

Special Focus

Modeling cell migration in 3D

Status and challenges

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Cell migration is a multi-scale process that integrates signaling, mechanics and biochemical reaction kinetics. Various mathematical models accurately predict cell migration on 2D surfaces, but are unable to capture the complexities of 3D migration. Additionally, quantitative 3D cell migration models have been few and far between. In this review we look and characterize various mathematical models available in literature to predict cell migration in 3D matrices and analyze their strengths and possible changes to these models that could improve their predictive capabilities.

Introduction

Cell migration is a key process involved in the immune response system, wound healing processes and development of tissues in an embryo.¹ Beyond these physiological processes cell migration is an important aspect of cancer metastasis where cancer cells exfoliate from the site of primary tumor formation and migrate to other organs within the body through the circulatory and lymphatic systems.² Cell migration is inherently a multi-scale and multi-disciplinary process that requires a thorough understanding of both the biochemical aspects of cell signaling and chemotaxis and the biophysical and mechanical aspects of cell-matrix interactions.³

Cell migration studies have focused primarily on migration in 2D environments, including a number of modeling endeavors⁴⁻¹⁷ over the past 20 years. Though these 2D analyses give us a fair insight on the mechanics of cell-substrate and cell-cell interactions, yet they still fall short of explaining the comprehensive in-vivo processes due to lack of the third dimension. A key deficiency is the lack of quantitative treatment of key components cell-matrix interactions (such as proteolysis) within a 2D framework.^{18,19}

Part of the problem resulting in fewer models of 3D motility is lack of high quality data of cell movement in 3D. However, this problem is increasingly become less of an issue with recent success in high resolution imaging in native and synthetic 3D matrices.^{3,20} Apart from experimental assays that quantify cell motility in 3D matrices we also need to develop quantitative mathematical models

to fully comprehend the role of matrix mechanics, matrix structure and cellular parameters (e.g., mechanical properties, receptor density and signaling) in regulating migration in 3D environments. These mathematical models, rooted in fundamentals of cell biology, mechanics and kinetics will have the power to determine the rate limiting parameters that regulate cell motility in 3D. Additionally, mathematical models also need to act in a co-operative manner with experiments. While experiments can supply the data required to build these models, the results of these models can help design better experiments that can give more meaningful results.

The Biology of 3D Cell Migration

The ECM interacts with the cell mainly through specific cell surface receptors.^{21,22} These receptors are responsible for the growth and differentiation of cell and also mediate cell attachment, polarization and migration. The integrins are the major cell surface receptors involved in cell-ECM interactions.²³⁻²⁵ When cells migrate through 3D matrices they experience resistance through biophysical interactions with the visco-elastic matrix. This resistance is a unique feature of motility in 3D, as in the case of 2D motility, resistance is limited to interfacial 2D friction. In order to overcome this resistance the cells follow a sequence of adhesion and detachment events facilitated by the cell surface receptors.^{18,20} Matrix degradation by serine and metalloproteinases also helps in reducing the resistance to cell motility. Recent studies have also suggested alternate mechanisms resulting in an amoeboid motility in the absence of MMPs within certain matrices.¹⁸⁻²⁰ The cross-talk mechanisms between MMP and integrin receptors during 3D migration, however, remain elusive. Similarly, the organization of integrins on cell surface to form focal adhesions in 3D is also a subject of debate and a definitive picture is yet to emerge.^{26,27} These unanswered questions of fundamental importance make 3D cell migration all the more interesting and challenging from a modeling perspective.

Typical migration process is characterized in a three step cycle including extension of lamellipod and attachment to substratum through cell surface receptors followed by constriction of cell cytoskeleton and subsequent detachment of cells to the substratum at the rear end.¹ While these processes in 3D may seem similar to processes in 2D migration, there are numerous differences.^{3,28} The cells typically do not polarize in the same manner in 3D migration as they do in 2D migration. Additionally, the rate limiting step may vary under different circumstances. While sometimes lamellipod

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extension and cell substratum attachment can govern cell migration speed, at other instances it's regulated by rear end detachment. Usually there is an asymmetry in the number of cell surface receptors in the front and the rear of the cell.²⁸ This leads to an asymmetry in the attachment and subsequent detachment between the front end and rear end. This establishes a force gradient between the two ends, hence leading to a subsequent motion. Cell constriction is thought to be provided by the insertion of myosin into actin filaments¹⁸ leading to cell displacement. Subsequently detachment occurs due to either exfoliation of surface receptors or internalization of these receptors. For the amoeboid motility in 3D, while the role of MMPs and integrins has been studied in some detail,^{18-20,29} the quantitative picture connecting the matrix with the cellular mechanics is yet to emerge. Hence there is an even greater need for mathematical and computational modeling of migration in 3D.

Existing Mathematical Models in 3D

While the number of mathematical models describing motility in 3D has increased, the number is still orders of magnitude lower than their 2D counterparts. While some of these models predict individual cell motility in a 3D environment that mimics the in vivo extra-cellular matrix, others predict population behavior. The level of detail and length-scales also varies significantly. Some of these migration models look at the effect of cell proliferation and death and at the same time there are some simplistic models that ignore these effects. In the following section, we provide a brief overview of the key parameters of the 3D models reported in literature as well as suggestions for future development and refinement of these models.

Force Based Dynamics Models

These models use internally generated traction forces and introduce parameters like matrix density and stiffness and cell-matrix adhesivity in generating cell motility tracks. Zaman et al.²⁸ have discussed this kind of model in detail. The dynamics of this model is accounted for by the traction forces at both the front and rear end of the cell and forces due to cell protrusion into the matrix and viscous drag due to cell motility in the visco-elastic Extra Cellular Matrix (ECM). The traction forces at front and rear ends are different and are dependent on the force per ligand-receptor complex and receptor adhesivity. The force-per ligand receptor is a function of the Young's modulus of the ECM. Adhesivity is a dimensionless parameter that varies with the number of receptors on each end of the cell, their binding constant and the concentration of ligand at each end. In order to simplify the model the authors assumed that the binding constants of the receptors at both ends were the same and that the concentration of ligand in the matrix was uniform.

The model then defines a drag force that arises due to the resistance to motility of cells in the viscous environment and a protrusion force that is present due to cell movement in the ECM. The drag force is proportional to the velocity of cell and is dependent on the cell shape and viscosity of the ECM. The protruding force has a magnitude that has been determined experimentally. But the direction of protrusion is randomly chosen after every timestep. The model assumes the only stable protrusions are responsible for cell migration and any retractions small protrusions are ignored. The cell velocity is calculated under the constraint that the net force acting on the cell is zero. Simulations of this model were carried out with a

timestep of 600 seconds. Owing to this large timestep, cell dynamics at the edges are excluded. These include actin waves and lamellipodial contractions.

At low and high adhesivity values the model predicts low cell velocity while cell velocity increases at intermediate adhesivity values. Also the asymmetry in receptor concentrations between the front and rear ends increases cell velocity. Similarly cell velocity is highest at intermediate values of cell detachment force. Experimental results are consistent with these findings.³ At low adhesivity values there is not sufficient traction hence cell velocity is low while at high values there is a steric hindrance from the matrix which reduces cell velocity. Also the model predicts a similar biphasic relationship between cell velocity and ligand concentration and matrix stiffness which is consistent qualitatively. The results of the model agree with the experimentally determined 2D cell motility assays. But since the model takes into account matrix stiffness and matrix viscosity it's a better indicator of the actual cell velocity in-vivo.

The drawback of this model is that it only predicts the movement of a single cell in the matrix, while in-vivo cell migration almost always includes a population of cells. In vivo these cells aggregate and sometimes dissociate and form clumps, this is especially true in the case of cancer metastasis. The model also does not account for the change in shape of cells as they protrude into the matrix. The change in shape of cells can affect the drag force and hence the cell velocity. In this model the matrix properties remain constant through the course of the simulation, while in real world conditions the matrix properties might not necessarily remain constant. Degradation by proteases can change the matrix stiffness and its visco-elastic nature which is not taken into account by the model. In spite of these drawbacks the results from this model can help design better experiments that take into account matrix properties and help find key parameters responsible for cell motility in vivo.

Stochastic Model of Persistent Random Walks

These models are extensions of the 2D migration models of Tranquillo^{5,7} and Stokes.^{30,31} The path taken by each cell is determined by solving the Langevin equation numerically. The model selects a cubic volume element and cells are distributed uniformly within this volume. A random velocity vector is assigned to each cell where each component of the velocity is selected randomly from a Gaussian distribution. This Gaussian distribution is directly proportional to the size of the timestep. Parkhurst et al.³² used this kind of model to predict neutrophil motility in a 3D environment. Parkhurst et al. in their studies performed their simulations on this model using a timestep of 0.1 seconds.

After each timestep the velocity and the location of each cell is updated. The model defines the root mean square displacement of the cell as a function of root mean square speed and persistence. Random motility coefficient (μ) and persistence values are available in literature and can be used in this model. After simulation over a period of time the path taken by each cell in three dimensions is generated. By comparing their computer simulations to experimental results Parkhurst et al. determined that the 3D paths generated by the simulation were similar to path taken by particles in a Brownian motion. By fitting the mean square displacement values for different population of cells to calculate μ and persistence they determined that at low cell population (around 10 cells) the variation in μ and

persistence was high while at higher population (greater than 50 cells) the estimates of μ and persistence approached experimental values. Hence they conclude that even a cell population of around 100 cells is enough to predict population behavior.

The strength of this model lies in the fact that population behavior can be predicted. Even though its individual cell paths that are predicted population effects are still visible. The downside to this model is that dynamic effects like traction and drag are not incorporated into the model. Also the effect of matrix stiffness and porosity are not apparent in the model. Even though the population as a whole is looked at in this model, it still doesn't account for the fact that aggregation of cells is a possibility. But this model is quite useful for validating the experimental results of a small population of cells in a 3D environment.

Multi-Cell Spheroid Migration

Some mathematical models look at the movement of cancer cell spheroids. Cell proliferation and death are a major parameter involved in cell motility in these models. Differential rates of cell proliferation and cell death lead to pressure gradients that induce cell locomotion. McElwain et al.³³⁻³⁶ used this kind of a model to show that pressure differences created by cell proliferation and death are responsible for cell locomotion and that this motion is governed by a Darcy's law kind of equation. They proposed that the rate of consumption of nutrients decreased with the concentration of nutrients within a critical range, with a constant rate of consumption when the concentration of nutrients is greater than a threshold and rate of consumption drops to zero when the concentration of nutrients drops below a lower threshold value.

In this model the rate of cell proliferation is assumed to be proportional to the rate of nutrient consumption and hence both rates follow the same distribution. Conversely rate of cell death increases with decreased concentration of nutrient concentration. These rates of proliferation and death are modeled as volume gain and loss respectively. The volume loss and volume gain functions are modified to make the distribution continuous. The model then applies a mass balance study of the cells within the multi-cell spheroid with parameters including diffusion coefficient, concentration of nutrient, velocity of cellular fluid (which is considered incompressible) and a chemotactic factor. In this model the diffusion coefficient and chemotactic factor are assumed to be constant.

Without calculating the actual pressure gradient the model calculates the gradient due change in volume (either gain or loss). The nutrient concentration gradient of the multi-cell spheroid is calculated with parameters including the radius of the spheroid, radius of region where nutrient is depleted (cell proliferation is limited) and the radius of the necrotic region (where cell death is maximum). The local particle velocity is related to the conservation of mass as particle velocity is proportional to pressure gradient. The model thus develops distributions for both spheroid velocity and nutrient concentration. This is used to calculate the movements due to random walk, movement due to a pressure gradient and motion due to chemotactic activity. Dimensionless parameters that are a combination of these movements are measured.

McElwain et al. show that when the multi-cell spheroid (MCS) is still expanding there is a net velocity outwards at the edge and when it's dormant there is a net velocity inwards at the inner edge

and zero at the outer edge i.e., there is no expansion of the MCS. By varying the values of the chemotactic parameter it was concluded that at higher values of the chemotactic parameter active migration is observed while at lower values a more dormant migration resulting from the passive pressure gradient is observed.

This model combines the motion due to random walk, pressure gradients and chemotactic activity of cell aggregates, making it a good model to study tumours. But it fails to take into account important parameters like matrix density, porosity and stiffness which are important factors in in-vivo migration. While nutrient concentration is part of the parameters, inclusion of ECM parameters can help model real world conditions better.

Monte Carlo Modeling Studies

Recent studies have also used monte carlo type models, particularly using rectangular or square lattices in 3D environments.^{37,38} This kind of approach allows for faster simulations, population effects as well as prediction of speed and persistence in 3D in a heterogeneous environment. A key advantage of this type of modeling initiative is the use of rather simple set of rules governing the process of motility and prediction of results for long time behavior. These simulations^{37,38} have shown good agreement with experimental studies. Similarly, lattice monte carlo approaches allow for mapping of migration in diverse and complex environments where the steric effects can change abruptly. Thus these types of models render themselves fairly well to model tumor invasion in vivo.

A key handicap of these models is the qualitative nature of the results. Kinetic effects at the cell matrix interface, mechanical effects of the matrix and cell polarity can only be studied qualitatively. Additionally, sensitivity on initial conditions and the rules governing movement from one lattice site to another can also affect the overall speed and persistence results. Nonetheless, these models provide are capable of handling issues at the multi-scale level that are beyond the reach of other modeling initiatives.

Summary

As evident from the overview of existing 3D models, there is a critical need for developing high resolution quantitative models of cell migration in 3D. A number of efforts in this area have shown highly promising results, yet a lot more needs to be done. Key among these are issues of quantitative comparisons with experiments and addressing the multi-scale nature of the problem. The current level of detail in 3D models lacks the incorporation of molecular events that define these processes and are sometimes responsible for most dramatic changes. The interface between mechanics and intra-cellular and inter-cellular signaling also needs to be addressed. Perhaps most significantly, a strong collaboration between experimentalists and modeling groups is the bottleneck for the development of the next generation of 3D migration models. Integration of more quantitative experimental data will undoubtedly create new platforms for rich, detailed and multi-scale models that have the mathematical foundations and are capable of predicting complex phenomenon in 3D. Mathematical models are only useful when they are predictive and quantitative, rather than qualitative and simply "postdictive." Such a level of mathematical modeling in 3D motility is yet to arrive, yet right steps in that direction have been taken and an integration of efforts from theoreticians and experimentalists will ensure that level of sophistication in the very near future.

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