



Faculty of Computer Science and Information Technology

**Three-dimensional Simulation of Invadopodia Formation Associated
with Ligand and Signal Stimulation**

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Three-dimensional Simulation of Invadopodia Formation Associated with Ligand and Signal Stimulation

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



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ABSTRACT

The simulation of invadopodia replication holds significance in establishing its biological relevance, as it contributes to a deeper comprehension of cancer invasion and metastasis. Additionally, it offers valuable insights into potential strategies for impeding the dissemination of this life-threatening disease. In this study, we present a mathematical model that describes the behaviour of an individual cell, with the aim of extending the simulation of invadopodia creation to a three-dimensional (3D) environment, thereby incorporating a higher level of realism and complexity. The formation of invadopodia is approached utilising the Stefan problem framework, wherein the cell membrane is defined by the Stefan free boundary condition, which entails that the boundary membrane is not predetermined. The proposed method for characterising the behaviour of the cell interface and the mobility of the plasma membrane is the level set method. The modelling process incorporated the following parameters: ECM c , MMPs f , ligands c_* , and signal σ . Furthermore, we enhanced the fidelity of the simulation results by employing an alternative simulation methodology and domain, along with a distinct dataset. This was achieved by applying the finite element method (FEM) and employing software tools such as FreeFem++ for simulation and Gmsh for mesh generation. The presence of singularities and instabilities was successfully eradicated and our comparison of the findings demonstrates their near resemblance to the actual ones. The attained outcomes have the potential to offer valuable insights for innovative methodologies and can be further applied to enhance the precision of numerical simulations.

Keywords: Invadopodia, individual cell model, free boundary problem, finite element method, level set method

Simulasi Tiga Dimensi Pembentukan Invadopodia berkaitan dengan Ligan dan Stimulasi Isyarat

ABSTRAK

Simulasi replikasi invadopodia selari dengan relevansi biologi amat penting kerana ia membantu kepada pemahaman yang lebih mendalam tentang penyusupan kanser dan metastasis. Selain itu, ia memberi pengetahuan penting tentang kaedah yang boleh digunakan untuk menghentikan penyebaran penyakit yang mengancam nyawa ini. Dalam kajian ini, saya membentangkan model matematik yang menggambarkan tingkah laku setiap sel individu dengan objektif untuk memperluaskan simulasi pembentukan invadopodia kepada persekitaran tiga dimensi (3D), dimana ia menggabungkan tahap realisme dan kompleksiti yang lebih tinggi. Pembentukan invadopodia menggunakan rangka kerja masalah Stefan, di mana membran sel ditakrifkan oleh keadaan sempadan bebas Stefan, yang bermakna membran sempadan tidak ditentukan terlebih dahulu. Tingkah laku antara permukaan sel dan mobiliti membran plasma boleh digambarkan dengan menggunakan kaedah set tahap. Proses pemodelan menggunakan parameter berikut: ECM c , MMPs f , ligan c_* , and isyarat sel σ . Saya juga meningkatkan hasil simulasi dengan menggunakan persekitaran dan metodologi simulasi yang berbeza, serta set data yang berlainan. Ini telah dicapai dengan menggunakan kaedah unsur terhingga dan menggunakan alat perisian seperti FreeFem++ untuk simulasi dan Gmsh untuk pembentukan mesh. Singulariti dan ketidakstabilan telah berjaya disingkirkan dan perbandingan temuan kami menunjukkan persamaan mereka dengan yang sebenar. Hasil yang dicapai mempunyai potensi untuk menawarkan matlamat yang berharga untuk metodologi inovatif dan boleh diterapkan lebih lanjut untuk meningkatkan ketepatan simulasi numerik.

Kata kunci: *Invadopodia, model sel individu, masalah sempadan bebas, kaedah unsur terhingga, kaedah set tahap*

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LIST OF ABBREVIATIONS

2D	two-dimensional
3D	three-dimensional
API	application programming interface
Arp 2/3	actin related protein 2/actin related protein 3
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EMT	epithelial to mesenchymal transformation
FDM	finite difference method
FEA	finite element analysis
FEM	finite element method
GUI	graphical user interface
MMP	matrix metalloproteinase
MT1-MMP	type 1-matrix metalloproteinase
N-WASP	neuronal Wiskott-Aldrich syndrome protein
ODE	ordinary differential equation
PDE	partial differential equation
UV	ultraviolet
WIP	WASP-interacting protein
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Research Background

Cancer is the second leading cause of mortality worldwide, accounting for one in every six fatalities, according to the World Health Organisation (WHO) (Ferlay et al., 2015). Cancer-related mortality is expected to rise by 70% in the next 20 years (Yaacob et al., 2019). The term "cancer" refers to a wide range of diseases that can be roughly grouped by abnormal cell behaviour, which differs from that of normal cells due to gene mutations that change the usual cellular instructions (Preziosi, 2003). According to Sporn (1996), the primary cause of death for cancer patients and one of the "hallmarks of cancer" is tissue invasion and metastasis (Hanahan & Weinberg, 2000, 2011).

In cancer research, mathematical modelling and simulation can complement conventional biological and experimental methods. In the last 25 years or so, there has been a surge in interest in the mathematical modelling of cancer growth and treatment, leading to the emergence of a new field: Mathematical Oncology (Vieira et al., 2023). Defined as the application of mathematics to cancer research, mathematical oncology integrates and overlaps with a number of other disciplines that rely on mathematics as a core methodology. As a result, it has a comprehensive scope - ranging from theoretical studies to mathematical model-based clinical trials (Rockne et al., 2019). Simultaneously, there has been an increase in interest in computational tools and simulation techniques, also known as *in silico* models, which seek to provide the biologist with additional insight without incurring potentially high financial, time, or ethical costs (Macnamara et al., 2020). Cancer research is a prominent example of a field where *in silico* modelling is gaining momentum.

1.2 Problem Statement

Numerous studies had been done over the last ten years to understand the mathematical basis of tumour growth and cancer invasion. Saitou et al. (2012) introduced mathematical models of a number of essential processes to examine the genesis of invadopodia. This discovery is very important since it sheds new light on the appearance of invadopodia in cancer cells. In several following research that followed, the model described here was used or modified to expand on this one, including Othman and Suzuki (2020).

This research aims to enhance the modelling of invadopodia development, in contrast to the study conducted by Othman and Suzuki (2020). The previous study utilised an in-silico model of a single cell and simulated the results in a two-dimensional (2D) format. To enhance the precision of outcomes and establish a setting that emulates the complexity of simulations, we conducted our experiments within a three-dimensional (3D) environment. It is crucial to precisely simulate invadopodia formation in order for it to be biologically relevant because it aids in our understanding of cancer invasion and metastasis. It also provides some insight into researching potential methods to halt the spread of this deadly disease.

In Othman and Suzuki's (2020) study, simulations demonstrated the presence of singularities and instability. Singularities are locations where the solution or its derivatives become infinitely large or indeterminate (Bruce, & Giblin, 1992) (Sonnerlind, 2015). They can originate from the geometric properties, physical characteristics, or numerical techniques employed in the problem. If singularities are not properly addressed, they can lead to numerical instability, convergence problems, or loss of precision. Several strategies for addressing singularities and instability include: implementing mesh refinement in the vicinity of the singularity, utilising specialised elements or schemes capable of capturing the

singularity, or employing regularisation techniques to smooth or eliminate the singularity and instability (Sonnerlind, 2015).

Therefore, in the context of this study, singularities refer to the fact that the invadopodia simulation should not have a sharp edge or cusp, whereas instabilities refer to the fact that there should not be more than two invadopodia simulated because, depending on the circumstances, this could result in singularities. For the solution to be more precise, it is essential to simulate the results while excluding the possibility of singularities and instabilities.

1.3 Objectives

This research goal is to investigate invadopodia formation in cancer cells from a mathematical standpoint. The aforementioned goal raises the following objectives:

- i. To simulate the formation of invadopodia in 3D using FreeFEM++ and Gmsh.
- ii. To accurately replicate the formation of invadopodia for biological significance.
- iii. To prevent the occurrence of singularities and instabilities by using different simulation techniques and dataset.

1.4 Thesis Organisation

The project is organised into the following sections. The formation of invadopodia and cancer cell mechanisms were discussed in Chapter 1. Chapter 2 will next provide a summary and analysis of the pertinent articles. In Chapter 3, which describes the mathematical model, all of the presuppositions are outlined before the model's rules are

specified. The Stefan problem will be used to model the system, and finite element method (FEM) will be used to solve it numerically. Results of numerical simulations are presented and examined in Chapter 4. Recommendations and conclusions are covered in Chapter 5.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer Cells

Cancer cells are erroneous cells. In other words, gene mutations cause normal cells to develop into cancer cells. These deoxyribonucleic acid (DNA) changes may be inherited, evolve over time as our genes deteriorate with age, or—more frequently—be brought on by carcinogens (substances that cause cancer) in our environment, such as tobacco smoke, alcohol, or ultraviolet (UV) radiation from the sun (National Cancer Institute, 2022).

Normal cells behave differently than cancer cells do. This is caused by gene changes in cancer cells that alter a cell's usual instructions. As a result, it prevents cells from dying when they ought to or causes them to expand out of control. The provided attributes pertain to malignant cells are as follows (Canadian Cancer Society, n.d.):

- i. Out of control division.
- ii. Immature cells that do not progress into mature cells with a particular purpose.
- iii. Impede the function of the immune system, which defends the body from diseases.
- iv. Disregard the signals that instruct the cells to stop dividing or undergo cell death.
- v. Disseminate to other bodily regions.

- vi. The resultant expansion induces damage to bodily organs and tissues.

Cancer cells share two characteristics in common: proliferation and metastasis. When cells exhibit these characteristics, a tumour known as a primary tumour is created but it has not yet acquired the potential to expand beyond its original location (metastasis has not occurred yet). It is a mass caused by an excessive cell proliferation. Tumours come in two varieties: benign and malignant. While a malignant tumour invades neighbouring tissues and becomes cancerous, a benign tumour does not spread and is thus not cancerous (Ruoslahti, 1996).

The beginning of the spread of cancer cells is a sign that metastasis has taken place. When cancer spreads from the main site to the secondary site through the bloodstream or lymphatic vessels, this is known as metastasis.

In most cases, there are four stages of cancer:

Table 2.1: Cancer Stages (Giuliano et al., 2017)

Stage	Explanation
I	The tumour is 2 cm or smaller and has not spread to other organs.
II	The tumour is larger than 2 cm but less than 5 cm and has not spread to the tissues adjacent.
III	The tumour is larger than 5 cm and has spread to the lymph nodes and tissues nearby.
IV	The tumour is any size and has spread to different parts of the body via the lymphatic or blood systems.

2.2 Cancer Cell Invasion

The ability of cancer cells to spread throughout the body via the interconnected mechanisms of invasion and metastasis is a fundamental trait. The extension and penetration of cancer cells into other tissues is referred to as invasion in this context. The growth of cancer cells results in their expansion, which causes a breach in the tissue barriers. As a result, it causes the cancer to spread into nearby tissue. Local invasion refers to the initial phase of the process leading to the development of secondary tumours or metastases, during which cancer cells spread by infiltrating lymphatic or blood vessels (Zijl et al., 2011).

Matrix metalloproteinases (MMPs) are triggered at the formation of a primary tumour by cancer cells that have undergone maturation. MMPs are enzymes that break down basement membranes, allowing cancer cells to penetrate nearby organs. Endothelial cells play a critical role in tumour angiogenesis by producing MMPs, particularly MMP-2 and MT1-MMP (Foda & Zucker, 2001).

Subsequently, the cancer cells undergo a process known as epithelial to mesenchymal transformation (EMT), which involves the conversion of epithelial cells into mesenchymal cells. Here, E-cadherins are eliminated and N-cadherins are produced erratically by the cells. It results from the synthesis of transcription factors that promote EMT and enable cells to adopt a mesenchymal phenotype that aids in intravasation of the cells into the bloodstream. Compared to E-cadherins, intracellular adhesion has been decreased as a result of N-cadherins, allowing for more local tissue invasion (Loh et al., 2019).

The next process is intravasation, where cancer cells squeeze through the ECM and enter the blood or lymphatic system. The cells dispersed throughout the body after entering

the circulatory system and remained in the capillaries of other organs. The cells then move through the ECM during extravasation to create micro-metastases. At this stage, it is beginning to develop a secondary tumour, which will then prompt more to angiogenesis that is brought on by hypoxia. In order to sustain ongoing expansion and spread to other parts of the body, a new vascular network is established to provide a fresh influx of blood (Reymond et al.,2013).

Due to the immune system's and the blood stream's hydrodynamic pressures, the majority of malignant cells will not survive this process. In order to avoid detection by the immune system, the malignant cells migrate by forming heterotypic clumps as a protective measure (Zijl et al., 2011).

2.3 Invadopodia Formation

Numerous tumours share the following common traits (Hanahan & Weinberg, 2011):

- i. Growth signals that are self-sufficient.
- ii. Intolerance to signals that would normally restrict growth (antigrowth).
- iii. Avoiding programmed cell death (apoptosis).
- iv. Potential for endless reproduction.
- v. Long-term angiogenesis.
- vi. Tissue invasion and metastasis.

With regard to the final characteristic, proteolysis of the ECM, which is fueled by actin-rich protrusions of the plasma membrane known as invadopodia, is a critical

component in cancer invasion and metastasis (Stylli et al., 2008) (Andasari & Chaplain, 2012) (Andasari et al., 2012).

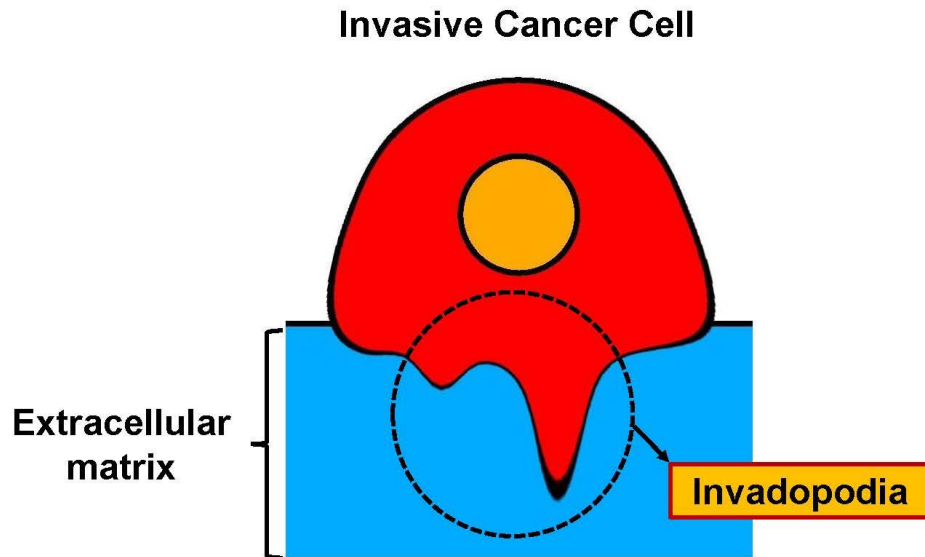


Figure 2.1: An invadopodia scheme

Metastatic carcinoma cells generate subcellular structures called invadopodia which contain in invasive cancer cells. The coordination of numerous cellular biological processes is required for the creation of invadopodia and the breakdown of ECM. This comprises actin cytoskeletal polymerisation and reorganisation, ligand and epidermal growth factor receptor (EGFR) signalling. These result in physical force being applied to membrane surface and MMPs localisation, activation, and secretion (Yamaguchi et al., 2005).

Invadopodia are actin-rich structures also referred to as "invasive feet." Its name was given because it is found at the basal surfaces of cells that were able to cross the ECM, which are typically found in invasive cancer cells (Weaver, 2008). Invadopodia primarily function

to selectively breakdown the ECM by the secretion of MMPs. It can be formed and described in terms of the stages of initiation, extension, ECM degradation, and disassembly.

Invadopodia typically have elongated or tiny, finger-like protrusions with punctures as their morphology (Gallinato & Poignard, 2017). Cancer cells can generate a few (1–10) invadopodia (Saitou et al., 2012) with a lifespan of a few tens to 60 minutes and a size range of 0.05 to 2 μm , according to Murphy and Courtneidge (2011). On the contrary, the F-actin core makes up the fundamental structure of invadopodia. Microtubules and intermediate filaments have also been found in mature invadopodia. Aside from that, this structure is also home to actin filament nucleators and their regulators, including the actin related protein 2/actin related protein 3 (Arp2/3) complex, N-WASP (neuronal Wiskott-Aldrich syndrome protein), WASP-interacting protein (WIP), and cortactin. However, invadopodia are rich in cross-linking proteins like fascin, and as a result, there is a substantial correlation between fascin expression and the ability of cancer cells to invade (Admon, 2015).

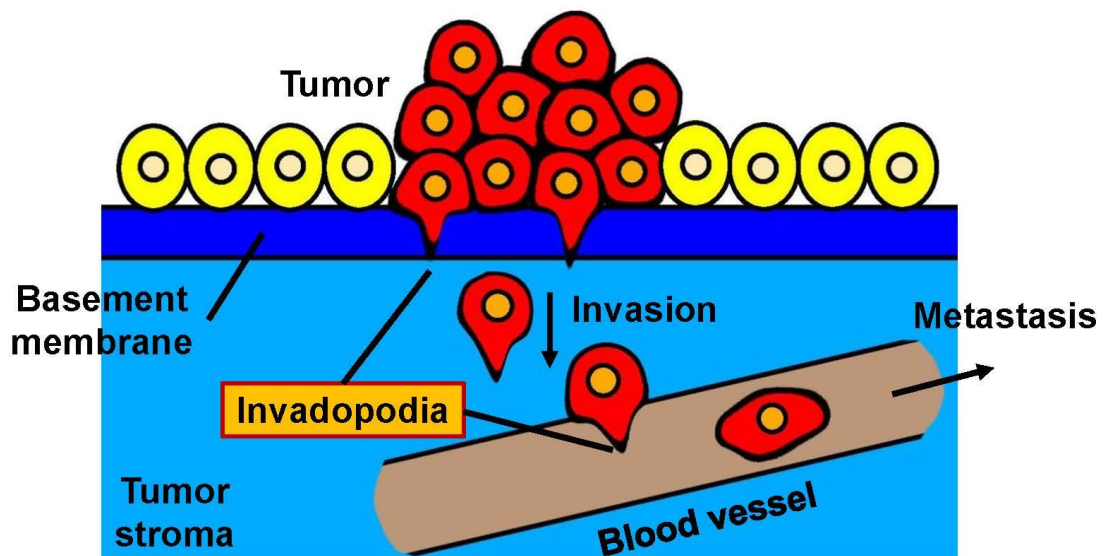


Figure 2.2: Cancer metastasis and invasion: the role of invadopodia

2.4 Previous Studies

Numerous studies have been conducted over the past decade to understand the mechanism of tumour growth and cancer invasion from a mathematical perspective, utilising continuous and discrete models, and focusing on ordinary partial differential equation (PDE) and ordinary differential equation (ODE). The structure of cancer at the tissue level has been successfully captured by certain studies. Currently, studies concentrating on the model of cancer cell invasion at the subcellular level (single cell) are of great interest such as Admon (2015), Gallinato et al. (2017), Gallinato and Poignard (2017), Othman and Suzuki (2020), while other studies also focused on signal transductions such as Yaacob et al. (2019). Additionally, a computational analysis of a free-boundary cancer cell invasion model also has been conducted.

The formation of invadopodia was examined by Saitou et al. (2012) by the utilisation of mathematical models that encompassed many essential processes. In their paper, they conducted an analysis of the spatio-temporal dynamics of the model in a 2D spatial domain using numerical computational method. They proposed the following model,

$$\begin{aligned}
 n_t &= d_n \Delta n + \nabla \cdot (n \nabla \phi(c)) - \gamma_n \nabla \cdot (n \nabla c_*), \\
 c_t &= -\kappa_c c f, \\
 c_{*t} &= d_{c_*} \Delta c_* + \kappa_c c f - \lambda_{c_*} c_*, \\
 f_t &= d_f \Delta f + \kappa_f c_* - \lambda_f f + \gamma_f \nabla \cdot f \nabla n,
 \end{aligned}
 \quad \text{in} \quad \bigcup_{0 < t < T} \partial \Omega \times \{t\} \quad \text{Equation 2.1}$$

with zero-flux boundary conditions

$$\begin{aligned}
v \cdot (d_n \nabla n + n \nabla (\phi(c) - \gamma_n c_*)) &= 0, \\
v \cdot (d_{c_*} \nabla c_*) &= 0, \\
v \cdot (d_f \nabla f + \gamma_f f \nabla n) &= 0,
\end{aligned}
\quad \text{on} \quad \bigcup_{0 < t < T} \partial \Omega \times \{t\} \quad \text{Equation 2.2}$$

where actin density is denoted by $n(t, x)$, ECM density is denoted by $c(t, x)$, ligand density is denoted by $c_*(t, x)$ and MMP density is denoted $f(t, x)$, respectively. They also marked the effect of MMP rate constant, κ_f in the invadopodia appearances. They took Ω as a rectangular domain that contains intra- and extracellular, diffusion coefficient of actin d_n , signal transmission rate of actin γ_n , chemical reaction rate of ECM κ_c , diffusion coefficient of ligands d_{c_*} , degradation rate of ligands λ_{c_*} , diffusion coefficient of MMPs d_f , chemical reaction rate of MMPs κ_f , degradation rate of MMPs λ_f , signal transmission rate of MMPs γ_f , which are all positive constants and v is the outer unit normal vector.

This discovery holds great significance as it provides novel insights into the existence of invadopodia in cancer cells. Subsequent research studies have further developed or modified the paradigm suggested in this initial study, as evidenced by the works of authors such as Admon (2015), Gallinato et al. (2017), Gallinato and Poignard (2017), as well as Othman and Suzuki (2020).

Invadopodia, which are actin-rich membrane protrusions, are responsible for degrading the ECM and enabling cancer cells to infiltrate surrounding tissue barriers. Actin polymerization activities that push the membrane of migrating cells are aided by signalling pathways that involve ligand and membrane-associated receptor binding. This is contrary to the findings of Saitou et al. (2012), who found that actin is not only dispersed but also pushed past the cell membrane into the ECM region. Consequently, Admon (2015) took into account

mathematical modelling for a single cancer cell. He looked into the one-dimensional Stefan-like signal processing problem (CM-I-B) and used the cell membrane as a free boundary surface to distinguish between any activity occurring within and outside of cells. By converting the Stefan-like problem into an initial-boundary value problem for the signal equation with penalty term, the CM-I-C approximation problem is introduced. Then, using the integration of the penalty term, the velocity pertaining to the motion of the free boundary is determined. For the aforementioned integrated penalty technique, the auxiliary problem is numerically solved using the finite-difference method, (CM-I-C0). The error, ε and distance, δx approaching zero is employed to analyse the convergence of CM-I-C and CM-I-C0 towards CMI-B. The placements of the free boundaries and the distribution of signals in his findings exhibited a strong concurrence with those achieved by the use of the alternative fixed domain method, which is widely recognised.

Gallinato et al. (2017) presents a theoretical and numerical investigation of a free boundary problem pertaining to the formation of cell protrusions. The precise characterisation of the cell membrane is facilitated by the utilisation of a level set function, which undergoes motion as a result of certain signalling pathways. The objective is to develop a computational model that represents the chemical interactions occurring between the cell and its surrounding environment during the formation of invadopodia or pseudopodia.

They simplified the model from Saitou et al. (2012) version. The cell, denoted by ω_n^t is the domain enclosed by the membrane, Γ_t and the ECM is the outer domain denoted by ω_c^t .

Degradation of the ECM:

$$\begin{aligned}
\Delta c^* &= 0, \quad x \in \omega_c^t, \\
c^*|_{\partial\Omega} &= 0, \\
-\partial_{\mathbf{n}} c^*|_{\Gamma_t} &= g|_{\Gamma_t}.
\end{aligned}
\tag{Equation 2.3}$$

Generation of the inner signal for actin polymerisation:

$$\begin{aligned}
\Delta \sigma &= 0, \quad t \in [0, T], \quad x \in \omega_n^t, \\
\sigma|_{\Gamma_t} &= c^*|_{\Gamma_t}.
\end{aligned}
\tag{Equation 2.4}$$

Motion of the cell membrane:

$$\begin{aligned}
\partial_t \gamma(t, \theta) &= \nabla \sigma(\gamma(t, \theta)), \quad \theta \in \mathbb{T}, \\
\Gamma_t &= \{\gamma(t, \theta), \quad \theta \in \mathbb{T}\}.
\end{aligned}
\tag{Equation 2.5}$$

The numerical simulations conducted by Gallinato et al. (2017) yield protrusion-like shapes, so confirming the validity of their model derivation (see Figure 2.3).

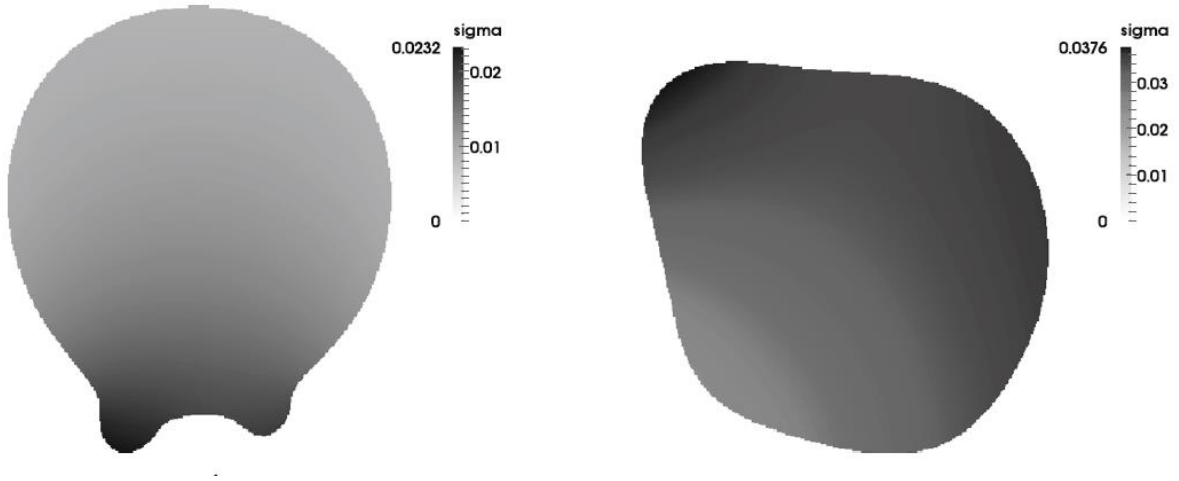


Figure 2.3: Plots for test-cases 1 and 2 (Gallinato et al., 2017)

The proposed model comprises the Laplace equation with Dirichlet boundary condition within the cell, which is connected with the Laplace equation including Neumann boundary condition in the outer domain. The process of actin polymerisation is attributed to the gradient of the internal signal, which propels the movement of the interface. The well-posedness of the free boundary problem is established through a proof, which is contingent upon a sign condition being satisfied by the datum. This criterion serves to maintain the coherence of the model and establishes certain factors to prioritise when enhancing the model. Therefore, a new method was proposed that uses a first order Cartesian finite difference method (FDM) to solve the given problem. The model successfully accounts for the primary biological characteristics observed, namely the development of slender and elongated protrusions like invadopodia, or bigger protrusions resembling pseudopodia, contingent upon the source term in the equation. The presented model offers a comprehensive framework for the theoretical and numerical analysis of single cell migration modelling, which is applicable in both 2D and 3D settings.

In the study by Gallinato and Poignard (2017), they provide a second order superconvergent Cartesian approach to solve a free boundary issue with two harmonic phases coupled through a moving interface. The model, which has been recently proposed by Gallinato et al. (2017), provides a description of the process involved in the production of cell protrusions. The dynamic interface is characterised by a level set function and is transported at a velocity determined by the gradient of the internal phase. The present study introduced a novel approach to the FDM, which involves the utilisation of a stabilised ghost fluid method and second order discretisation for the Laplace operator. This method is capable of accommodating various boundary conditions, including Dirichlet, Neumann, and Robin conditions. It is noteworthy that the approach employed to address the harmonic subproblems exhibits a phenomenon known as superconvergence on two levels. This implies that the first and second order derivatives of the numerical solutions are found with a second order level of accuracy, mirroring the accuracy achieved in the solution itself. Numerical criteria are employed to assess the accuracy of the data, and these criteria are subsequently validated by numerical simulations. Furthermore, they suggested a suitable expansion of the velocity of the level-set in order to prevent any compromise in consistency and achieve second-order accuracy for the entire free boundary problem. It is noteworthy that they emphasised the transmission of the superconvergent features in the static subproblems and their preservation through the dynamical method. Their proposed methodology is highly appropriate for addressing quasistatic issues that resemble the Hele-Shaw or Muskat phenomena.

Othman and Suzuki (2020) proposed a novel solution to the free boundary problem of invadopodia formation and cancer invasion. The interaction across the plasma membrane cell is combined in the invasion and invadopodia modelling, which is referred to as a free

boundary problem. It must be taken into account that the alterations will cause cell membrane protrusions. The boundary membrane is a priori undetermined (free boundary) in the Stefan problem approach formulation of the formation. The level set approach was used to visually represent the dynamic movement of the plasma membrane and the behaviour of the cell interface. After that, the phase change problem is employed to efficiently and simply describe the diffusion of the cell membrane. Invadopodia formation and single cell movement are successfully demonstrated.

CHAPTER 3

METHODOLOGY

3.1 Overview

The modelling approach was utilised throughout this project. The model was constructed based on the Stefan problem and afterwards solved by the implementation of the finite element method (FEM) for numerical analysis. The Stefan problem is a well-known example of an initial boundary value problem in the field of parabolic partial differential equations (PDEs). The mathematical model under consideration represents the most elementary framework for studying phase change phenomena, specifically the distribution of heat during phase transitions. Given that invadopodia formation is a dynamic process including phase shift, it is appropriate to employ the Stefan problem as a model for studying and subsequently solving invadopodia formation. FEM is commonly employed as the predominant approach for solving engineering and mathematical problems. This method is classified as a numerical technique used to solve PDEs in two or three spatial dimensions.

3.2 Mathematical Model

As this study builds upon the work of Othman and Suzuki (2020), the modelling approach employed remains consistent. Individual cancer cell model, free boundary problem, and weak form derivation are the three components of this section, in line with Othman and Suzuki (2020).

3.2.1 Individual Cancer Cell Model

Based on the simplified signalling pathways version of Saitou et al. (2012) by Gallinato et al. (2017), ECM is defined as the domain out of the cell. Given at any time on

the cell membrane, type 1-matrix metalloproteinase (MT1-MMP) enzymes flux is assumed and denoted by $g(x, t)$. It generates a flux of degraded matrix which called ligands and denoted by c_* on the cell boundary. These ligands will diffuse in the extracellular medium and generate a signal σ when they bind to the cell membrane. Then, signal will diffuse inside the cell. The membrane is then carried at the interface by the normal velocity.

Figure 3.1 shows the degradation of ECM by MMPs only happens on the cell plasma membrane Γ_t and produces ligands (ECM fragment). Also, there is no change in the density of MMPs on the boundary since MMPs play the role of cutting the fiber-proteinases of ECM that caused it to produces ligands. Simply put, MMPs will not consume itself in degradation of ECM.

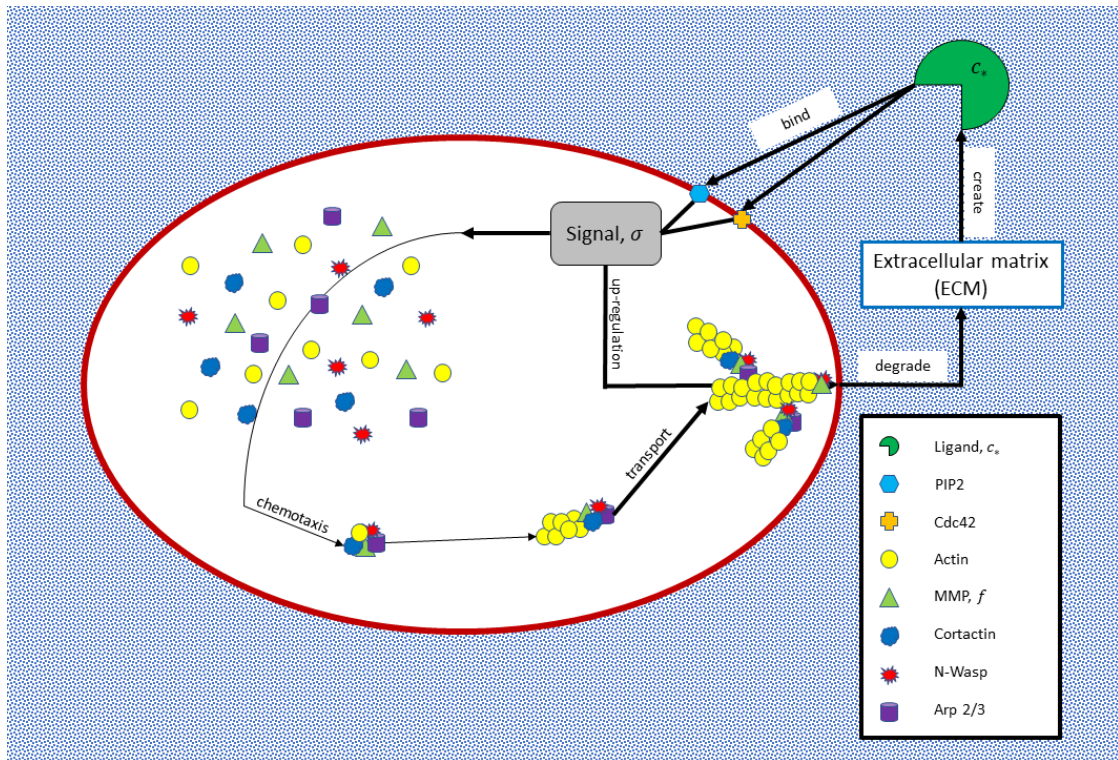


Figure 3.1: Diagrammatic representation of the molecular interactions

The level set approach is used to indicate the plasma membrane as

$$\Gamma_t = \{x \in \Omega | \psi(x, t) = 0\}, \quad \text{Equation 3.1}$$

where $\Omega \subset \mathbb{R}^N$ ($N = 3$) is the domain indicating a cancer cell with smooth boundary $\partial\Omega$ while x is the particle position on the membrane surface, Γ_t . Assume the smooth level set function, ψ that detects the plasma membrane location which satisfies

$$\psi_t(x, t) + \frac{dx}{dt} \cdot \nabla \psi(x, t) = 0. \quad \text{Equation 3.2}$$

Then $\frac{dx}{dt} = v(x, t)$ is the driving force for the movement of plasma membrane and is defined by

$$v = \gamma_n(\nabla \sigma - \nabla c_*) \quad \text{on } \Gamma_t \quad \text{Equation 3.3}$$

where γ_n , σ , and c_* are positive constant, gradient of signal inside the cell, and gradient of ligand outside the cell, respectively.

Therefore, from Equation 3.3,

$$\psi_t + v \cdot \nabla \psi = 0 \quad \text{on } \Gamma_t. \quad \text{Equation 3.4}$$

In accordance with Stefan problems, the membrane interface, Γ_t can undergo either contraction, which results in the inward movement of the plasma membrane towards the inner part of the cell, or expansion, which leads to the outward movement of the plasma membrane towards the outer region of the cell. Here, the domain inside the cell is defined as

$$\omega_n^t = \{x \in \Omega | \psi(x, t) < 0\} \subset \subset \Omega. \quad \text{Equation 3.5}$$

Therefore,

$$Q_n = \bigcup_{0 < t < T} \omega_n^t \times \{t\} \quad \text{Equation 3.6}$$

and

$$\omega_c^t = \{x \in \Omega | \psi(x, t) > 0\} \quad \text{Equation 3.7}$$

as the domain outside the cell

$$Q_c = \bigcup_{0 < t < T} \omega_c^t \times \{t\} \quad \text{Equation 3.8}$$

According to Liouville's theorem, the cell membrane Γ_t undergoes decay, and a reaction constant is set to a value of 1, resulting in

$$\begin{aligned}
c_t + v \cdot \nabla c &= -fc, \\
c_{*t} + v \cdot \nabla c_* &= fc
\end{aligned}
\tag{Equation 3.9}$$

on Γ_t .

Signal is generated by ligands which is diffuse inside the cell. However, this is only occurred when it bound to the plasma membrane and lead to actin polymerisation. Actin polymerisation is vital for protrusion to happen at the leading edge and it is counted by the gradient of inner signal. The aforementioned will results in the subsequent equations

$$\begin{aligned}
\sigma_t &= d_\sigma \Delta \sigma && \text{in } \mathcal{Q}_n, \\
\sigma|_{\Gamma_t} &= c_* && \text{on } \Gamma, \\
f_t &= d_f \Delta f + \kappa_f \sigma - \lambda_f f.
\end{aligned}
\tag{Equation 3.10}$$

According to the law of mass conservation, mass for any closed system must remain constant in time regardless of matter or energy transferring activity. To put it differently, total mass inside and outside the cell of the actin must equal. Subsequently, the total mass of conservation of f is conserved, where this is based on Liouville's theorem. Hence,

$$d_f \frac{\partial f}{\partial v} + (v \cdot v)f = 0 \quad \text{on} \quad \bigcup_{0 < t < T} \Gamma_t \times \{t\}
\tag{Equation 3.11}$$

From the biological perspective, there are also no activity on the membrane. According to Grecco et al. (2011), this is due to the existence of receptors on the membrane which bind to ligands in the extracellular region before signals are generated in the

intracellular region. Therefore, the movement of ligands are not directly bind to the membrane but merely with the help of receptors.

As mentioned before, there is no changes in the density of MMPs on the boundary when cutting the fiber-proteinases of ECM, it holds that

$$\begin{aligned} c_{*t} &= d_{c_*} \Delta c_* && \text{in } Q_c, \\ \frac{\partial c_*}{\partial \nu} &= g && \text{on } \Gamma. \end{aligned} \tag{Equation 3.12}$$

3.2.2 Free Boundary Problem

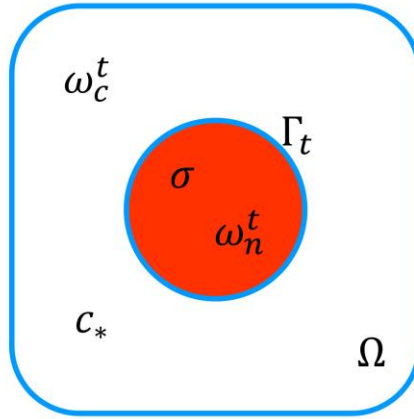


Figure 3.2: A simple cell model

In order to simulate the formation of invadopodia, the domain of applications is exhibited as free boundary problem. Free boundary is those described by PDEs that show a priori unknown (free) interfaces or boundaries (Ros-Oton & Serra, 2010). In this particular scenario, the utilisation of a free boundary problem enables the accurate localisation of the

cell membrane. However, the velocity at which invadopodia formation occurs is not imposed, and remains undetermined within the PDE systems. Thus, the cell membrane is positioned as a free boundary surface to effectively segregate the intracellular and extracellular regions, hence facilitating the regulation of cellular activities.

Therefore, signal generation for actin polymerisation is represented by

$$\begin{aligned} d_{\sigma}^{-1} \sigma_t &= \Delta \sigma && \text{in } Q_n, \\ Q|_{\partial \omega_c^t} &= c_* \end{aligned} \tag{Equation 3.13}$$

and degradation of ECM is

$$\begin{aligned} d_{c_*}^{-1} c_{*t} &= \Delta c_* && \text{in } Q_c, \\ \frac{\partial c_*}{\partial \nu} &= 0 \end{aligned} \tag{Equation 3.14}$$

where $d > 0$ is the diffusion coefficient.

Let $\sigma = c_* = g(x, t)$ where the value is known. Define

$$\theta = \begin{cases} \sigma & \text{in } Q_n = \{\psi < 0\} \\ c_* & \text{in } Q_c = \{\psi > 0\} \end{cases} \tag{Equation 3.15}$$

This model is based on the biological facts of cell communication. According to Bartee et al. (2017), a ligand is a molecule that binds to a certain molecule (receptors), occasionally sending a signal as a result. Hence, can be viewed as signalling molecules. The

signal travels across the membrane and into the cytoplasm once a ligand connects to a receptor.

However, one of the biological facts that initiate this model is that ligands do not cross the cell membrane (Bartee et al., 2017). Therefore, only signal is considered in the modified velocity model since ligands does not exist inside the cell. Besides, no jump of process should happen in the cell communication and signalling process.

Accordingly, Stefan problem also exhibit similar condition to the biological fact stated before. Thus, the free boundary condition is defined as Stefan condition.

$$\begin{bmatrix} \cdot \\ \cdot \end{bmatrix}_{-}^{+} = \begin{bmatrix} \cdot \\ \cdot \end{bmatrix}_{\psi < 0}^{\psi > 0}$$

If g is continuous and diffuse where $\pm\psi > 0 \Leftrightarrow \pm g > 0$, it holds that

$$\theta = g, \tag{Equation 3.16}$$

$$v = -[\nabla\theta]_{\pm}^{\pm} \tag{Equation 3.17}$$

Hence,

$$\begin{aligned} \psi_t &= -(v \cdot v) \frac{\partial\psi}{\partial v} \\ &= -(\nabla\sigma - \nabla c_*) \frac{\partial\psi}{\partial v} \\ &= (\nabla c_* - \nabla\sigma) \frac{\partial\psi}{\partial v} \end{aligned}$$

$$= \left[\frac{\partial \theta}{\partial \nu} \right]_+^+ \frac{\partial \psi}{\partial \nu} \quad \text{Equation 3.18}$$

where $\theta = g = c_*$ on Γ .

3.2.3 Weak Form Derivation

Let $\Omega \subset \mathbb{R}^N, N = 3$ be a smooth bounded domain. Let

$$\begin{aligned} H(u)_t &= \Delta u & \text{in } Q = \Omega \times (0, T) \\ u|_{\partial\Omega} &= 0 \\ u|_{t=0} &= u_0(x) \end{aligned} \quad \text{Equation 3.19}$$

where H is the enthalpy function and $u = u(x, t)$ is the temperature distribution. Let $\varphi \in H^1(\Omega)$ be trial function,

$$- \int_{\Omega} \left(\frac{\partial}{\partial x} \frac{\partial u}{\partial x} + \frac{\partial}{\partial y} \frac{\partial u}{\partial y} \right) \varphi - \int_{\Omega} H(u)_t \varphi = 0 \quad \text{Equation 3.20}$$

Using integration by parts,

$$\begin{aligned} - \int_{\Omega} \left(\frac{\partial}{\partial x} \frac{\partial u}{\partial x} \right) + \left(\frac{\partial}{\partial y} \frac{\partial u}{\partial y} \right) - \varphi \, d\Omega &= - \int_{\Omega} f g \, d\Omega \\ &= - \int_{\Omega} g \frac{\partial}{\partial x} \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right) - g \frac{\partial}{\partial y} \left(\frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial x^2} \right) d\Omega \end{aligned}$$

$$= - \int_{\Omega} g \frac{\partial}{\partial x} \left(\frac{\partial^2 u}{\partial x^2} \right) - g \frac{\partial}{\partial y} \left(\frac{\partial^2 u}{\partial y^2} \right) - g \frac{\partial}{\partial x} \left(\frac{\partial^2 u}{\partial y^2} \right) - g \frac{\partial}{\partial y} \left(\frac{\partial^2 u}{\partial x^2} \right) d\Omega$$

Equation 3.21

Using product rule,

$$- \int_{\Omega} \left(\frac{\partial}{\partial x} \frac{\partial u}{\partial x} + \frac{\partial}{\partial y} \frac{\partial u}{\partial y} \right) \varphi d\Omega = - \int_{\Omega} g \frac{\partial^2 u}{\partial x^2} - g \frac{\partial^2 u}{\partial y^2} + g \frac{\partial^2 u}{\partial x^2} + g \frac{\partial^2 u}{\partial y^2} d\Omega$$

Equation 3.22

Let $f = H(u)_t$ and $g = \varphi$. Then

$$\frac{df}{dt} = H'(u)_t, \quad \frac{dg}{dt} = 0$$

Equation 3.23

$$- \int_{\Omega} H(u)_t \varphi d\Omega = - \int_{\Omega} fg d\Omega = - \int_{\Omega} g H'(u) u_t d\Omega = 0$$

Equation 3.24

$$- \int_{\Omega} H'(u) u_t d\Omega = 0$$

Therefore,

$$- \int_{\Omega} \left(\frac{\partial}{\partial x} \frac{\partial u}{\partial x} + \frac{\partial}{\partial y} \frac{\partial u}{\partial y} \right) \varphi d\Omega - \int_{\Omega} H(u)_t \varphi d\Omega = 0$$

Equation 3.25

$$- \int_{\Omega} \frac{\partial}{\partial x} \left(\varphi \frac{\partial u}{\partial x} \right) + \frac{\partial}{\partial y} \left(\varphi \frac{\partial u}{\partial y} \right) - \left(\frac{\partial u}{\partial x} \frac{\partial \varphi}{\partial x} + \frac{\partial u}{\partial y} \frac{\partial \varphi}{\partial y} \right) - H(u)_t \varphi d\Omega = 0$$

Hence,

$$\int_{\Omega} (\nabla u \cdot \nabla \varphi) - H(u)_t \varphi \, d\Omega - \int_{\partial\Omega} (\nabla u) \cdot \varphi \nu \, d\Omega = 0. \quad \text{Equation 3.26}$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overview

In order to validate the methodologies employed, numerical simulations are initiated, and the ensuing outcomes will be deliberated about in this section. The PDEs were numerically solved using FreeFem++ (version 4.10) (Hecht, 2012), while the meshes were created using Gmsh (version 4.9.3) (Geuzaine and Remacle, 2009).

4.2 Finite Element Method (FEM)

FEM is a mathematical tool used to analyse a wide range of problems. The approach is widely used in the examination of solids and structures, as well as heat transmission and fluids. FEM is applicable in virtually every academic field. The finite element approach is employed in diverse fields of analysis and design to address physical difficulties. The idealisation of a physical problem to a mathematical model necessitates specific assumptions, which result in differential equations that control the mathematical model.

The mathematical model is approximated using finite element analysis (FEA). Because the FEM is a numerical method, the solution accuracy must be evaluated. A frequently used criterion for convergence is to assess the error against a predetermined tolerance, which is a small positive value that signifies the most permissible error. When the error is less than the tolerance, the iteration is halted and the approximate value is considered as the answer. Therefore, if the accuracy standards are not reached, the numerical solution must be repeated using modified solution parameters (such as a finer mesh) until the required

precision is achieved. Furthermore, the selection of an approximate mathematical model is critical, as it entirely impacts the insight into the actual physical situation that we can acquire from the study. Some of the benefits of FEM over other numerical approaches are as follows:

- i. The body under consideration can have any shape, load, or support conditions
- ii. It is a flexible method
- iii. The matrix mesh can combine elements of many types
- iv. Its flexibility can be encapsulated in a single programme
- v. The actual structure and its finite element model are very similar

4.3 Platforms

4.3.1 FreeFEM++

FreeFEM++ is a computational tool designed to solve PDEs in the context of non-linear multi-physics systems. It is capable of handling problems in one, two, and three dimensions, as well as in domains with borders, such as surfaces and curves. The resolution of PDEs in several fields of physics, such as fluid-structure interactions, necessitates the interpolation of data over multiple meshes and their manipulation inside a single programme. The FreeFEM++ software package incorporates a highly efficient interpolation method and a versatile programming language designed for the processing of data across many meshes. FreeFEM is implemented using the C++ programming language and adheres to the C++ idiom.

As an introductory guide, learning through the use of examples is employed. Consider the Poisson equation on the unit disc, with zero Dirichlet boundary conditions. The classical version of the problem is as follows:

$$\begin{cases} -\Delta u = 0 & \text{in } \{(x, y) \in \mathbb{R}^2: x^2 + y^2 < 1\} \\ u = 0 & \text{on } \partial\Omega \end{cases} \quad \text{Equation 4.1}$$

where $u = u(x, y)$ is the unknown and f is the source term.

This problem is a valid mathematical model for a variety of physical problems. For example, Equation 4.1 represents the shape of a soap film that is attached to a ring in a plane. The shape is defined as the graph $(x, y, u(x, y))$ of the vertical displacement $u(x, y)$ under a vertical pressure p (force per unit area) and an initial tension μ (force per unit length). In Equation 4.1, $f = p/\mu$. The boundary condition $u = 0$ on $\partial\Omega$ in Equation 4.1 implies the soap film is adhered to the ring. Equation 4.1 can also be used in electrostatics, where u represents an electrostatic potential and $f = \rho/\epsilon$ represents the space charge density and dielectric coefficient, respectively. Similar applications exist in fluid mechanics and stationary heat conduction.

The variational formulation of Equation 4.1 reduces to find $u \in H_0^1(\Omega)$ such that

$$\int_{\Omega} \nabla u \cdot \nabla v \, dx dy = \int_{\Omega} f v \, dx dy \quad \forall v \in H_0^1(\Omega). \quad \text{Equation 4.2}$$

Next, we include and comment on the FreeFem++ code that solves this problem (Figure 4.1). For comparison purposes, we assume $f = 1$. Hence, Equation 4.1 has an accurate solution, which is provided by $u(x, y) = \frac{1-(x^2+y^2)}{4}$. This expression can be used to analyse the evolution of the error (in the L^2 -norm) as a function of finite element type

and/or mesh node count. All script files in FreeFem++ must be saved with the extension .edp. Figure 4.1 shows the picture of the solution obtained with the code below. As for the error, we obtain $\|u - u_h\|_{L^2(\Omega)} = 5.19123 \times 10^{-6}$.

```

1 // Poisson's equation on the 2D unit disk
2 border C(t=0,2*pi){x=cos(t); y=sin(t);} // boundary of the domain mesh
3 Th=buildmesh(C(1000)); // mesh with 100 points on the boundary
4 savemesh(Th,"mesh_malla1.msh"); // to save the mesh data
5 plot(Th,ps="malla.eps"); // to plot and save the mesh
6 fespace Vh(Th,P1); // P1 Lagrange finite elements
7 Vh uh,vh; // uh,vh belong to Vh
8 func f=1; // source term
9 func u=0.25*(1-x^2-y^2); // exact solution
10 real L2error;
11 // solving the variational formulation of the problem
12 solve Poisson(uh,vh)= int2d(Th) (dx(uh)*dx(vh)+dy(uh)*dy(vh))
13 -int2d(Th) (f*vh)
14 +on(C,uh=0);
15 real [int] viso=[0.,0.05,0.1,0.15,0.2,0.25];
16 plot(uh,value=true,fill=true,ShowAxes=0,ColorScheme =1,
17 viso=viso,ps="fig_poisson2d.eps"); //to plot and save the solution
18 L2error=sqrt(int2d(Th) ((u-uh)^2)); // error in L^2-norm
19 cout<<"L2error = "<<L2error<<endl; // to show the error

```

Figure 4.1: FreeFEM++ code for the problem Equation 4.1 (Font & Peria, 2013)

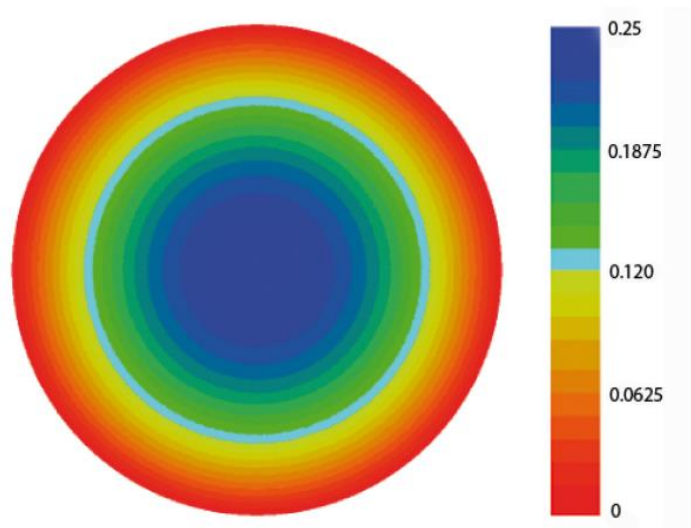


Figure 4.2: Picture of the solution of problem Equation 4.1 obtained with the code in Figure 4.1 (Font & Peria, 2013)

4.3.2 Gmsh

Gmsh is a freely available software tool that is utilised for generating 3D finite element meshes. It incorporates a built-in computer-aided design engine and post-processing capabilities. The primary objective of this tool is to offer a meshing solution that is efficient, lightweight, and intuitive for users. It aims to incorporate parametric input functionality and adaptable visualisation capabilities. Gmsh is structured into four distinct modules, namely geometry, mesh, solver, and post-processing. These modules can be managed through various means, such as the graphical user interface, command line interface, Gmsh's scripting language (.geo files), or the application programming interfaces (APIs) provided for C++, C, Python, Julia, and Fortran programming languages.

Additionally, examples are utilised as a method of instruction in this basic tutorial. We will create a 3D membrane of length l_m ($l_m=1$), width w_m ($w_m=1$), and thickness th ($th=0.2$). We shall refer to the element size near points as lc ($lc=0.1$). First, we'll establish the parameters we'll use. We define them in *Geometry* $\rightarrow$ *Elementary entities* $\rightarrow$ *Add* $\rightarrow$ *Parameter*. Once we've defined the parameter's various fields, we can simply click the Add button.

To construct the required points, simply click on the Point sheet on the same window or *Geometry* $\rightarrow$ *Elementary entities* $\rightarrow$ *Add* $\rightarrow$ *Point*. In our example, we only need four points to form the membrane's four boundary lines. Points can be formed by clicking on the graphics window where we wish to set the point or by entering the coordinates into the Point window. Be careful not to position the mouse on the graphics windows after you've filled out the fields and before hitting the Add button, as the mouse's coordinates will overwrite

the coordinate field values. If you want to do it by clicking on the graphics window, press the Shift key to fix the coordinates in the windows even if we move the mouse.

After creating all four points (click q to finish), as seen in Figure 4.3, we must generate the lines. To create a straight line, select *Geometry* → *Elementary entities* → *Add* → *Straight line*, and click the two end points to define the line. The line is drawn in a certain direction, from the first picked point to the second. Figure 4.4 depicts the four lines that will result from this operation. Click q to finish creating lines.

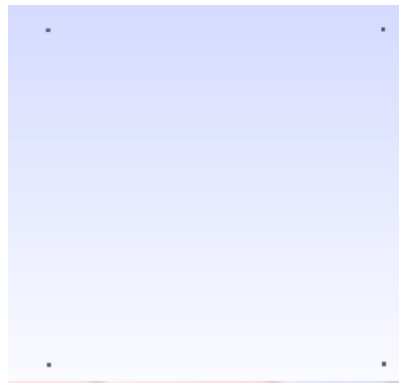


Figure 4.3: The four points (Carmona et al., 2013)

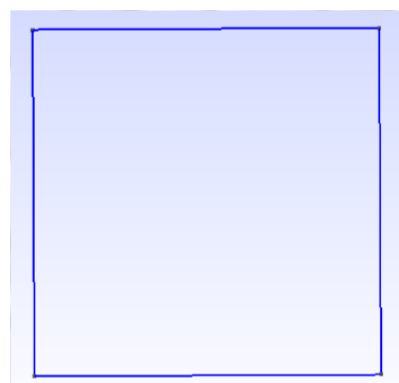


Figure 4.4: The four lines created for the membrane (Carmona et al., 2013)

We shall now create the surface. In this scenario, we'll make a ruled surface. To add a ruled surface, go to *Geometry* → *Elementary entities* → *Add* → *Ruled surface*. In this scenario, we only need to click on one line and GMSH will locate the rest (there are no other options for constructing a surface in our case). To complete the line selection for the surface, hit e (or q if we did not select the correct set of lines), and then press q to create the surface. The surface appears as depicted in Figure 4.5. The dashed lines depict the creation of the surface.

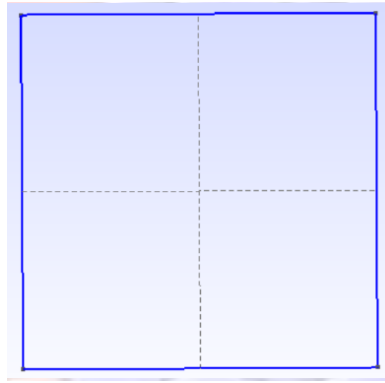


Figure 4.5: Created rule surface of the membrane (Carmona et al., 2013)

We will mesh the surface first, before constructing the volume. In this approach, when we build the volume (by translation of the already created surface), we also create the volume mesh. For this, we need to indicate two things:

- We want to use transfinite interpolation to generate a regular mesh. To select a surface, navigate to *Mesh* → *Define* → *Transfinite* → *Surface* and click on the dashed lines. (We can pick all connected things in an area by holding down the Ctrl key and defining a region with the mouse.)

- We want to generate square elements rather than rectangles. To do this, choose the same surface and click *Mesh* $\rightarrow$ *Define* $\rightarrow$ *Recombine*.

To preview surface elements, construct a mesh by clicking *Mesh* $\rightarrow$ *2D*. The results should be as displayed in Figure 4.6 with a mesh of 10×10 square components. To clear them, use the bar menu option *File* $\rightarrow$ *Clear*.

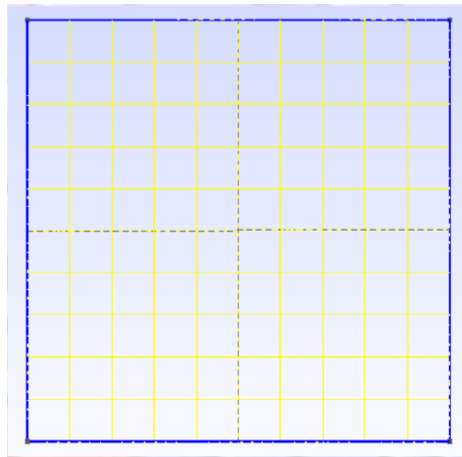


Figure 4.6: Meshed surface (Carmona et al., 2013)

Let us now create the volume. To use the extrude option, navigate to *Geometry* $\rightarrow$ *Elementary entities* $\rightarrow$ *Translate* $\rightarrow$ *Extrude surface*. We must input the *th* value in the Z Component field. Unfortunately, not all choices are available through the graphical user interface (GUI). To define the number of elements in the extruded Z direction and cube-shaped elements, modify the geo file and include *Layers{3}; Recombine;* within the last field of the extrude command. The instruction might look like this: *Extrude {0, 0, th} { Surface{5}; Layers{3}; Recombine; }*. Save the file and select *File* $\rightarrow$ *Open*.

To generate a 3D model, select *Mesh* $\rightarrow$ *3D*. The result should be similar to Figure 4.7.

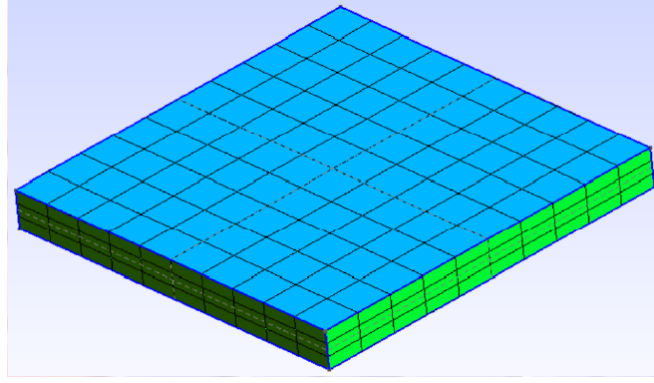


Figure 4.7: Meshed volume (Carmona et al., 2013)

4.4 Results and Discussion

The numerical simulations found major difficulty while dealing with moving boundaries in the free boundary problem of this model mainly due to a constantly changing physical domain. Hence, the use of the level set method is applied in the context of a single cell model, whilst the level set approach is adopted to depict the plasma membrane. This is done to effectively address the challenge associated with handling moving boundaries. Consider the function $\psi(\cdot, t) = \psi$, which represents a smooth level set.

Consider that there is no signal density at the start of the computation. As a result of ECM degradation, dispersed ligands outside of the cell attach to receptors and generate an internal signal. Signal densities diffuse within the cell, activating actin polymerisation. We have devised two tests: Test 1 simulates a single invadopodia, while Test 2 simulates two invadopodia. The values for tests 1 and 2 are derived from the references provided by Othman and Suzuki (2020). These values are then adjusted through a trial-and-error process. Based on Equations 3.2 and 3.3, we defined the boundary data as follows:

1. Test 1: $\sigma = c_* = g = 0.01(2 + \sin(2\pi(x + y)^2) \cos(\pi(x + 0.5)))$ and level set solution as $\psi = 0.05 \times \sinh(((x - y)^{1/2} - R)/(\epsilon h)^2)$ where $\epsilon = 5$ and $h = 0.2$.
2. Test 2: Modifications of equations from Test 1. $\sigma = c_* = g = 0.01(2 + \sin(4\pi(x + y)^2) \cos(\pi(2x + 0.5)))$ and level set solution as $\psi = 0.05 \times \tanh(((x - y)^{1/2} - R)/(\epsilon h)^2)$ where $\epsilon = 5$ and $h = 0.2$.

The PDEs were numerically solved using FreeFem++ with a P1 FEM with a computation maximum time of 1000s. Figures 4.8 and 4.9 illustrates the initial shape of cell while Figures 4.10 – 4.13 depict the process of invadopodia formation and protrusion at the cell's boundary. The formation of protrusion occurred due to the displacement of the membrane caused by the existence of a signal gradient, which was initiated by interactions between the membrane and the surrounding ECM.

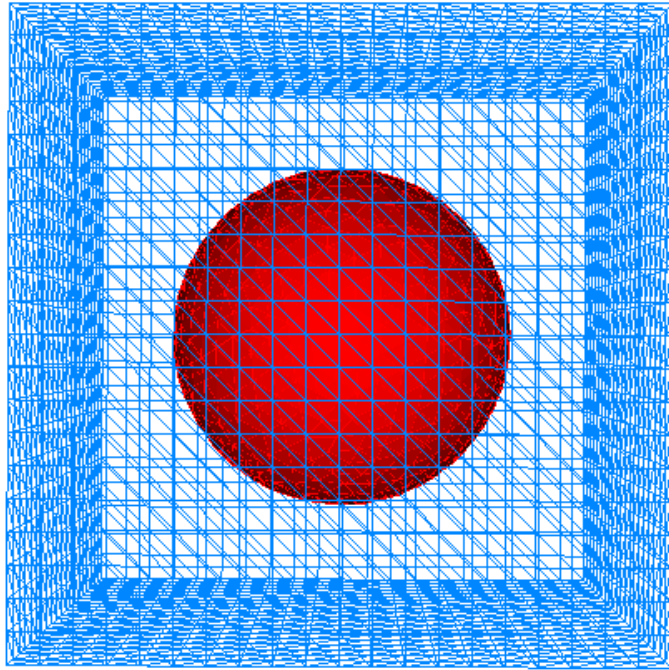


Figure 4.8: Initial cell configuration with an outer domain (box)

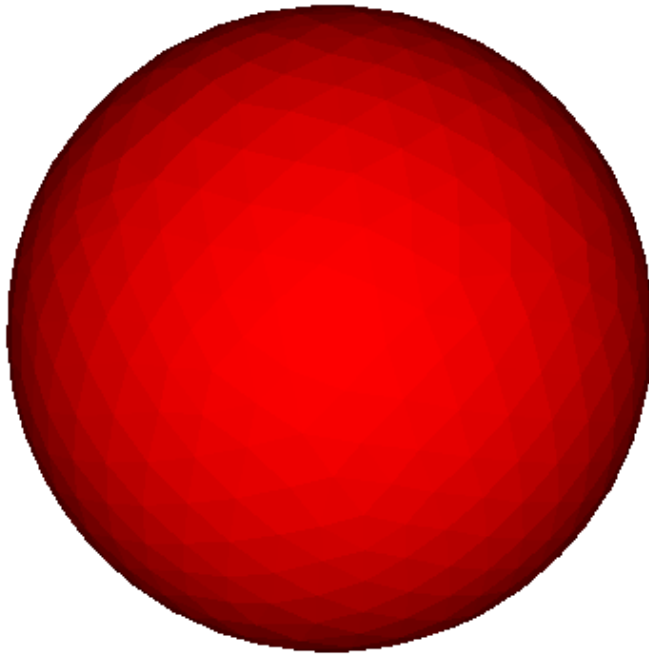


Figure 4.9: Initial cell configuration without an outer domain (box)

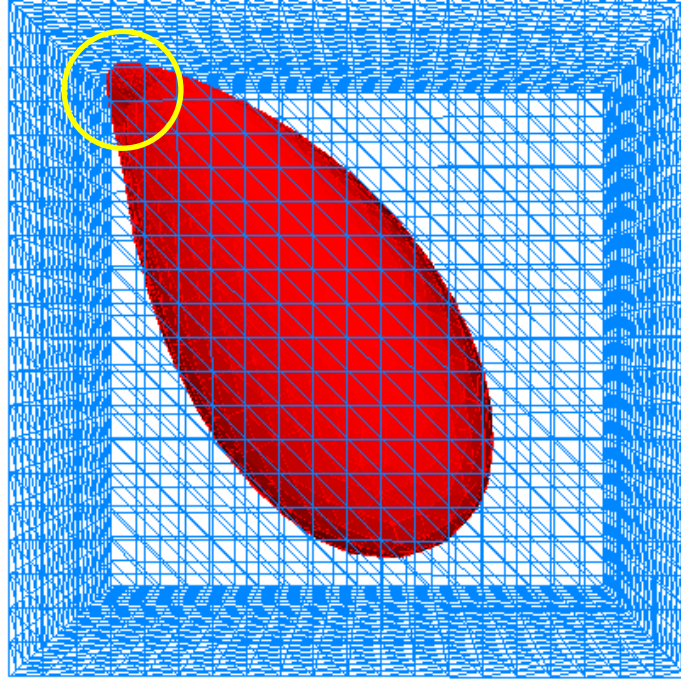


Figure 4.10: Invadopodia formation simulation with an outer domain (box) using Test 1

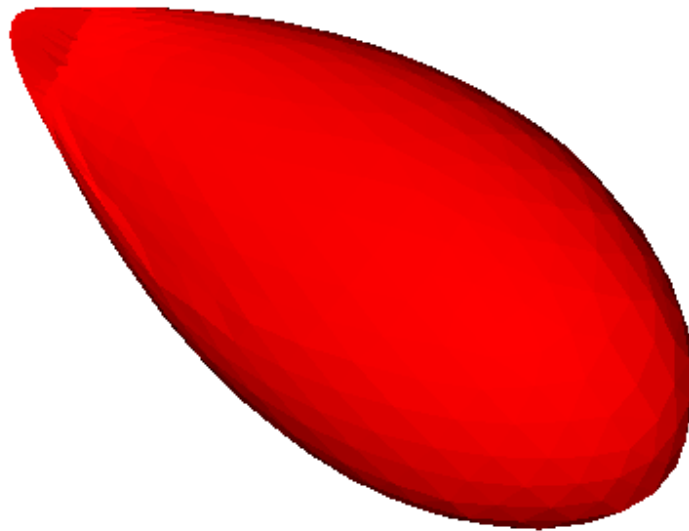


Figure 4.11: Invadopodia formation simulation without an outer domain (box) using Test 1

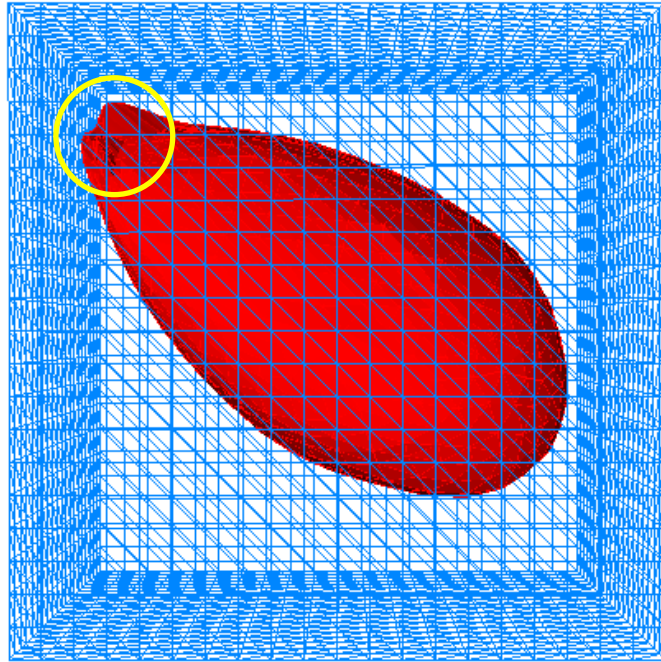


Figure 4.12: Invadopodia formation simulation with an outer domain (box) using Test 2

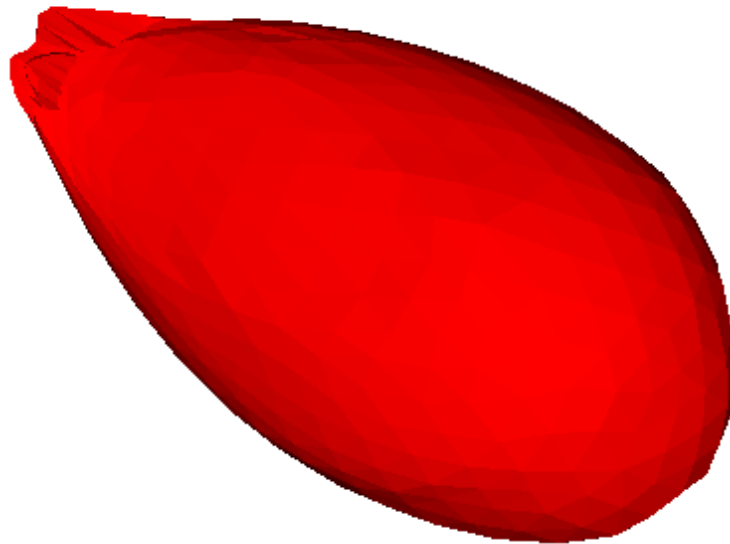


Figure 4.13: Invadopodia formation simulation without an outer domain (box) using Test 2

Level sets are advantageous in the context of cell membranes as they are straightforward to implement and do not require significant computer resources. They prove to be valuable in representing membrane interfaces as free boundaries. The presence of a discontinuity in the derivatives of the level set function at the interfaces between consecutive outer and inner cells can have an effect on the computation of velocity and curvature. Consequently, the utilisation of the level set approach facilitates the straightforward determination of the curvature and velocity of an interface, thereby enabling the localisation of the plasma membrane interface. Consequently, the velocity extension is specified across the entire domain in order to mitigate the singularities and instabilities.

To improve the accuracy of invadopodia formation simulation and address the issues identified in Othman and Suzuki (2020), as outlined in Section 1, 3D simulations are computed. The outcomes of these simulations are depicted in Figures 4.8 – 4.13. The shape of cells in Figures 4.8 and 4.9 illustrates the initial configuration of the cell. Figures 4.10 and 4.11 depict the process of invadopodia and protrusion creation at the cellular boundary employing Test 1 where it is used to simulate a single invadopodia. While Test 2 is employed to simulate two invadopodia which are shown in Figures 4.12 and 4.13. Protrusion occurs as a result of the displacement of the membrane produced by the existence of a signal gradient induced by the interaction between the membrane and the ECM.

As mentioned earlier, Othman and Suzuki (2020) provide evidence for the presence of singularities and instabilities. However, in the context of this research, the sole method to quantify singularities and instabilities is by visual observation. Based on the findings depicted in Figures 4.10 – 4.13, it can be inferred that the simulation effectively averted the occurrence of both instabilities and singularities. As mentioned in Chapter 1, singularities refer to the fact that the invadopodia simulation should not have a sharp edge or cusp,

whereas instabilities refer to the fact that there should not be more than two invadopodia. Hence, according to our analysis of the simulation, it exhibits neither singularity nor instabilities. This discrepancy may be attributed to the utilisation of distinct simulation techniques and datasets in Tests 1 and 2.

Based on Equation 3.4 and level set solutions in Tests 1 and 2, graphs of level set values were generated as shown in Figures 4.14 and 4.15. The graphs displayed a similarity in the fluctuation of the level set values during the deformation of the cell in Tests 1 and 2. These were created solely to demonstrate the impact of the signal gradient within the cell and the ligand gradient outside the cell on the cell membrane.

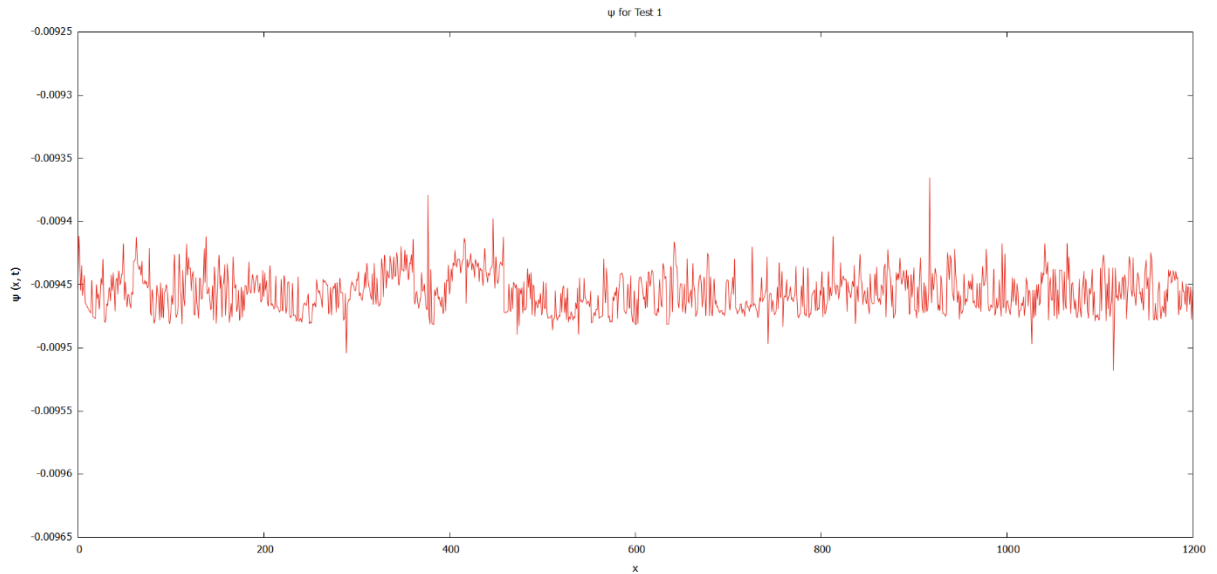


Figure 4.14: Graph of level set function, ψ for Test 1

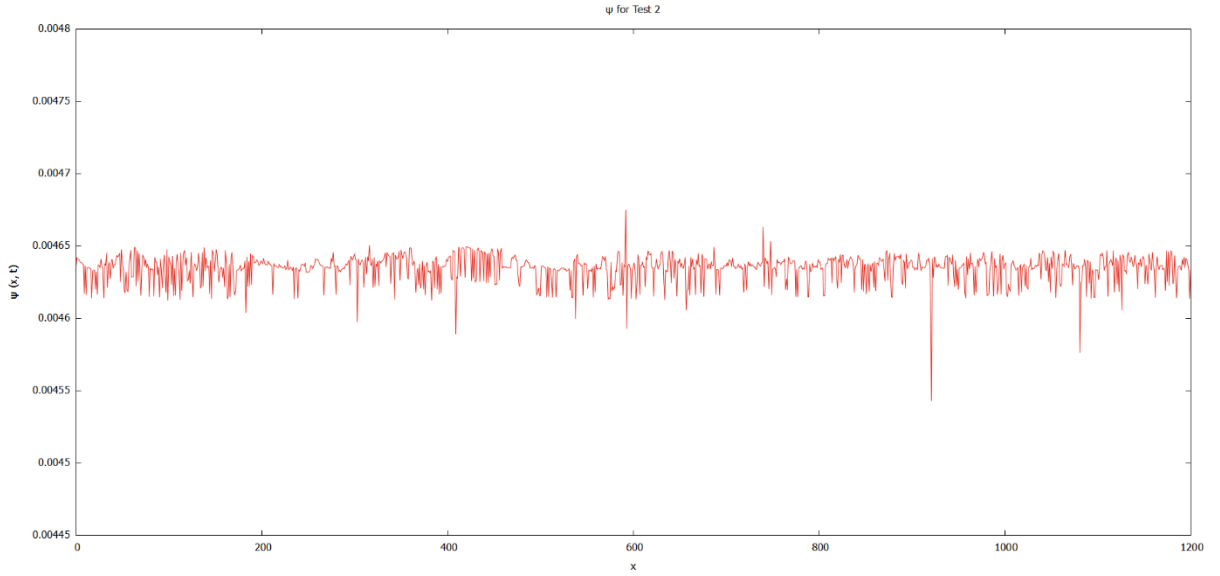


Figure 4.15: Graph of level set function, ψ for Test 2

Figure 4.16 (a) (Xue et al., 2022) depicts the biological imaging of a single invadopodium in cancer cells while Figure 4.16 (b) shows the result of invadopodia formation simulation using Test 1. In this context, it is evident that there exists a resemblance of one invadopodia between the observed phenomenon and our simulation from Test 1.

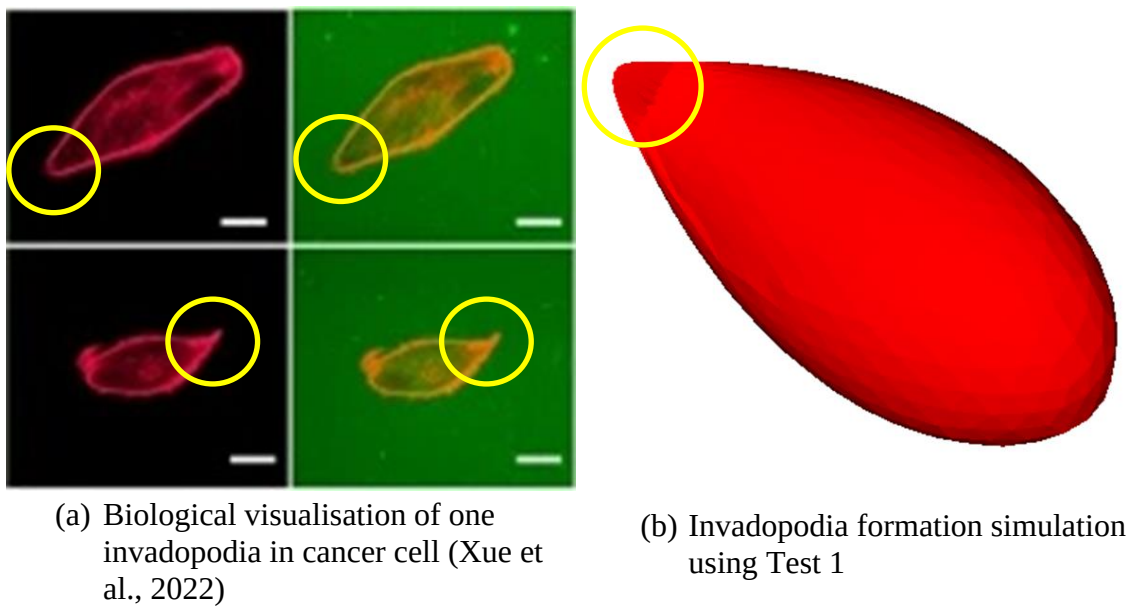
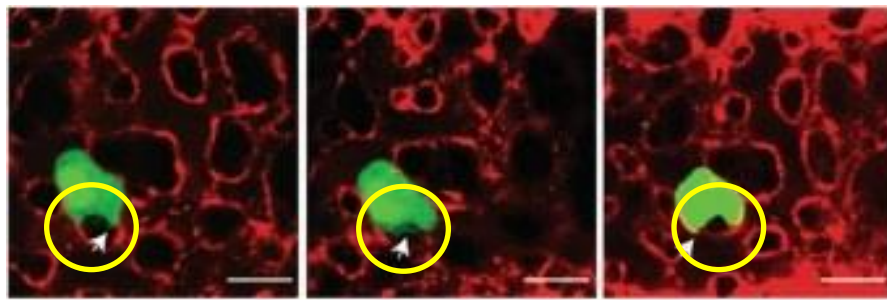
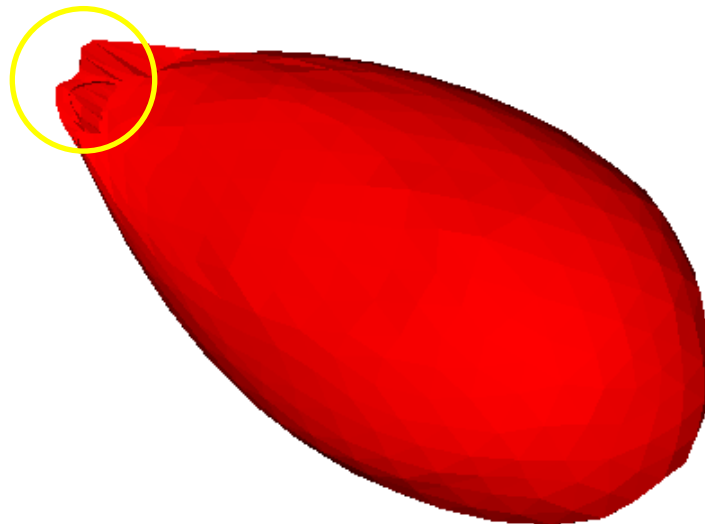


Figure 4.16: Comparison of biological visualisation and Test 1 simulation of invadopodia formation

Similarly, Figure 4.17 (a) (Williams et al., 2019) depicts the biological imaging of two invadopodia in a cancer cell while Figure 4.17 (b) shows the result of invadopodia formation simulation using Test 2. The object closely resembles the simulation carried out during Test 2, where the morphology of the two invadopodia appears identical.



(a) Biological visualisation of two invadopodia in cancer cell (Williams et al., 2019)



(b) Invadopodia formation simulation using Test 2

Figure 4.17: Comparison of biological visualisation and Test 2 simulation of invadopodia formation

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

A mathematical model that accurately represents the actions of a single cell is proposed. The objective is to enhance the simulation of invadopodia formation by introducing a 3D setting, which adds a greater level of authenticity and intricacy. The creation of invadopodia is analysed using the Stefan problem framework, which considers the cell membrane as described by the Stefan free boundary condition. This condition implies that the boundary membrane is not preset. The modelling technique included the following parameters: ECM concentration (c), MMPs function (f), ligands concentration (c_*), and signal strength (σ).

A latest numerical solution of cancer invasion is used in this study, utilising the level set technique to effectively tackle the Stefan problem associated with the formation of invadopodia. The level set method is employed to ascertain the precise position of the membrane by utilising the zero-level function of ψ . This methodology is employed to quantitatively address the phenomenon of deformation and invasion of cancer cells. Protrusions are induced by the existence of invadopodia, whereas invasion occurs when the cancer cell initiates dissemination to a different region of the organism.

Furthermore, we have improved the results of the simulation conducted by Othman and Suzuki (2020) by incorporating a more sophisticated simulation technique where the software tools utilised for implementing all the aforementioned approaches in 3D are FreeFem++ and Gmsh. Other than that, distinct data sets were utilised for Test 1, which

involved one invadopodia, and Test 2, which involved two invadopodia. The occurrence of singularities was successfully eradicated, while the occurrence of instabilities was well mitigated. In addition, our simulations exhibit a resemblance to the biological visual representations of invadopodia in cancer cells, as depicted in Figure 4.16 and 4.17.

5.2 Recommendations

There are a handful of suggestions for forthcoming endeavors that will significantly aid in advancing this subject of research. One approach is to develop a simulation that models the concentration of ligand and signal within the cell. This is crucial for future studies in which we aim to investigate the impact of concentration on invadopodia development. In addition, determining the signal and ligand densities could also contribute to the progress of the research.

Besides that, it is necessary to simulate the findings using various mesh values in order to determine the convergence of the solutions. This information can aid in obtaining the most accurate solutions that closely approximate the real solution. Other than that, the sole method of investigating singularity and instability in this research is through observation. It is hoped that in the future, alternative means of quantifying the occurrence of singularity and instability will become available. Nevertheless, the findings presented in this study hold promise for the advancement of fresh methodologies and the expansion of existing techniques in the pursuit of enhanced precision in numerical simulations.

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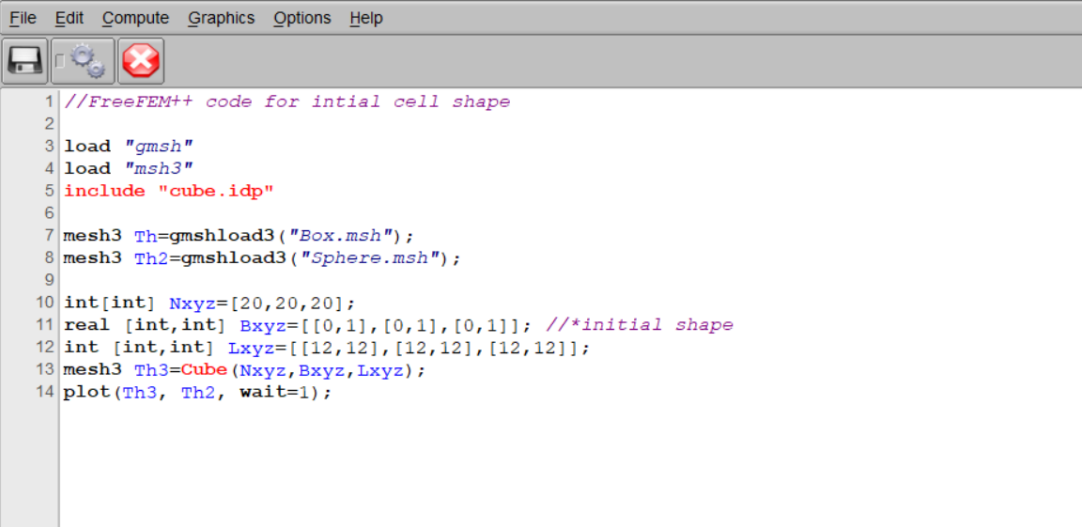
APPENDICES

Appendix A

```
//Gmsh code for mesh generation

//+
SetFactory("OpenCASCADE");
Box(1) = {0, 0, 0, 1, 1, 1};
Sphere(1) = {0.5, 0.5, 0.5, 0.3, -Pi/2, Pi/2, 2*Pi};
```

Appendix B



```
1 //FreeFEM++ code for intial cell shape
2
3 load "gmsh"
4 load "msh3"
5 include "cube.idp"
6
7 mesh3 Th=gmshload3("Box.msh");
8 mesh3 Th2=gmshload3("Sphere.msh");
9
10 int[int] Nxyz=[20,20,20];
11 real [int,int] Bxyz=[[0,1],[0,1],[0,1]]; /*initial shape
12 int [int,int] Lxyz=[[12,12],[12,12],[12,12]];
13 mesh3 Th3=Cube(Nxyz,Bxyz,Lxyz);
14 plot(Th3, Th2, wait=1);
```

Appendix C

```

File Edit Compute Graphics Options Help
[Icons]

1 //FreeFEM++ code for Test 1 (1 invadopodia)
2
3 load "gmsh"
4 load "msh3"
5 include "cube.idp"
6
7 mesh3 Th=gmshload3("Box.msh");
8 mesh3 Th2=gmshload3("Sphere.msh");
9
10 int[int] Nxyz=[20,20,20];
11 real [int,int] Bxyz=[[-0.0001,-0.03],[-0.0001,-0.03],[-0.0001,-0.03]];
12 int [int,int] Lxyz=[[12,12],[12,12],[12,12]];
13 mesh3 Th4=Cube(Nxyz,Bxyz,Lxyz);
14
15 real R=0.3;
16 real E=5;
17 real H=0.2;
18 real eps1=0.001;
19 real eps2=0.001;
20 real dt=0.005;
21 real weak=0.0001;
22 real T=0.01;
23 real T2=0.01;
24 real TT=0.01;
25 int N=1000;
26 int Q=1000;
27 int iterinit;
28
29 int nbTriangles=Th.nt;
30 int nbtriangles=Th2.nt;
31 int iplt=nbtriangles/100;
32
33 fespace Uh(Th, P1);
34 fespace Uh1(Th, P1);
35 fespace Vh(Th2, P1);
36 fespace Vh1(Th2, P1);
37
38 Uh u=0.01*(2+sin(2*pi*(x+y)^2)*cos(pi*(x+0.5))), v, ligand0;
39 Uh1 S, H1, H2, H3, X1, Xapprox1, nabla1, N1, N2, N3;
40 Vh uu=0.01*(2+sin(2*pi*(x+y)^2)*cos(pi*(x+0.5))), vv, signal0;
41 Vh1 phi, nabla0, phiinit, nabla2, X2, Xapprox2, M1, M2, M3, Tx, Ty, Tz, phim;
42
43 func phi0=0.05*sinh(((x-y)^1/2)-R)/(E*H)^2);
44 phi=phi0;
45 plot(Th4, phi, wait=1);
46
47 Vh h1=hTriangle;
48 real h=h1[].max;
49 cout<<"maximal size of a mesh triangle h="<<h<<endl;
50
51 for (iterinit=1; iterinit<nbTriangles; iterinit=iterinit+1)
52 int j;
53 {
54 nabla0=(dx(phi))^2+(dy(phi))^2+(dz(phi))^2;
55 S=phi/(sqrt(phi^2+h*nabla0));
56 H1=dx(phi)/(sqrt(nabla0+eps1^2));
57 H2=dy(phi)/(sqrt(nabla0+eps2^2));
58 H3=dz(phi)/(sqrt(nabla0+eps2^2));
59 phiinit=convect([-S*H1,-S*H2,-S*H3],TT,phi);
60 phiinit=phiinit+TT*S;
61 phi=phiinit;
62 };
63
64 X1=(phi>0);
65 Xapprox1=X1+weak*(1-X1);
66

```

```

67 problem ligand(u, v, solver=LU)=
68 int3d(Th) ((u*v/dt)+Xapprox1*(dx(u)*dx(v)+dy(u)*dy(v)+dz(u)*dz(v)))
69 -int3d(Th)(ligand0*v/dt)
70 -int2d(Th,1)(0*v);
71
72 //Computation for ligand normal vector
73 nablal=(dx(u))^2+(dy(u))^2+(dz(u))^2;
74 N1=dx(u)/(sqrt(nablal+eps1^2));
75 N2=dy(u)/(sqrt(nablal+eps1^2));
76 N3=dz(u)/(sqrt(nablal+eps1^2));
77
78 X2=(phi<0);
79 Xapprox2=X2+weak*(1-X2);
80
81 problem signal(uu, vv, solver=LU)=
82 int3d(Th2) ((uu*vv/dt)+Xapprox2*(dx(uu)*dx(vv)+dy(uu)*dy(vv)+dz(uu)*dz(vv)))
83 -int3d(Th2)(signal0*vv/dt)
84 +on(2, uu=u);
85
86 //Computation for signal normal vector
87 nablal2=(dx(uu))^2+(dy(uu))^2+(dz(uu))^2;
88 M1=dx(uu)/(sqrt(nablal2+eps2^2));
89 M2=dy(uu)/(sqrt(nablal2+eps2^2));
90 M3=dz(uu)/(sqrt(nablal2+eps2^2));
91
92 Tx=N1-M1;
93 Ty=N2-M2;
94 Tz=N3-M3;
95
96 for(int f=1; f<N; f=f+1)
97 {
98   phim=convect([-Tx,Ty,-Tz],-dt,phi);
99   T=T+dt;
100 };
101
102 mesh3 Th3=movemesh(Th2, [x*phim, y*phim, z*phim]);
103
104 fespace Vh2(Th3, P1);
105
106 Vh2 phim2;
107
108 for(int g=1; g<Q; g=g+1)
109 {
110   phim2=convect([-Tx,Ty,-Tz],-dt,phi);
111   T2=T2+dt;
112   if(!(j%iplt))
113     plot(phim2, Th4, cmm="t="+T2+"", dim=3, fill=true, value=true, wait=true, bw=1);
114 };

```

Appendix D

```

File Edit Compute Graphics Options Help
[Icons]

1 //FreeFEM++ code for Test 2 (2 invadopodia)
2
3 load "gmsh"
4 load "msh3"
5 include "cube.idp"
6
7 mesh3 Th=gmshload3("kotakk.msh");
8 mesh3 Th2=gmshload3("bulatt2.msh");
9
10 int[int] Nxyz=[20,20,20];
11 real [int,int] Bxyz=[[0.00003,0.0055],[0.00003,0.0055],[0.00003,0.0055]];
12 int [int,int] Lxyz=[[12,12],[12,12],[12,12]];
13 mesh3 Th4=Cube(Nxyz,Bxyz,Lxyz);
14
15 real R=0.3;
16 real E=5;
17 real H=0.2;
18 real eps1=0.001;
19 real eps2=0.001;
20 real dt=0.005;
21 real weak=0.0001;
22 real T=0.01;
23 real T2=0.01;
24 real TT=0.01;
25 int N=1000;
26 int Q=1000;
27 int iterinit;
28
29 int nbTriangles=Th.nt;
30 int nbtriangles=Th2.nt;
31 int iplt=nbtriangles/100;
32
33 fespace Uh(Th, P1);
34 fespace Uh1(Th, P1);
35 fespace Vh(Th2, P1);
36 fespace Vh1(Th2, P1);
37
38 Uh u=0.01*(2+sin(4*pi*(x+y)^2)*cos(pi*((2*x)+0.5))), v, ligand0;
39 Uh1 S, H1, H2, H3, X1, Xapprox1, nabla1, N1, N2, N3;
40 Vh uu=0.01*(2+sin(4*pi*(x+y)^2)*cos(pi*((2*x)+0.5))), vv, signal0;
41 Vh1 phi, nabla0, phiinit, nabla2, X2, Xapprox2, M1, M2, M3, Tx, Ty, Tz, phim;
42
43 func phi0=0.05*tanh(((x-y)^1/2)-R)/E*H)^2;
44 phi=phi0;
45 plot(Th4, phi, wait=1);
46
47 Vh h1=hTriangle;
48 real h=h1[.max];
49 cout<<"maximal size of a mesh triangle h="<<h<<endl;
50
51 for (iterinit=1; iterinit<nbTriangles; iterinit=iterinit+1)
52 int j;
53 {
54 nabla0=(dx(phi))^2+(dy(phi))^2+(dz(phi))^2;
55 S=phi/(sqrt(phi^2+h*nabla0));
56 H1=dx(phi)/(sqrt(nabla0+eps1^2));
57 H2=dy(phi)/(sqrt(nabla0+eps2^2));
58 H3=dz(phi)/(sqrt(nabla0+eps2^2));
59 phiinit=convect([-S*H1,-S*H2,-S*H3],TT,phi);
60 phiinit=phiinit+TT*S;
61 phi=phiinit;
62 };
63
64 X1=(phi>0);
65 Xapprox1=X1+weak*(1-X1);
66

```

```

67 problem ligand(u, v, solver=LU)=
68 int3d(Th) ((u*v/dt)+Xapprox1*(dx(u)*dx(v)+dy(u)*dy(v)+dz(u)*dz(v)))
69 -int3d(Th) (ligand0*v/dt)
70 -int2d(Th,1) (0*v);
71
72 //Computation for ligand normal vector
73 nabla1=(dx(u))^2+(dy(u))^2+(dz(u))^2;
74 N1=dx(u)/(sqrt(nabla1+eps1^2));
75 N2=dy(u)/(sqrt(nabla1+eps1^2));
76 N3=dz(u)/(sqrt(nabla1+eps1^2));
77
78 X2=(phi<0);
79 Xapprox2=X2+weak*(1-X2);
80
81 problem signal(uu, vv, solver=LU)=
82 int3d(Th2) ((uu*vv/dt)+Xapprox2*(dx(uu)*dx(vv)+dy(uu)*dy(vv)+dz(uu)*dz(vv)))
83 -int3d(Th2) (signal0*vv/dt)
84 +on(2, uu=u);
85
86 //Computation for signal normal vector
87 nabla2=(dx(uu))^2+(dy(uu))^2+(dz(uu))^2;
88 M1=dx(uu)/(sqrt(nabla2+eps2^2));
89 M2=dy(uu)/(sqrt(nabla2+eps2^2));
90 M3=dz(uu)/(sqrt(nabla2+eps2^2));
91
92 Tx=N1-M1;
93 Ty=N2-M2;
94 Tz=N3-M3;
95
96 for(int f=1; f<N; f=f+1)
97 {
98   phim=convect([-Tx,Ty,-Tz],-dt,phi);
99   T=T+dt;
100 };
101
102 mesh3 Th3=movemesh(Th2, [x*phim, y*phim, z*phim]);
103
104 fespace Vh2(Th3, P1);
105
106 Vh2 phim2;
107
108 for(int g=1; g<Q; g=g+1)
109 {
110   phim2=convect([-Tx,Ty,-Tz],-dt,phi);
111   T2=T2+dt;
112   if(!(j%iplt))
113     plot(phim2, Th4, cmm="t="+T2+"", dim=3, fill=true, value=true, wait=true, bw=1);
114 };

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Journal Publication

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Article

Invadopodia Formation in Cancer Cell: The Mathematical and Computational Modelling Based on Free Boundary Problem

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Abstract: We present a mathematical model of an individual cell to expand the simulation of invadopodia formation to a three-dimensional (3D) domain for a more realistic complexity. Simulating invadopodia replication in order for it to be biologically relevant is important since it helps us to understand cancer invasion and metastasis better as well as giving some insight into investigating ways to stop the spread of this fatal disease. Invadopodia formation is formulated using the Stefan problem approach, where the free boundary is characterised by the Stefan free boundary condition, in which the boundary membrane is not known in advance. Level set method is proposed to indicate the behaviour of the cell interface and the motion of the plasma membrane. An enthalpy method (phase-transition problem) is used to describe the cell membrane diffusion. In addition to this, we were able to improve the simulation outcome, giving it a more realistic complexity by using a different simulation technique and domain as well as a different data set. Singularities and instabilities were eliminated. The results that were achieved have the potential to be helpful for novel approaches or to be extended to other methods in the development of a more accurate numerical simulation.

Keywords: invadopodia; individual cell model; free boundary problem; finite element method; Stefan problem; level set method; phase transition; enthalpy

MSC: 92-08; 92-10

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1. Introduction

The term cancer encompasses a variety of diseases that are broadly categorised by abnormal cell behaviour which act differently from normal cells due to the gene mutations that alter the normal cellular instructions [1]. Tissue invasion and metastasis are one of the “hallmarks of cancer” [2,3] and the leading cause of death among cancer patients [4]. Invasion of cancer cells is the capacity of the cells to disrupt the basement membrane (BM) and secondary tumours arise when it penetrates the surrounding tissue or extracellular matrix (ECM), also referred to as metastasis.

The key factor in tumour invasion and metastasis is proteolytic deterioration of the ECM [5,6] which are propelled by actin-rich protrusions of the plasma membrane, also called invadopodia [7]. Invadopodia is also called ‘invasive feet’ as it is most commonly detected on the basal surfaces of invasive cancer cells; invadopodia have the ability to invade through the ECM [8]. The morphology of invadopodia is a small punctuated finger-like protrusion or elongated shapes [9]. According to Saitou et al. [10], cancer cells can form one to ten invadopodia with a lifespan of several tens of minutes to 60 min and a size ranging from 0.05 to 2 μm [11]. Numerous cell biological processes must be coordinated for the formation of invadopodia and the degradation of ECM. The formation of invadopodia

and the degradation of ECM require the coordination of numerous cell biological processes. The molecular dynamics and biological phenomena related to invadopodia formation and ECM degradation are further detailed in [12]. Figure 1 depicts the scheme of invadopodia in invasive cancer cell while Figure 2 shows the role of invadopodia in cancer invasion and metastasis.

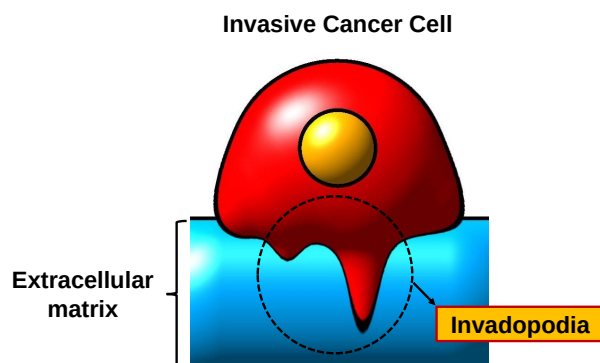


Figure 1. A scheme of invadopodia.

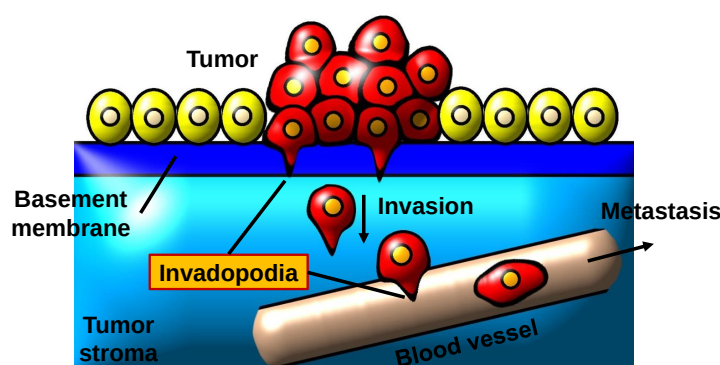


Figure 2. Invadopodia's role in cancer invasion and metastasis.

Over the past decade, numerous studies have been carried out to comprehend the mechanism of tumour growth and cancer invasion from a mathematical point of view using continuous and discrete models, particularly in partial differential equations (PDEs) and ordinary differential equations (ODEs) [13–16]. Some studies have been successful in capturing the structure of cancer at the tissue level [17,18]. At present, the model of cancer cell invasion at the subcellular level (single cell) is of particular interest [9,10,12,19–21], while numerical analysis on the cancer cell invasion model with the free boundary problem was conducted in [22].

Saitou et al. [10] investigated the formation of invadopodia by introducing mathematical models of several fundamental processes. In [10], they analysed the spatio-temporal dynamics of the model in two spatial dimensions by using the numerical computational approach. In their results, the dynamics of the model is explored, particularly in space and time for two spatial dimensions by using the Monte-Carlo approach. This study is extremely significant because it offers new insight on how invadopodia exist in cancer cells. Numerous subsequent studies built on this one by using or modifying the model presented here such as [9,12,19–21].

For instance, ref. [20] investigates a free boundary problem for cell protrusion formation where the aim of the paper is to model the chemical interactions between the cell and its environment during the process of invadopodia formation. They prove the free boundary problem is well-posed. They eventually exhibit the main biological feature that can be accounted for via the model which is the formation of invadopodia. Ref. [9] present

a superconvergent second order Cartesian method to solve a free boundary problem with two harmonic phases coupled through the moving interface. The model was proposed to describe the formation of cell protrusions. The finite difference method (FDM) proposed in this paper consists of second-order discretisation and a new stabilised ghost fluid method.

This paper aims to improve the simulation of invadopodia formation with respect to the previous research of [12] in which the results were simulated in a two-dimensional (2D) domain and an in silico model of an individual cell was presented. Therefore, we simulated our results in a 3D domain for accurate results and conditions where the complexities of simulations are more realistic. Simulating invadopodia formation accurately in order for it to be biologically relevant is important since it helps us to understand cancer invasion and metastasis better, as well as giving some insight into investigating ways to stop the spread of this fatal disease. We note that in the previous work of [12], the simulations demonstrate the existence of singularities and instabilities. For that reason, eliminating the existence of singularities and instabilities is crucial in simulating the results for the solution to be more accurate.

2. Materials and Methods

There are two parts to this section, individual cell model and numerical scheme, in accordance with [12].

2.1. Individual Cell Model

2.1.1. Classical Solution Scheme

This subsection describes the mathematical cell-deformation model that explains the invadopodia-formation process, as depicted in Figure 3. According to [12,20], ECM is in the outer domain of the cell. We define the flux of type 1 matrix metalloproteinase (MT1-MMP) enzymes, denoted by $g(x, t)$. MT1-MMP generates ligands c_* which is a flux-degraded matrix at the cell boundary which then diffuses into the ECM. Ligands will then activate signals that diffuse within the cell when bound to the cell membrane.

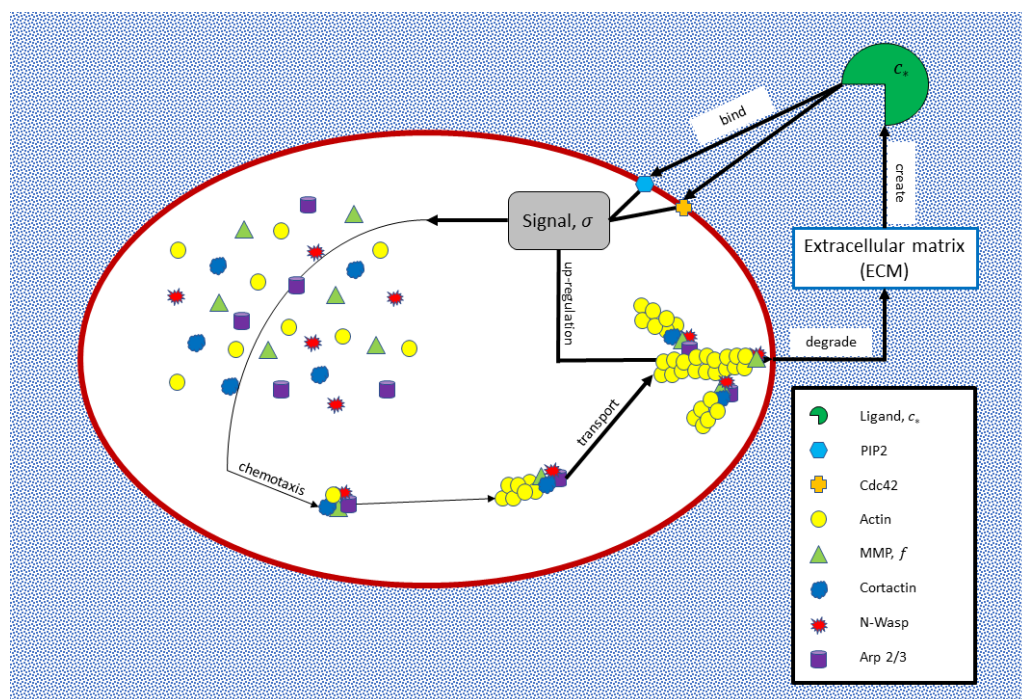


Figure 3. An individual cell model.

The model of a single cell is described by using the Eulerian approach. Let $\psi(x, t)$ be defined across the entire domain Ω to detect the cell membrane. The plasma membrane is defined as:

$$\Gamma_t = \{x \in \Omega | \psi(x, t) = 0\}, \quad (1)$$

where Ω is the Lipschitz domain indicating the cancer cell with smooth boundary, $\partial\Omega$, and a particle on the moving interface Γ_t is defined by x .

While the zero level set function, ψ detects the location of the plasma membrane which satisfies:

$$\psi_t(x, t) + v \cdot \nabla \psi(x, t) = 0 \quad (2)$$

which is a smooth level set function, whereas at the interface, v is defined as the velocity of the plasma membrane:

$$v = \gamma_n (\nabla \sigma - \nabla c_*) \quad \text{on } \Gamma_t \quad (3)$$

where γ_n is a positive constant and σ is the signal gradient inside the cell. Keep in mind that it would be impossible for the domain to grow the shape's volume, which can lead to discontinuity, because velocity v is only defined on the boundary. Since the zero level set function takes the plasma membrane into account, velocity extension is defined throughout the entire domain to prevent discontinuity as:

$$\begin{aligned} (\nabla \psi \cdot \nabla) w &= 0, \quad \forall x \in \Omega, \quad w \in (x, y) \\ w &= v \quad \Gamma \end{aligned} \quad (4)$$

where:

$$v|_{\psi(x,t)=0} = \nabla \sigma|_{\psi(x,t)=0} - \nabla c_*|_{\psi(x,t)=0}.$$

Hence, substituting (3) into (2), we obtain:

$$\psi_t + v \cdot \nabla \psi = 0 \quad \text{on } \Gamma_t. \quad (5)$$

The cell's plasma membrane may shrink or expand. On the plasma membrane, Γ_t and MMPs, f degraded ECM and c and produced an ECM fragment that is ligands. Henceforth, the chemical reactions that occur between ECM and MMPs are derived by:

$$c_t + \nabla \cdot vc = -\kappa_c fc, \quad c_{*t} + \nabla \cdot vc_* = \kappa_c fc \quad \text{on } \bigcup_{0 < t < T} \Gamma_t \times \{t\} \quad (6)$$

where κ_c is the reaction rate of the diffusion coefficient. Then, f and σ are defined inside the cell as:

$$\begin{aligned} \sigma_t &= d_\sigma \Delta \sigma \quad \text{in } \bigcup_{0 < t < T} \omega_n^t \times \{t\}, & \sigma|_{\Gamma_t} &= c_* \quad \text{on } \bigcup_{0 < t < T} \omega_n^t \times \{t\} \\ f_t &= d_f \Delta f + k_f \sigma - \lambda_f f \quad \text{in } \bigcup_{0 < t < T} \omega_n^t \times \{t\}. \end{aligned} \quad (7)$$

Since the density of MMPs does not change during ECM fibre proteinase cutting, it can be concluded that:

$$c_{*t} = d_{c_*} \Delta c_* \quad \text{in } \bigcup_{0 < t < T} \omega_c^t \times \{t\}, \quad \frac{\partial c_*}{\partial \nu} = g \quad \text{on } \bigcup_{0 < t < T} \omega_c^t \times \{t\}. \quad (8)$$

We define $\omega_n^t = \{x \in \Omega | \psi(x, t) < 0\} \subset \subset \Omega$ as a one-domain inside cell. Hence:

$$Q_n = \bigcup_{0 < t < T} \omega_n^t \times \{t\} \quad (9)$$

and $\omega_c^t = \{x \in \Omega | \psi(x, t) > 0\}$ as an outside cell. Thus:

$$Q_c = \bigcup_{0 < t < T} \omega_c^t \times \{t\}. \quad (10)$$

If the solution of $y = y(t)$ to:

$$\frac{dy}{dt} = v(y, t), \quad y|_{t=s} = x$$

is denoted by $y = U(t, s)x$, then:

$$\frac{D\psi}{Dt} = \psi_t + v \cdot \nabla = -(\nabla \cdot v)\psi.$$

Substituting (9) and (10) into (7), we obtain:

$$\begin{aligned} \sigma_t &= d_\sigma \Delta \sigma \quad \text{in } Q_n, & \sigma|_{\Gamma_t} &= c_* \quad \text{on } \Gamma \\ f_t &= d_f \Delta f + k_f \sigma - \lambda_f f & \text{in } Q_n. \end{aligned} \quad (11)$$

The total mass of conservation of f guarantees, by Liouville's Theorem, that the following derivations on the boundary will be produced:

$$d_f \frac{\partial f}{\partial \nu} + (v \cdot \nu) f = 0 \quad \text{on } \Gamma = \bigcup_{0 < t < T} \Gamma_t \times \{t\}. \quad (12)$$

As the MMPS density at the boundary will not change during cutting through the fiber-proteinases of ECM, it holds that:

$$c_{*t} = d_{c_*} \Delta c_* \quad \text{in } Q_c, \quad c_*|_{\partial\Omega} = 0, \quad \frac{\partial c_*}{\partial \nu} = g \quad \text{on } \Gamma. \quad (13)$$

Figure 4 shows a simplified version of the signalling pathways depicted in Figure 3. From Figure 4, the MMP generates ligands on the cell membrane which then diffuses into the ECM as described in (13). Ligands will then activate signals that diffuse within the cell when bound to the cell membrane as in (11). The membrane is then pushed by the interface's normal velocity (3).

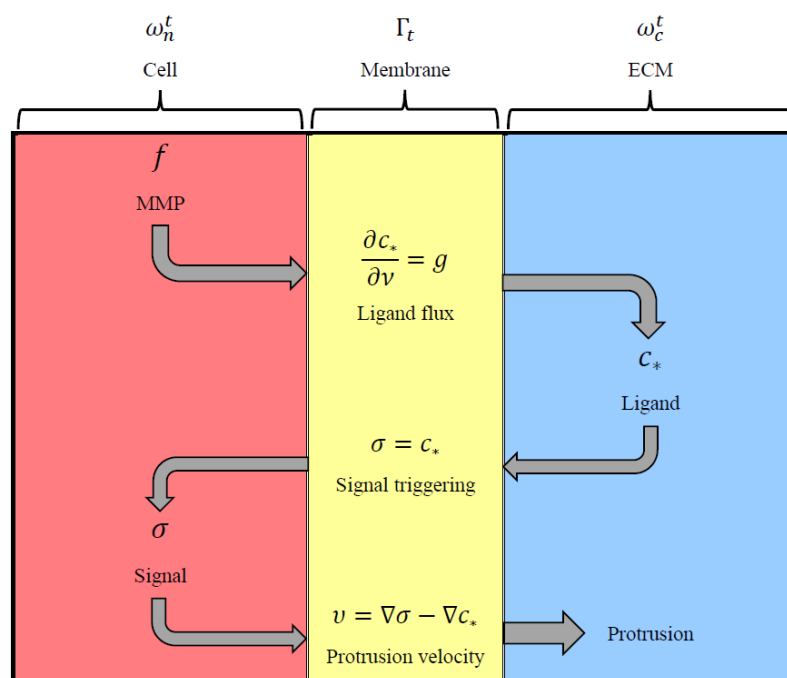


Figure 4. A schematic representation of the molecular interactions.

2.1.2. Free Boundary Problem

In order to simulate the formation of invadopodia, we proposed velocity extension to the entire domain of the cell. The cell model from Figure 3 was simplified, as shown in Figure 5. According to [12,20], when the velocity of protrusion is not imposed, the free boundary problem enables accurate membrane localisation even when it is unknown with

respect to the PDE systems. As a result, we used the cell membrane as a free boundary to distinguish between any activity occurring within and outside of cells.

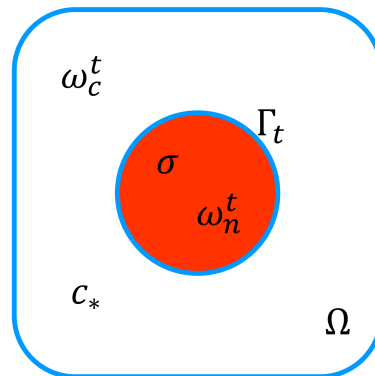


Figure 5. A simple cell model.

Given MT1-MMP at any time on the cell membrane and signal generation for actin polymerisation is represented by:

$$d_{\sigma}^{-1} \sigma_t = \Delta \sigma \quad \text{in } Q_n, \quad \sigma|_{\partial \omega_c^t} = c_* \quad (14)$$

while degradation of ECM is:

$$d_{c_*}^{-1} c_{*t} = \Delta c_* \quad \text{in } Q_c, \quad \frac{\partial c_*}{\partial \nu} = 0 \quad (15)$$

where $d > 0$ is a diffusion coefficient. Let $\sigma = c_* = g(x, t)$, considering the cell's primary problem. Define:

$$\theta = \begin{cases} \sigma & \text{in } Q_n = \{\psi < 0\} \\ c_* & \text{in } Q_c = \{\psi > 0\} \end{cases}. \quad (16)$$

Next, define the Stefan condition. Suppose that g is continuous and diffuse where $\pm \psi > 0$ if and only if $\pm g > 0$; it holds that:

$$\theta = g, \quad \nu = -[\nabla \theta]_{-}^{+}. \quad (17)$$

Therefore:

$$\begin{aligned} \gamma_n^{-1} \psi_t &= -\gamma_n^{-1} (\nu \cdot \nu) \frac{\partial \psi}{\partial \nu} \\ &= \left(\frac{\partial c_*}{\partial \nu} - \frac{\partial \sigma}{\partial \nu} \right) \frac{\partial \psi}{\partial \nu} \\ &= \left[\frac{\partial \theta}{\partial \nu} \right]_{-}^{+} \frac{\partial \psi}{\partial \nu} \end{aligned} \quad (18)$$

where $\theta = g = c_*$ on Γ .

2.2. Numerical Scheme

2.2.1. Weak Form Derivation

In this subsection, we will solve the modelling of invadopodia formation and migration of a single cell. Based on the phase-transition formulation, a mathematical model was developed to address these problems. According to [12,23,24], the problems are characterised as time-dependent differential equations with boundary conditions at an unknown interface concerning which it has to be part of the solution. Let $\Omega \subset \mathbb{R}^N$, $N = 1, 2, \dots$ be a smooth bounded domain and:

$$\begin{aligned} H(u)_t &= \Delta u & \text{in } Q = \Omega \times (0, T) \\ u|_{\partial \Omega} &= 0, & u|_{t=0} = u_0(x) \end{aligned} \quad (19)$$

where $u = u(x, t)$ represents the temperature distribution and H represents the enthalpy function.

Let $\varphi \in H^1(\Omega)$ be trial function:

$$-\int_{\Omega} \left(\frac{\partial}{\partial x} \frac{\partial u}{\partial x} + \frac{\partial}{\partial y} \frac{\partial u}{\partial y} \right) \varphi - \int_{\Omega} H(u)_t \varphi = 0.$$

Using integration by parts:

$$-\int_{\Omega} \frac{\partial}{\partial x} \left(\varphi \frac{\partial u}{\partial x} \right) + \frac{\partial}{\partial y} \left(\varphi \frac{\partial u}{\partial y} \right) - \left(\frac{\partial u}{\partial x} \frac{\partial \varphi}{\partial x} + \frac{\partial u}{\partial y} \frac{\partial \varphi}{\partial y} \right) - H(u)_t \varphi \, d\Omega = 0$$

Hence:

$$\int_{\Omega} (\nabla u \cdot \nabla \varphi) - H(u)_t \varphi \, d\Omega - \int_{\partial\Omega} (\nabla u) \cdot \varphi \nu \, d\Omega. \quad (20)$$

2.2.2. Stefan Problems—Phase-Transition Formulation

Generally, Stefan problems is the emergence of smooth boundaries or interfaces between different phases of a substance that result from a phase transformation [12,25]. Therefore, for this research, Stefan problems are involved in the deformation of the cancer cell membrane or interfaces between inner and outer cell domains that arise from the molecular interactions to produce protrusions called invadopodia. Defining the temperature field is represented by Q and its evolution is given by:

$$\begin{aligned} H(u)_t &= \Delta u && \text{in } Q \setminus \Gamma \\ [u]_{-}^{+} &= 0, && [H(u)]_{-}^{+} = \ell \quad \text{on } \Gamma \end{aligned} \quad (21)$$

where $\Gamma = \{\Phi = 0\}$. On jump condition Γ , it is taken between the inner and outer cell or otherwise the following holds:

$$\ell v = - \left[k_{+} \frac{\partial u_{+}}{\partial \nu} - k_{-} \frac{\partial u_{-}}{\partial \nu} \right] \quad (22)$$

where ℓ is the heat transfer of the cell, $k_{\pm}$ are the constant for thermal diffusivities on Ω and ν is the outward normal vector. Setting $\ell \neq 1$, the problem is then simplified into finding $u(x, t)$ as well as Γ_t , such that:

$$v = -\frac{1}{\ell} \left[\frac{\partial u}{\partial \nu} \right]_{-}^{+}, \quad x \in \Gamma. \quad (23)$$

The outward normal vector, ν , is defined by:

$$\nu = \nabla \Phi / |\nabla \Phi| \quad (24)$$

and the curvature term, κ , by:

$$\kappa = \nabla \nu = \nabla \cdot \left(\frac{\nabla \Phi}{|\nabla \Phi|} \right). \quad (25)$$

From (24) and (25), expression v is rewritten as:

$$v = -[\nabla u] \cdot \nu = -[\nabla u] \cdot \left(\frac{\nabla \Phi}{|\nabla \Phi|} \right) \quad (26)$$

where $|\nabla u|$ is the jump taken between two regions—inner and outer cell or otherwise. As a result from (5), we obtained:

$$\Phi_t + v \cdot \nabla \Phi = 0, \quad v = (v \cdot \nu) \nu \quad (27)$$

where $v \cdot \nu$ is the Stefan condition.

During the process of physical or chemical transformations, Liouville's Theorem of the first volume of enthalpy H between two regions where it must be equal guarantees the total mass of conservation, which is in line with [26].

Based on Theorem 1 (Liouville's Theorem) from [12], the volume of ligands that diffuse outside the cell which then bind to the cell membrane are equal to the volume of signals generated inside the cell to produce invadopodia during the molecular interactions of cell membrane deformation.

2.2.3. Cell Deformation: Free Boundary Conversion

Based on degenerate parabolic equations, we describe the boundary of the cell as free boundary. See details on this in [12]. A cell's phase interfaces may experience sudden shifts during the phase-transition process that happen between the outer and inner cell, which can result in singularities and instabilities. During this process, invadopodia will pierce and move through the complex-appearing collagen walls.

The simulation procedure is carried out in discrete time steps as the phase-transition formulation is built on a time-dependent scheme. Phase transition begins when invadopodia start to break through a cell, a process known as invasion that is easily observed in 2D rather than 3D. Let:

$$\begin{aligned} H_g(\theta)_t &= \Delta\theta & \text{in } Q = \Omega \times (0, T) \\ \frac{\partial\theta}{\partial\nu}\Big|_{\partial\Omega} &= 0, \\ \theta|_{t=0} &= \theta_0(x) \geq 0 \end{aligned} \quad (28)$$

where

$$H_g(\theta) = \begin{cases} \alpha_+\theta + \ell & \text{in } \{\theta > g\} \\ \alpha_-\theta - \ell & \text{in } \{\theta < g\} \end{cases}.$$

We discretise the problem based on Euler time discretisation such that:

$$\begin{aligned} \frac{\theta^{n+1}}{\delta t} - \nabla \cdot (\kappa \nabla \theta^{n+1}) &= f^{n+1} + \frac{\theta^n}{\delta t}, & \text{in } Q = \Omega \times (0, T) \\ \frac{\partial\theta^{n+1}}{\partial\nu} &= 0, & \text{on } \partial\Omega_t \\ \theta^{n+1}|_{t=0} &= \theta_0(x) \geq 0. \end{aligned} \quad (29)$$

2.2.4. Phase-Transition Formulation

For this subsection, the density of signals, ligands and plasma membrane are time-dependent. However, only the plasma membrane is treated as free boundary. From (28), we define enthalpy, $v = H_g(\theta)$ by:

$$f(v) = \begin{cases} \alpha_-^{-1}(v + \ell), & v < \ell \\ 0, & -\ell \leq v \leq \ell \\ \alpha_+^{-1}(v - \ell), & \ell < v \end{cases} \quad (30)$$

if and only if $\theta = f_g(v) + \Delta g$. Based on Theorem 2 in [12]:

$$\begin{aligned} v_t &= \Delta f(v) + \Delta g & \text{in } Q \\ \frac{\partial}{\partial\nu} f(v)\Big|_{\partial\Omega} &= 0 & \frac{\partial g}{\partial\nu}\Big|_{\partial\Omega} = 0 \\ v|_{t=0} &= v_0(x) \geq 0. \end{aligned} \quad (31)$$

Likewise as in Subsection 2.2.3, problem (31) is discretised into:

$$\begin{aligned}
\frac{v^{n+1}}{\delta t} - \nabla \cdot (\kappa \nabla f^{n+1}) - \nabla \cdot (\kappa \nabla g^{n+1}) &= h^{n+1} + \frac{v^n}{\delta t}, & \text{in } Q = \Omega \times (0, T) \\
\frac{\partial f^{n+1}}{\partial \nu} &= 0, & \frac{\partial g^{n+1}}{\partial \nu} &= 0 & \text{on } \partial \Omega_t \\
v^{n+1} \Big|_{t=0} &= v_0(x) \geq 0.
\end{aligned} \tag{32}$$

3. Results

To verify the methods, we begin our numerical simulations. We divide our results into two parts: level set method and enthalpy method (phase-transition formulation), where the results obtained will be discussed in Section 4. The PDEs are solved numerically using FreeFem++ (v 4.10) [27] using a P1 finite element method (FEM) and meshes were generated using Gmsh (v 4.9.3) [28].

3.1. Level Set Method

When dealing with moving boundaries, numerical simulations for the free boundary problem of this model encountered significant challenges. Therefore, the level set method is carried out for a single cell model, while the level set approach is used to represent the plasma membrane, in order to overcome the difficulty in dealing with moving boundaries. Let $\psi(\cdot, t) = \psi$ be a smooth level set function, as you may recall from Section 2.

Consider that at the start of the computation there is no signal density. ECM will degrade, create diffused ligands outside of the cell, bind to receptors and generate an internal signal as a result. Inside the cell, signal densities spread and activate actin polymerisation. We defined the boundary data as follows based on (14) and (15):

1. Test 1: $\sigma = c_* = g = 0.01(2 + \sin(2\pi(x + y)^2) \cos(\pi(x + 0.5)))$ and level set solution as $\psi = 0.05 \times \sinh(((x - y)^{1/2}) - R)/(\epsilon h)^2$ where $\epsilon = 5$ and $h = 0.2$.
2. Test 2: Modifications of equations from Test I. $\sigma = c_* = g = 0.01(2 + \sin(4\pi(x + y)^2) \cos(\pi((2x) + 0.5)))$ and level set solution as $\psi = 0.05 \times \tanh(((x - y)^{1/2}) - R)/\epsilon h)^2$ where $\epsilon = 5$ and $h = 0.2$.

Figures 6 and 7 illustrate the formation of invadopodia and protrusion at the cell's boundary. Protrusion developed as a result of membrane displacement brought on by the presence of a signal gradient that was sparked by interactions between the membrane and the surrounding ECM.

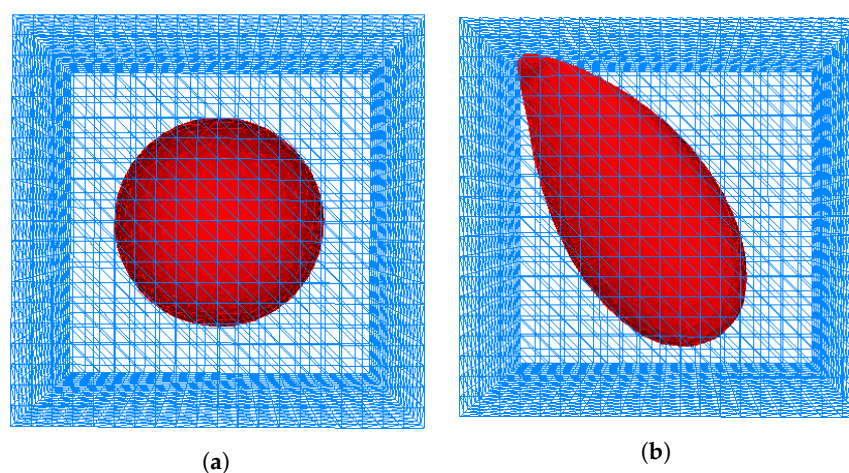
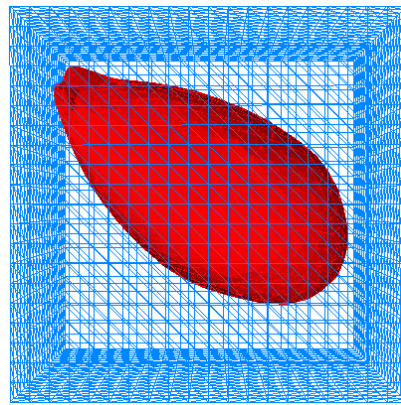
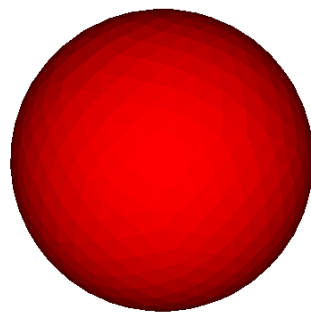


Figure 6. Cont.

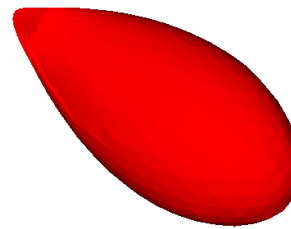


(c)

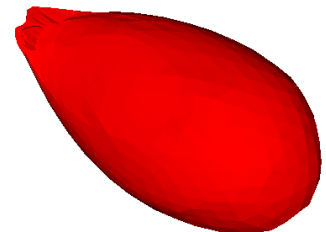
Figure 6. Simulation of invadopodia formation with outer domain (box). (a) Initial cell shape. (b) Invadopodia formed based on Test 1. (c) Invadopodia formed based on Test 2.



(a)



(b)

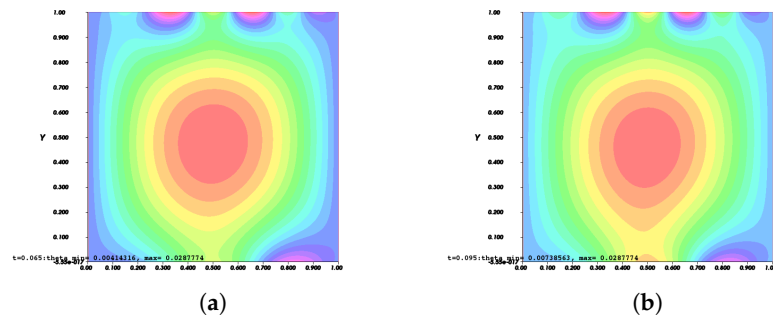


(c)

Figure 7. Simulation of invadopodia formation without outer domain (box). (a) Initial cell shape. (b) Invadopodia formed based on Test 1. (c) Invadopodia formed based on Test 2.

3.2. Enthalpy Method

Phase transition begins when invadopodia start to break through a cell, a process known as invasion. As described previously, the plasma membrane is regarded as a free boundary and is time-dependent in this problem, although the density of signals and ligands does not change over time. Figures 8 and 9 show the invasion of invadopodia and cell migration from Tests 1 and 2 based on (28).



(a)

(b)

Figure 8. Cont.

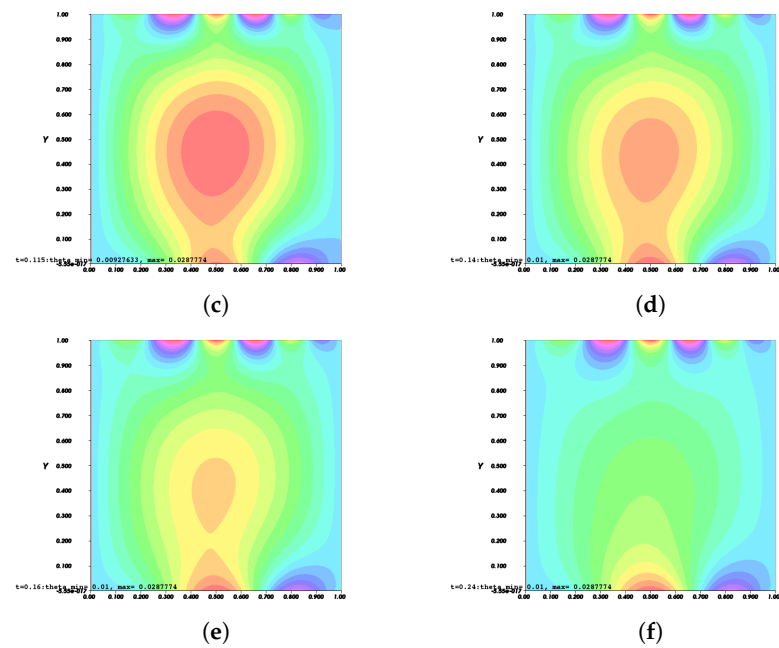


Figure 8. Invasion of invadopodia and cell migration from Test 1 based on (28).

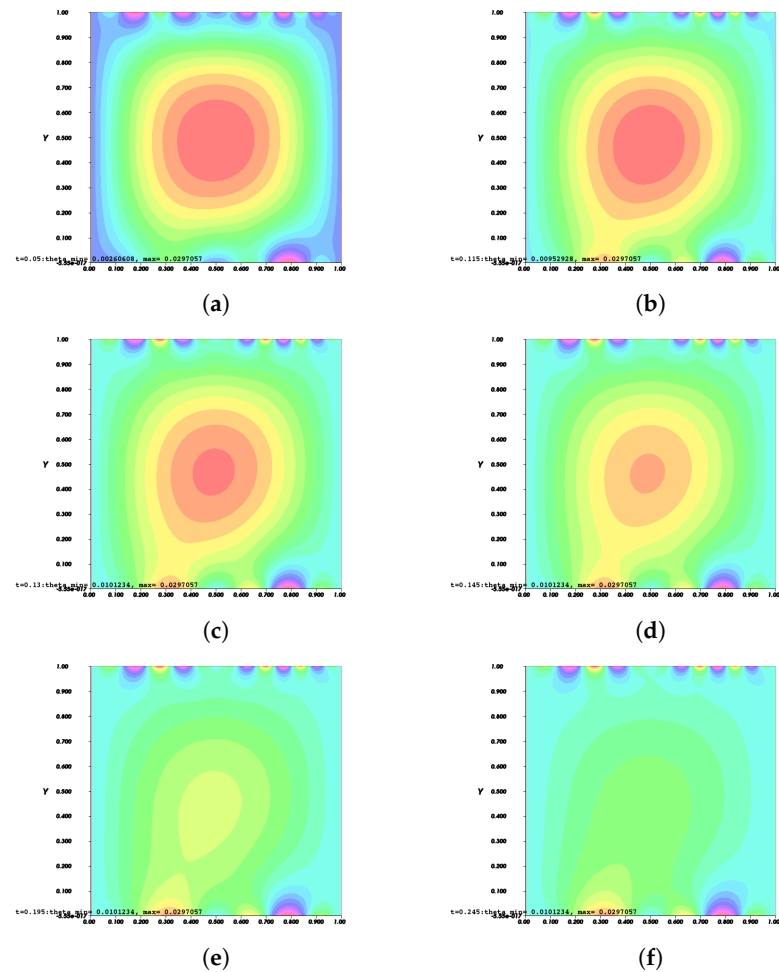


Figure 9. Invasion of invadopodia and cell migration from Test 2 based on (28).

On the other hand, Figures 10 and 11 show the invasion of invadopodia and cell migration from Tests 1 and 2 based on (31).

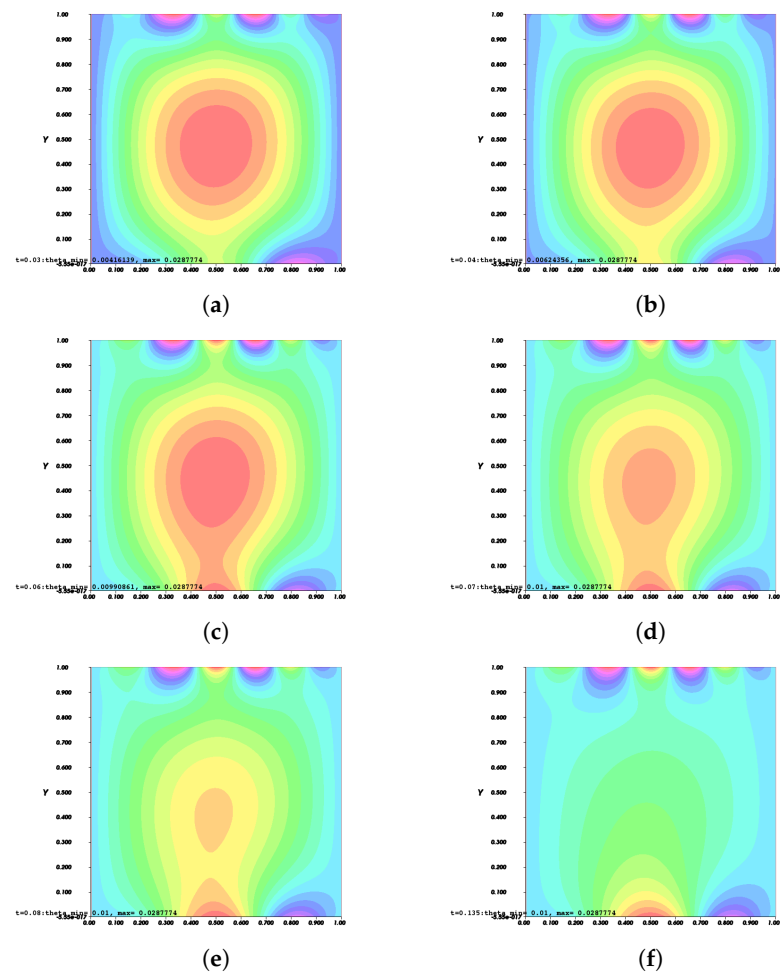


Figure 10. Invasion of invadopodia and cell migration from Test 1 based on (31).

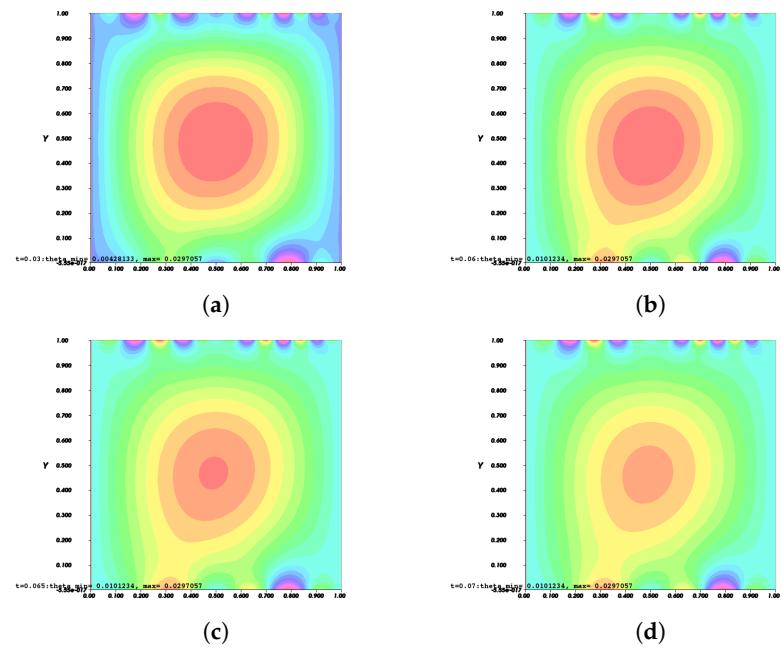


Figure 11. Cont.

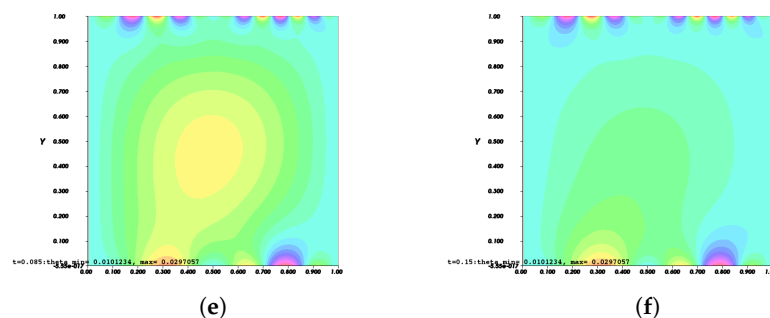


Figure 11. Invasion of invadopodia and cell migration from Test 2 based on (31).

4. Discussion

Level set serves the purpose of dealing with cell membranes in order to denote the membrane interfaces when the problem is investigated as a free boundary because they are simple to execute and computationally inexpensive. The discontinuity in the level set function derivatives between two outer and inner cell interfaces that are near to one another can affect the estimation of velocity and curvature. As a result, the level set approach makes it straightforward to determine an interface's curvature and velocity in order to locate the plasma membrane interface. Therefore, we define the velocity extension to the entire domain in order to prevent singularities and instabilities as described in Section 3.

In order to enhance the simulation of invadopodia formation and also to surmount singularities and instabilities that occurred in [12] as discussed in Section 1, 3D simulations were used, resulting in Figures 6 and 7. The initial shape of a cell is depicted in Figures 6a and 7a. Figures 6a and 7b illustrate the formation of invadopodia and protrusions at the cell's boundary using Test 1 while Figures 6c and 7c used Test 2. Protrusion is caused by the membrane's displacement due to the presence of a signal gradient induced by contact of the membrane with the surrounding ECM.

As previously discussed, the authors of [12] demonstrate the existence of singularities and instabilities. On the basis of the results shown in Figures 6 and 7, both instabilities and singularities have been prevented from occurring in the simulation. This could be due to the fact that Tests 1 and 2 used a different simulation technique and set of data. The graphs for level set function, ψ for Tests 1 and 2 in Figure 12 demonstrate that the values of the level set during the deformation of the cell in both tests fluctuated.

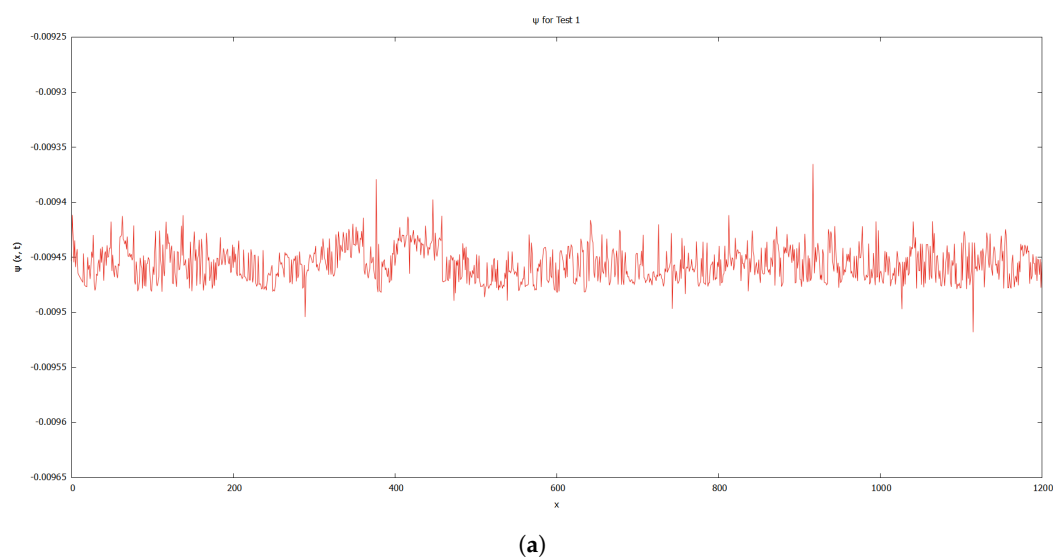


Figure 12. Cont.

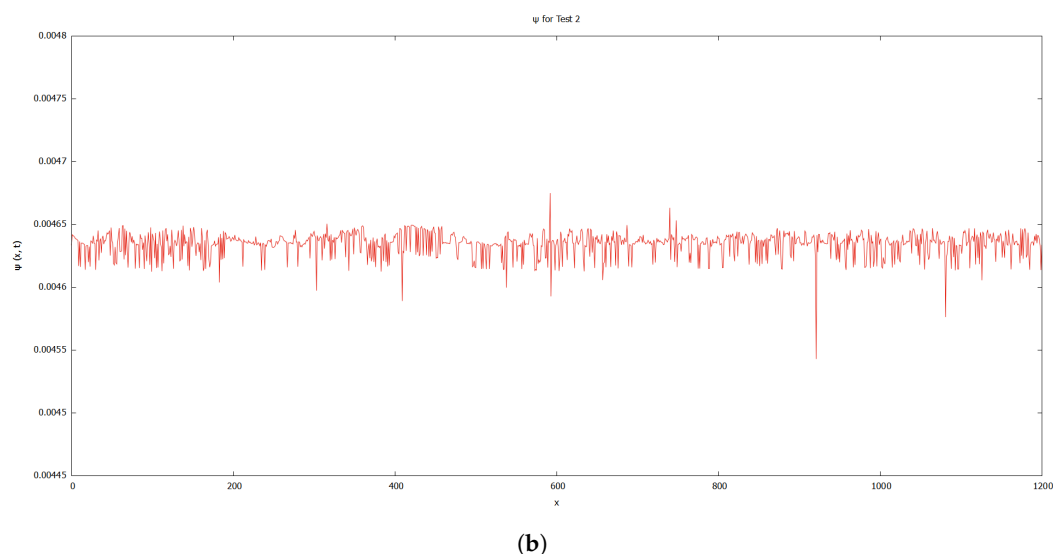


Figure 12. Graphs for level set function, ψ . (a) Test 1. (b) Test 2.

ECM is broken down by MT1-MMP, which also generates ligands that diffuse and attach to membrane receptors. The signal is then generated inside the cell which will spread and cause the actin molecule to polymerise. The morphology of the plasma membrane was harmed by this process. During this process, the plasma membrane begins to undergo phase transition, which can result in singularities and instabilities. Invadopodia begin to invade through the intricately patterned collagen walls in the cell's interfaces between outer and inner cells and migration occurs.

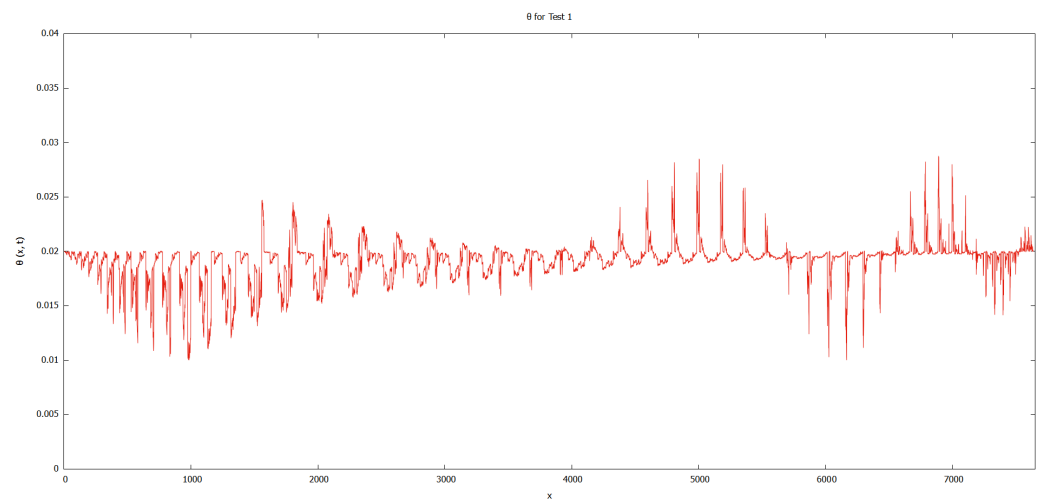
The phase field variable in the enthalpy method (phase-transition formulation) smoothly varies between two phases over a small yet numerically resolvable thickness on the diffused interface area. As a result, it offers a hazy depiction of the thinned-out membrane interface between the outer and inner cell.

Figures 8–11 depict the process of cell deformation that occurs when a normal cell undergoes mutation before it develops into a cancer cell. Invasion begins in a cancer cell after invadopodia protrude and begin to separate from the primary cell. The invasion process can be seen starting with Figure 8a and the movement of the invasive cancer cell is depicted in Figure 8b–e. These can also be seen in Figures 9a–e, Figure 10a–e, and Figure 11a–e. The interactions between the cell membrane and the surrounding ECM causing the cell to change and move, which in turn promotes the existence of ligands and activates the signal gradient. As time passes, the cell disperses to nearby regions as illustrated in Figures 8f–11f which then grows in various parts of the body.

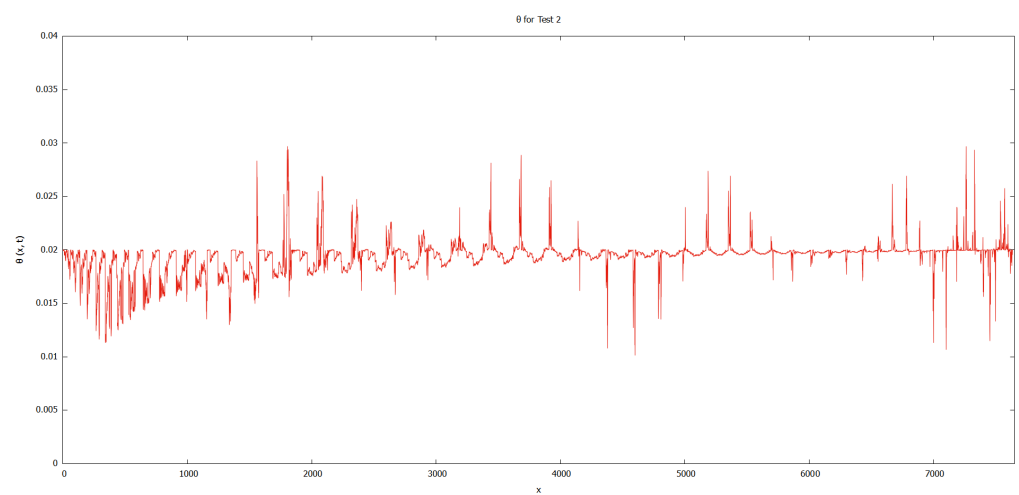
The results of Figures 8 and 9 indicate that the phase transition of the cell membrane based on (31) is significantly faster than (28). Moreover, (31) describes why the phase-transition problem depicted in Figures 10 and 11 occurred more rapidly than in Figures 8 and 9. In (31), it is presumed that the concentration of MT1-MMP, f , embedded in the cell membrane degrades the ECM by contact and that the signal density equals the function $g(x, t)$, which also represents MT1-MMP at any time, t , at the interface based on (16), while (28) only evaluates the signal or ligand density.

When compared to [12], our findings demonstrate that phase transition occurred more slowly. The simulation results of Figures 8–11 show that as protrusion took place, the cell began to migrate through the ruptured membrane and as protrusion velocity decreased, it showed that the formation of invadopodia had stabilised. As the amount of time increases and the distance from the primary cell increases, the position of the phase-transition interface diminishes, as well as the density of the signal. Figure 13 depicts the graphs for density of signals and ligands, θ , during the cancer cell's phase transition for Tests 1 and 2, where the values fluctuated in both tests. Figure 14 displays the graphs

also for density of signals and ligands during phase transition of cancer cell, θ , but with MT1-MMP enzyme flux, g , also for Tests 1 and 2 which likewise fluctuate.

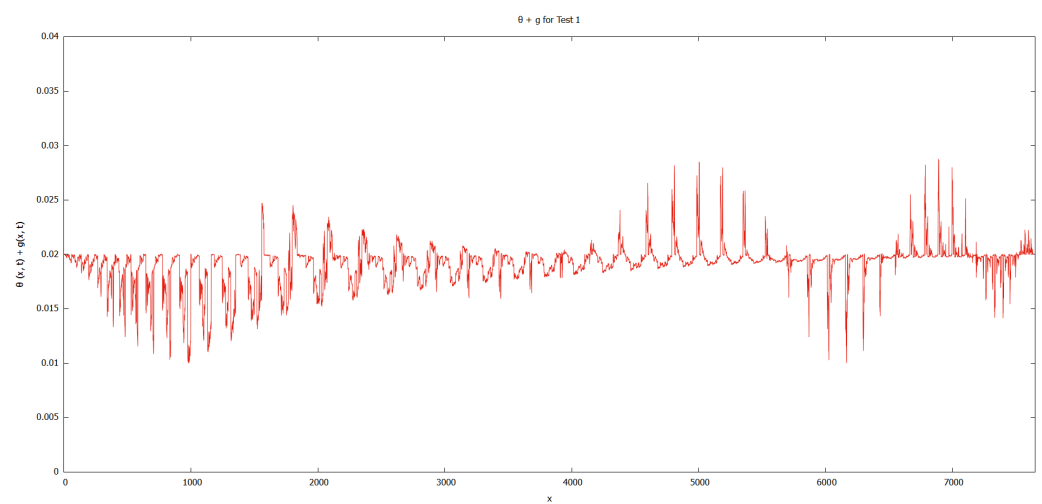


(a)



(b)

Figure 13. Graphs for density of signals and ligands, θ . (a) Test 1. (b) Test 2.



(a)

Figure 14. *Cont.*

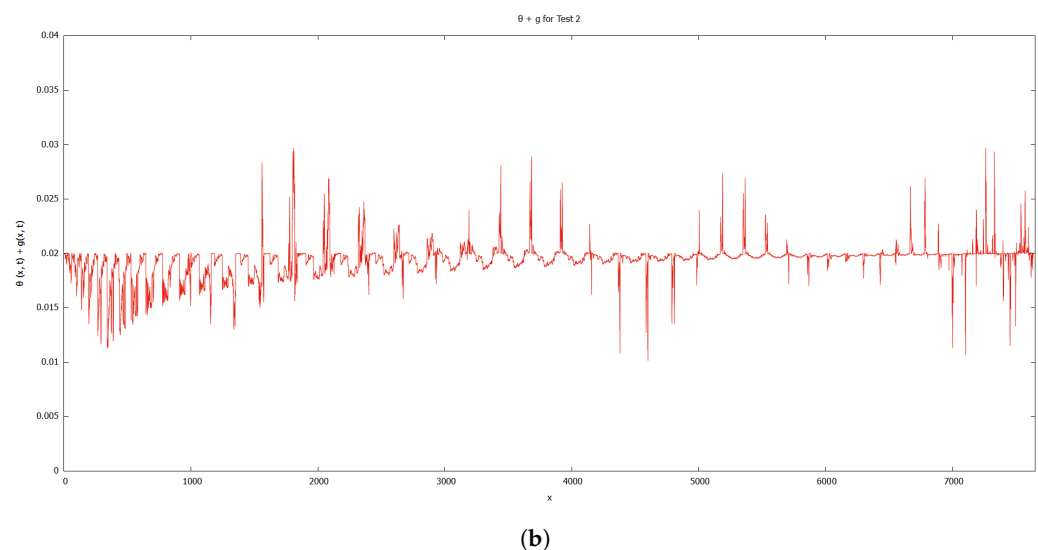


Figure 14. Graphs for density of signals and ligands with MT1-MMP enzyme flux, $\theta + g$. (a) Test 1. (b) Test 2.

5. Conclusions

We created a numerical method, based on the level set technique and the phase-transition formulation (enthalpy method), to address the Stefan problem of invadopodia formation. FreeFem++ and Gmsh were used to carry out all described methods. Phase-transition problems in cancer cells were created using phase-transition formulation and level set was used to determine the location of the membrane using the zero level function of ψ . The enthalpy technique was shown to be effective and precise in solving this kind of issue. Both approaches were used to mathematically resolve the deformation and invasion of cancer cells, where protrusions were caused by the presence of invadopodia and invasion was caused when the cancer cell began to disseminate to another area of the body. In addition to this, we were able to enhance the simulation outcome from [12] to a more realistic complexity for the solution to be accurate by using a different simulation technique and domain as well as a different data set for Tests 1 and 2. Singularities and instabilities were eliminated. The results that were achieved in this paper have the potential to be helpful for novel approaches or extended to other methods in the development of a more accurate numerical simulation.

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Abbreviations

The following abbreviations are used in this manuscript:

BM	basement membrane
ECM	extracellular matrix
PDE	partial differential equation
ODE	ordinary differential equation
2D	two-dimensional
3D	three-dimensional

TLA	Three letter acronym
LD	Linear dichroism
FDM	finite difference method
FEM	finite element method
MMP	matrix metalloproteinases
MT1-MMP	type 1-matrix metalloproteinases

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