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Numerical Computation of Ligand and Signal Related to Invadopodia Formation by Level Set Approach

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Abstract
The invasion of cancer cells during metastasis, marked by structures called invadopodia has contributes significantly to the high death rate among cancer patients. Invadopodia are finger-like protrusions in invasive cancer cells that break down the extracellular matrix (ECM) using enzymes called matrix metalloproteinases (MMPs) aiding in tumor spread. This study investigated how MMP density affects ligand distribution, signal transduction, and invadopodia formation at the plasma membrane. MMP density is modeled as a trigonometric function and actin polymerization drives membrane movement which described as membrane velocity. A level set approach is used to detect plasma membrane movement which combining linear extrapolation and ghost fluid methods. The results show that higher MMP density leads to more aggressive invadopodia, with the highest density of ligands and signals at the membrane interface. Over time, invadopodia size increases, highlighting the important role of MMP density in cancer cell invasion.
Keywords: Cancer cell invasion; invadopodia formation; level set method; free boundary interface.

1. Introduction

Understanding the formation of invadopodia is key to studying invasive cancer cells and their spread which is a major cause of cancer death that is projected to increase by 70% in the next two decades [1]. Cancer which potentially claimed 9.6 million lives in 2018 is the second leading cause of death worldwide. Genetic mutations can change cell regulators to oncogenes, leading to uncontrolled cell division without tumor suppressor genes [2]. Invadopodia, small projections on cancer cell membranes play an important role in cell invasion by accumulating proteins that enhance ECM degradation [3].

Research shows that studying invadopodia is important for preventing metastasis where it is the initial phase of cancer spread. [3] detailed actin reorganization, ECM degradation, receptor signaling and MMP transmission but had limitations with actin connectivity. [4] and [5] introduced a one-dimensional signal transduction model to address this which treats the plasma membrane as a free boundary for continuous actin connections. [6] further explores actin polymerization and boundary conditions using Laplace's equation. Combining these approaches improves our understanding of invadopodia formation and cancer metastasis.

The main purpose of this study is to investigate the effect of MMP density towards the distribution profiles of ligand and signal transduction with the formation of invadopodia at the location of the plasma membrane. This study focused on modeling invadopodia formation using a two-dimensional approach. The model examined how ligand and signal transduction over time influence invadopodia. It employed a first-order Cartesian finite difference technique and a second-order upwind technique to solve the level set method, represented by ψ for a consistent finite difference approximation of the PDEs. The forward difference method was used for computing the time derivative and numerical methods to detect invadopodia on the plasma membrane were developed and solved using Matlab software. Understanding invadopodia is crucial as they help cancer cells spread by breaking down the extracellular matrix (ECM) which allows metastatic cells to invade others. The study highlighted the role of ligand and signal transduction in invadopodia formation and suggesting new therapies could target these factors to prevent invadopodia. Mathematically, understanding the free boundary interface provided insights into how the plasma membrane shifted, with the level set method effectively addressing this issue. This research is significant for developing strategies to prevent metastasis by stopping invadopodia formation.

2. Literature Review

2.1. Metastasis Process

Cancer cells form due to genetic changes that lead to uncontrolled growth and tumor formation [7]. These cells ignore signals that regulate normal cell behavior which results in unchecked growth, invasion of normal tissues, and spread throughout the body [8]. This uncontrolled growth is due to abnormalities in various cell regulatory systems causing cancer cells to avoid growth suppression, resist cell death, support blood vessel growth, invade surrounding tissues, and metastasize. Genetic instability triggers cancer and leads to uncontrolled cell growth, invasion, and metastasis. Key studies by [9] and [10] identified traits of cancer cells like evading growth suppression and resisting cell death. [11] highlighted the role of proteolytic enzymes, particularly matrix metalloproteinases (MMPs), and their interactions with the extracellular matrix (ECM). [12] modeled cancer cell behavior within the ECM, considering chemical and physical responses. [13] and [14] used partial differential equations (PDEs) to describe the dynamics of cancer cell movement within the ECM, while [15] used finite element models to predict tumor location and shape which shows the impact of matrix-degrading enzymes on ECM breakdown.

Further research into metastasis has deepened understanding of cancer spread about molecular variations and treatment approaches. [16] developed a model involving the enzyme lysyl oxidase (LOX) to describe interactions among cancer cells, collagen fibers, and enzymes like MMP and LOX. [17] studied environmental factors affecting breast cancer invasion and [18] used differential equations to model the transition from early cancer stages to invasive phases. These studies have significantly advanced our understanding of cancer cell spread. Using advanced models and experiments, they have provided valuable tools for researchers to test new theories and develop better ways to manage cancer.

2.2. Invadopodia Formation and the Binding of Ligand with Epidermal Growth Factor Receptor

Invadopodia are specialized structures in cancer cells that help them invade tissues and spread to other parts of the body. They form through complex processes involving actin cytoskeleton reorganization and are aided by the enzyme MT1-MMP which breaks down the extracellular matrix (ECM), allowing cancer cells to move through tissues. [3] introduced a mathematical model to understand invadopodia dynamics, focusing on actin reorganization, ECM degradation, and signaling pathways. [4] added signal transduction to address issues like actin disconnection, offering a more detailed understanding of the interaction between signals and structural changes in invadopodia.[6] further refined this by addressing the free boundary problem in cell protrusion formation by using mathematical models and the level set method to manage membrane movement and avoid discontinuities.

The interaction between ligands and the Epidermal Growth Factor Receptor (EGFR) is key in cellular communication and affects processes in both normal and cancer cells. [19] showed how EGFR activation by ligands triggers signaling pathways. [20] studied the structural changes during ligand binding and receptor dimerization, providing insights into EGFR activation. EGFR is essential for cell growth and proliferation, activated by specific ligands like EGF. Mutations in the EGFR pathway can lead to its overexpression, increasing signaling and contributing to tumor growth. Understanding these processes helps identify potential targets for cancer treatment.

3. Methodology

3.1. Mathematical Modeling

The mathematical model explains how cancer cells form structures called invadopodia. It assumes there are no ligands at the boundary of the area being studied. At the interface Γ, the ligand concentration is the same as the MMP concentration, g, and the signal concentration matches the ligand concentration. The plasma membrane moves based on the difference in gradients between the ligand and the signal. The model focuses on ligand density $(c^*(x,t))$, signal transduction $(\sigma(x,t))$, and plasma membrane velocity $(v(x,t))$.

MMPs like MT1-MMP breaks down the extracellular matrix (ECM) at the cell boundary, producing ligands that bind to EGFR on the cell membrane, which triggers signaling within the cell. This signaling causes the cell to change shape due to actin filament growth. Chemoattractants like EGF binding to EGFR control the reorganization of the actin cytoskeleton. In invasive cancer cells, MMPs at the leading edge break down the ECM and creats ligands that move the membrane. The study focuses on ECM density, ligand density, signal transduction, and MMP density to understand the behavior of cancer cells during invadopodia formation. Although invadopodia formation involves various processes, this study focuses only on specific aspects which are ECM density, ligand density, signal transduction and MMP density. As seen in Figure 1, the domain defines the interaction between the variables.

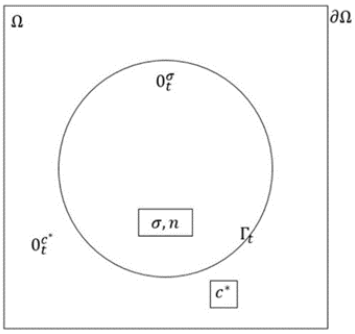


Figure 1 The geometrical setting of the complete domain for two-dimensional invadopodia formation

The mathematical model is represented by the following partial differential equations:

- Ligand (c^*):

$$\begin{aligned} c_t^* &= \Delta c^*, \boldsymbol{x} \in O_t^{c^*}, \boldsymbol{x} \in (x,y), \\ c^*(\boldsymbol{x},0) &= c_0^*(\boldsymbol{x}), \boldsymbol{x} \in O_t^{c^*}, \boldsymbol{x} \in (x,y), \\ c^*|_{\partial\Omega} &= 0, \\ c^*|_{\Gamma_t} &= g|_{\Gamma_t}, t \in [0,T]. \end{aligned} \tag{1}$$

- Signal transduction (σ):

$$\begin{aligned} \sigma_t &= \Delta\sigma, \boldsymbol{x} \in O_t^{\sigma}, \boldsymbol{x} \in (x,y), \\ \sigma(\boldsymbol{x},0) &= \sigma_0(\boldsymbol{x}), \boldsymbol{x} \in O_t^{\sigma}, \boldsymbol{x} \in (x,y), \\ \sigma|_{\Gamma_t} &= c^*|_{\Gamma_t}, t \in [0,T]. \end{aligned} \tag{2}$$

- Velocity of the interface (v):

$$v = \nabla\sigma - \nabla c^*, \Gamma_t. v \in (x,y). \tag{3}$$

- Extension velocity (w):

$$\begin{aligned} (\nabla\psi.\nabla)w &= 0 \text{ on } \Omega, w \in (x,y), \\ w &= v \text{ on } \Gamma_t. \end{aligned} \tag{4}$$

3.2. Numerical Computation and Discretization

Figure 2 depicts the horizontal distance θ_x in between a neighboring point on the right (intracellular) and the interface, Γ_t.

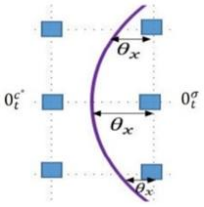


Figure 2 Interface located in between two horizontal meshes with left θ_x to the neighboring point

Based on Figure 2, the discretization is as follow

$$\sigma_t = \sigma_{xx} + \sigma_{yy} , \tag{5}$$

$$\frac{1}{k}\sigma_{i,j}^{n+1} - \frac{1}{k}\sigma_{i,j}^{n} = \frac{2}{(1+\theta_x)h^2}\sigma_{i+1,j}^{n+1} - \frac{2}{\theta_x h^2}\sigma_{i,j}^{n+1} + \frac{2}{\theta_x(1+\theta_x)h^2}\sigma_{i-\theta_x h,j}^{n+1} + \frac{1}{h^2}\sigma_{i,j+1}^{n+1} - \frac{2}{h^2}\sigma_{i,j}^{n+1} + \frac{1}{h^2}\sigma_{i,j-1}^{n+1} , \tag{6}$$

$$\sigma_{i,j}^{n+1} - \sigma_{i,j}^{n} = \frac{2d}{1+\theta_x}\sigma_{i+1,j}^{n+1} - \frac{2d}{\theta_x}\sigma_{i,j}^{n+1} + \frac{2d}{\theta_x(1+\theta_x)}\sigma_{i-\theta_x h,j}^{n+1} + d\sigma_{i,j+1}^{n+1} - 2d\sigma_{i,j}^{n+1} + d\sigma_{i,j-1}^{n+1} , \tag{7}$$

Since the initial and boundary conditions, $\sigma_{i-\theta_x h,j}^{n+1} = (g_{\Gamma_t})_{i,j}^{n+1}$, the equation becomes

$$-\frac{2d}{1+\theta_x}\sigma_{i+1,j}^{n+1} + \left(1 + \frac{2d}{\theta_x} + 2d\right)\sigma_{i,j}^{n+1} - d\sigma_{i,j+1}^{n+1} - d\sigma_{i,j-1}^{n+1} = \sigma_{i,j}^{n} + \frac{2d}{\theta_x(1+\theta_x)}\left(g_{\Gamma_t}\right)_{i,j}^{n+1} . \tag{8}$$

Figure 3 illustrates the horizontal distance θ_{Lx} in between a neighboring point on the left (extracellular) and the interface, Γ_t.

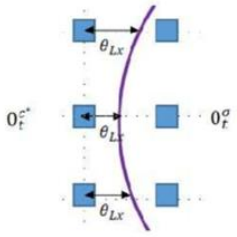


Figure 3 Interface located in between two horizontal meshes with left θ_{Lx} to the neighboring point

Based on Figure 3, the discretization is as follow

$$c_t^* = c_{xx}^* + c_{yy}^* , \tag{9}$$

$$\frac{1}{k}c^{*n+1}_{i,j} - \frac{1}{k}c^{*n}_{i,j} = \frac{2}{(1+\theta_{Lx})h^2}c^{*n+1}_{i-1,j} - \frac{2}{\theta_{Lx}h^2}c^{*n+1}_{i,j} + \frac{2}{\theta_{Lx}(1+\theta_{Lx})h^2}c^{*n+1}_{i+\theta_{Lx}h,j} + \frac{1}{h^2}c^{*n+1}_{i,j+1} - \frac{2}{h^2}c^{*n+1}_{i,j} + \frac{1}{h^2}c^{*n+1}_{i,j-1}\,, \tag{10}$$

$$c^{*n+1}_{i,j} - c^{*n}_{i,j} = \frac{2d}{1+\theta_{Lx}}c^{*n+1}_{i-1,j} - \frac{2d}{\theta_{Lx}}c^{*n+1}_{i,j} + \frac{2d}{\theta_{Lx}(1+\theta_{Lx})}c^{*n+1}_{i+\theta_{Lx}h,j} + dc^{*n+1}_{i,j+1} - 2dc^{*n+1}_{i,j} + dc^{*n+1}_{i,j-1}\,, \tag{11}$$

Since the boundary conditions, $c^{*n+1}_{i+\theta_{Lx}h,j} = (g_{\Gamma_t})^{n+1}_{i,j}$, the equation becomes

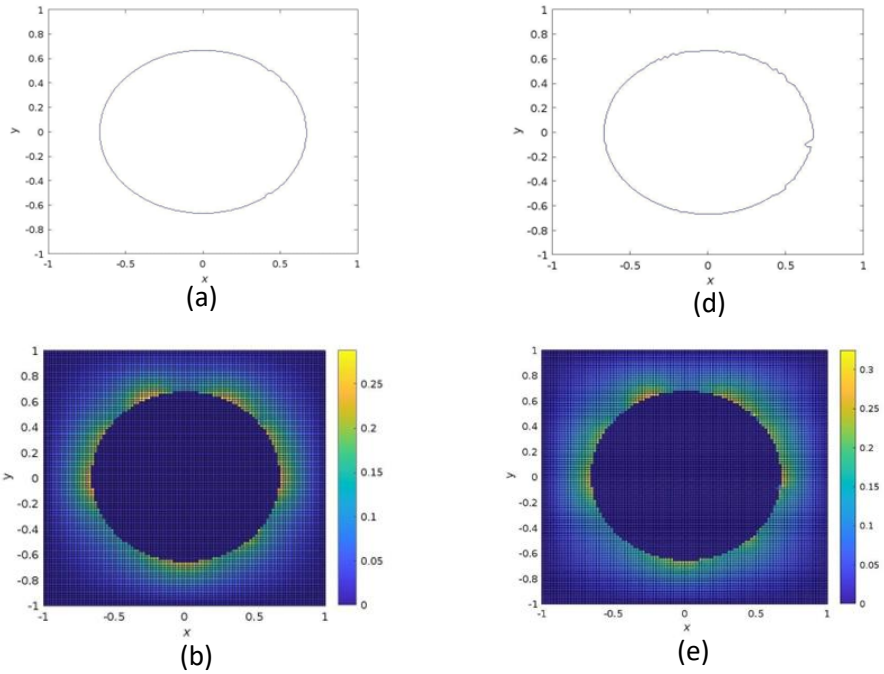
$$-\frac{2d}{1+\theta_{Lx}}c^{*n+1}_{i-1,j} + \left(1 + \frac{2d}{\theta_{Lx}} + 2d\right)c^{*n+1}_{i,j} - dc^{*n+1}_{i,j+1} - dc^{*n+1}_{i,j-1} = c^{*n}_{i,j} + \frac{2d}{\theta_{Lx}(1+\theta_{Lx})}\left(g_{\Gamma_t}\right)^{n+1}_{i,j}\,. \tag{12}$$

4. Results and Discussion

The behavior of protrusions in the plasma membrane of invasive cancer cells changes over time. This section discusses the unsteady nature of ligands and signals and how membrane movement causes these protrusions. The interface location, ligand density, and signal density are analyzed and shown in graphs. Based on the unsteady model, the ligand and signal density follow a heat-like equation.

Observing the protrusions on the plasma membrane helps understand the formation of invadopodia. Graphs showing the interface position, ligand density, and signal density are used to discuss the simulation results for the unsteady model. This model considers cell protrusions on the plasma membrane using the jump velocity approach. Initially, the interface is assumed to be a circle, and the ligand and signal distributions in both intracellular and extracellular regions are set to zero.

4.1. Effect of Large MMP Density



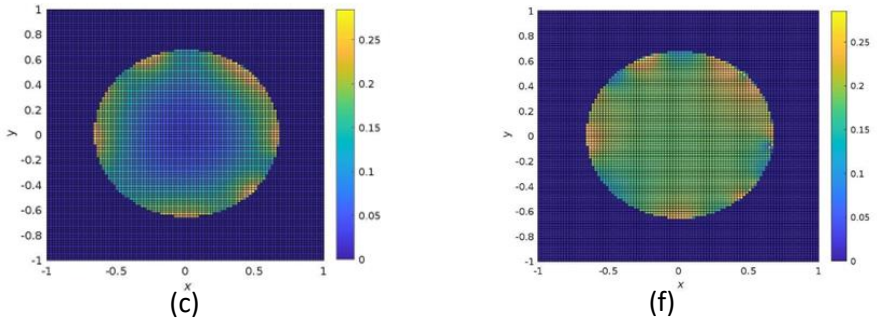


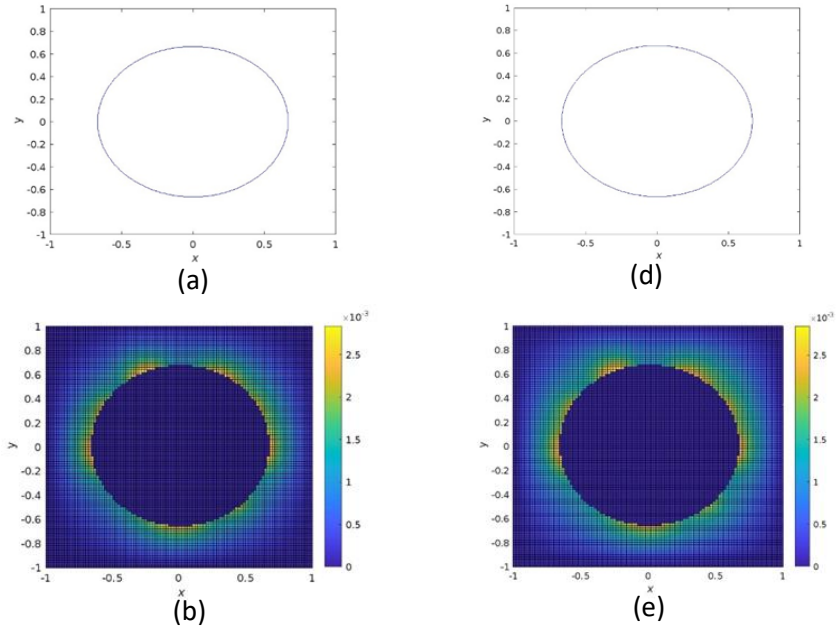
Figure 4 Results for (a) and (d) are interface position, (b) and (e) are ligand density, (c) and (f) are signal density with $t = 5$ and $t = 25$ respectively.

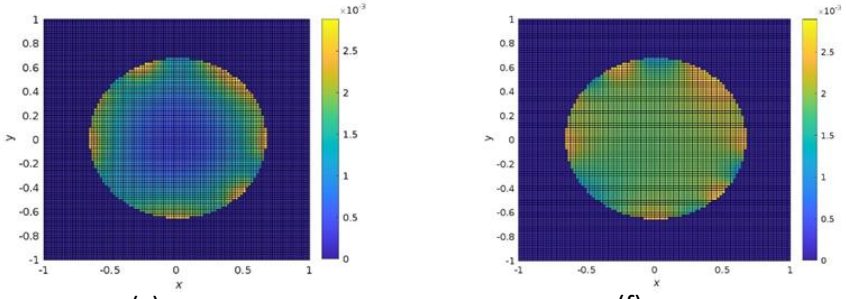
Figure 4(a) shows the formation of the invadopodia through the existence of two small actin-rich protrusions at the bottom right and top right of the plasma membrane. At the location of the protrusions are detected, the ligand density as shown in Figure 4(b) shows increases. Since the MMPs degrade the extracellular matrix and then create ligands, hence the ligand density is spotted higher at the invasion front. As time increased, the ligand slowly diffused throughout the extracellular region and almost all the extracellular region is occupied with the ligand. Nevertheless, a higher density of ligand is noticed at the membrane area where the degradation takes place.

Furthermore, the signal density also shows changes as shown in Figure 4(c). It is because the signal transduction is stimulated through the binding process of the ligand with the membrane-associated receptor such as EGFR. As with ligand, the position of signal transduction is determined from the graphical results where signal spreads only in the intracellular region. To sum up, the stimulation of signal transduction leads to the formation of invadopodia.

In addition, Figure 4(d) depicts the clearly noticed actin-rich protrusions at the bottom right and top right of the interface of the plasma membrane. This protrusions are basically from the small protrusion spotted at the same location of the plasma membrane (Figure 4(a)) and becomes apparent as time increases. In this part, the motion of the free boundary plasma membrane is observed within time. Moreover, the increment of ligand density as shown in Figure 4(e) and signal density (Figure 4(f)) are spotted at the location of the prominent protrusions are located (compared to Figure 4(b) and Figure 4(c)).

4.1. Effect of Small MMP Density





(c) (f)

Figure 6 Results for (a) and (d) are interface position, (b) and (e) are ligand density, (c) and (f) are signal density with $t = 5$ and $t = 25$ respectively.

Figure 6(a) and Figure 6(d) shows an identical graphic of the movement of the free boundary of plasma membrane. It is noticed that, as the epsilon become smaller with the same period of time, the changes of the movement of plasma membrane become diminished and invisible. A similar pattern noticed in both those Figures 6(b) and 6(e) where the ligand densities corresponding to the behavior of protrusion compared to the previous profile (Figure 5(b) and Figure 5(e)).

Conclusion

The graphical results depicts that the protrusion from the movement of the free boundary plasma membrane is detected to represent the presence of the formation of invadopodia. This result is relevant to show that the cancer cell invasion process through metastasis at the sub-cellular level. The size of the invadopodia become obvious as time increases and no movement is visible as the epsilon become smaller. In the meantime, the ligand density and signal profile also shows high density mainly at the location of the protrusions are formed. This truly showed that the ligand and signal densities play an crucial role in forming the invadopodia. The profiles of interface position, ligand and signal densities are graphically presented, which proves the movement of the plasma membrane that form protrusions (invadopodia) can support the invasion of cancer cells, the ligand and signal transduction under the effects of with velocity jump are corresponding to the location of the formation of invadopodia, the existence of the protrusions is higher at $\varepsilon = 0.1$ compared to $\varepsilon = 0.01$ and $\varepsilon = 0.001$. In addition, comparing at two different time $t = 5$ and $t = 25$, the size of the protrusions increased in size as time increased.

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