long times, the mean-squared displacement becomes linear with the number of steps, hence the average velocity over the course of the simulation is simply root-mean-squared displacement divided by the total number of time steps.

Experimental studies on 3D migration have classified cellular motion in three categories:[7,8,12] cells migrate through the pores present in the extracellular matrix without any deformation, deform and squeeze through regions of high steric hindrances, or degrade the surrounding matrix by secreting proteases to reduce steric obstacles. A cell can employ any of these three mechanisms based upon its external environment, and the underlying mechanism for transition from one mechanism to another remains an open-ended question.

In our simulation, we examine all of the above mentioned possibilities, namely cells with MMP and deformation ability, cells with MMP and no deformation, cells that have deformation ability but are devoid of MMPs and cells that can neither deform nor degrade the matrix. For a lattice site with pores bigger than the cells, the cell does not need to deform or use MMPs to move in that direction. On the other hand, if a cell is surrounded by matrix with pore size smaller than the cell, it must deform *or* use its proteolysis (MMP) machinery to migrate. A cell's ability to deform depends upon the cell type and the number of available lattice sites that a cell can enter with that given deformation. A cell can also move forward using its proteolysis machinery, and in which case it depletes the surrounding lattice sites of the ligand protein.

Our method allows us to calculate the directional persistence time as a function of deformation ability of the cell, average pore size and protein concentration and presence or absence of proteolysis. Thus in effect, we can simulate the effects of cellular stiffness, matrix structure and presence or absence of matrix digesting machinery.

In this study we report the results of gels with uniform distribution of pore sizes, within a 10% variation. This variation is built in the simulation routine to mimic minor heterogeneities often seen in *in vitro* and *in vivo* experimental studies where the pore sizes of the scaffold are constant within a small range.[13,14] In other words the protein ligand concentration in each lattice site is within 5% of other lattice sites. The lattice space used for our simulation contains $10^4 \times 10^4 \times 10^4$ lattice spacings. The simulation protocol uses periodic boundary conditions in all three dimensions. Simulations are carried for 10^4 time-steps and are repeated 10^4 times with varying initial conditions (initial location of the cell). The velocity and persistence results shown are an average over these distinct simulations and the error bars indicate the standard deviation.

RESULTS AND DISCUSSION

To study the role of MMP activity and cell deformation in a number of biologically relevant conditions, Monte Carlo simulations were performed on cells that had active MMP machinery as well as on cells without the ability to degrade the matrix. Figure 2 shows the random-walk like behavior of sample trajectories. The sample trajectories for cells with MMP suggest increased persistence over cells with no MMP activity.

In addition to cells with or without proteolysis machinery Monte Carlo simulations were also performed on cells that were rigid and could not deform at all as well as cells that could deform by up to 50%
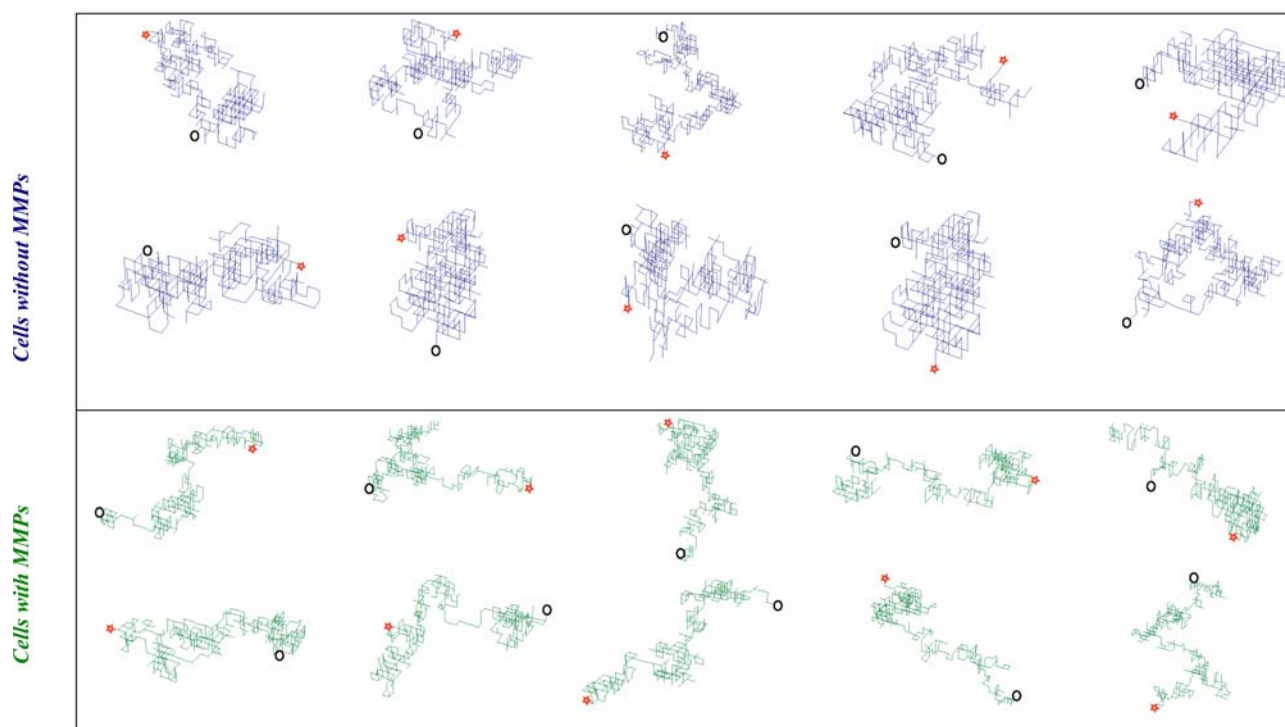
FIGURE 2. A small sample of trajectories used in persistence calculation. Top two rows are for cells with no MMP activity (lower persistence) and the bottom two are for cells with MMP activity (higher persistence). The black circle and the red start show the starting and ending location of each trajectory, respectively.

(i.e. a decrease in cell cross-section by 50%). These simulations were performed in 3D environments of varying degrees of protein concentration (and pore sizes). The mean squared displacement (MSD) of cells simulated in 3D matrices shows that for a given pore size and cell deformation, MMP activity increases the mean-squared displacement significantly (Fig. 3). This is primarily due to the ability of the MMP active cells to degrade the matrix and move greater distances. The MSD of both cell types (with and without MMPs) show an initial "burst-phase" which is non-linear, but at longer times MSD becomes linear and shows time invariance with the number of simulation steps, suggesting that the cells show ergodic behavior over long times. In order to avoid any non-ergodic behavior in our analysis, we ignore the first 5000 time steps and calculate all parameters from steps 5001 to 10000, where the mean-squared displacement is time invariant.

Our Monte Carlo simulations predict a bimodal behavior of cell velocity and persistence as a function of pore sizes (Fig. 4a–c). At very high pore size, the extracellular protein concentration is too low for any binding, and all cells, regardless of their MMP activity or deformation abilities are unable to migrate. On the other hand, at high protein concentration (small pore size), any migration over long distances is only either due MMP activity or very high deformation, and cells

without any MMP activity tend to sample smaller areas. Both of these constraints have the strongest impact on cells without MMP machinery or deformation ability (i.e. the black curve in Fig. 4a). These cells show a step-function like behavior, suggesting
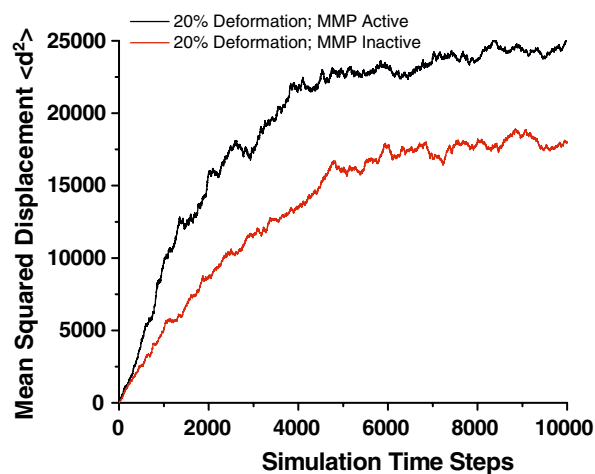
FIGURE 3. Mean-squared displacement for cells with and without MMP activity at a given deformation (20%). Results for other deformations are similar (data not shown). The mean-squared displacement shows an initial "burst-phase" non-linear behavior (inset) but becomes linear and invariant with time at longer times.
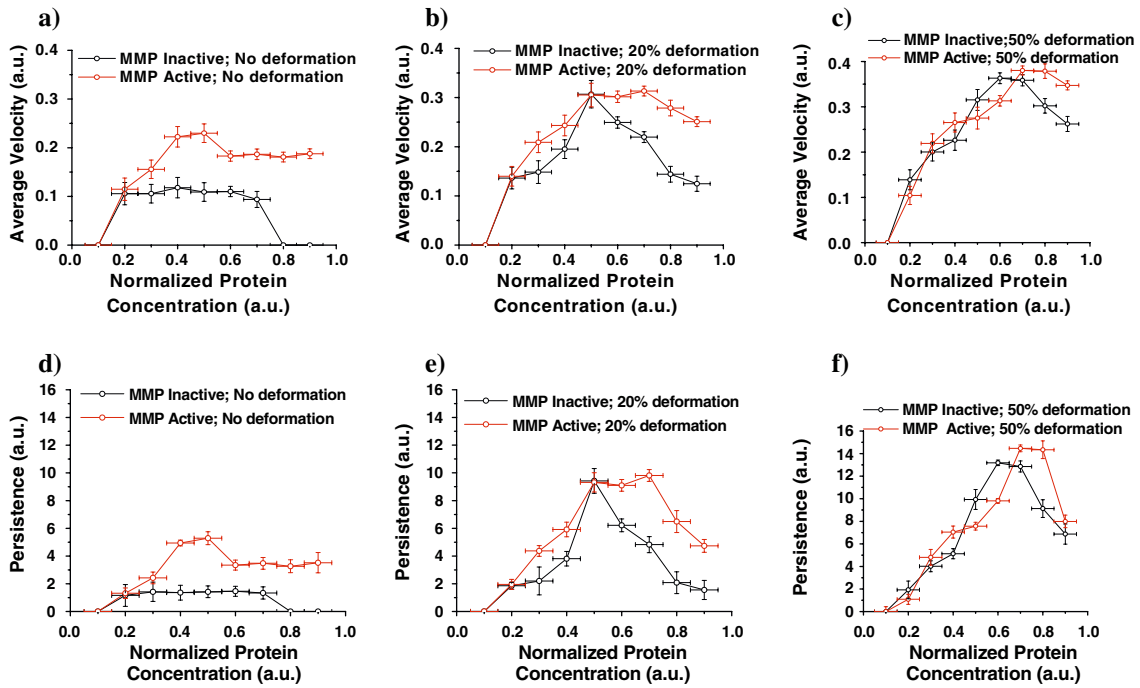
FIGURE 4. Mean-velocity for cells with varying proteolytic and deformation abilities. The cells without any MMP or deformation have a step-function like velocity profile (Fig. 4a). Increasing deformations result in increasing speeds of the cells (Fig. 4b, c). The bimodal behavior, where the cell speed first increases, reaches a maximum and then decreases, is very similar to previous experimental and theoretical studies in 3D (see text for details), suggesting that maximum velocity occurs at intermediate pore sizes and intermediate ligand densities (Fig. 4a, b). The *y*-error bars represent standard deviation over all (i.e. 2×10^4) simulations starting with different initial conditions, while the *x*-error bars show 5% variation in ligand concentration between different lattice sites. Persistence of rigid and deformable cells, with and without MMP machinery in an ECM of varying protein concentration (and pore sizes) is shown in Fig. 4d–F. In the absence of MMP or cell deformation, cells do not exhibit significant persistence (Fig. 4d). Maximum persistence corresponds to intermediate protein concentration, which is in good agreement with results of Burgess *et al.* [15]. (Fig. 4d–f) Additionally, lack of MMP activity corresponds to lower overall persistence (black curves in Fig. 4d–f), and cells with no MMP show negligible movement and persistence in matrices of high protein concentration and low pore sizes.

that apart from the extreme cases of no protein present or high steric hindrance, these cells do not respond significantly to changes in ligand concentration or pore sizes (black curves in Fig. 4a). While MMP activity allows a cell to migrate through pores of any sizes, migration through realistic deformations (such as deformations seen in amoeboid motility and can lead to formation of constriction rings of $\sim$3–5 μm[7]) can only occur in larger or intermediate pore sizes. This effect, combined with the presence of a finite amount of protein needed for binding results in maximum displacement at intermediate pore sizes, where both deformation and MMP activity contribute significantly. On the other hand, in very small pore sizes, contribution to sampling greater distances comes only from MMP activity, and hence the overall displacement (and persistence) of cells without MMP activity decreases.

In addition to the bimodal response of cell speed and persistence to matrix structural properties, we also note that the loss of MMP activity leads to lower persistence (Fig. 4d–f). At high pore sizes the persistence remains the same regardless of MMP activity,

however as the pore size starts to decrease the cells need to either deform or degrade the matrix proteins. As the pore size decreases further, even 50% deformation cannot result in a ψ value that is less than 1, and the cell is confined to smaller sample space. While 50% deformation does lead to high persistence at intermediate pore size in cells with no MMP activity, persistence in MMP deficient cells decreases to lower values at very high protein concentration, while cells that have MMP activity show relatively higher persistence even at very high protein concentration.

As cell migration in 3D simultaneously depends on multiple cellular (e.g. stiffness, receptor density) and extracellular (e.g. pore size, ligand conc.) parameters we study the behavior of "migration landscapes" where cellular response such as speed and persistence are plotted simultaneously as a functions of key variables. Such plots capture the collective effect of coupled parameters and hence show a more detailed picture than the one that would emerge from plotting just speed or persistence against a single variable. Figure 5 shows the behavior of average cell velocity in the absence (Fig. 5a) or presence (Fig. 5b) of MMP

activity simultaneously as a function of pore size and percent deformation. The left panel shows a 3D landscape plot and the right panel shows a contour plot showing regions of highest and lowest velocity. This landscape quantitatively and qualitatively captures the differences as a function of cellular and extracellular parameters in cells with and without active proteolysis machinery. The color-coded regions show maximum and minimum velocity as a function of protein concentration and cell deformation. The speed shows a
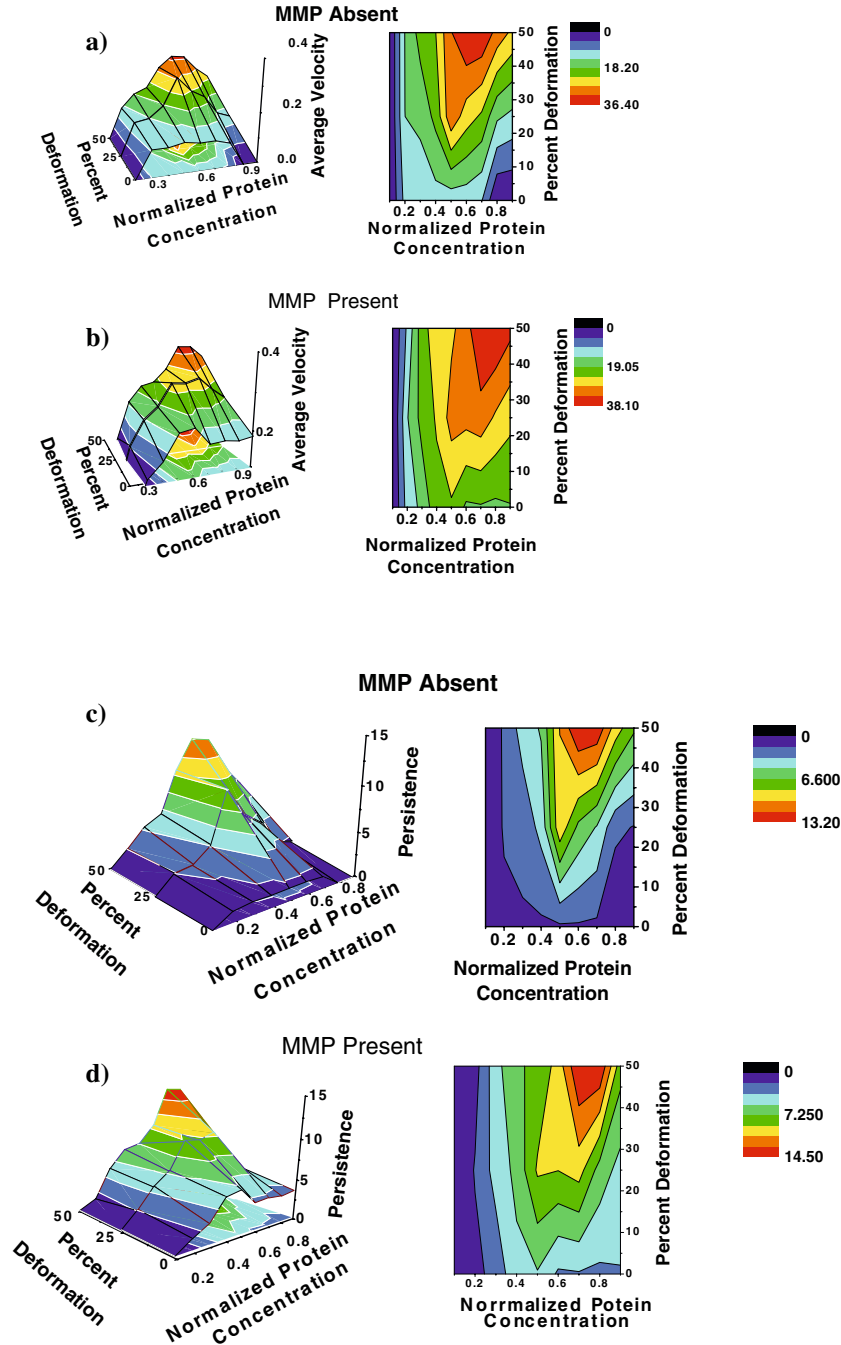
FIGURE 5. Three-Dimensional average velocity surface and contour plots as a function of pore size and percent deformation, for cells (a) without and (b) with MMP activity show highest velocities at intermediate pore sizes but cells with MMP show a gradual decrease in overall velocity as compared to cells without MMPs which show a sharp decrease as the pore size decreases. Similar to 3D velocity surfaces persistence surface as a function of pore size and percent deformation, for cells (c) without and (d) with MMP activity are shown. Cells with no MMP show a sharp decrease in persistence as the pore size increases, and show only significant persistence for cells with high degree of deformability.

maximum at high deformation and intermediate pore sizes, but decreases sharply to zero for cells with no MMP but decreases at a slower rate for cells with MMP. Additionally, the regions of lowest speed a much smaller in cells with MMP as compared to cells without the proteolysis machinery. Similar behavior is observed in persistence (Fig. 5c, d) where cells without MMP show only significant persistence in the case of highly deformable cells, while cells with MMP activity show a gradual decrease with pore size increase and decrease in cell deformability.

COMPARISON WITH EXPERIMENTAL RESULTS

Our results suggesting a bimodal response of persistence and speed with matrix structure, MMP activity and ligand density are in good agreement with recent experimental findings. Using melanoma cells cultured in 3D collagen scaffolds, Dickinson and co-workers observed a bimodal dependence to persistence time (and length) as a function of RGD concentration.[15] They observed that as the concentration of integrin binding peptide, RGD, is increased the persistence length first increases and then decreases. This result is very similar to our predicted result (Fig. 4d–f) where we observe calculated persistence to vary non-linearly with protein concentration.

In another study, Kuntz et al.[16] reported that Neutrophil migration (velocity as well as random motility coefficient) in 3D environments varied in a bimodal manner with the pore size of the 3D gel. Figure 4a–c capture this experimentally observed behavior. For all of the conditions studied, we note that the cell speed first increases, reaches a maximum and then decreases. The exact magnitude of this bimodal behavior depends upon the cellular and extracellular parameters. Similar behavior is observed in persistence, where we observe first an increase, followed by a maximum at intermediate protein concentration, and then a decrease in overall persistence.

Our mean velocity results also correlate very well with the experimental results of Lutolf et al.[17] in 3D environments. Lutolf et al. observed that cell speed varies non-linearly with variations in ligand concentration, and with increasing adhesion ligand concentration in the matrix, the cell speed first increases, reaches a maximum, and then decrease. Our mean velocity results shown in Fig. 4a–c capture this observation accurately. In addition our simulations also agree well with previous theoretical studies of published by our group[18] suggesting a non-linear dependence of velocity on ligand concentration.

Finally, in a recent study, Raeber et al. studied the role of MMP activity on cell migration in novel molecularly engineered PEG hydrogels. They observed that the 3D motility of dermal fibroblasts was dependent on MMP activity, and that the number of migrating cells increased significantly upon up-regulation of MMP. These results are in very good agreement with our findings that mean-squared displacement (Fig. 3) and persistence (Fig. 4) show an increase in magnitude upon increase in proteolytic activity. Our results show that cells that are devoid of MMP activity show lower speeds and persistence as compared to cells with MMP activity.

It must be noted that the studies of Dickinson and co-workers[15] as well as those of Lutolf et al.[17] and Raeber et al.[11] are in systems where the 3D environments had near constant pore sizes and included both proteolytically degradable and non-degradable environment. Therefore all of our results cannot be compared directly with these experimental findings. In these constant pore size systems, sterics do not affect a cell's ability to migrate, but in fact ligand concentration modulates the cell speed and persistence. Our simulations with cells that do not have MMP machinery and are able to deform significantly are, in principle, similar to the experimental studies conducted in proteolytically non-degradable environments. Like the experimental scenario, these cells are not affected by steric hindrances but move or don't move based upon the concentration of the available ligands. The presence of a bimodal behavior in these simulations does in fact suggest that our simulations are able to capture a wide variety of experimental scenarios. Thus our simulations are not only able to capture the behavior of cells in degradable environments with varying pore sizes, but our simulations also show good agreement with experimental studies on cells moving in non-degradable matrices fortified with extracellular ligands.[19]

LIMITATIONS OF OUR MODEL

Despite the ability of our simulations to predict the effect of matrix sterics, MMP activity, and cell stiffness on overall persistence in 3D matrices, and our good comparison with experimental results, our simulations have several limitations. First of all, our simulations are blind to sub-cellular events such as protrusions and lamellipodial waves that occur on time scales that are much shorter than the timescale of migration.[20] We also do not incorporate individual receptor-ligand interactions, as have been addressed in previous computational models.[18] Another important feature of migrating cells (especially in tumor cell lines) is the

secretion of extra-cellular matrix by the cells. Migrating cells often lay down their own ECM and use it for traction generation as well as contact guidance. At the moment, our simulations cannot account for ECM secretion by the cells. Another key factor ignored in our simulations is the fine balance the effect of soluble and insoluble ligands. Our model, in its current form, ignores the possibility of revealing new insoluble ligands as a result of proteolysis. This may increase adhesion as a result of proteolysis. Such effects would require a more explicit spatial treatment of the extra cellular matrix, which upon degradation would expose new insoluble ligands previously buried. Since our model does not explicitly simulate the spatial location of the extracellular ligands, capturing the above mentioned effect is beyond the scope of our model. Similarly, we only model insoluble ligands, and once the extracellular matrix is degraded, there are no soluble ligands available for cell adhesion. Soluble and insoluble ligands are known to compete for integrin binding and hence solubilization of ligands by proteolysis will affect overall effective concentration of the insoluble ligands. Modeling such an effect would require a detailed description of numerous soluble and insoluble ligands, which constitute the extracellular matrix. Our current model only assumes generic insoluble extracellular ligands and does not explicitly simulate various soluble and insoluble ligands, and is therefore incapable of addressing this aspect of cell adhesion and competitive binding.

Finally, parameters such as cell deformation are only considered implicitly. Thus while our model is able to capture the migration behavior of cells in a number of extracellular environments, our comparison is largely qualitative at this stage.

Resolving these limitations will provide further insights into cell migration in 3D matrices and we are working on further extensions of the current and previous computational models to address these issues. In spite of these and other limitations, our model presents a novel approach to understand the role of matrix and cellular biophysical and biochemical properties on overall speed and persistence in 3D matrices.

CONCLUSIONS

Understanding the mechanism of tumor cell migration during metastasis will undoubtedly lead to designing better therapeutics for cancer treatment. While recent research on certain cancer cell lines has shown that migration speed of tumor cells remains unchanged even in the absence of MMP machinery,[7] it is still unclear whether the cells will have any change in persistence as a function of MMPs or not. Using lattice Monte Carlo simulations, we predict that blocking MMPs will have an effect on the ability of the cell to move greater distances and depending upon the ECM structure, loss of MMP can restrict the movement of tumor cells to a smaller area.

So far, to our knowledge, only a handful of quantitative experiments have been carried out to study cell's speed and persistence in 3D matrices.[11,15,16,21] With technological advancements in high resolution *in vivo* imaging, a further extension of such studies to understand the role of MMPs in persistence of cells is now possible. We believe such studies will not only test the predictions of our model but will also aid to improve the overall understanding of subtle aspects of tumor cell migration.

We hope that the results of these experiments will help in the development of a thorough understanding of the migration process and will lead to better manipulation of migrating cells in physiological and biotechnological processes.

REFERENCES

[1]Burgess, B. T., J. L. Myles, and R. B. Dickinson. Quantitative analysis of adhesion-mediated cell migration in three-dimensional gels of RGD-grafted collagen. *Ann. Biomed. Eng.* 28(1):110–118, 2000.

[2]Cukierman, E., R. Pankov, D. R. Stevens, and K. M. Yamada. Taking cell-matrix adhesions to the third dimension. *Science* 294(5547):1708–1712, 2001.

[3]Cukierman, E., R. Pankov, and K. M. Yamada. Cell interactions with three-dimensional matrices. *Curr. Opin. Cell Biol.* 14(5):633–699, 2002.

[4]Dickinson, R. B. and R. T. Tranquillo. Optimal Esitmation of Cell Movement Indices from the Statistical Analysis of Cell Tracking Data. *AIChEJ*. 39(12):1995–2010, 1993.

[5]Discher, D. E., P. Janmey, and Y. L. Wang. Tissue cells feel and respond to the stiffness of their substrate. *Science* 310(5751):1139–1143, 2005.

[6]Dunn, G. A. Characterising a kinesis response: time averaged measures of cell speed and directional persistence. *Agents Actions Suppl.* 12:14–33, 1983.

[7]Friedl, P. and E. B. Brocker. The biology of cell locomotion within three-dimensional extracellular matrix. *Cell Mol. Life Sci.* 57(1):41–64, 2000.

[8]Friedl, P. and K. Wolf. Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat. Rev. Cancer* 3(5):362–374, 2003.

[9]Giannone, G., B. J. Dubin-Thaler, H. G. Dobereiner, N. Kieffer, A. R. Bresnick, and M. P. Sheetz. Periodic lamellipodial contractions correlate with rearward actin waves. *Cell* 116(3):431–443, 2004.

[10]Harms, B. D., G. M. Bassi, A. R. Horwitz, and D. A. Lauffenburger. Directional persistence of EGF-induced cell migration is associated with stabilization of lamellipodial protrusions. *Biophys J.* 88(2):1479–1488, 2005.

[11]Kuntz, R. M. and W. M. Saltzman. Neutrophil motility in extracellular matrix gels: mesh size and adhesion affect speed of migration. *Biophys. J.* 72(3):1472–1480, 1997.

[12]Lauffenburger, D. A. and A. F. Horwitz. Cell migration: a physically integrated molecular process. *Cell* 84(3):359–369, 1996.

[13]Lutolf, M. P., J. L. Lauer-Fields, H. G. Schmoekel, A. T. Metters, F. E. Weber, G. B. Fields, and J. A. Hubbell. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. *Proc. Natl. Acad. Sci. U S A.* 100(9):5413–5418, 2003.

[14]O'Brien, F. J., B. A. Harley, I. V. Yannas, and L. Gibson. Influence of freezing rate on pore structure in freeze-dried collagen-GAG scaffolds. *Biomaterials* 25(6):1077–1086, 2004.

[15]Pek, Y. S., M. Spector, I. V. Yannas, and L. J. Gibson. Degradation of a collagen-chondroitin-6-sulfate matrix by collagenase and by chondroitinase. *Biomaterials* 25(3):473–482, 2004.

[16]Raeber, G. P., M. P. Lutolf, and J. A. Hubbell. Molecularly engineered PEG hydrogels: a novel model system for proteolytically mediated cell migration. *Biophys. J.* 89(2):1374–1388, 2005.

[17]Ridley, A. J., M. A. Schwartz, K. Burridge, R. A. Firtel, M. H. Ginsberg, G. Borisy, J. T. Parsons, and A. R. Horwitz. Cell migration: integrating signals from front to back. *Science* 302(5651):1704–1709, 2003.

[18]Shreiber, D. I., V. H. Barocas, and R. T. Tranquillo. Temporal variations in cell migration and traction during fibroblast-mediated gel compaction. *Biophys. J.* 84(6):4102–4114, 2003.

[19]Wolf, K., I. Mazo, H. Leung, K. Engelke, U. H. von Andrian, E. I. Deryugina, A. Y. Strongin, E. B. Brocker, and P. Friedl. Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J. Cell Biol.* 160(2):267–277, 2003.

[20]Zaman, M. H., R. D. Kamm, P. Matsudaira, and D. A. Lauffenburger. Computational model for cell migration in three-dimensional matrices. *Biophys. J.* 89(2):1389–1397, 2005.

[21]Zaman, M. H., L. M. Trapani, A. Siemeski, D. Mackellar, H. Gong, R. D. Kamm, A. Wells, D. A. Lauffenburger, and P. Matsudaira. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc. Natl. Acad. Sci. U S A* 103(29):10889–10894, 2006.