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NUMERICAL SIMULATION OF UNSTEADY SIGNAL TRANSDUCTION DURING THE FORMATION OF INVADOPODIA USING LEVEL SET METHOD

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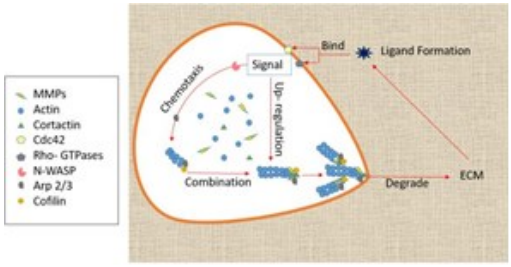
Graphical abstract



Abstract

The finger-like protrusions formed by an invasive cancer cell known as invadopodia is actively observed recently because of this formation on the cancer invasion. The signal on the interface which is stimulated upon contact between epidermal growth factor receptor and ligand, is investigated to be the start of invadopodia formation. In this research, a model of invadopodia formation with signal variable is formulated in two dimensions. The plasma membrane is assumed to be free boundary. The signal is in an unsteady state. The equation of signal is represented by heat-like equation with time-dependent boundary condition. Trigonometry types of the boundary condition which are sine and cosine function are tested to identify the most suitable boundary condition represented the free boundary. The plasma membrane is considered as zero level set function. The membrane moves by the velocity of the signal inside the cell. To handle the free boundary problem, the level set method combining features of ghost method, interpolation and extrapolation method is applied to solve the model numerically. Our result shows that the free boundary is moved at different positions and seen to move inward meaning that the boundary has shrunk. Cosine function is discovered to fit the boundary conditions since the boundary solutions are stable across the time. The computation of the signal density profiles displayed highest density on the membrane.

Keywords: Invadopodia formation, signal transduction, unsteady case, free boundary problem, level set method

Abstrak

Bonjolan berbentuk jejari terhasil dari invasif sel kanser dikenali sebagai invadopodia sedang aktif diperhatikan kerana memberi kesan kepada penghasilan pencerobohan kanser. Signal dipermukaan yang dirangsang dari persentuhan antara reseptor faktor pertumbuhan lapisan sel dan ligan diselidik menjadi pemula penghasilan invadopodia. Di dalam kajian ini, model penghasilan invadopodia bersama pemboleh ubah signal dirumuskan di dalam dua dimensi. Membran plasma diandaikan sebagai sempadan bebas. Signal dipertimbangkan di dalam keadaan tidak stabil. Persamaan signal diwakili dengan persamaan berbentuk haba berserta

dengan pergantungan masa bagi syarat sempadan. Trigonometri bagi syarat sempadan di mana fungsi sinus dan kosinus diuji untuk mengenalpasti syarat sempadan yang paling bersesuaian mewakili sempadan bebas. Membran plasma dipertimbangkan sebagai fungsi sifar set aras. Membran digerakkan oleh halaju signal di dalam sel. Untuk mengawal masalah sempadan bebas, kaedah set aras yang menggabungkan kaedah titik bayang, penentudalaman dan penentuluaran digunakan untuk menyelesaikan model secara berangka. Keputusan kami menunjukkan sempadan bebas bergerak dengan kedudukan yang berbeza dan dilihat bergerak ke dalam menunjukkan sempadan mengecut. Fungsi kosinus didapati memenuhi syarat sempadan kerana hasil sempadan kekal stabil sepanjang masa. Penghitungan profil ketumpatan signal memaparkan ketumpatan tertinggi di sempadan.

Kata kunci: Penghasilan invadopodia, Transduksi signal, Kes keadaan tidak stabil, Masalah sempadan bebas, Kaedah set aras

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1.0 INTRODUCTION

Cancer is characterised by the evasion of growth inhibitors, release of pro-angiogenic signals, apoptosis, , invasion of cancer cells, and metastasis [1,2]. This invasion and migration, known as metastasis, is reported to be one of the leading causes of mortality among cancer patient [3]. Hence, in the past decade, intensive studies have been done through biological [4,5] or mathematical [6,7] approaches to acknowledge the molecular mechanism of cancer cell invasion since there is a chance for treating cancer patients with an anti-invasion therapy.

The metastasis from a subcellular perspective, through vivo and vitro studies [8,9] found the small punctuated finger-like protrusions called invadopodia [10] existed on the cancer cell's ventral membrane. Invadopodia play a vital role in the cancer cells invasion where they able to degrade the extracellular matrix complex that consists of many proteins [11]. Studies on signal transduction factor is important since it may mediate the invadopodia formation [12].

The numerical simulation and mathematical modeling of invasion of cancer from the perspective of subcellular are active but limited. Saitou *et al*. [6] were first to highlight the fundamental processes of invadopodia formation with a fixed plasma membrane, such as actin reorganisation, extracellular membrane (ECM), degradation, ligand synthesis, membrane receptor signalling, and matrix metalloproteases (MMP) distribution in the invasion front. With the lower rate of MMP, their work can result in protrusions. However, as time passes, the actin area becomes disconnected.

In correspondence to the problem, an additional variable was proposed by Admon [13] where the researcher took signal transduction into account in the prior model. His study concentrated on the relationship between signal existence and the output of invadopodia in one-dimensional space due to the model's complexity and the assumption that the signal variable is independent of other variables. By using the fixed domain approach, the boundary was described as a free boundary and this work was validated using the integrated penalty method. Hence, the boundary shifted slowly and steadily over time, indicating the elongation of the membrane and invadopodia had emerged. In investigating the signal concentration, Admon and Suzuki [7] also did a signal distribution profile simulation and this leads to a discovery that on the membrane location, the concentration is the highest. However, studies in higher dimensions are required to fully understand the relationship between signal transduction and invadopodia development, particularly the problem that involves free boundaries.

Recently, reserchers focus on the free boundary of invadopodia for two and three dimension case. Azhuan *et al*. [14] conducted a study on signal transduction in two dimensions for steady case with Dirichlet boundary condition. They simulated the model numerically using the level set method. The result shows that the boundary moves as time increases, which implies that the protrusion exists. They also discovered that on the interface, signal density is the highest. Yaacob et. al [15] proposed a mathematical modelling of the free boundary of invadopodia formation including ligand and signal transduction variables. From their results, it is observed that the stimulated signal transduction from the membrane-associated receptor and ligand binding results in the motion of plasma membrane thanks to the level set approach where the free boundary is represented with the level set function. Recently, Ramlee et. al [16] considered five variables in their computation to represents the formation of invadopodia. Entalphy method was considered to solve the model numerically and they able to resolve the deformation and invasion of cancer cells. Most recently, Hamidi et. al [17] investigate the formation of invadopodia with three variables which are ligand, epidermal growth factor receptor (EGFR) and signal

transduction. They able to demostrated the formation protrusions on the plasma membrane.

There is no study in literature considering the unsteady case of signal transduction in two dimension case to investigate the free boundary positions (plasma membrane location) and the signal distribution profiles during the formation of invadopodia with time dependent boundary condition. Hence, this study highlight the model of invadopodia formation with the signal is represented by a heat-like equation. The level set method with combined features of ghost method, interpolation and extrapolation method is applied to discretize the model and the result is simulated using Matlab software. In the next section, the formulation of the mathematical model and the level set approach was applied throughout this work are described.

2.0 MATHEMATICAL FORMULATION

A Eulerian approach was applied where a square domain, Ω is considered with smooth boundary, $\partial\Omega$. A circle shape representing the cell is embedded inside the domain with the plasma membrane, Γ_t as the interface. The domain is divided into three parts; signal domain (inner), $\Omega_t^\sigma \subset\subset \Omega$, interface, $\partial\Omega \equiv \Gamma_t$ and the dense proteins domain (outer), $\Omega_t^c = \Omega \setminus \Omega_t^\sigma$ where $\Omega = \mathbf{x} \subset \mathbb{R}^2$, Figure 1 depicts the geometrical setting of the complete domain.

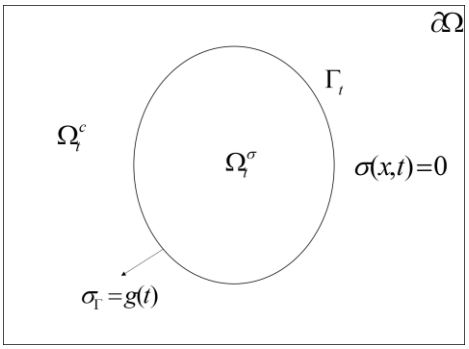


Figure 1 Schematic diagram of invadopodia formation

Here, the actin assembly necessary for the development of invadopodia is facilitated by the signal, which is triggered after ligand and membrane-associated receptors interaction. Hence, the governing equation for the unsteady case of signal transduction is as follows;

$$\sigma_t = d_\sigma(\Delta\sigma), \quad \mathbf{x} \in \Omega_t^\sigma, \tag{1}$$

where $\sigma(\mathbf{x},t)$ and d_σ are the signal density and signal diffusivity coefficient, respectively. On the interface, $\sigma(\mathbf{x},t)$ is taken as a function, $g(\mathbf{x},t)$ to portrays the flux of MT1-MMP enzymes at any time t. Besides, the membrane moves at the normal velocity on the interface and its velocity is assumed to be similar as the signal gradient inside the cell, $\mathbf{V} = \gamma_n(\nabla\sigma)$, $\mathbf{x} \in \Gamma_t$, $t > 0$, where γ_n is a positive constant. For the simplification, we consider constants γ_n and d_σ are equal to 1. Accordingly, during the invadopodia formation, the two-dimensional unsteady-case of the signal transduction (STU) is governed by the following STU model,

$$STU = \begin{cases} \sigma_t = \sigma_{xx} + \sigma_{yy}, & \mathbf{x} \in \Omega_t^\sigma, \\ \sigma(\mathbf{x},0) = 0, & \mathbf{x} \in \Omega_0^c, \\ \sigma(\mathbf{x},t) = g(t), & \mathbf{x} \in \Gamma_t, \\ \mathbf{V} = \left\langle \dfrac{\partial\sigma}{\partial x}, \dfrac{\partial\sigma}{\partial y} \right\rangle, & \mathbf{x} \in \Gamma_t, \end{cases} \tag{2}$$

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

Several numerical methods were conducted to handle the free boundary problem. One of the known methods is the level set where this method trails the moving boundary directly. There are several steps in applying the level set method such as level set function initialization, computation of the velocity, extension of the velocity and updating the new level set function. An improvised version of level set method proposed by [18] and is employed in this study because it is simpler to use and more constructive in capturing moving interfaces.

Throughout this computation, the plasma membrane, Γ_t is set as zero level set function, $\psi(\mathbf{x},t)$ for all time, t. Thus, the STU model is solved as stated in the following algorithm:

1. The initial condition of the level set function is fixed by the implicit function.
$$\psi(\mathbf{x},0) = x^2 + y^2 - r^2, \tag{3}$$

2. The initial condition of signal density, $\sigma(\mathbf{x},0)$ on the interface is considered as
$$\sigma(\mathbf{x},0) = f(\mathbf{x}), \qquad \mathbf{x} \in \Gamma_0, \tag{4}$$

3. The Laplace operator of the signal is calculated only in Ω_t^σ to solve for $\sigma(\mathbf{x})$ by using equation,
$$\sigma_{xx} + \sigma_{yy} = 0, \qquad \mathbf{x} \in \Omega_t^\sigma, \tag{5}$$

to obtain initial signal distribution.

4. Afterwards, in the signal region, Ω_t^σ the velocity, $\mathbf{V}$ is computed using the following equation,

$$\mathbf{V} = \nabla\sigma, \qquad \mathbf{x} \in \Gamma_t,\ t > 0. \tag{6}$$

5. Next, the velocity is extended away from the interface, $\mathbf{W}$ where a point which is located next to the interface point is considered as near the interface, otherwise the point is considered away from the interface and is computed.

$$(\nabla\psi \cdot \nabla)\mathbf{W} = 0, \qquad \mathbf{x} \in \Omega_t^c, \tag{7}$$

where $\mathbf{W} = \mathbf{V}$ on the interface. Note that, the extension of velocity to the exterior region is needed to avoid discontinuities next to the interface.

6. Following that, the level set function, $\psi(\mathbf{x},t)$ is reformed.

$$\psi_t + \mathbf{V} \cdot \nabla\psi = 0. \tag{8}$$

7. Then, the heat-like equation is computed by using equation as follows,

$$\sigma_t = \sigma_{xx} + \sigma_{yy}, \qquad \mathbf{x} \in \Omega_t^\sigma, \tag{9}$$

to get an updated signal density.

8. Finally, steps 4-7 are recommenced to get the updated solution of $\psi(\mathbf{x},t)$ and $\sigma(\mathbf{x},t)$ until a maximum time computation.

3.0 RESULTS AND DISCUSSION

In the following simulation, level set method with first order Cartesian finite difference scheme was conducted as mentioned in the previous section. The domain is assumed as a square box of $[L,L]\times[L,L]$ and the number of grids is considered up to $(M+1)^2$. The model is examined using initial condition and time dependent boundary condition on the interface, respectively, where the trigonometric function is considered initially as,

$$f(\boldsymbol{x}) = 0.1\left[2 + \cos\left(3\pi\left(x+y\right)\right) + \cos\left(\pi\left(x+0.3\right)\right)\right] \times 0.1 \tag{10}$$

[18] and followed by two proposed trigonometric functions: sine and cosine functions to identify the most suitable boundary condition. Here, the initial condition provided by Gallinato *et al*. [18] is multiplied by 0.1 to guarantee the stability of the solutions. Initially, the signal densities are distributed on the interface (see Figure 2). The boundary conditions are multiplied to the initial condition to avoid wide jump in the signal values.

$$g(\boldsymbol{x},t) = m\rho(t)f(\boldsymbol{x}), \qquad t > 0, \boldsymbol{x} \in \Gamma_t \tag{11}$$

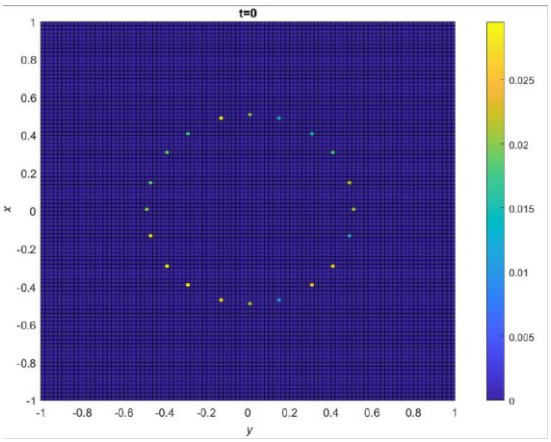


Figure 2 Initial signal density profiles following [18]

where $p(t)$ represented the trigonometric function. A constant of *m* is introduced in Eq 11 because for the case of sine function, if *t* is too small, the value of $\sin t$ approaches 0, hence lead to static interface movement. In contrast, for cosine function, if *t* is equal to 0, the value of $\cos t$ becomes 1. Consequently, there exist wide jump of signal values on the boundary (between $t = 0.0005$ and $t = 0.001$) and hence leads to the unstable solution. Note that, *m* must be chosen properly to ensure the stability of the solutions. Trial and error technique is applied since there is no available reference in the literatures. The list of parameters values used in the simulation is shown in Table 1.

Table 1 Parameters values used to solve STU

PARAMETER	VALUE
Ω	$[-1,1]\times[-1,1]$
M	100
N	1000
L	4
$t_{\max}$	1
r	0.5
h	L/M
dt	$t_{\max}/N$

This study is aimed to investigate three main hypotheses; (i) the effect of the unsteady signal transduction process on the formation of invadopodia (protrusion formation), (ii) the suitable boundary condition, $g(x,t)$ to represent the stimulation of signal on the membrane and (iii) the distribution of the signal densities.

3.1 Trigonometric Function of Boundary Condition

Two types of trigonometric function are considered which are sine and cosine functions since these functions consist only values in between −1 and 1. Note that the basic trigonometric functions are considered for simplification signal densities computation purpose.

3.1.2 Sine Function

Figure 3 displays the interface position at the first iteration ($t = 0.0005$). The existence of a small protrusion on the interface implies that there are invadopodia formation due to the presence of signal with stability value,

$$\eta = \max\left(\sigma^x, \sigma^y\right)\left(2dt / h\right) \tag{12}$$

is recorded as 0.3267. As time increases, several other protrusions are formed at different location on the interface (Figure 4).

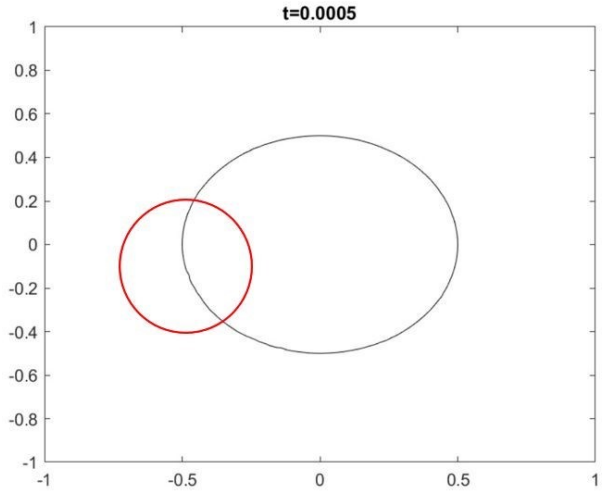


Figure 3 Interface position at $t = 0.0005$

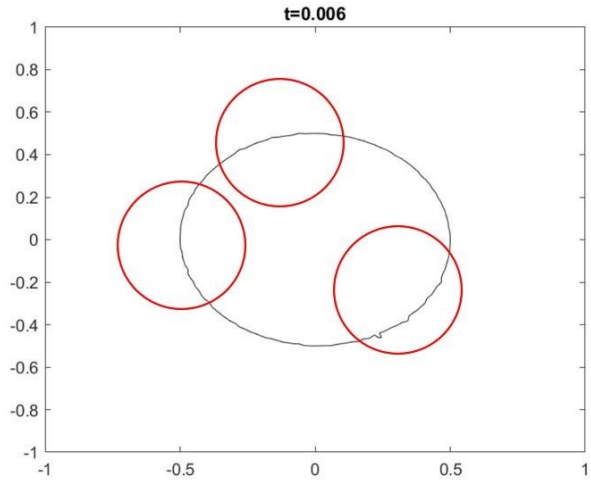
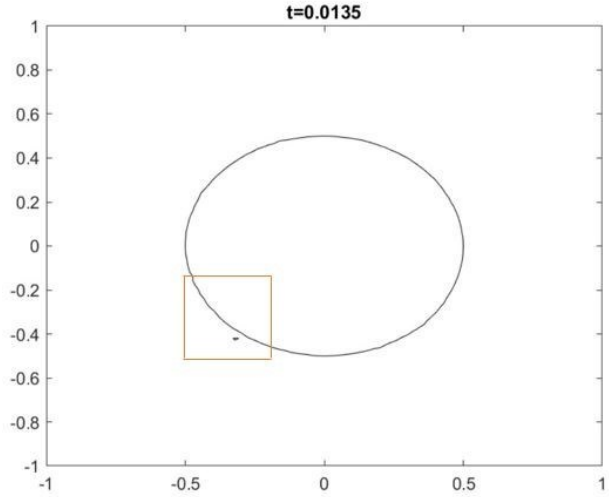
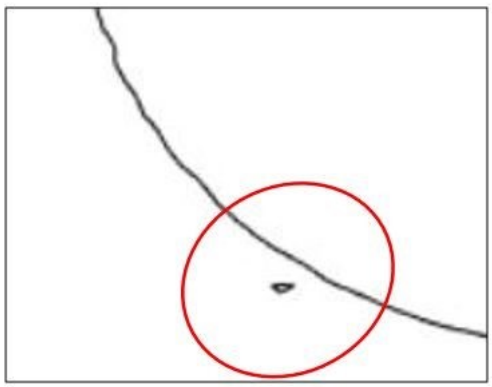


Figure 4 Interface position at $t = 0.006$



5(a)



5(b)

Figure 5 (a) Interface position at $t = 0.0135$ (b) Close up disconnection of interface

However, at $t = 0.0135$, the interface becomes disconnected (see Figure 5). This is because, the solutions become unstable as time increases (see Figure 6) where the stability value reached 58.01. The solution become unstable since there exists higher value of the velocity on the exterior points in both components as time progressed. Note that, for the case two off-grid interface, the velocity on the off-grid interfaces are high, so thus the velocity value on the exterior points. Therefore, sine function is not suitable to represent the boundary condition of the signal densities on the interface of the cell membrane due to the instability solutions.

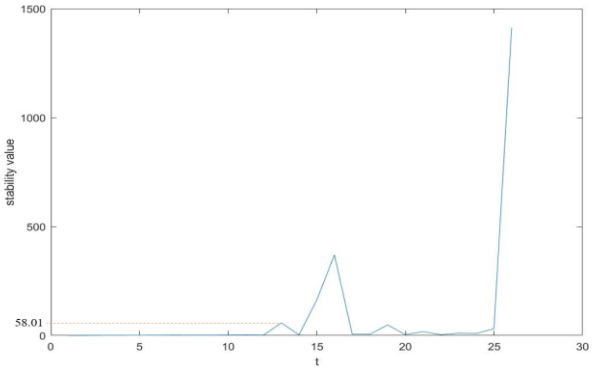
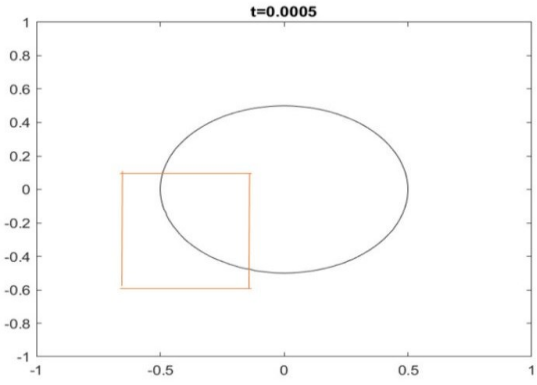


Figure 6 Stability values across time computation with sine boundary condition

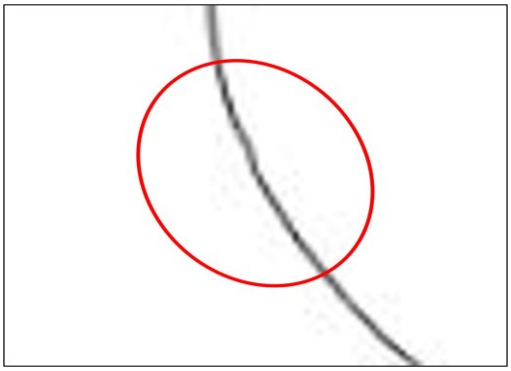
3.1.3 Cosine Function

Initial signal solutions ($t = 0.0005$) are obtained (see Figure 7) where the stability value is recorded as 0.3267. The interface position is discovered to move randomly where the interface is seen to move slightly inward, meaning that the plasma membrane is shrunk and indicate that invadopodium may exist.

As the time increases, the interface continues to move inward, up to $t = 0.005$. as shown in Figure 8. However, after $t = 0.0055$ the interface starts to remain unchanged (see Figure 9) since the solution is in stable state and the value of the stability played in the range of 0 to 0.1 as time increases as shown in Figure 10.

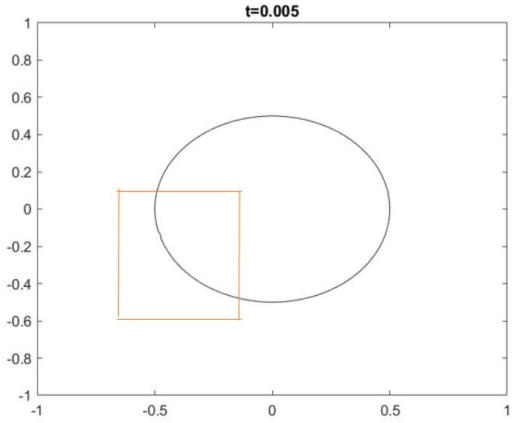


7 (a)

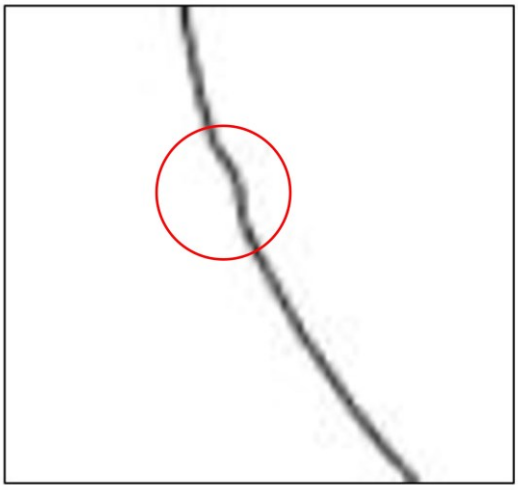


7 (b)

Figure 7 (a) Interface position at $t = 0.0005$, (b) The slightly movement of interface



8 (a)



8 (b)

Figure 8 (a) Interface position at $t = 0.005$, (b) the interface continues to move inward

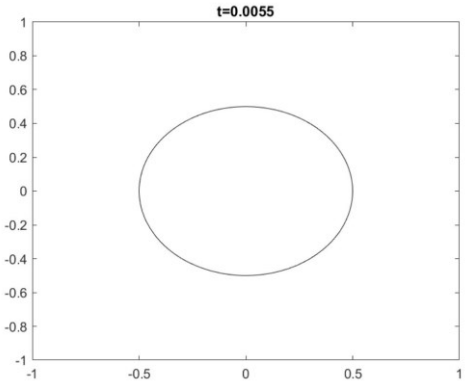


Figure 9 Interface position at $t = 0.0055$

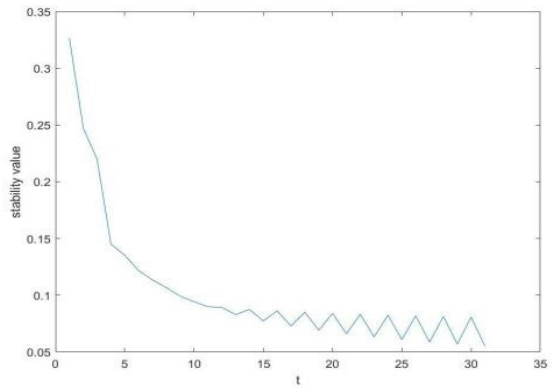
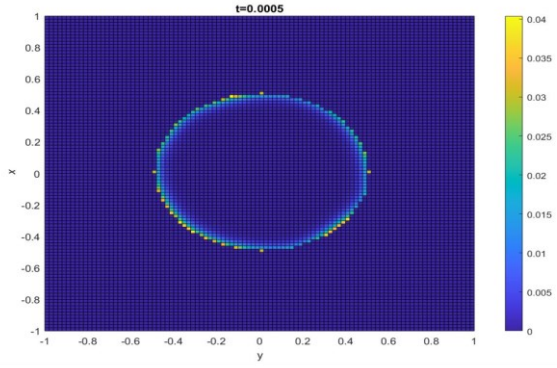


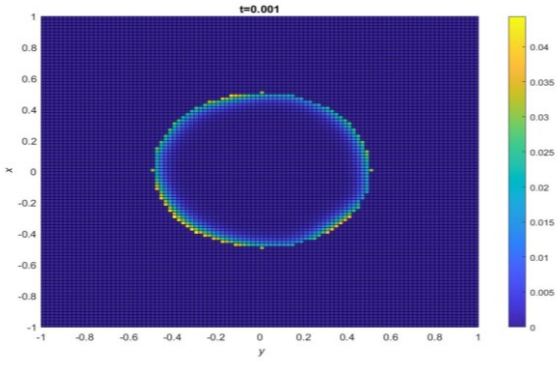
Figure 10 Stability values across time taken with cosine boundary condition by using Eq 12

Since the moving interface is not disconnected at any time, the cosine function can be considered as one of the options to represent the boundary condition of the signal on the interface of plasma membrane.

Third hypothesis is conducted to study the behaviour of the signal distribution. The signal started to diffuse inside the cell at $t = 0.0005$ (see Figure 11 (a)). The yellow and light blue colors indicate the highest and lowest signal density that exists inside the cell. Meanwhile, dark blue color indicates that there is no signal density distribution. The signal continues to diffuse further inside the cell as time increases (see Figure 10 (b)).



11 (a)



11 (b)

Figure 11 Signal density profile, (a) at $t = 0.0005$ and (b) at $t = 0.001$

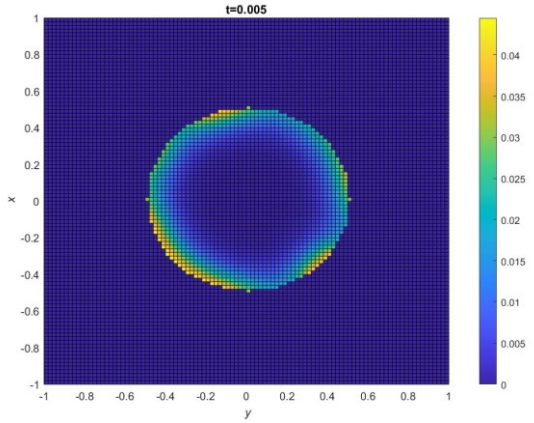


Figure 12 Signal density profile at $t = 0.005$

As $t = 0.005$, the interface is shrunk as shown in Figure 8, and the signal density increases inside the cell where there exists increment of green region inside the cell as shown in Figure 12 compare to the initial signal density as in Figure 11. In addition, there is no signal density outside the cell since the signal only exists inside the cell as mentioned earlier [19-20]. The highest signal densities (yellow color) are also discovered only on the membrane.

4.0 CONCLUSION

This paper aimed to investigate the invadopodia creation using the unsteady model of signal transduction. The membrane is taken as a free boundary and moves with a velocity that was assumed to be like the gradient of intracellular signal. Besides, the heat-like equation with time-dependent boundary condition is considered for the signal equation. Numerical simulations were conducted using the level set method combining ghost method, interpolation, and extrapolation method.

Throughout this study, three important results were obtained. First, cosine function is discovered to fit the boundary conditions since the boundary solutions are stable across the time. Second, the protrusion started to exist and moves randomly as time increases. This indicates that the presence of the signal in an unsteady state inside the cell is significant in the invadopodia formation. Third, the signal density remained highest on the boundary. From the observation, the signal density exists inside the cell due to the diffusion activity of the signal from the boundary to the inner region.

Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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