Malaysian Journal of Mathematical Sciences 13(2): 155–164 (2019)

MALAYSIAN JOURNAL OF MATHEMATICAL SCIENCES

Journal homepage: http://einspem.upm.edu.my/journal

Two-Dimensional Signal Transduction during the Formation of Invadopodia

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Received: 6 November 2018
Accepted: 7 April 2019

ABSTRACT

Signal transduction is an important process associated with invadopodia formation which consequently leads to cancer cell invasion. In this study, a two-dimensional free boundary problem in a steady-case of signal transduction during the formation of invadopodia is investigated. The signal equation is represented by a Laplace equation with Dirichlet boundary condition. The plasma membrane is taken as zero level set function. The level set method is used to solve the complete model numerically. Our results showed that protrusions are developed on the membrane surface due to the presence of signal density inside the cell.

Keywords: Invadopodia formation, Signal transduction, Free boundary problem and Level set method.

1. Introduction

Normally, human cells grow and divide to form new cells as required by the body. Cells grow old or become damaged and die, and new cells take their place. However, this orderly process breaks down when a cancer cell formed through multiple mutation in an individual's normal cell key genes. Consequently, avascular cancer cells is formed after the cells continue to proliferate themselves in the primary site. In order to expand their colony, metastatics cancer cells break out of the tissue compartment and invade locally. This is because Urokinase-type plasminogen activator (uPA) and matrix metalloproteinases (MMPs) enzymes break down the surrounding tissue or extracellular matrix (ECM) protein (Chaplain and Lolas (2005), Vivi et al. (2011)). This invasion known as metastasis process is a major cause of death among cancer patients. Preventing this secondary spread of the cancer cells is able to increase survival rate. The molecular mechanism for metastasis process consist of five main steps:

- Cancer cells attach to other cells and detach from the primary site.
- Cancer cells penetrate and invade the surrounding tissue.
- Cancer cells penetrate the dense basement membrane extracellular matrix (ECM) surrounding blood vessel walls (intravasation).
- Cancer cells transport through blood stream and lymphatic vessel.
- Cancer cells escape from the stream (extravasation), proliferate and hence form new colony in a secondary site.

Recently, studies on intravasation process in subcellular view become a main concern since the formation of actin rich protrusions in the cell leads to cancer cells invasion and migration (Hideki et al. (2005a), Helicia et al. (2014) and Or-Yam and Benjamin (2014)). Invadopodia is the specialized filamentous (F)-actin rich protrusions localized on the ventral membrane of a cell and has the ability to penetrate the ECM proteins (Hadas and Hava (2012)). The formation of invadopodia and degradation of ECM key processes are signal transduction, actin polymerization and assembly (chemotaxis), up regulation of matrix metalloproteinases (MMPs), degrading of extracellular matrix (ECM) and ligand formation.

In the intracellular membrane, epidermal growth factor receptor (EGFR) which provides signalling molecules such as Nck and Rho GTPases cdc42 and

N-WASP is activated (Hideki et al. (2005b)). N-WASP induces actin polymerization through the activation of Arp 2/3 complex. Arp 2/3 complex nucleates the actin filaments (F-actin) and forms branched filament network (assembly) (Kazuhiro et al. (2015)). Next, the EGFR induces cortactin to promote protease secretion matrix metalloproteases (MMPs). MMPs complex push the membrane and degrades the ECM proteins. Low density lipoprotein (LDL) receptor eliminates excess active proteinases from tissue and body liquid, and ligand is formed instead (Mark and Georgios (2005)). The ligand binds to extracellular membrane with the help of signalling receptor called low receptor-related protein 1 (LRP1). Once again, the process of actin polymerization happen with activation from the signal transduction. All the processes are correlated with one another and EGFR processes shows to be the starting point of the invadopodia formation (Aron and Alissa (2015)). Now, researchers need to focus on the study of signal transduction during invadopodia formation before proceeding with other processes.

In mathematical studies, many continuous and discrete models have been proposed to visualize the behavior of cancer cell invasion. Some studies are able to capture the cancer structure successfully at the tissue level (Vivi et al. (2011) and Mark and Georgios (2005)). Currently, model of the cancer cell invasion at sub cellular level has become the main interest since the cancer cell begins to migrate from the primary site through the formation of invadopodia around $1 - 10\mu m$.

Saitou et al. (2012) derived a continuous model based on partial differential equations (PDEs) describes the formation and maturation of invadopodia. The model consists of actin polymerization, movement of matrix metalloproteinases (MMPs), degrading of extracellular matrix (ECM) and ligand formation. Their work is able to generate protrusions with small value of the effect of MMP rate constant. But, Saitou's model faced a problem where the region of actin, $n > 0$ becomes disconnected as time progresses.

Due to that, Admon (2015) proposed a new model where the author considered the signal transduction (inside the cell) that leads the invadopodia formation which was not taken into account in Saitou et al. (2012) and treated the cancer cell membrane as free boundary using fixed domain method in one dimension. Hence, the region of actin continuous to be connected as time advances (Admon and Suzuki (2017)).

Since the formation involves a free boundary problem, a two dimensional simulation is needed to gain a clear picture of invadopodia formation with presence of signal transduction. In this paper, a simulation of the moving boundary

problem of steady-case of signal transduction associated with invadopodia formation in two dimension had been investigated by using level set approach. The membrane is set to be free boundary membrane where it may shrink when the membrane is pulled inside or expands if the membrane is pushed outside the cell region. The algorithm proposed by Olivier et al. (2017) is considered in this study where level set method with first order Cartesian finite difference scheme is used. In the next section, the mathematical formulation of the model and the method of solution used throughout this study is provided.

2. Mathematical Formulation

Consider the square domain, Ω with smooth boundary $\partial\Omega$. A cell is embedded inside the domain with the plasma membrane, Γ_t be the interface. The cell consists of interior region, $\Omega_t^\sigma \subset\subset \Omega$, interface, $\partial\Omega_t^\sigma \equiv \Gamma_t$ and exterior region, $\Omega_t^c = \Omega \backslash \Omega_t^\sigma$ where $\Omega \subset \mathbb{R}^2$. Figure 1 shows the geometrical setting of the complete domain.

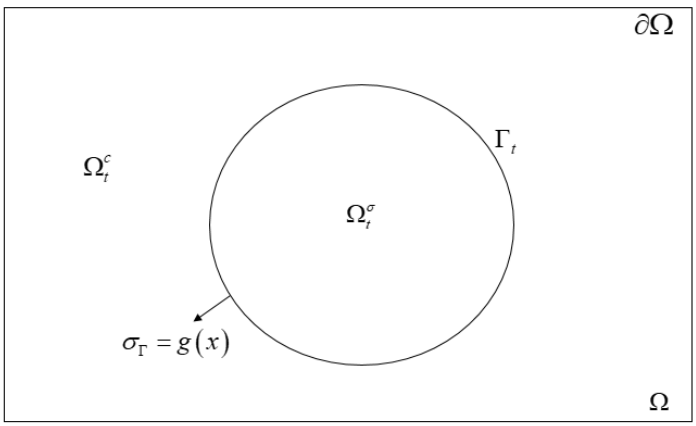

Figure 1: Two dimensional invadopodium formation with signal transduction.

Here, the signal, $\sigma(\mathbf{x})$ is stimulated upon contact between ligand, $c(\mathbf{x})$ and membrane-associated-receptors in order to enhance the actin assembly corresponding to the formation of invadopodia. The governing equation for the

steady-case of signal transduction is as follows (Admon and Suzuki (2017));

$$\underbrace{d_\sigma(\triangle\sigma)}_{\text{diffusion}} = 0, \quad \mathbf{x} \in \Omega_t^\sigma, \tag{1}$$

where $\sigma(\mathbf{x})$ and d_σ are the signal density and signal diffusivity coefficient, respectively. $\sigma(\mathbf{x})$ is taken to be equal to a function, $g(\mathbf{x})$ that represents the flux of MT1-MMP enzymes at any time t on the interface. There is no signal density in the exterior region since the signal is only stimulated inside the cell (Karla and Marc (2014)). The membrane moves with normal velocity on the interface. The velocity is assumed to be equal to the gradient of the signal inside the cell,

$$\mathbf{V} = \gamma_n(\nabla\sigma) \quad \mathbf{x} \in \Gamma_t,\, t > 0, \tag{2}$$

where γ_n is a positive constant. For the simplification, constants γ_n and d_σ are considered to be equal to 1. Consequently, the two dimensional steady-case of the signal transduction during the formation of invadopodia is governed by the following,

$$ST \begin{cases} \triangle\sigma = 0, & \mathbf{x} \in \Omega_t^\sigma, \\ \sigma(\mathbf{x}) = 0, & \mathbf{x} \in \Omega_t^c, \\ \sigma(\mathbf{x}) = g(\mathbf{x}), & \mathbf{x} \in \Gamma_t, \\ \mathbf{V} = \nabla\sigma, & \mathbf{x} \in \Gamma_t, \end{cases}$$

for all $t > 0$ where $\mathbf{x} = (x, y)^T$.

Numerous numerical approaches to solve free boundary have been conducted in the past. There exist two common ways to handle the moving boundary problem; first, by transforming the original equation to an approximation model; second, trailing directly the free boundary domain. An example for the approximating the original model is grid deformation using finite element method. This method is a powerful tool since it is able to simulate the free boundary problem up to three dimensional case (Sashikumaar and Shangerganesh (2017)). However, this adaptive technique tends to be more complicated and computationally expensive.

Trailing directly the free boundary or by using fixed grid method provides great flexibility to represent topologically complicated moving interface. Level set method is a well suited method to simulate the free boundary problem. This is because, instead of tracking the boundary or front using Lagrange approach, one can capture the front on a fixed grid (Eulerian approach). Osher and Fedkiw (1992) were the first to introduce this method. Improvements of this method has been observed in the past few years (Chen et al. (1997)), Sethian

and Straint (1992)). In this study, the method proposed by Olivier et al. (2017) is used. This method is easy to implement and more effective in order to capture the moving interface since they are approximated by method of first order of accuracy.

Firstly, the plasma membrane, Γ_t is taken to be equal to zero level set function $\psi(\mathbf{x}, t)$ for all time, t such that,

$$\Gamma_t = \{\mathbf{x} \in \Omega : \psi(\mathbf{x}, t) = 0\}, \tag{3}$$

which satisfies the equation of motion,

$$\psi_t + \mathbf{V} \cdot \nabla\psi = 0. \tag{4}$$

An implicit function for the initial interface, $\psi(\mathbf{x}, t)$ is considered and is defined by

$$\psi(\mathbf{x}, 0) = x^2 + y^2 - r^2, \tag{5}$$

where r is the radius of a circle. The velocity needs to be extended to the exterior region, Ω_t^c from the interface in order to avoid discontinuities near the interface. The equation of velocity extension, $\mathbf{W}$ is

$$(\nabla\psi \cdot \nabla)\mathbf{W} = 0, \quad \mathbf{x} \in \Omega_t^c, \tag{6}$$

where $\mathbf{W} = \mathbf{V}$ on the interface. Note that the level set function is updated using (4). The algorithm to solve ST is outlined as follows:

1. The initial condition for level set function, $\psi(\mathbf{x}, 0)$ is set by using (5).
2. The initial condition for signal, $\sigma(\mathbf{x})$ is considered as $\sigma(\mathbf{x}) = g(\mathbf{x})$ on the interface.
3. The laplace operator of signal is computed in Ω_t^σ to get the solution of $\sigma(\mathbf{x})$ as stated in (1).
4. The velocity, $\mathbf{V}$ is computed in the signal region, Ω_t^σ denoted by (2).
5. The velocity inside the interface is extended to the outer region and is computed as in (6).
6. The forward Euler scheme is used to update the level set function, $\psi(\mathbf{x}, t)$ by following (4).
7. Steps 2 to 6 are repeated to get the updated solution of $\sigma(\mathbf{x})$ and $\psi(\mathbf{x}, t)$.

3. Result and Discussion

As stated in Section 2, ST model is computed by using level set method with first order Cartesian finite difference scheme. The domain Ω is assumed as a square box of $[-L, L] \times [-L, L]$ and the number of grid is considered up to $(M+1)^2$. The model is tested with Dirichlet boundary condition on the interface where the trigonometric function is considered as $g(\mathbf{x}) = 0.1[2 + \cos(3\pi(x+y))\cos(\pi(x+0.3))]$ (Olivier et al. (2017)). Here are the list of parameter values used in the simulation.

Table 1: Parameters values used to solve ST.

Parameter	Value
Ω	$[-1, 1] \times [-1, 1]$
M	100
N	1000
L	2
h	L/M
dt	t_{max}/N
t_{max}	10
r	0.5

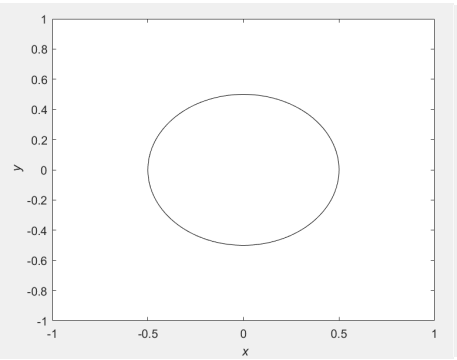

Figure 2: Initial interface of a cancer cell

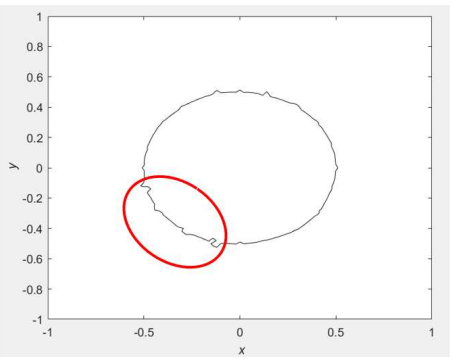

Figure 3: New interface after one timestep

Theoretically, the signal distribution should exist only inside the cell(Danielle et al. (2011), Karla and Marc (2014)), hence the initial signal profile is shown as in Figure 4.

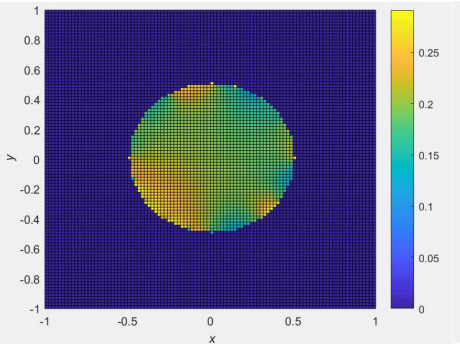

Figure 4: Initial signal distribution

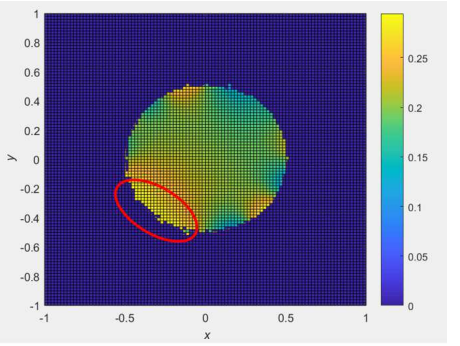

Figure 5: New signal distribution

A simple hypothesis is conducted where the invadopodia will form with the presence of signal distribution. Figure 3 showed protrusion structures appeared on the membrane after the first timestep. The structures indicate the invadopodia on the cancer cell membrane exists.

Besides that, the signal distribution profile is also computed in this study. Admon and Suzuki (2017) proved that the maximum concentration of signal is located on the interface. Hence, this study also aims to validate their result. Figure 4 displays the signal distribution profile after first timestep. The yellow colour near the position of the membrane (red circle) indicates the highest density of the signal. Consequently, there are some protrusions formed at the high signal density as shown in Figure 3.

4. Conclusions

This study is an extension from the work done by Admon and Suzuki (2017)). The signal transduction variable is considered in two dimension due to the fact that signal also plays an important role during invadopodia formation. The signal is stimulated upon contact between ligand and membrane-associated-receptor which consequently, lead to the formation of invadopodia. The movement of the plasma membrane is driven by the gradient of the signal inside the cell. Here, the membrane is treated as free boundary where the boundary is considered as zero level set function.

The model of signal transduction is solved numerically by using level set method with first order Cartesian finite difference scheme. Based on numerical results after the first timestep, the protrusions appeared demonstrated the

existence of invadopodia. In addition, through the signal distribution profiles, there exist different concentration of signal distribution inside the cell. As a result, at the highest concentration of signal distribution visualized the formation of some protrusion. As a conclusion, the protrusions are developed on the membrane surface due to the presence of signal density inside the cell.

Acknowledgement

The author would like to thank the Ministry of Education Malaysia, GE STEM grant (07397), MyBrainSC, UTM Research Management Centre through FRGS grant (5F098) and JSPS Core-to-Core Program grant for funding.

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