

**A MATHEMATICAL STUDY ON THE IMPACTS OF OXYGEN
HETEROGENEITY ON TUMOR GROWTH AND EMT
DYNAMICS**

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April, 2025**



**A MATHEMATICAL STUDY ON THE IMPACTS OF OXYGEN
HETEROGENEITY ON TUMOR GROWTH AND EMT
DYNAMICS**

*A thesis submitted
in partial fulfilment of the requirements
for the degree of*

DOCTOR OF PHILOSOPHY

by

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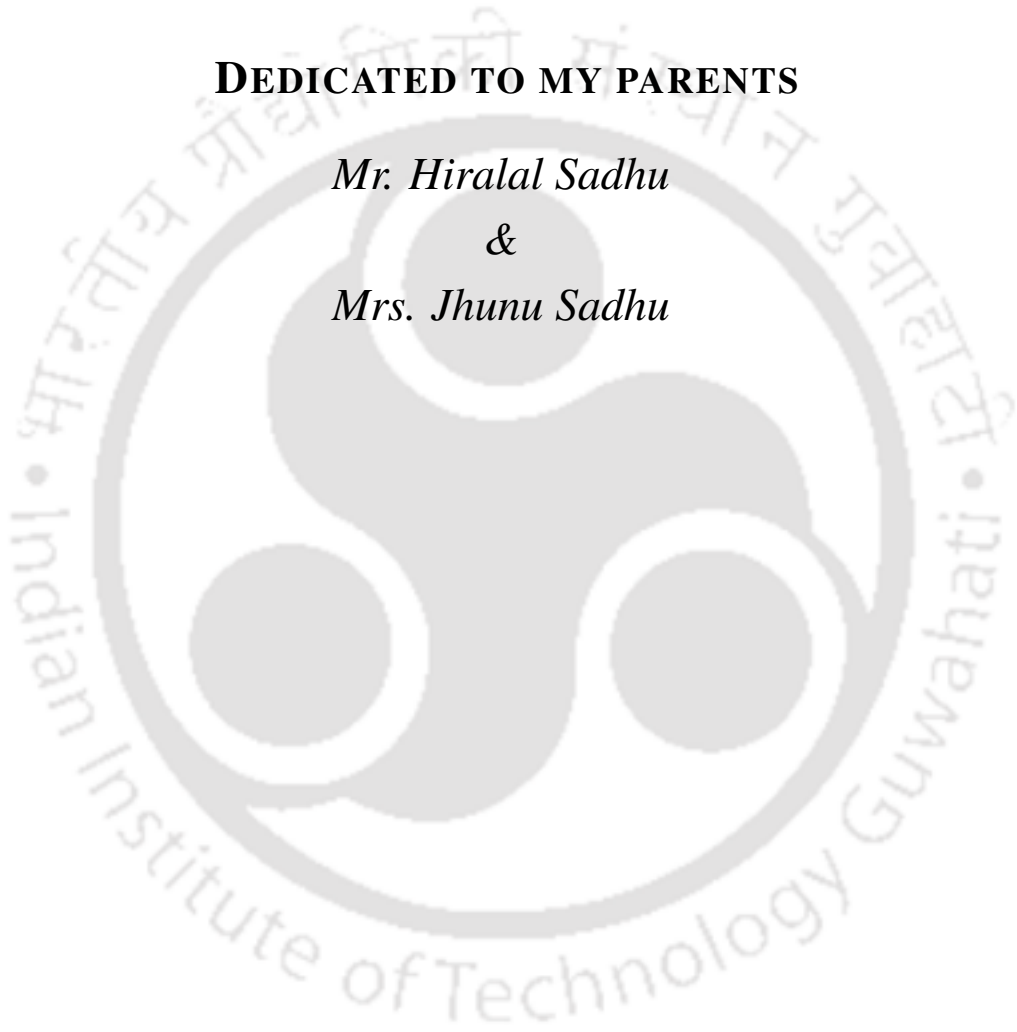


DEDICATED TO MY PARENTS

Mr. Hiralal Sadhu

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Mrs. Jhunu Sadhu





Declaration

I hereby declare that the work presented in this thesis, titled “**A Mathematical Study on the Impacts of Oxygen Heterogeneity on Tumor Growth and EMT Dynamics,**” is accomplished under the supervision of **Dr. D. C. Dalal**, Professor, Department of Mathematics, Indian Institute of Technology Guwahati for the award of the degree of Doctor of Philosophy, and that it has not been submitted for a degree elsewhere.

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Certificate

It is to certify that the work contained in this thesis entitled “**A Mathematical Study on the Impacts of Oxygen Heterogeneity on Tumor Growth and EMT Dynamics**” has been carried out by **Mr. Gopinath Sadhu**, a student in the Department of Mathematics, Indian Institute of Technology Guwahati, under my supervision for the award of the degree of Doctor of Philosophy, and that this work has not been submitted for a degree elsewhere.

April, 2025

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ABSTRACT

Oxygen heterogeneity in the tumor microenvironment refers to the non-uniform distribution of oxygen levels within a tumor due to disorganized blood vessels and variable blood flow. Oxygen has a significant role in tumor necrosis and the evolution of tumor growth. Also, experimental literature suggests that oxygenation stress can alter tumor cells' energy production mechanism. Hypoxic tumor cells adopt anaerobic glycolysis in place of aerobic metabolism of glucose. In anaerobic glycolysis, lactate, H^+ ions, etc., are produced as metabolic wastes. These metabolic wastes play a crucial role in tumor progression. The role of oxygen heterogeneity is not limited to metabolic switch and necrosis; it has the potential to initiate the invasion process. Experimental evidence suggests that oxygen stress helps transition the epithelial phenotype to the mesenchymal phenotype. Experimental studies on animal models are both expensive and time-consuming. A mathematical model may be a good substitution for visualizing these scenarios. In this thesis, a continuum mathematical modeling framework has been employed to illustrate the underlying biological phenomena. The models consist of a system of coupled partial and ordinary differential equations with appropriate boundary and initial conditions. The governing equations are solved using finite difference methods such as forward time-centered space (FTCS) schemes.

The first aim of the present dissertation is to determine how unequal oxygen concentrations at the boundaries of a tumor affect the evolution of the necrotic core in solid tumors using the multiphase framework. We have a departure from the assumption of symmetric growth, as asymmetry is a characteristic feature of tumors in vivo. To solve this model, we have adopted the "Semi-Implicit Method for Pressure Linked Equations" to address the coupled pressure-velocity equations. A unique feature of this proposed model is its ability to represent both in

vivo and in vitro growth environments.

The second goal of this work is to elucidate the oxygen and lactate interplay for both in vivo and in vitro tumors through a mathematical model. The proposed model is solved analytically with the appropriate choice of the initial and boundary conditions. In order to establish the potency of the model, the simulated results are compared with the existing experimental data. A quantitative analysis is also carried out on the lactate accumulation and necrotic core length in response to unequal oxygen concentration at the tumor boundaries. Moreover, the model offers to measure the length of the necrotic core in the post-necrotic situation of in vivo and in vitro tumors.

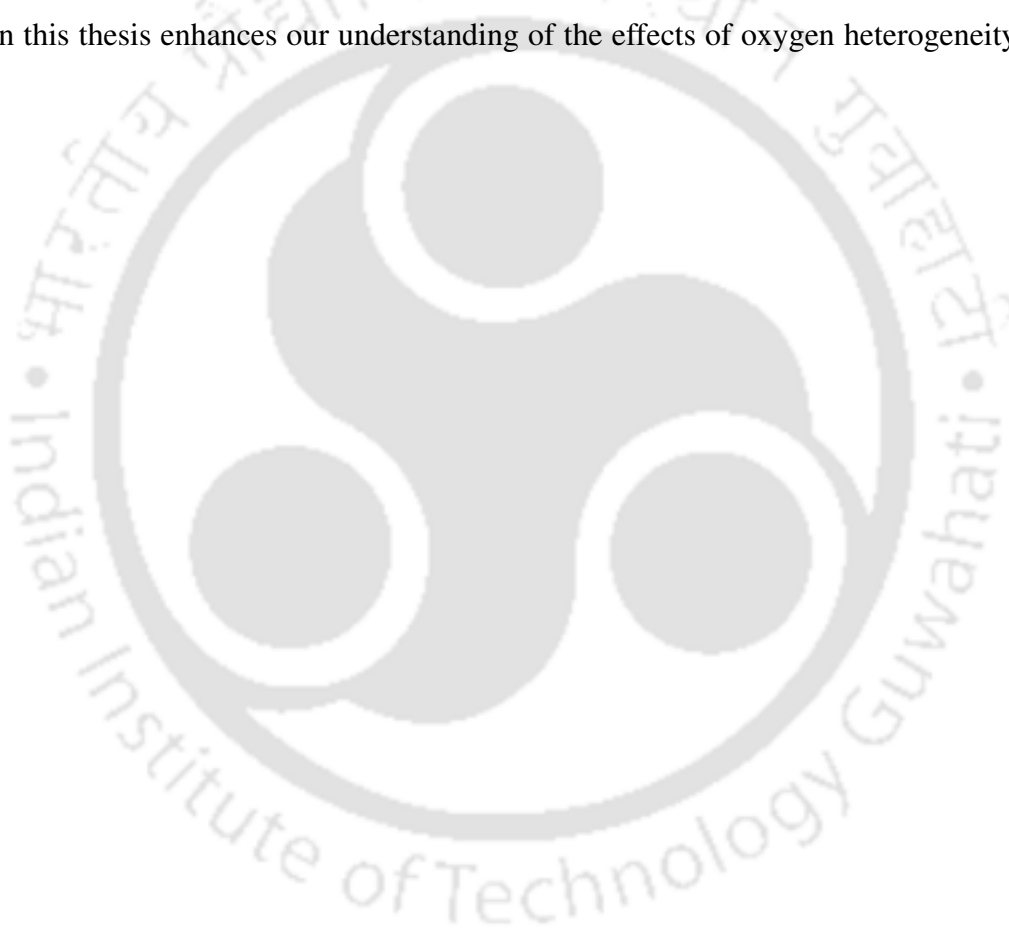
The third target of this dissertation is concentrated on the formulation of a mathematical model elucidating the tumor growth pattern in response to the non-linear interaction between oxygen and lactate in an oxygen fluctuation environment in a two-dimensional framework. This model is a generalized version of the second work. The spatio-temporal dynamics of tumor cell density are incorporated in the current study. Also, in this work, the growth patterns for different types of initial shapes of tumors are investigated along-with the impacts of tumor initial shape on lactate status in the tumor microenvironment. In addition, a series of numerical simulations are conducted to explore the influences of oxygen fluctuation in the tumor region on the growth patterns of the tumor.

The goal, in the next chapter, focuses on how exosomes contribute to the progression of tumor growth in an acidic microenvironment. Tumor cells secrete exosomes to communicate with both local environments and distant body parts. Experimental investigation indicates that exosome secretion is enhanced in the tumor's acidic microenvironment, and it also supports various activities in cancer progression, such as cell proliferation, angiogenesis, EMT, and escaping from immune destruction. In this study, it is considered that lactate plays a major role in the acidification of tumor microenvironment and that exosomes contribute to tumor cell proliferation. A system of coupled PDEs is used to model the underlying phenomena. A quantitative analysis is conducted through numerical simulations to illustrate how the density of tumor cells changes over time under different situations.

The final aim of this dissertation is to study the impacts of oxygen heterogeneity on epithelial-mesenchymal transition (EMT). EMT is a process by which epithelial cells acquire mesenchymal traits, marking the initial step in the metastasis cascade. In this study, a comprehensive mathematical model is used to elucidate the oxygen dependency of EMT and reverse EMT,

i.e., MET processes in the tumors. Mesenchymal cells achieve motile properties. In this work, the influence of mesenchymal motility on the EMT process is investigated. Cyclic hypoxia is a unique feature in an in vivo tumor. This biological character can be mimicked by controlling the oxygen supply in the tumor growth medium in an in vitro study. This feature is also captured through the proposed mathematical model.

In conclusion, this thesis provides a comprehensive understanding of how varying oxygen levels affect tumor dynamics. It explains how oxygen heterogeneity influences tumor growth, promotes lactate accumulation, and facilitates exosome-mediated progression. Additionally, it highlights the role of oxygen heterogeneity in driving EMT mechanisms. The research presented in this thesis enhances our understanding of the effects of oxygen heterogeneity inside tumors.





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Superscripts

$'$	Dimensional quantity
$\hat{\cdot}$	Characteristic variable

Notations

Ω	Computational domain
$\partial\Omega$	Computational domain boundary
∇	Gradient operator
Γ	Cell membrane (interface)
μ	Tumor cell phase viscosity
Δx	Spatial-step sizes
Δt	Time-step size
C	Oxygen concentration
C_N	Critical oxygen concentration for necrosis
C_∞	Normal physiologic oxygen concentration in the tissue
L	Lactate concentration
E	Exosome concentration
T	Tumor cellular density
m	Mesenchymal cell density
e	Epithelial cell density
n	Necrotic cell density
D_L	Lactate's diffusion coefficient
D_E	Exosome's diffusion coefficient

D_T	Tumor cell's diffusion coefficient
D_m	Mesenchymal cell's diffusion coefficient
i	Indices of the Cartesian spatial grid in x -direction
J	Index set
N	Grid points in the x -direction
$\mathbf{n}$	Outward unit normal vector to the domain
t	Time
$\mathbf{x}$	Spatial position vector (x, y)
x, y	Spatial coordinate variables

Units

μm	Micrometer
s	second
h	hour
nm	Nanometre
cm	centimetre
M	Molar

Abbreviations

1D	One-dimensional
2D	Two-dimensional
ODE	Ordinary differential equation
PDE	Partial differential equation
ABM	Agent-based modeling
CPM	Cellular pot model
FDM	Finite difference method
EMT	Epithelial-mesenchymal transition
MET	Mesenchymal-epithelial transition
DNA	Deoxyribonucleic acid
ATP	Adenosine triphosphate
FTCS	Forward time and central space
VEGF	Vascular endothelial growth factor
TIP	Tumour interstitial fluid pressure

Glossary

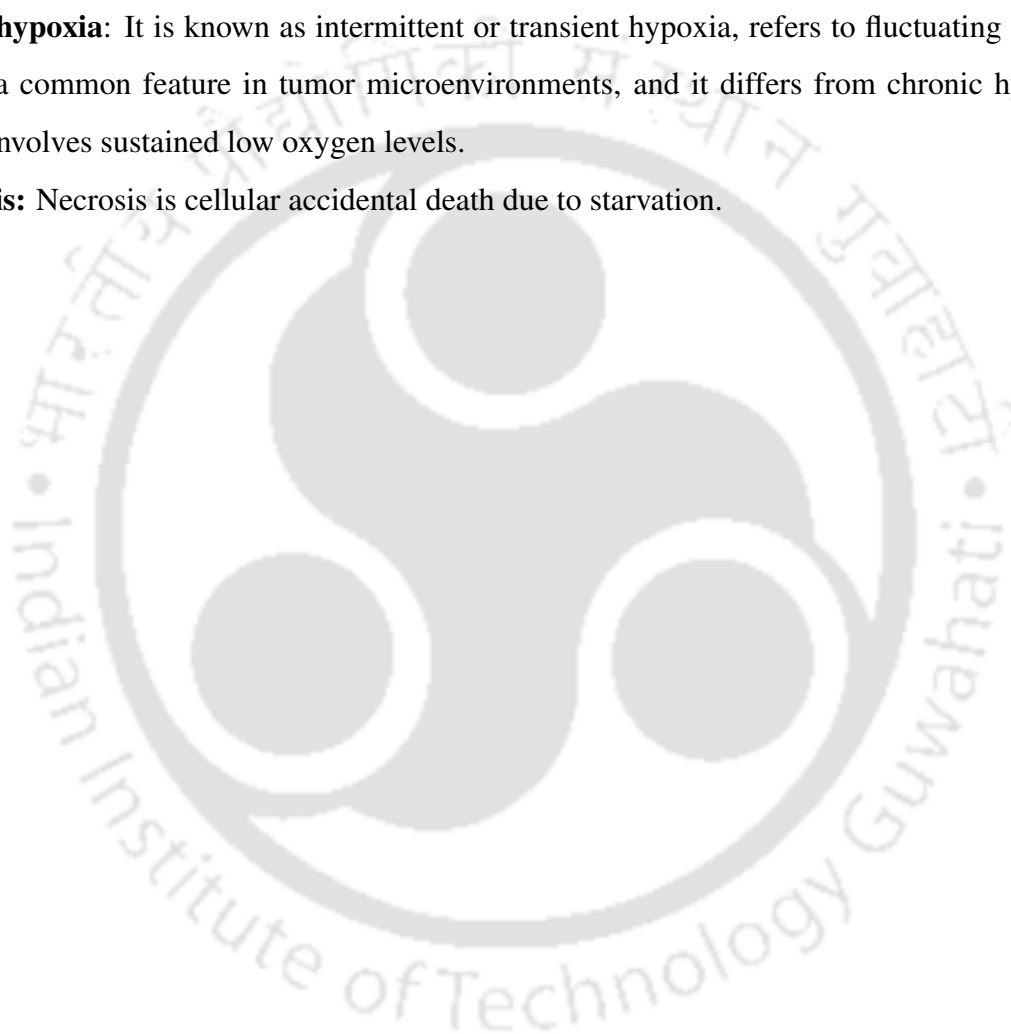
in vivo experiment: Experimental study is carried out in living organism.

in vitro experiment: A study conducts in the controlled environment, outside of a living organism.

Hypoxia: Hypoxia is a condition where the body or a specific region lacks sufficient oxygen at the tissue level, potentially leading to organ damage or death if left untreated.

Cyclic hypoxia: It is known as intermittent or transient hypoxia, refers to fluctuating oxygen levels, a common feature in tumor microenvironments, and it differs from chronic hypoxia, which involves sustained low oxygen levels.

Necrosis: Necrosis is cellular accidental death due to starvation.





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CHAPTER 1

INTRODUCTION

1.1 Motivation

According to the World Health Organization (W.H.O.), cancer is one of the leading causes of death worldwide, accounting for nearly 19.3 million new cases and 10 million deaths in 2020.

The most common types in 2020 (in terms of new cases of cancer) were:

- breast cancer (2.26 million cases);
- lung cancer (2.21 million cases);
- colon and rectum cancer (1.93 million cases);
- prostate cancer (1.41 million cases);
- skin cancer (1.20 million cases); and
- stomach cancer (1.09 million cases)

W.H.O. also estimated that by 2040, the number of cancer patients will be nearly 30.2 million. So, cancer is a burden to the society and is global threat to healthcare system. Cancer is a complex disease and not completely understood till date. A lot of resources is spent every year on cancer research, resulting in a small, yet sustained reduction in cancer death rates. However, there is widespread concern about the slow pace at which these discoveries in fundamental biology are translated into safe and effective clinical interventions. Hence cancer research attracts broad resources and scientists from various disciplines to make some impressive advances in

the process of understanding the disease. The role of mathematicians in “the war of cancer” is very important.

Biological phenomena of cancer progression are quite complex. It becomes a necessity to explain the concerned biological processes in terms of mathematics. Cancer research is one of the most challenging areas that has given rise to an enormous opportunity for new researchers to be a part of this global war. Biomedical research relies on the experimental models with all its strengths and limitations. Mathematical models can complement expensive, time-consuming experimental and clinical studies, but they also challenge current paradigms, redefine our understanding of the mechanisms driving tumorigenesis, and shape future research in cancer biology. Models describe a system through abstraction and mathematical formalism. They enable extrapolation beyond the originally analyzed situations, quantitative predictions, inference of mechanisms, validation of underlying biological hypotheses, and a quantitative description of relationships between different system components. This integration of mathematics and biology represents a powerful tool in advancing our understanding of cancer and developing more effective treatment strategies.

1.2 Biological background

The human life starts from an embryo (single-cell zygote) and becomes an adult human body (100 trillion cells) over the time. The proliferation and death of cells in our body are a continuous process, maintained by our regulatory system. The proliferation and death of cells should be in the homeostatic state for the maintenance of normal and healthy life. This mechanism is controlled by signals and cellular checkpoints, which determine whether cells will be divided, die, or differentiate. Cancer is the phenomenon that leads to the lack of this co-operation. This loss of co-operation is driven by several reasons. Deoxyribonucleic acid (DNA, genetic material of cell residing in the cell nucleus) alternation is one of them. When DNA is altered, the cell loses its normal proliferation functions. Cells give rise to clusters of cells as a result of uncontrolled, indefinite proliferation, leading to the development of a tumor. Tumors are generally two kinds: benign, a tumor that is not capable of indefinite growth and does not invade surrounding tissues and malignant tumor, a tumor that continues to grow and becomes progressively more invasive. The term cancer refers specifically to malignant tumors. Figure 1.1 describes the phenomena of malignant tumor. In addition to uncontrolled growths, malig-

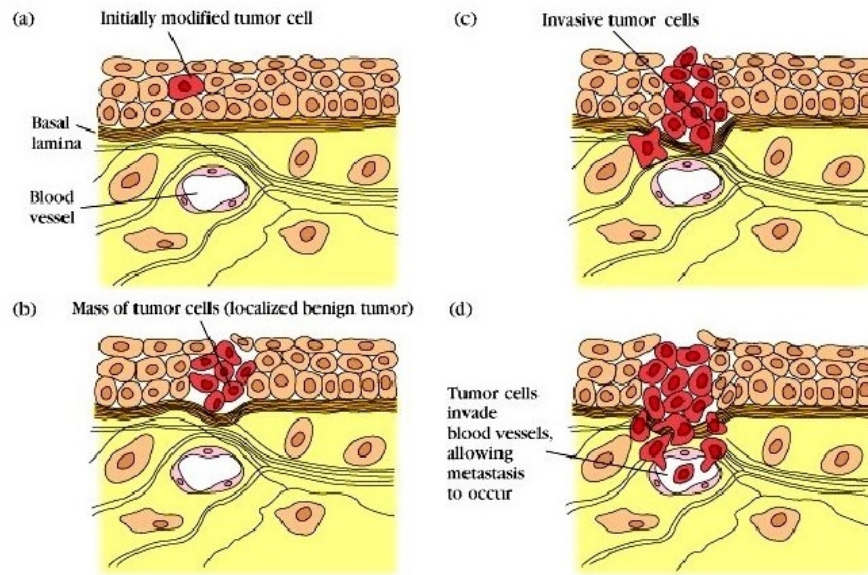


Figure 1.1: This illustrates the process of tumor growth and metastasis. (a) shows a tumor cell placed in tissue. (b) depicts the tumor cell proliferating, forming a cluster of cells, and increasing in size over time. (c) portrays the tumor beginning to spread into the surrounding healthy region, and (d) displays some cells detaching from the primary tumor and penetrating a blood vessel, facilitating the establishment of a new tumor at a secondary site.

nant tumors exhibit metastasis, whereby small clusters of cancerous cells dislodge from the original tumor, invade the blood or lymphatic vessels, and are carried to other distant area via this circulation, where they take shelter and continue to proliferate. In this way, a primary tumor at one site can give rise to a secondary tumor at another site. This process is termed as metastasis, and most deaths occur as a result of it. This metastasis process is initiated by epithelial-mesenchymal transition (EMT). Epithelial cells in tissue are attached through highly adhesive molecules, such as E-cadherin. During EMT, epithelial cells lose cell-cell adhesion and acquire mesenchymal traits. Mesenchymal cells take spindle shape from nice cobblestone-shaped epithelial cells. The change in shape helps the invasion process.

Oxygen is crucial for cellular respiration. Oxygenation pressure may alter many cellular behaviors in tumors. Also, it plays prominent roles in various events of cancer progression, such as metabolic switch, lactate accumulation, and invasion process. In an *in vitro* situation, oxygen is readily available at a constant level in the tumor growth medium, which does not reflect the actual dynamics of *in vivo* tumor. In an *in vivo* tumor, the oxygen levels may fluctuate spatio-temporally. As a result, the oxygen level in the tumor is sometimes above the hypoxic

level, and at subsequent times, it declines to a level below the hypoxic level. This phenomenon is termed as cyclic hypoxia. Many experimental investigations reveal that the duration of cyclic hypoxia varies significantly, ranging from mere seconds to several hours, depending on the specific type of tumor.

1.2.1 Classification of cancer

Cancer is classified according to the origin of the tumor. The main categories include:

- **Carcinoma:** cancer that begins epithelial cells, is the most common type of cancer (80-90%). The common carcinomas are kidney cancer, colon cancer, breast cancer, prostate and lung cancer.
- **Leukemias** is cancer of hematopoietic cells derived from the bone marrow that do not form solid tumors.
- **Lymphomas** is a cancer of the lymphatic system, including Hodgkin and non-Hodgkin lymphoma.
- **Melanomas** are cancers that arise in the cells that make pigments on skin.
- **Sarcomas** are derived from mesodermal connective tissues, such as bone, fat and cartilage. This kind of cancer is very rare.
- **Gliomas & other brain tumors** are cancers that arise in the brain or spinal cord.
- **Germ cell tumors** originate in reproductive cells (testicular or ovarian cancer).
- **Neuroendocrine tumors** arise from neuroendocrine cells, which produce hormones.

Proliferation capacity of tumor cells is much higher compared to that of healthy cells. To sustain this rapid growth, tumor cells require greater amounts of nutrients and oxygen. As a tumor increases in size, the cells in its central area often experience nutrient deprivation, leading to cell death in that region, which is known as the necrotic core. At the edge of tumor, oxygen and nutrients are readily available. Therefore, tumor cells proliferate in this region and build proliferating rim [49]. In between proliferating rim and necrotic core, a region is developed where oxygen is limited, tumor cells cannot proliferate, but they can still manage to survive. This particular region is commonly referred to as hypoxic area (Figure 1.2). In

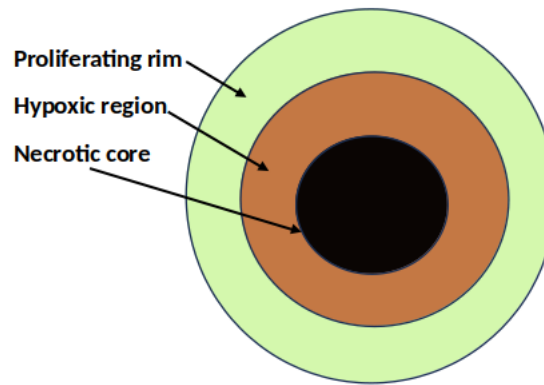


Figure 1.2: The structure of a tumor comprises a necrotic core at the center, a hypoxic layer surrounding it, and a proliferating rim on the outermost side.

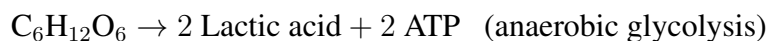
the early development of avascular tumor, cancer cells solely rely on the supplied oxygen and nutrients from the primary blood vessel. When the diffusional distance from the blood vessel to the tumor site increases, nutrient gradients develop [101]. It restricts the tumor growth process. To overcome such challenge, tumor cells secrete vascular endothelial growth factor (VEGF), which initiates angiogenesis [70]. In angiogenesis, VEGF stimulates the formation of a tumor-specific vascular network from the nearby existing blood vessel. The neovasculatures fulfill the need of oxygen and nutrients supply at the tumor site. As a result, tumor can grow larger and metastasizes [35].

Tumor cells need to produce energy quickly to support rapid proliferation. They generate energy through two pathways: aerobic and anaerobic glycolysis. In aerobic glycolysis, glucose is metabolized in the presence of oxygen. During this process, one unit of glucose combines with six units of oxygen to produce six units of water, six units of carbon dioxide, and thirty-eight units of adenosine triphosphate (ATP). These thirty-eight ATP units are readily available for cellular use. However, this process involves a series of chemical reactions. The chemical reaction equation for aerobic glycolysis is given as



In contrast, anaerobic glycolysis is a rapid process that facilitates quick energy availability for tumor cells. However, it generates metabolic waste, such as lactate, which acidifies the tumor microenvironment (TME). This metabolic process is particularly suitable for tumor cells in

hypoxic regions. The chemical reaction equation for anaerobic glycolysis is given as



Lactate is not merely a metabolic waste product; it is also consumed by tumor cells in normoxic regions and contributes to the invasion process. Tumor cells secrete various growth factors that promote aggressive tumor growth, with exosomes being one of them [59]. Exosomes facilitate communication not only between nearby tumor cells but also with distant regions of the body. They play a role in several cancer-related processes, including angiogenesis, immune regulation, drug resistance, invasion, and epithelial-mesenchymal transition (EMT) (Figure 1.3) [113].

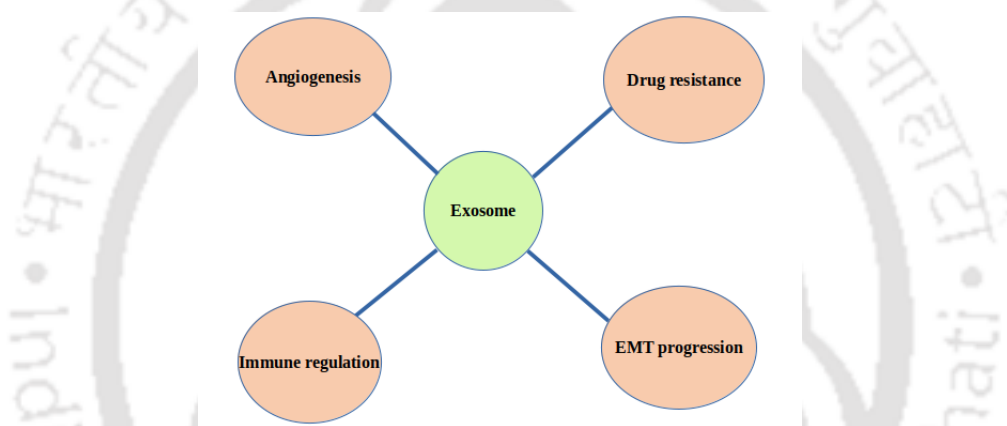


Figure 1.3: Exosome activities in various events of tumor biology.

A tumor is composed of many subpopulations, which can be categorized in various ways. Based on proliferation characteristics, they can be classified as proliferative, quiescent, or senescent. Tumor cells may acquire motile properties; however, not all cells within a tumor are capable of movement. Based on motility, tumor cells are categorized into two types: epithelial and mesenchymal. Mesenchymal cells are motile, whereas epithelial cells remain static. Interestingly, epithelial cells acquire mesenchymal phenotype through EMT. EMT and reverse EMT i.e., mesenchymal epithelial transition (MET) are contributing in many biological processes, such as embryonic development, tumor progression. Both EMT and MET are not binary processes. Epithelial cells first undergo a transformation into hybrid epithelial/mesenchymal types before fully acquiring mesenchymal traits. Similarly, during MET, mesenchymal cells first attain a hybrid phenotype before transitioning into the epithelial type (Figure 1.4). In epithelial tissue, epithelial cells are connected by highly adhesive molecules. Various biological factors

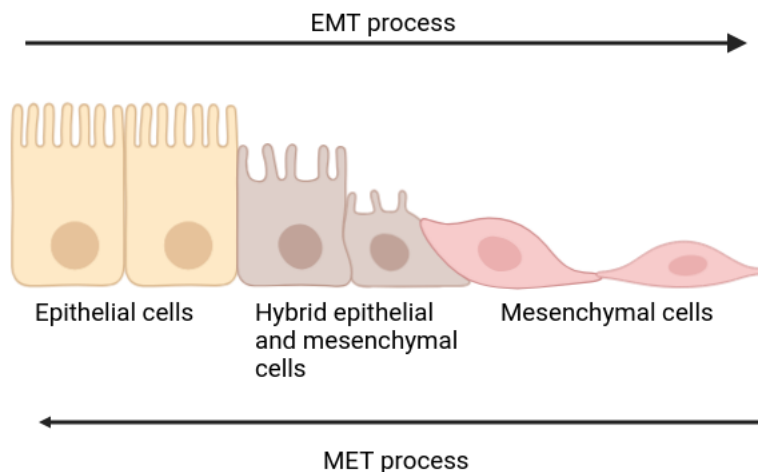


Figure 1.4: This figure illustrates EMT and MET process. (It is created in BioRender)

in the tumor microenvironment (TME) contribute to breaking these strong adhesive bonds, enabling the cells to acquire invasive characteristics—the first step in the metastasis cascade. Experimental studies evidence that when oxygen levels fall below a critical level, EMT starts. In an *in vivo* setting, oxygen delivery in the tumor microenvironment (TME) may fluctuate over time and space, a phenomenon known as cyclic hypoxia. The effects of cyclic hypoxia on the cancer cell cycle have been studied. However, its impact on tumor growth dynamics, EMT progression, and lactate accumulation remains not fully understood in the literature.

1.3 Mathematical modeling

A mathematical modeling framework provides an alternative approach to the complex and time-consuming experimental processes in cancer biology. Results from mathematical models help to generate biologically testable hypotheses, guiding experimentalists in designing new experiments and potentially opening new avenues in cancer research. Mathematical modeling approaches are categorized into three types: continuum, discrete, and hybrid models.

Continuum modeling: This modeling approach treats variables, particularly in biological contexts, such as cell populations, chemical concentrations, and other variables, as continuum species. To construct the model, the dynamics of specific phenomena are expressed mathematically using differential equations. Here, biological laws are described with the help of laws of mass, momentum and energy conversations. In addition, some researchers studied various

interactions of a tumor with its microenvironment. A continuum model captures larger-scale volumetric tumor growth dynamics, and it is easier to analyze tumor growth both analytically and computationally. However, it fails to capture the details of cell-level behaviors.

Discrete modeling: This modeling approach is used to illustrate the interactions between individual cells and components of the tumor microenvironment. Unlike continuum models, discrete modeling treats time and space as separate systems where cells adhere to specific rules for motility and proliferation. The computational cost of a discrete model increases as the system size grows. Several discrete modeling strategies are currently available to describe inherent biological phenomena, such as the Cellular Potts Model (CPM), Agent-Based Models (ABM), and Cellular Automata Models (CAM). In general, the advantages of continuum models are drawbacks in discrete models, and vice versa.

Hybrid modeling: The combination of continuous and discrete modeling approaches is known as a hybrid framework. This approach has recently gained popularity in the scientific community for modeling the multi-scale nature of biological problems. By integrating cellular interactions with global morphological changes, hybrid approaches allow for the representation of cancerous growth across multiple spatial and temporal scales. These approaches are used to explore the effects of gene expression and mutations, intracellular and extracellular signaling, cellular migration, multicellular proliferation, and tumor architecture. Hybrid models consider discrete agents into continuous fields, providing both a local and global perspective of tumor dynamics. These modeling approaches reduce the limitations of both continuous and discrete models. Therefore, the hybrid modeling framework provides a formalism for understanding the complex biological mechanisms involved in cancer biology.

Although cancer progression is a multi scale problem, all the issues addressed in this thesis are formulated using a continuum mathematical modeling framework. This approach represents the tumor as a spatial density field that evolves continuously due to factors, such as cell proliferation, random motility, and competition for resources and space. In contrast, a discrete approach, which tracks the exact positions and states of individual cells, becomes computationally infeasible for large-scale systems. Since our objective is to gain a qualitative understanding of the underlying biology at the macroscopic level, the continuum mathematical modeling

framework serves as an appropriate and effective tool for this study.

In the field of mathematical oncology, Greenspan [33] proposed a mathematical model to study solid tumor growth based on oxygen concentration. This paper is considered as a seminal work in this field. Reaction-diffusion-based modeling techniques are used to illustrate the mechanism of evolution of tumor growth under the influence of various chemicals, nutrients, and growth factors. In this framework, most models are DZM (different zone models); here, all the layers are sharply distinguished. However, from a biological point of view, a tumor is actually a mixture of all kinds of cancer cells, thus not creating sharp discrimination among the involved layers. This kind of model is known as MZM (mixed zone model). Unlike DZM, this model does not consider different layers for different types of cells in an avascular tumor. Instead, it presumes a mixture of live and dead cells and describes the variation of live and dead cell density with the variation of the nutrients. Usually, reaction-diffusion partial differential equations (PDEs) are frequently used to model these phenomena. The general three-dimensional reaction-diffusion equation that describes the rate of change of a quantity of interest $c_i(x, y, z; t)$ (e.g., cell density, growth factors etc) is

$$\frac{\partial c_i}{\partial t} = \nabla(\mathcal{D}\nabla c_i) + \mathcal{G}(c_1, c_2, c_3, \dots, c_n) \text{ in } \Omega$$

in which the rate of change of c_i for $i = 1, 2, \dots, n$ (i.e., $c_{i,t}$) is a function of diffusion, modeled by the function $\mathcal{D}$, and reaction or growth, modeled by the function $\mathcal{G}$. Ω denotes the domain of the problem. Depending on the physics of the problem, $\mathcal{D}$ could be highly non-linear in terms of time, space, and $c_1, c_2, c_3, \dots, c_n$. $\mathcal{G}$ may be depend on $c_1, c_2, c_3, \dots, c_n$ and it could also be highly non-linear. To close the model equation, appropriate boundary and initial conditions are imposed without violating the biology of the problem.

Apart from diffusion phenomena, more complex frameworks have been developed to study the spatio-temporal dynamics of tumor growth [16]. Multiphase models provide a continuum framework which may be used to investigate interactions between different components of growing tissue, modeling the microenvironment as mixture of two or more constituent phases [12, 40, 80]. In this modeling framework, mass and momentum conservation equations need to be prescribed for each phase [4]. Also, multiphase models are able to describe more mechanical insights into the tumor growth process [84, 55].

In order to solve the governing equations in mathematical models numerically, several methods are commonly used in the scientific community, including the finite difference method (FDM), the finite volume method (FVM), and the finite element method (FEM). In this thesis,

the finite difference method is extensively employed to simulate the mathematical models. Additionally, another study utilizes a FEM-based COMSOL Multiphysics interface to simulate these governing equations.

1.4 Thesis objectives

This thesis aims to study the effects of oxygen heterogeneity on solid tumor growth and on epithelial-mesenchymal transition (EMT) process. The distribution of oxygen concentrations inside the tumor changes spatio-temporally. This feature is observed in an *in vivo* tumor. The objectives of this thesis are listed as follows.

1. The oxygen concentration at the tumor boundary near to blood vessel and oxygen concentration at the tumor boundary distant from blood vessel may not be same always. This hypothesis is considered to analyse the tumor growth dynamics. To elucidate the underlying biological phenomena, a multiphase mathematical modeling framework is used. The FDM is employed to simulate the proposed model. Furthermore, simulated results are examined and compared with corresponding existing experimental data to manifest the efficaciousness of the advocated model.
2. Tumor cells in a hypoxic region switch the metabolic process from oxidative phosphorylation to anaerobic glycolysis, which leads to secrete lactate in the tumor microenvironment. The effects of unequal oxygen concentration at tumor boundaries on lactate accumulation are investigated. A system of coupled reaction-diffusion equations is employed to illustrate the biological laws. This system is solved analytically. In order to establish the potency of the proposed model, the simulated results are compared with corresponding experimental data to look for any remarkable agreement so as to validate the applicability of the model considered.
3. Tumor may hold smooth and non-smooth geometrical shape. The impact of initial shape of tumor on lactate accumulation under the non-linear interaction between oxygen and lactate in cyclic hypoxia is explored. A continuum mathematical model, which consists of a system of reaction-diffusion equations, is used to do this investigation. The model is implemented in the FEM-based COMSOL multiphysics interface. A qualitative analysis is carried out through numerical simulations in order to understand the cyclic hypoxia on

tumor growth dynamics and lactate status.

4. Tumor secretes many growth promoting factors, exosome is one of them. Exosome enhances the tumor proliferation rate, and its secretion increases in a lactate environment. The effects of exosome on tumor growth dynamics in lactate acidic environment are analyzed. The model in this work is an extension of the model mentioned in previous objective. To solve the model, the FDM is employed. The proposed model and the simulated results act as a tool to examine the effects of exosome on tumor growth dynamics.
5. Tumor cells undergo epithelial to mesenchymal transition (EMT) in order to initiate invasion and metastasis processes. Oxygen concentration plays a crucial role in order to start EMT process. The impact of oxygen heterogeneity on EMT dynamics is investigated. The effects of cyclic hypoxia on EMT dynamics are also explored.

1.5 Organization of the thesis

The thesis is organized as follows.

- In **Chapter 2**, the effects of unequal oxygen concentration at tumor boundary on tumor growth dynamics are studied (Objective 1).
- In **Chapter 3**, the impacts of unequal oxygen concentration on tumor boundary on lactate accumulation inside the tumor are discussed (Objective 2).
- The effects of non-linear interaction between oxygen and lactate on tumor growth pattern in cyclic hypoxic environment are examined in **Chapter 4** (Objective 3).
- **Chapter 5** focuses on understanding the role of exosome on tumor progression in acidic tumor microenvironment (Objective 4).
- The impacts of oxygen heterogeneity on EMT dynamics is investigated in **Chapter 6** (Objective 5).
- Finally, **Chapter 7** presents general conclusions of the thesis work and suggests potential future directions for further research.



CHAPTER 2

EFFECTS OF UNEQUAL OXYGEN CONCENTRATIONS AT TUMOR BOUNDARIES ON ITS GROWTH

This chapter focuses on studying the growth of a tumor with unequal oxygen concentrations at the tumor boundaries using a mathematical model, aligning with the first objective outlined in the Chapter 1.

2.1 Introduction

For proliferation and survival, tumor cells need a continuous supply of oxygen, glucose, and other nutrients [35]. At an early stage of development of a tumor, these requirements are met from the surrounding tissues and blood vessels. However, as a tumor grows in size, the nutrient supply to the tumor's central region gradually decreases. As a result, cells die in large quantities due to starvation, and owing to the lack of a proper clearance system, dead cells are gathered in the central region, which is known as necrotic core.

In order to explore the dynamics of a tumor growth, researchers mostly rely on experimental approaches [1, 46, 109]. Not only experimental models are costly and their process is time-consuming, but they also often fail to explain the underlying growth phenomena. So, mathematical models complement the experimental studies. The outcomes of the models help clinicians and biologists to understand the disease progression and growth factors in detail. Over the years, many mathematical models have been proposed to gain insights into tumor growth [21, 33, 36]. In 1972, Greenspan [33] proposed a mathematical model of tumor growth.

The model based on the reaction-diffusion equation was developed to investigate oxygen transport in an avascular tumor. Casciari et al. [18] studied the effects of various essential nutrients, like oxygen, glucose, H^+ ions, and extracellular pH, on tumor growth. They found that the center of the tumor has a lower level of oxygen and glucose concentrations, considerably reducing the proliferation rate.

To elaborate the bio-physical factors for tumor growth, researchers proposed multiphase models [11, 12, 40, 16, 4, 55]. In a multiphase model, a tumor is treated as a mixture of more than one phase, like the tumor cells phase, extracellular matrix (ECM), and dead cells. In 2002, Byrne et al. [16] developed a two-phase model to elaborate on the bio-physical factors of tumor growth. They considered tumors to be a mixture of two phases: tumor cellular phase and ECM (which includes components other than the tumor cells). In the multiphase framework, various physical properties, like cellular stress, phase pressure, cell-cell interaction force, cellular viscosity, and ECM phase properties, are generally taken into account in avascular tumor growth models [4, 11, 80, 84]. Droniou et al. [25] studied tumor growth in two dimensions using the multiphase model. They developed a finite element-based numerical method to cater to the irregular and asymmetric initial tumor geometries.

As the oxygen supply in a tissue depends on the diffusional distance from the nearest blood vessel, the tumor region near a blood vessel is rich in oxygen supply compared to the one far from the blood vessel [24]. As a result, the oxygen concentration at the tumor boundary near the blood vessel is not the same as that at the tumor boundary far from the blood vessel. Therefore, oxygen concentrations at the tumor boundaries may not always be uniform. The main limitation of the existing models is that these were formulated to capture the tumor growth under uniform oxygen supply through its boundaries [12, 11, 16, 84]. In this chapter, we aim to explore the tumor growth dynamics under unequal oxygen concentration at the tumor boundaries. To elucidate the growth dynamics, a multiphase one-dimensional (1D) continuum-based approach as proposed by Breward et al. [11] is adopted. The tumor is assumed to be composed of two phases: tumor cellular phase and ECM as the other phase.

Symmetrical oxygen concentration at the boundaries helps establish an explicit relationship between the velocities of tumor cellular and ECM phases [12, 11, 16, 84]. In contrast, when the oxygen concentrations at the tumor boundaries differ, such a relationship no longer holds, and the pressure-velocity equations are coupled. To address this, we adopt the ‘Semi-Implicit Method for Pressure-Linked Equations’ (SIMPLE) algorithm for velocity and pressure cou-

pling in the tumor growth model. A finite difference-based numerical method is employed, incorporating the SIMPLE algorithm on a staggered grid. The model is then simulated to investigate tumor growth patterns under different oxygen concentration at the tumor boundary.

2.2 Model formulation

Figure 2.1 demonstrates an *in vivo* situation, where a tumor is situated, and oxygen is delivered through a preexisting primary blood vessel. As we go from the blood vessel to a distant part of the tissue, an oxygen gradient develops. This work explores the effects of non-uniform oxygen concentrations at the tumor boundaries on tumor growth. In order to obtain a mathematical

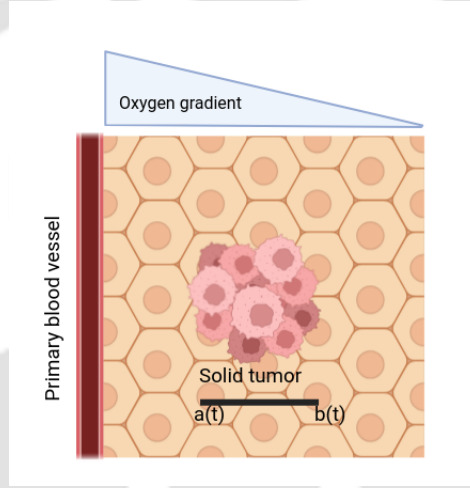


Figure 2.1: An *in vivo* situation of tumor tissue. This picture is created in BioRender

model, we have adopted a continuum multiphase mathematical modeling framework in one dimension. Here, the mass and momentum balance equations for tumor and ECM phases and oxygen transport equations are developed with the help of existing literature [16, 11]. The cancer cell proliferation and death depend on oxygen concentration. The oxygen supply is considered to be distinct at different ends of the tumor.

It is assumed that the mixture of two phases is saturated, and it constitutes the whole tumor (Figure 2.1). So, no void condition is imposed as,

$$\alpha' + \beta' = 1, \quad (2.1)$$

where $\alpha'(x, t)$ and $\beta'(x, t)$ denote the volume fractions for tumor cellular phase and ECM, respectively. The fluid density ρ' is assumed to be constant and equal for both the phases.

By applying the conservation of mass as well as of momentum to each phase, the governing equations are obtained as [11],

$$\frac{\partial \alpha'}{\partial t'} + \frac{\partial(\alpha' u'_c)}{\partial x'} = S'_c, \quad (2.2)$$

$$\frac{\partial \beta'}{\partial t'} + \frac{\partial(\beta' u'_w)}{\partial x'} = S'_w, \quad (2.3)$$

$$\rho' \alpha' \frac{\partial u'_c}{\partial t'} = F'_c + \frac{\partial(\alpha' \sigma'_c)}{\partial x'}, \quad (2.4)$$

$$\rho' \beta' \frac{\partial u'_w}{\partial t'} = F'_w + \frac{\partial(\beta' \sigma'_w)}{\partial x'}. \quad (2.5)$$

Here, c' and w' in the subscripts denote tumor cellular phase and ECM, respectively. u denotes the velocity, σ' is the stress tensor, S' is the mass source or sink, and F' momentum source/sink. F'_c represents the force applied by ECM phase on cellular phase. Initially (i.e., at $t' = 0$), it is assumed that the tumor is not growing, so $u'_c = u'_w = 0$. The initial volume fraction of tumor cellular phase is considered to be $\alpha' = \alpha'_0$.

The oxygen transport is given as [55],

$$\frac{\partial C'}{\partial t'} = D'_c \frac{\partial^2 C'}{\partial x'^2} - \Gamma' \alpha' C' H(C' - C'_N), \quad (2.6)$$

where C' is the oxygen concentration, D' is the diffusion coefficient, C'_N the necrotic threshold value of oxygen below which tumor cells cannot survive. The tumor cells consume oxygen with a consumption rate Γ' . H is the Heaviside function defined as,

$$H(C' - C'_N) = \begin{cases} 1, & \text{if } C' > C'_N, \\ 0, & \text{otherwise.} \end{cases}$$

It is considered that the oxygen is available at the tumor boundaries, so the oxygen concentrations are given as,

$$\begin{aligned} C' &= C'_l \quad \text{at } x' = a'(t'), \\ C' &= C'_r \quad \text{at } x' = b'(t'). \end{aligned} \quad (2.7)$$

The initial oxygen distribution is considered to be linear over the tumor region $[a', b'] = [a'_0, b'_0]$ at $t' = 0$, and it is given as,

$$C'(x') = C'_r + (b'_0 - x') \frac{C'_l - C'_r}{b'_0 - a'_0} \quad \text{for } a'_0 \leq x' \leq b'_0. \quad (2.8)$$

Calculation of mass source/sink terms

The volume fraction (α') of tumor cells increases due to cell division and decreases with cell death. So, the source term is defined as [11],

$$S'_c = \frac{\alpha' \beta' S'_0 C'}{1 + S'_1 C'} - \frac{S'_2 + S'_3 C'}{1 + S'_4 C'} \alpha', \quad (2.9)$$

where the first term on the right-hand-side accounts for the cell proliferation under nourished conditions with parameters S'_0 and S'_1 , and the last-term for necrotic cell death due to shortage of oxygen supply with parameters S'_2 , S'_3 , and S'_4 . The deceased cells are thought to dissolve into the ECM and are ready for mitosis. One can note that if the relation $S'_2 < \frac{S'_3}{S'_4}$ holds, cell proliferation increases with an increase in C and cell death increases with a decrease in C' [11]. This restriction is taken into account to investigate the tumor growth dynamics in the present study. However, if this restriction does not hold, tumor may shrink in size.

The mass source/sink for the ECM phase (S'_w) is calculated based on the conservation of mass. So, any loss/gain in the volume of one phase via source/sink is balanced by an equal volume change in the other phase. This can be incorporated with the condition

$$S'_c + S'_w = 0. \quad (2.10)$$

Calculation of momentum source/sink terms

The momentum source/sink terms F'_c and F'_w are computed using the relations as follows [40],

$$\begin{aligned} F'_c &= p'_c \frac{\partial \alpha'}{\partial x'} + K' \alpha' \beta' (u'_w - u'_c), \\ F'_w &= p'_w \frac{\partial \beta'}{\partial x'} - K' \alpha' \beta' (u'_w - u'_c), \end{aligned} \quad (2.11)$$

where p'_c and p'_w are pressures in cellular and ECM phases, respectively. K' is the drag coefficient associated with the relative movement between the phases.

Let μ'_c be the viscosity of tumor cellular phase. The stresses are given as [11],

$$\begin{aligned} \sigma'_c &= -p'_c + 2\mu'_c \frac{\partial u'_c}{\partial x'}, \\ \sigma'_w &= -p'_w, \text{ with } p'_c = p'_w + \Sigma'_c. \end{aligned} \quad (2.12)$$

Here, Σ'_c is the cell-cell interaction force. In healthy tissue, cells are sparsely distributed, and hence Σ'_c is negligible. Due to the rapid proliferation, tumor cells come in their close contact, which results in a net cell-cell interaction force. If $0 < \alpha' < \alpha'_{min}$, the cells are sparsely

distributed and do not interact with one another. For $\alpha_{min} < \alpha' < \alpha^*$, $\Sigma'_c < 0$, which signifies that the cell experiences attraction force. For $\alpha' > \alpha^*$, the tumor cell phase exerts a net repulsive force. So, Σ'_c is calculated as [11],

$$\Sigma'_c = \begin{cases} \gamma' \frac{(\alpha' - \alpha^*)}{(1 - \alpha')^2}, & \alpha' > \alpha_{min}, \\ 0, & \text{otherwise,} \end{cases} \quad (2.13)$$

where γ' is the tension force constant.

2.2.1 Boundary propagation tracking equations

We assume that tumor boundaries propagate with the tumor cellular phase velocity. So, the equations are given as,

$$\frac{da'}{dt'} = u'_c(a'), \quad (2.14)$$

$$\frac{db'}{dt'} = u'_c(b'). \quad (2.15)$$

2.2.2 Model simplification

The momentum equations (2.4)–(2.5) (using Eqs. (2.11) and (2.12)) reduce to

$$\rho' \alpha' \frac{\partial u'_c}{\partial t'} = -\alpha' \frac{\partial p'_c}{\partial x'} + 2\mu'_c \frac{\partial(\alpha' \frac{\partial u'_c}{\partial x'})}{\partial x'} + K' \alpha' \beta' (u'_w - u'_c), \quad (2.16)$$

$$\rho' \beta' \frac{\partial u'_w}{\partial t'} = -\beta' \frac{\partial p'_w}{\partial x'} - K \alpha \beta (u'_w - u'_c), \quad (2.17)$$

with $p'_c = p'_w + \Sigma'_c$. We assume that tumor grows in a physical obstacle-free medium, which mimics the *in vitro* experimental setup. This growth environment is resembled by imposing zero stress condition at the boundaries. These conditions are given as,

$$\begin{aligned} \sigma'_c = \sigma'_w = 0 \quad \text{at} \quad x' = a'(t') \quad \text{and} \quad b'(t'), \\ 2\mu'_c \frac{\partial u'_c}{\partial x'} = \Sigma'_c \quad \text{at} \quad x' = a'(t') \quad \text{and} \quad b'(t'). \end{aligned} \quad (2.18)$$

2.2.3 Non-dimensionalized model

Let R_m be the maximal tumor length, $\hat{C}$ be the characteristic oxygen concentration (which is taken as the oxygen concentration at the left boundary of tumor ($C_l = 10 \text{ mmHg}$)), and the

characteristic time $\hat{t} = \frac{1 + S_1 \hat{C}}{S_0 \hat{C}} \approx 18 \text{ hr}$, representing the time scale of proliferation rate under a well-nourished condition [84]. So, the dimensionless variables are given as,

$$x = \frac{x'}{R_m}, C = \frac{C'}{\hat{C}}, \text{ and } t = \frac{t'}{\hat{t}}. \quad (2.19)$$

The parameters and variables are made dimensionless in the following way: $a = \frac{a'}{R_m}$, $b = \frac{b'}{R_m}$, $a_0 = \frac{a'_0}{R_m}$, $b_0 = \frac{b'_0}{R_m}$, $\alpha = \alpha'$, $\beta = \beta'$, $u_c = \frac{\hat{t} u'_c}{R_m}$, $u_w = \frac{\hat{t} u'_w}{R_m}$, $S_1 = S'_1 \hat{C}$, $S_2 = S'_2 \hat{t}$, $S_4 = S'_4 \hat{C}$, $S_3 = S'_3 \hat{t} \hat{C}$, $p_c = \frac{\hat{t}^2 p'_c}{R_m^2 \rho'}$, $p_w = \frac{\hat{t}^2 p'_w}{R_m^2 \rho'}$, $\mu_c = \frac{\mu'_c \hat{t}}{R_m^2 \rho'}$, $K = \frac{K' \hat{t}}{\rho'}$, $\gamma = \frac{\hat{t}^2 \gamma'}{R_m^2 \rho'}$, $D = \frac{D' \hat{t}}{R_m^2}$, $\Gamma = \hat{t} \Gamma'$, $C'_N = C_N \hat{C}$, and $C_r = \frac{C'_r}{\hat{C}}$.

Then non-dimensional mass conservation equations are obtained as,

$$\frac{\partial \alpha}{\partial t} + \frac{\partial(\alpha u_c)}{\partial x} = \frac{(1 + S_1) \alpha \beta C}{1 + S_1 C} - \frac{S_2 + S_3 C}{1 + S_4 C} \alpha = S_c, \quad (2.20)$$

$$\frac{\partial \beta}{\partial t} + \frac{\partial(\beta u_w)}{\partial x} = -\frac{(1 + S_1) \alpha \beta C}{1 + S_1 C} + \frac{S_2 + S_3 C}{1 + S_4 C} \alpha = -S_c. \quad (2.21)$$

The non-dimensional momentum equations (using the relation $p_c = p_w + \Sigma_c$) are given as,

$$\alpha \frac{\partial u_c}{\partial t} = -\alpha \frac{\partial p_c}{\partial x} + 2\mu_c \frac{\partial(\alpha \frac{\partial u_c}{\partial x})}{\partial x} + K \alpha \beta (u_w - u_c), \quad (2.22)$$

$$\frac{\partial u_w}{\partial t} = -\frac{\partial p_c}{\partial x} + \frac{\partial \Sigma_c}{\partial x} - K \alpha (u_w - u_c). \quad (2.23)$$

The zero stress conditions at boundaries in the non-dimensional form are given as,

$$\begin{aligned} \sigma_c = \sigma_w = 0 \quad \text{at} \quad x = a(t) \quad \text{and} \quad b(t), \\ 2\mu_c \frac{\partial u_c}{\partial x} = \Sigma_c \quad \text{at} \quad x = a(t) \quad \text{and} \quad b(t). \end{aligned} \quad (2.24)$$

Velocities and cellular volume fraction at initial time ($t = 0$) are given as,

$$u_c = u_w = 0 \quad \text{and} \quad \alpha = \alpha_0. \quad (2.25)$$

The non-dimensional oxygen diffusion equation becomes

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - \Gamma \alpha C H(C - C_N) \quad (2.26)$$

with boundary conditions

$$\begin{aligned} C = C_l \quad \text{at} \quad x = a(t), \\ C = C_r \quad \text{at} \quad x = b(t), \end{aligned} \quad (2.27)$$

and initial concentration distribution profile

$$C(x) = C_r + (b_0 - x) \frac{C_l - C_r}{b_0 - a_0} \quad \text{for} \quad a_0 \leq x \leq b_0. \quad (2.28)$$

The non-dimensional boundary propagation equations can be written as,

$$\frac{da}{dt} = u_c(a), \quad (2.29)$$

$$\frac{db}{dt} = u_c(b). \quad (2.30)$$

2.3 Numerical method

This section presents the numerical procedure to solve the mathematical model (Eqs. (2.20) – (2.26)). We use the staggered grid framework to discretize the governing equations, since, the staggered framework avoids the possibility of spurious results in case of checkerboard-like pressure distribution [75]. In staggered grid framework, the scalar variables (in our case, oxygen, volume fractions, and pressure for each phase) are considered between two adjacent vector quantities (here, velocity for each phase). The staggered grid is chosen in such a way that the velocity u_c is on the boundary nodes as depicted in Figure 2.2. The domain $[a, b]$ is discretized with step size $\Delta x = \left(\frac{b-a}{N-1}\right)$, where N is the number of grid points. The spatial grid points are numbered as $i = 0, 1, \dots, N-1$. N is odd number, which ensures that velocities u_c and u_w exist only for even value of i , and C, p, α exist at odd value of i .

At the interior nodes, a central difference scheme is used for spatial discretization. While for boundary nodes, a special treatment is adopted as discussed below. The first-order forward difference scheme is adopted for temporal derivatives.

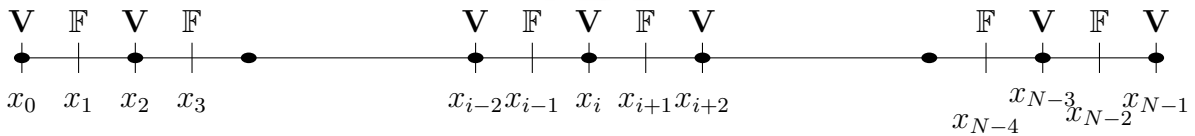


Figure 2.2: Schematic diagram of the grid points and the staggered arrangement of variable on the computational domain. Here, $\mathbb{F}$ denotes the scalar quantities $\{C, p_c, \alpha\}$ and $\mathbb{V}$ denotes the velocities $\{u_c, u_w\}$.

2.3.1 Discretization of momentum conservation equations

The discretized form of the Eq. (2.23) is given as,

$$u_{w,i}^{n+1} = \frac{1}{1 + A_1 \alpha_i} (u_{w,i}^n + A_1 \alpha_i u_{c,i}^{n+1} - B_1 (p_{c,i+1} - p_{c,i-1}) + B_1 (\Sigma_{c,i+1} - \Sigma_{c,i-1})), \quad (2.31)$$

where $A_1 = K \Delta t$, $B_1 = \frac{\Delta t}{2 \Delta x}$, and $\alpha_i = \frac{\alpha_{i+1} + \alpha_{i-1}}{2}$ for $i = 2, 4, \dots, N - 3$. $u_{c,i}^{n+1}$ and $u_{w,i}^{n+1}$ are the discrete counterparts of u_c and u_w respectively at time $t_{n+1} = (n + 1) \Delta t$ for time-step Δt and at position $x_i = i \Delta x$.

Using the expression of $u_{w,i}^{n+1}$, the discretized form of Eq. (2.22) can be written as,

$$\begin{aligned} & -\frac{B_1 \mu_c \alpha_{i-1}}{\Delta x^2 \alpha_i} u_{c,i-2}^{n+1} + \left(\frac{A_2}{1 + A_1 \alpha_i} + \frac{B_1 \mu_c (\alpha_{i-1} + \alpha_{i+1})}{\Delta x \alpha_i} \right) u_{c,i}^{n+1} - \frac{B_1 \mu_c \alpha_{i+1}}{\Delta x \alpha_i} u_{c,i+2}^{n+1} \\ & = -\frac{A_2 B_1}{1 + A_1 \alpha_i} (p_{c,i+1}^n - p_{c,i-1}^n) + \frac{A_1 (1 - \alpha_i)}{1 + A_1 \alpha_i} (u_{w,i}^n + B_1 (\Sigma_{c,i+1} - \Sigma_{c,i-1})), \end{aligned} \quad (2.32)$$

where $A_2 = 1 + A_1$ and $\Sigma_{c,i} = \gamma \frac{\alpha_i - \alpha^*}{(1 - \alpha_i)^2}$.

At the left boundary (i.e., for $i = 0$), the discretized form of Eq. (2.24) is obtained as,

$$u_{c,0} = u_{c,2} - \frac{\Delta x \Sigma_{c,0}}{\mu_c}. \quad (2.33)$$

At the right boundary (i.e., for $i = N - 1$), the discretized form of Eq. (2.24) is obtained as,

$$u_{c,N-1} = u_{c,N-3} + \frac{\Delta x \Sigma_{c,N-1}}{\mu_c}. \quad (2.34)$$

The boundary information required in the Eq. (2.32) are supplied by Eqs. (2.33) and (2.34).

2.3.2 Discretization of mass conservation equation

Mass conservation Eq. (2.20) is discretized using FTCS scheme. The discretized equations are given as,

$$\alpha_i^{n+1} = \alpha_i^n + \Delta t S_{c,i}^n - (f_{i+1}^n - f_{i-1}^n) B_1, \quad (2.35)$$

where $f_{i+1}^n = u_{c,i+1}^n \frac{(\alpha_{i+2}^n + \alpha_i^n)}{2}$ and $f_{i-1}^n = u_{c,i-1}^n \frac{(\alpha_i^n + \alpha_{i-2}^n)}{2}$ with $f_0^n = u_{c,0}^n \alpha_0^n$ and $f_{N-1}^n = u_{c,N-1}^n \alpha_{N-1}^n$ for $i = 1, 3, \dots, N - 2$.

Since, we are using the explicit scheme for mass conservation equation, we must check at each time step that Eq. (2.35) satisfy the following stability condition

$$\Delta t \leq \max_i \left(\frac{4 \Delta x |u_{c,i+1} - u_{c,i-1}|}{u_{c,i+1}^2 + u_{c,i-1}^2} \right) \quad \text{for } i = 1, 3, \dots, N - 2. \quad (2.36)$$

This relation is obtained by performing the Fourier stability analysis [78]. Once α is obtained, β can be calculated from $\alpha + \beta = 1$.

2.3.3 Discretization of oxygen diffusion equation

The oxygen diffusion equation (3.8) is discretized as,

$$\frac{D_1}{4}C_{i-2}^{n+1} - \left(1 + \frac{D_1}{2} + \Delta t \Gamma \alpha_i^{n+1} H(C_i^n - C_N)\right) C_i^{n+1} + \frac{D_1}{4}C_{i+2}^{n+1} = -C_i^n, \quad (2.37)$$

where $i = 3, 5, \dots, (N-4)$ and $D_1 = \frac{D\Delta t}{\Delta x^2}$. C_N is the non-dimensional oxygen concentration of necrotic threshold value.

Note that the Eq. (2.37) is obtained by using central differencing with spatial step size $2\Delta x$. For $i = 1$, the central differencing is used with spatial size Δx . The resulting equation involves C_2 , which is calculated by taking the average of C_1 and C_3 . The equation for $i = 1$ is obtained as,

$$-\left(1 + \frac{3D_1}{2} + \Delta t \Gamma \alpha_1^{n+1} H(C_1^n - C_N)\right) C_1^{n+1} + \frac{D_1}{2}C_3^{n+1} = -C_1^n - D_1 C_0^{n+1}. \quad (2.38)$$

By following the similar approach, the equation for $i = N-2$ is obtained as,

$$\frac{D_1}{2}C_{N-4}^{n+1} - \left(1 + \frac{3D_1}{2} + \Delta t \Gamma \alpha_{N-2}^{n+1} H(C_{N-2}^n - C_N)\right) C_{N-2}^{n+1} = -C_{N-2}^n - D_1 C_{N-1}^{n+1}. \quad (2.39)$$

2.3.4 SIMPLE algorithm for momentum equations

In Eq. (2.32), the pressure and velocity are coupled, so SIMPLE algorithm [75] is adopted to solve for velocity. The algorithm is as follows.

Let us write the Eq. (2.32) in the form

$$a_i u_{c,i}^{n+1} = \sum_{nb} a_{nb} u_{c,nb}^{n+1} - \frac{A_2 B_1}{1 + A_1 \alpha_i} (p_{c,i+1} - p_{c,i-1}) + \phi(\alpha_i, \Sigma_{c,i}), \quad (2.40)$$

where $u_{c,nb}$ are the neighborhood velocities around the grid point i .

Let p_c^* be the guessed pressure field and take $p_c = p_c^*$ in Eqs. (2.40). The resulted system of equations is obtained as,

$$a_i u_{c,i}^{*n+1} = \sum_{nb} a_{nb} u_{c,nb}^{*n+1} - \frac{A_2 B_1}{1 + A_1 \alpha_i} (p_{c,i+1}^* - p_{c,i-1}^*) + \phi(\alpha_i, \Sigma_{c,i}). \quad (2.41)$$

Here, $u_{c,i}^*$ can be called the pseudo velocity field based on a guessed pressure field p_c^* .

If u'_c and p'_c are the velocity and pressure correction terms, then

$$u_c = u_c^* + u'_c, \quad (2.42)$$

$$p_c = p_c^* + p'_c. \quad (2.43)$$

Subtracting Eq. (2.41) from (2.40), we have

$$a_i u'_{c,i}{}^{n+1} = \sum_{nb} a_{nb} u'_{c,nb}{}^{n+1} - \frac{A_2 B_1}{1 + A_1 \alpha_i} (p'_{c,i+1} - p'_{c,i-1}). \quad (2.44)$$

The omission of the term $\sum a_{nb} u'_{c,nb}{}^{n+1}$ in deriving the pressure correction equations is of no consequence as far as the final converged results are concerned. Detailed information on this is available in the book of S.V. Patankar [75]. So by dropping the term $\sum a_{nb} u'_{c,nb}{}^{n+1}$, Eq. (2.44) can be written as,

$$u'_{c,i}{}^{n+1} = -\frac{A_2 B_1}{a_i (1 + A_1 \alpha_i)} (p'_{c,i+1} - p'_{c,i-1}), \quad (2.45)$$

where $a_i = \left(\frac{A_2}{1 + A_1 \alpha_i} + \frac{B_1 \mu_c (\alpha_{i-1} + \alpha_{i+1})}{\Delta x \alpha_i} \right)$, $\alpha_i = \frac{\alpha_{i+1} + \alpha_{i-1}}{2}$ for $i = 2, 4, \dots, N - 3$. nb denotes neighboring points of i . Eq. (2.45) is called the velocity-correction formula. Therefore, Eq. (2.42) can be rewritten as,

$$u_{c,i}^{n+1} = u_c^* - \frac{A_2 B_1}{a_i (1 + A_1 \alpha_i)} (p'_{c,i+1} - p'_{c,i-1}). \quad (2.46)$$

Upon summing up the mass conservation Eqs. (2.20) and (2.21) and using $\alpha + \beta = 1$, the pressure correction equation can be obtained from

$$\frac{\partial (\alpha u_c + \beta u_w)}{\partial x} = 0. \quad (2.47)$$

By using the central difference scheme in Eq. (2.47) and by substituting the values of u_c from (2.32) and u_w from (2.31), the pressure correction equation is obtained as,

$$B_1 (H_{i-1} - E_{i-1}) p'_{c,i-1} - B_1 (H_{i-1} - E_{i-1} + H_{i+1} - E_{i+1}) p'_{c,i+1} + B_1 (H_{i+1} - E_{i+1}) p'_{c,i+3} = Q_{i-1} - Q_{i+1}, \quad (2.48)$$

where $H_{i-1} = -\frac{A_2^2 \alpha_{i-1}}{a_{i-1} (1 + A_1 \alpha_{i-1})}$, $E_{i-1} = \frac{\beta_{i-1}}{1 + A_1 \alpha_{i-1}}$, and

$Q_{i-1} = \frac{\beta_{i-1}}{1 + A_1 \alpha_{i-1}} (u_{w,i-1}^n + B_1 (\Sigma_{c,i} - \Sigma_{c,i-2}) - B_1 (p_{c,i}^* - p_{c,i-2}^*)) + \frac{\alpha_{i-1} A_2}{1 + A_1 \alpha_{i-1}} u_{c,i-1}^{n+1}$ for $i = 3, 5, \dots, N - 4$.

For $i = 1$ and $i = N - 2$, the pressure correction equations are obtained as,

$$\begin{aligned}
 & \left(\frac{2\beta_0}{1 + A_1\alpha_0} + E_2 - H_2 - \frac{\alpha_0 A_2^2}{a_2(1 + A_1\alpha_0)(1 + A_1\alpha_2)} \right) B_1 p_{c,1}'^{n+1} \\
 & + \left(H_2 - E_2 + \frac{\alpha_0 A_2^2}{a_2(1 + A_1\alpha_0)(1 + A_1\alpha_2)} \right) B_1 p_{c,3}'^{n+1} \\
 & = \frac{2\beta_0}{1 + A_1\alpha_0} (u_{w,0}^n + 2B_1 \Sigma_{c,1}) - E_2 (u_{w,2}^n + B_1 (\Sigma_{c,3} - \Sigma_{c,1})) \\
 & \quad - \left(\frac{\alpha_2 A_2}{1 + A_1\alpha_2} - \frac{\alpha_0 A_2}{1 + A_1\alpha_0} \right) u_{c,2}^* \\
 & \quad - \frac{\alpha_0 A_2}{1 + A_1\alpha_0} \frac{\Delta x \Sigma_{c,0}}{\mu_c} + E_2 B_1 (p_{c,3}^* - p_{c,1}^*) - \frac{\beta_0 B_1}{1 + A_1\alpha_0} p_{c,1}^*, \tag{2.49}
 \end{aligned}$$

and

$$\begin{aligned}
 & \left(H_{N-3} - E_{N-3} + \frac{\alpha_{N-1} A_2^2}{a_{N-3}(1 + A_1\alpha_{N-1})(1 + A_1\alpha_{N-3})} \right) B_1 p_{c,N-4}'^{n+1} \\
 & + \left(\frac{2\beta_{N-1}}{1 + A_1\alpha_{N-1}} + E_{N-3} - H_{N-3} - \frac{\alpha_{N-1} A_2^2}{a_{N-3}(1 + A_1\alpha_{N-1})(1 + A_1\alpha_{N-3})} \right) B_1 p_{c,N-2}'^{n+1} = \\
 & - \frac{2\beta_{N-1}}{1 + A_1\alpha_{N-1}} (u_{w,N-1}^n - 2B_1 \Sigma_{c,N-2}) + E_{N-3} (u_{w,N-3}^n + B_1 (\Sigma_{c,N-2} - \Sigma_{c,N-4})) \\
 & \quad - \left(\frac{\alpha_{N-1} A_2}{1 + A_1\alpha_{N-1}} - \frac{\alpha_{N-3} A_2}{1 + A_1\alpha_{N-3}} \right) u_{c,N-3}^* \\
 & \quad - \frac{\alpha_{N-1} A_2}{1 + A_1\alpha_{N-1}} \frac{\Delta x \Sigma_{c,N-1}}{\mu_c} - E_{N-3} B_1 (p_{c,N-2}^* - p_{c,N-4}^*) - \frac{\beta_{N-1} B_1}{1 + A_1\alpha_{N-1}} p_{c,N-2}^*. \tag{2.50}
 \end{aligned}$$

The system of equations for pressure correction is solved using the tridiagonal matrix algorithm (TDMA). Once the pressure correction is obtained, the pressure is updated using Eq (2.43). With this corrected pressure, the velocity $u_{c,i}^{**n+1}$ is obtained from Eq. (2.32). Also, it can be obtain $u_{w,i}^{**n+1}$ from Eq. (2.31) by substituting $u_{c,i}^{n+1} = u_{c,i}^{**n+1}$ and the corrected pressure for $p_{c,i}$. If $u_{c,i}^{**n+1}$ and $u_{w,i}^{**n+1}$ satisfy the discretized version of the continuity equation (2.47), the convergence criteria is given by,

$$\alpha_{i+1} u_{c,i+1}^{**n+1} - \alpha_{i-1} u_{c,i-1}^{**n+1} + \beta_{i+1} u_{w,i+1}^{**n+1} - \beta_{i-1} u_{w,i-1}^{**n+1} = 0 \quad \text{for } i = 1, 3, \dots, N-2, \tag{2.51}$$

where $\alpha_{i+1} = \frac{\alpha_{i+2} + \alpha_i}{2}$ and $\beta_i = 1 - \alpha_i$. The absolute value of the right-hand side of Eq. (2.51) is achieved during simulation with a tolerance value tol (in this study $tol = 10^{-10}$).

The SIMPLE algorithm is followed in the following sequence:

1. Guess a pressure field p_c^* .
2. Solve the momentum Eq. (2.40) to obtain u_c^* .

3. Solve for pressure correction p'_c using Eq. (2.48).
4. Correct the pressure field by Eq. (2.43).
5. Correct the velocity field by Eq. (2.42).
6. Test the convergence using Eq. (2.51).
7. If solution converges, stop. Else return to step 1 with p_c as the new guessed pressure p_c^* and repeat the process until converges.

2.3.5 Algorithm for solving tumor growth model

The governing equations of the present model are solved with the following algorithm.

Step 1: Initialization, i.e., at $t = 0$,

$\alpha_0 = 0.8, a_{old} = -R_{old}, b_{old} = R_{old}, R_{old} = 0.05, u_c = 0$, and $u_w = 0$, and solve the oxygen diffusion equation to obtain the initial concentration distribution.

For each time-step until $t = T$ do

Step 2: Solve the momentum equations to get the velocity field using the initial volume fractions.

Step 3: Solve mass conservation equations using the velocities obtained in Step 2.

Step 4: Update boundaries a_{new}, b_{new} by

$$a_{new} = a_{old} + u_{c,0}\Delta t,$$

$$b_{new} = b_{old} + u_{c,N-1}\Delta t.$$

Step 5 : Update the oxygen field.

end

2.4 Results

2.4.1 Experimental validation

The numerical results obtained from the present model are validated with the experimental results of Mueller-Klieser and Sutherland [68]. They studied the oxygen distribution for var-

ious sizes of tumors using oxygen-sensitive microelectrodes. In order to perform numerical simulations, the values of the model parameters are taken from the existing literature and are presented in Table 2.1. We consider $\alpha = \alpha_{min} = \alpha^* = 0.8$ as the initial volume fraction [84]. So, no cell-cell interaction is considered at the beginning of tumor growth (follows from Eq. (2.13)). Let $R_0 = b_0 - a_0$ be the initial dimensionless diameter of the tumor (i.e., at $t = 0$). An experimental study by Shirinifard et al. [91] suggests that the maximum diameter of a solid tumor is approximately $200\mu m$ before the angiogenesis process. In this study, the initial tumor diameter is considered to be $20\mu m$ for simulation. Therefore, $R_0 = \frac{1}{10}(= \frac{20}{200})$ is chosen, and the model is simulated up to $t = 100$. The comparisons are shown in Figure 2.3. It can be seen that the numerical results are in excellent agreement with the experimental data. This comparison ensures that the model is a realistic and reliable one.

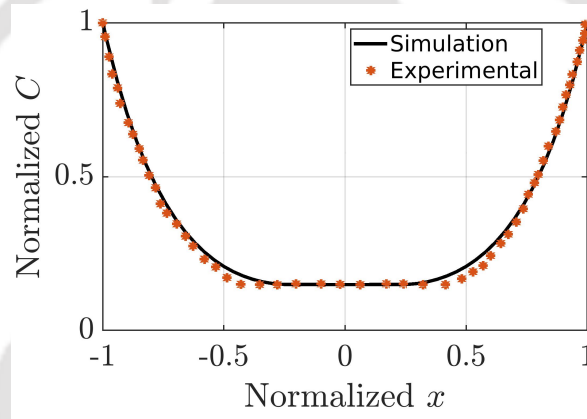


Figure 2.3: Comparison of predicted results with the results of Mueller-Klieser and Sutherland [68].

The results are presented in non-dimensional form. However, these results can be interpreted through the relations between dimensional and non-dimensional variables. These relations are $t' = 18t \text{ hr}$, $x' = 4x \text{ mm}$ and $C' = 10C \text{ mmHg}$.

2.4.2 Effects of oxygen supply on tumor cellular volume fraction

To understand the effects of oxygen supply on tumor cellular volume fraction, the four cases are considered as (a) $C_l = C_r = 1$, (b) $C_l = 1, C_r = 0.5$, (c) $C_l = 1, C_r = 0.25$, and (d) $C_l = 1, C_r = 0.1$. Changes in the volume fraction of tumor cellular phase and oxygen distribution over tumor region are displayed as subfigures (a) and (b), respectively, in each of Figs. 2.4–2.7.

Table 2.1: Parameter values.

Parameter	Non-dimensional value	Reference
Γ	0.1	[84]
D	$\frac{1}{1600}$	[84]
C_N	0.1	[55]
μ_c	1.0	[11]
γ	500	[84]
K	1600	[84]
S_1	10	[11]
S_2	0.5	[11]
S_3	0.5	[11]
S_4	10	[11]

The volume fraction of cellular phase (α) for the first case (i.e., $C_l = C_r = 1$) is displayed in Figure 2.4a. It can be noticed that α increases rapidly with time at the initial stage (for $0 < t \leq 10$) due to the sufficient supply of oxygen in the small tumors. Therefore, the proliferation rate is very high initially. However, as time progresses, though the tumor size increases, the volume fraction of the cellular phase starts falling in the central region of the tumor. It can be seen that the volume fraction falls below the initial fraction, as shown by the horizontal dashed line. The oxygen profile for the growing tumor is displayed in Figure 2.4b. Clearly, the oxygen concentration falls inside the tumor. As the tumor grows in size, tumor cells die owing to the lack of oxygen supply in the central region. Also, it can be noted that the oxygen concentration does not fall below the necrotic level, i.e., $C_N = 0.1$, as shown by the horizontal dashed line (Figure 2.4b).

As a result, the necrotic region is developed inside the core of the tumor. Also, it can be noticed that the volume fraction of tumor cellular phase is distributed symmetrically about the center $x = 0$, and a proliferating rim is spotted near the tumor boundaries. In Figure 2.5a, the cellular volume fraction (α) is displayed for oxygen concentrations $C_l = 1$ and $C_r = 0.5$. It can be observed that the cell volume is lower near the right boundary as compared to the left one, and cells decrease faster at the right boundary. This is because a larger amount of oxygen is available at the left boundary. The distribution of oxygen concentration is displayed

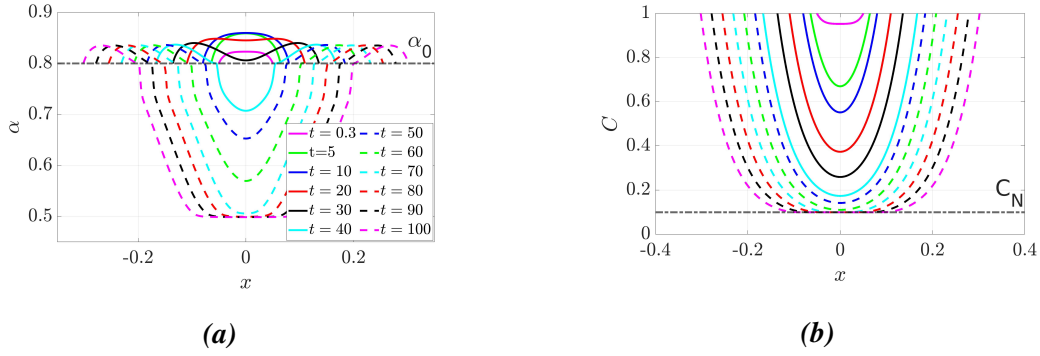


Figure 2.4: (a) Volume fraction profile of tumor cellular phase, and (b) oxygen distribution over tumor region ($C_l = 1$, $C_r = 1$). Horizontal dashed line in (a) shows the initial volume fraction of cancer cells (i.e., $\alpha_0 = 0.8$) whereas the horizontal dashed line in (b) shows the necrotic threshold for oxygen concentration (i.e., $C_N = 0.1$).

in Figure 2.5b. One can observe that the oxygen concentration falls to the threshold value at near $t = 80$. The necrotic region starts to develop inside the core, but the shape of this region is asymmetric. Also, α is distributed asymmetrically. Therefore, the tumor morphology is asymmetric. The findings are quite different from the first case where oxygen concentrations on tumor boundaries are same (Figure 2.4a). One may notice that the layer of proliferating tumor cells is less wide near the right boundary compared to that of the left.

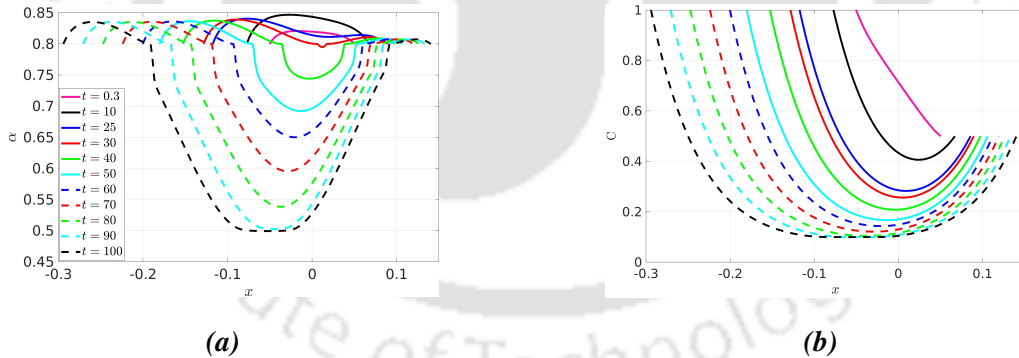


Figure 2.5: (a) Volume fraction profile of tumor cellular phase, and (b) oxygen distribution over tumor region ($C_l = 1$, $C_r = 0.5$).

Next, the numerical experiments are conducted for $C_l = 1$ and $C_r = 0.25$. The cellular volume fraction (α) is portrayed in Figure 2.6a, whereas the oxygen distribution is displayed in Figure 2.6b. It is seen that the tumor growth is negligible towards the right boundary, and α decreases during the initial growth. This is due to the insufficient oxygen supply at the right boundary. As a result, tumor cells start to die there, and the necrotic region is placed in the

vicinity of the same boundary. Tumors mostly grow toward the left side due to the sufficient oxygen supply there, and the cells' proliferating layer also appears only at the left boundary.

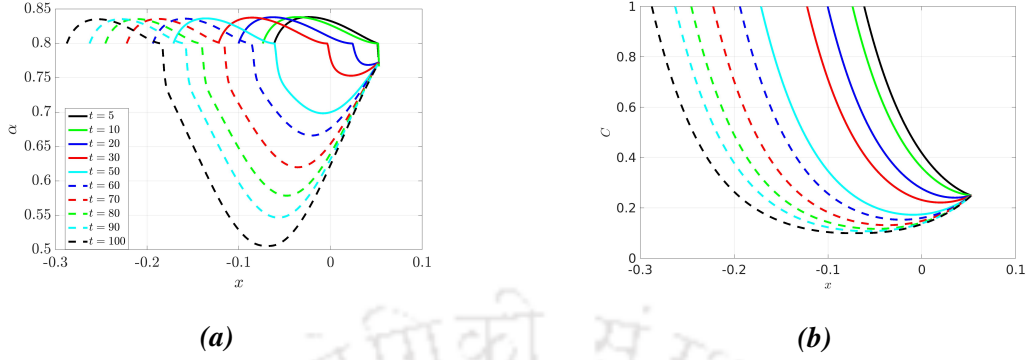


Figure 2.6: (a) Volume fraction profile of tumor cellular phase, and (b) oxygen distribution over tumor region ($C_l = 1, C_r = 0.25$).

In the following case, the oxygen concentration is considered to be the same as the necrotic threshold value at the right boundary (i.e., $C_r = C_N = 0.1$). The oxygen profile for the growing tumor is portrayed in Figure 2.7b, and the volume fraction of the cellular phase (α) is shown in Figure 2.7a. One can see that α starts declining near the right boundary as time progresses. It achieves the lowest value $\alpha = 0.5$ at the time $t = 20$. It can be noticed that the tumor growth is negligible towards the right boundary as a result of the fact that oxygen is insufficient to sustain the tumor cells. So, tumor cells are unable to proliferate owing to the tumor cells death.

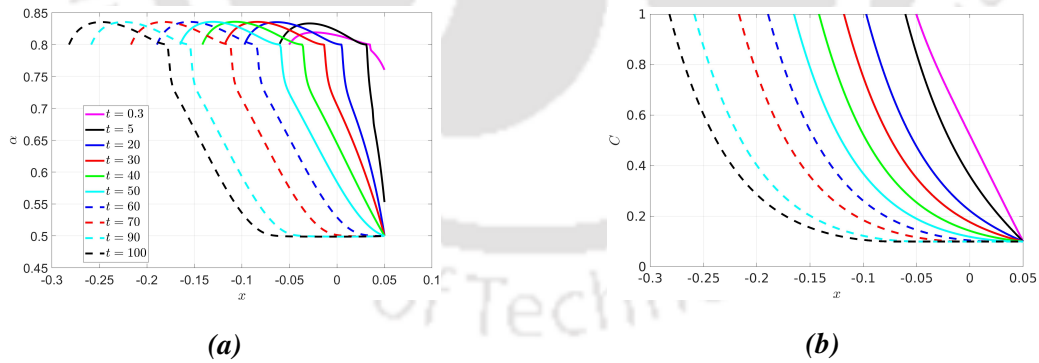


Figure 2.7: (a) Volume fraction profile of tumor cellular phase, and (b) oxygen distribution over tumor region ($C_l = 1, C_r = 0.1$).

Comparison between the volume fraction of tumor cellular phase for different oxygen concentrations at the right boundary is portrayed in Figure 2.8. The snapshots Figs. 2.8a, 2.8b, 2.8c, and 2.8d are taken at $t = 0.3, 30, 50$, and 100 , respectively. It can be noticed that tumor volume fraction increases throughout the domain for the cases $C_r = 1$ and $C_r = 0.5$ at

$t = 0.3$. On the other hand, tumor cells start to die at the boundary having oxygen concentration $C_r = 0.25$ and $C_r = 0.1$. This is due to insufficient oxygen supply to fulfill the demand for the livelihood of cells (Figure 2.8a). However, at subsequent times ($t = 30, 50$, and 100), tumor cells also die in the central region for each combination of oxygen concentration at the right boundary. One may observe that the proliferation rim is spotted at the right edge of the tumor only for $C_r = 1$ and $C_r = 0.5$ (Figs. 2.8b–2.8d). It is concluded that tumor volume fraction decreases at the right boundary with the decreasing value of oxygen concentration at that boundary, and the shape of the tumor strongly depends on the oxygen supply.

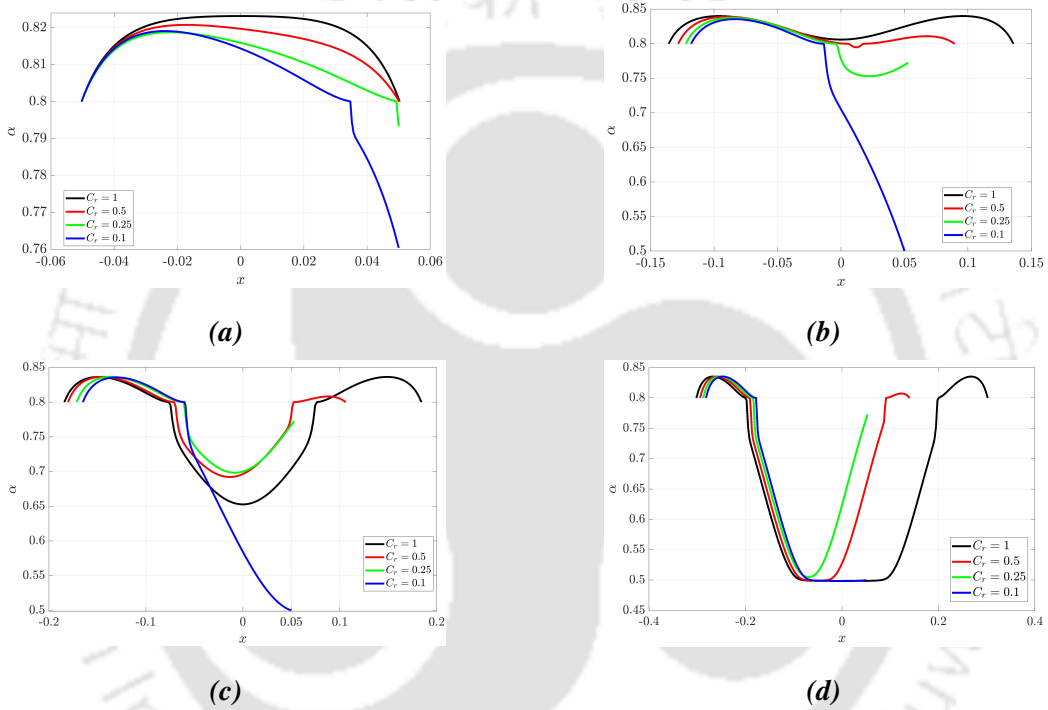


Figure 2.8: Comparison between volume fraction of tumor cellular phase for different oxygen concentration at the right boundary at (a) $t = 0.3$, (b) $t = 30$, (c) $t = 50$, and (d) $t = 100$.

2.4.3 Effects of oxygen supply on cellular phase velocity

The cellular phase velocity determines the aggressive nature of the tumor and its progression. So, its understanding is vital for developing new therapies for cancer disease. In this subsection, the effects of oxygen on tumor cellular phase velocity are investigated.

The velocity profiles of the cellular phase with boundary oxygen concentrations $C_l = 1, C_r = 1$ are plotted at different times in Figure 2.9a. The velocity is anti-symmetric about $x = 0$, and it increases with x during the initial stage of tumor development ($0 < t \leq 5$). How-

ever, as time progresses, the velocity decreases mainly in the central region of the tumor. In the time interval $5 < t \leq 30$, on the right side of $x = 0$, the velocity changes from negative to positive. The velocity changes from positive to negative while we move to the left from $x = 0$. This is due to the dead cells, which are converted into the ECM during tumor progression. For $t > 30$, the velocity becomes zero at the central region of the tumor, which is termed as the necrotic core of the tumor, which grows in size as the tumor progresses. Owing to the sufficient oxygen supply, the tumor spreads through its boundaries, and it grows symmetrically about $x = 0$ with the same magnitude of velocity on both sides of the tumor.

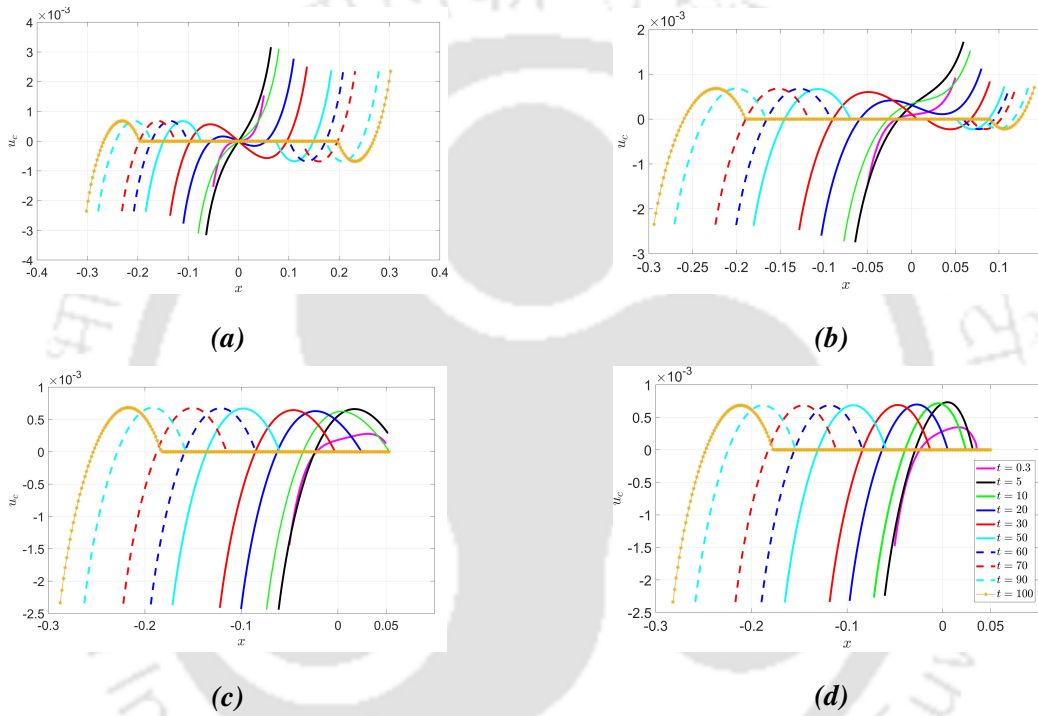


Figure 2.9: Velocity of tumor cellular phase over the tumor region with oxygen boundary concentrations (a) $C_l = C_r = 1$, (b) $C_l = 1$, $C_r = 0.5$, (c) $C_l = 1$, $C_r = 0.25$, and (d) $C_l = 1$, $C_r = 0.1$.

For the second case ($C_l = 1$ and $C_r = 0.5$), the velocity profile of the cellular phase is portrayed in Figure 2.9b. The magnitude of the velocity of the cellular phase at the left boundary is approximately 1.5 times the velocity at the right boundary for $t = 0.3$. The velocity increases continuously on both sides of the boundary up to $t = 10$. It shows the rapid proliferation of tumor cells initially. However, cellular velocity decreases as time progresses until $t = 50$ and becomes stagnant from $t = 60$ onward. The magnitude of velocity of the cellular phase at the left boundary is higher than that at the right boundary.

The velocity profile for the case $C_r = 0.25$ and $C_l = 1$ is displayed in Figure 2.9c. In this case, a minimal progression of the right boundary is spotted during the initial stage of tumor growth (i.e., for $0 < t \leq 5$). The velocity of the tumor cellular phase becomes zero at the right boundary $t = 20$ onward. Whereas the cellular phase velocity at the left boundary increases over time for $0 < t \leq 10$ and achieves a steady state for $t \geq 10$.

The case with $C_l = 1$ and $C_r = C_N = 0.1$ reflects the growth dynamics under a poor oxygen supply at the right boundary. This situation mimics an in-vivo situation when some part of the tumor is far from the blood vessels. The velocity profile of the cellular phase over time is displayed in Figure 2.9d. There is no movement of the tumor cellular phase near the right boundary. This is owing to the oxygen supply that remains below the threshold value of necrotic cell death. Whereas the velocity near the left boundary increases over time up to $t = 30$ and achieves steady-state when $t \geq 30$. So, it is concluded that the tumor grows under a sufficient oxygen supply, and the tumor growth could be significant near the blood vessels.

2.4.4 Effects of oxygen supply on diameter of a tumor

The diameter of a tumor is an important parameter for the tumor size and helps to identify the rate of its growth. The size of a tumor is used as a tool to recognize the stage of cancer, which is critical for the choice of treatment and predicting the patient's prognosis. In this subsection, the effects of oxygen supply on tumor size are investigated. The diameter is measured as the distance of the position of the right boundary to the left boundary.

The results on the effects of oxygen supply on the tumor diameter over time are shown in Figure 2.10. The oxygen concentration varies at the right boundary but is kept fixed ($C_l = 1$) at the left boundary. It is seen that the diameter of the tumor increases with the increase in oxygen concentration at the right boundary. It is concluded that the diameter of the tumor strongly depends on the oxygen supply through the surrounding medium. Tumor diameter is larger for the tumor having equal oxygen concentration at the boundaries compared to that for other choices of oxygen concentration at the right boundary.

2.5 Conclusions

In this study, we have investigated the effects of unequal oxygen supply at the tumor boundaries on its growth. It leads to asymmetric growth of the tumor. Due to the inherent asymmetry,

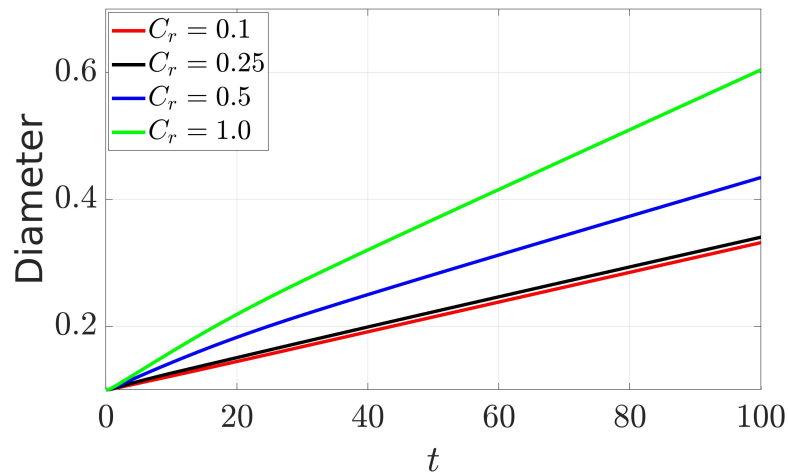


Figure 2.10: Propagation of diameter of a tumor with different oxygen supplies at the right boundary.

the pressure, cellular, and ECM velocities are coupled. To solve the model, a numerical method based on the Semi-Implicit Method for Pressure-Linked Equations (SIMPLE) framework is employed. The following key findings are observed from the simulated results.

1. If the concentration available at one boundary is lower than that at the other boundary, the tumor and its necrotic core grow asymmetrically about the tumor center. Also, the proliferating rims are of different widths at the edge of the tumor.
2. When a tumor grows with an unequal oxygen supply at tumor boundaries, the magnitude of cellular phase velocity also becomes unequal at the boundaries. Otherwise, it is the same for equal oxygen concentrations at the boundaries.
3. It is noticed that a tumor with a larger necrotic core grows slowly compared to a tumor containing a smaller necrotic core.



CHAPTER 3

EFFECTS OF UNEQUAL OXYGEN CONCENTRATIONS AT TUMOR BOUNDARIES ON LACTATE ACCUMULATION

Oxygen gradients inside a tumor develop a hypoxic region. The cells in the hypoxic region change their metabolic process from aerobic to anaerobic glycolysis. As a consequence, lactate is generated and diffuses out, acidifying the tumor microenvironment. Having the second objective underlined in Chapter 1, an attempt is made here to carry out a thorough study on the impacts of unequal oxygen concentrations at tumor boundaries on lactate accumulation through mathematical models.

3.1 Introduction

For glucose metabolism, tumor cells in the oxygen-rich region depend primarily on oxidative phosphorylation (OXPHOS), whereas cells in the hypoxic region of a tumor depend on anaerobic phosphorylation and generate lactate abundantly. As a result, hypoxic region is the primary source of lactate in tumor tissue [85, 115]. The oxygen concentration in the tissue is high in the vicinity of the oxygen source site (e.g., the local pre-existing blood vessel in the case of an avascular tumor) and gradually decreases with the diffusional distance from the oxygen source. Many mathematical models in existing literature tend to overlook crucial and realistic scenarios when it comes to the dynamics of tumor growth. These models generally make the simplifying assumption that tumor tissues uniformly maintain a constant oxygen concentration at its boundaries. Consequently, they have explored the intricate interplay of acidosis within tumors

and its impact on both tumor growth and the generation of metabolic waste [18, 98, 11, 106]. Fiandaca et al. [28] recently developed a mathematical model to study the effects of acidosis on tumor growth under unequal oxygen concentrations at the tumor boundaries and they assumed a lactate concentration threshold above which cell death occurs. However, as tumor biology advances, scientists have discovered that lactate is more than just a byproduct of glucose synthesis, and the role of lactate is not limited to healthy tissue damage. Instead, lactate acts as a metabolic fuel for well-oxygenated tumor cells or a signaling molecule for spreading network, as discussed in the articles [6, 41]. According to Sonveaux et al. [92], lactate is a major substrate that fuels the oxidative metabolism of oxygenated tumor cells. The relationship between key regulators of hypoxia and lactate production is established by Shabnam et al. [37]. The quantitative relationship between tumor metabolism and the function of transporter and enzymes is established by Q Wang et al. [105]. In literature, the interplay between oxygen and lactate for various sizes of *in vivo* and *in vitro* tumors remains to be explored.

In this chapter, we have proposed a one-dimensional mathematical model to understand the lactate and oxygen interplay under the assumption that lactate acts as an alternative fuel in the oxygen-rich region of a tumor for both *in vivo* and *in vitro* situations. This model comprises coupled steady-state reaction-diffusion equations for oxygen and lactate. It is solved analytically. The proposed model has the ability to compute the sizes of both pre-necrotic and fully developed tumors. Also, in the current study, the lactate and oxygen dynamics with various size of tumor are analyzed qualitatively. The effects of the presence of the necrotic core in tumor on lactate accumulation is examined.

3.2 Model formulation

3.2.1 Physical background

Oxygen is a pivotal component of cell respiration. Oxygen diffuses into the tissue domain from blood vessels. In case of an avascular tumor, the oxygen demand is fulfilled by the nearby blood vessels. As the tumor grows, oxygen shortage is sensed in the region away from blood vessels. In that region, tumors have a threshold value of oxygen concentration C'_H ; below that, tumor cells lose their proliferation ability but they remain viable. This region is known as hypoxic region (i.e., $C' \leq C'_H$, where C' represents oxygen concentration). For large tumors, oxygen concentration reaches a threshold value C'_N ; as a results, tumor cells become dead. In

a region, where $C' \leq C'_N$ is maintained, is known as the necrotic region. So, it can be assumed that oxygen is consumed by tumor cells that are present in a viable state. In the hypoxic region, glucose metabolism of tumor cells shifts toward anaerobic metabolism, producing lactate as the main byproduct. So, lactate can be considered as a pivotal contributor to the acidification of the tumor ecosystem. Tumor cells in well-oxygenated regions uptake that lactate as an alternative metabolic fuel. Though tumors are generally three-dimensional in shape, but in our study we have considered a one-dimensional model, which may be applied in a tumor looks like one-dimensional such as spinal-cord tumor. In order to model the above description on the piece of tumor tissue with length R (the tissue domain $[0, R]$, along x-axis), we have used system of coupled reaction-diffusion equations of oxygen and lactate in one dimensional cartesian coordinate. The schematic view of a one-dimensional *in vitro* tumor is given in Figure 3.1.

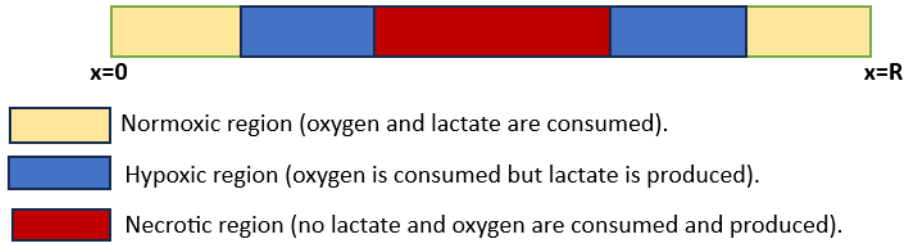


Figure 3.1: A typical one dimensional representation of a tumor.

3.2.2 Governing equations

In order to formulate mathematical model, we use a continuum mathematical modeling framework in one dimension to elucidate the interplay between oxygen and lactate for various sizes of *in vivo* and *in vitro* tumors. We do not consider the time evolution of oxygen and lactate in a fixed tumor tissue once they reach a steady state. Hence, the behavior of their respective concentrations is analyzed at steady state. Therefore, the governing equations are given as:

Oxygen diffusion equation: The steady-state oxygen transport is given as [55]

$$0 = D'_c \frac{d^2 C'}{dx'^2} - \gamma'_c C' H(C' - C'_N), \quad (3.1)$$

where C' is the oxygen concentration, D'_c is the diffusion coefficient and γ'_c is the rate at which the tumor cells consume oxygen. Last term on the right-hand side (RHS) of Eq. (3.1) incorporates the oxygen consumption that happens before oxygen concentration reaches the necrotic

threshold value C'_N . Because upon reaching C'_N , tumor cells are assumed to die. $H(x)$ is the Heaviside function that is defined as,

$$H(x') = \begin{cases} 1, & x' > 0, \\ 0, & \text{otherwise.} \end{cases}$$

Lactate diffusion equation: The steady-state lactate transport is given as

$$0 = D'_l \frac{d^2 L'}{dx'^2} + k' L' H(C' - C'_N) H(C'_H - C') - \gamma'_l L' H(C' - C'_H) + d' H(C' - C'_N), \quad (3.2)$$

where L' is the lactate concentration, D'_l is the diffusion coefficient. The second term on the RHS represents that lactate acid is produced by tumor cells in hypoxic region i.e, when oxygen concentration C' is in the range $C'_N \leq C' \leq C'_H$ only with the rate k' . The third term on the RHS of Eq. (3.2) denotes that lactate is consumed by the tumor cells in the rich-oxygen area (i.e., $C' \geq C'_H$) of the tumor with the rate γ'_l . Lactate can be produced even in aerobic conditions [14] and also through other means by live cells. The last term in the equation, represented by d' , accommodates this observation.

3.2.3 Boundary conditions

The boundary conditions of the model equations are guided by *in vitro* and *in vivo* circumstances.

For *in vivo* tumor: We consider that the primary blood vessel is situated near the left tumor boundary (at $x' = 0$), and oxygen enters into the solid tumor domain through this boundary. An oxygen gradient develops as we go from the blood vessel to the distant part of the tissue. Therefore, oxygen concentration at the tumor boundary near the blood vessel is higher than the other boundary far from the blood vessel, i.e., the right boundary at $x' = R$.

$$C'(0) = C'_l, \quad C'(R) = C'_r \quad \text{with } C'_l > C'_r \quad (3.3)$$

The lactate level at the tumor boundary near the blood vessel (at $x = 0$) is assumed to be constant, which signifies the normal physiological level outside the tumor region. Experimental studies have shown that lactate accumulates at the other end of the tumor (at $x = R$) due to insufficient drainage system [28]. This physical characteristic may be ideally mimicked by the “no flux” boundary condition at $x = R$.

$$L'(0) = L^*, \quad \frac{dL'}{dx'} = 0 \quad \text{at } x' = R, \quad (3.4)$$

where C'_l, C'_r are oxygen concentrations at left and right boundaries and L^* is the lactate concentration in blood vessel.

For *in vitro* tumor: When a tumor grows in an *in vitro* environment, an ample amount of oxygen is always present in the surrounding culture medium. Therefore, we fixed the oxygen concentration at both the tumor boundaries, i.e., at $x' = 0$ and $x' = R$.

$$C'(0) = C'(R) = C'_l, \quad (3.5)$$

We kept fixed lactate concentration in the culture medium, which can resemble an *in vitro* situation where the culture medium itself can neutralize or stabilize pH changes caused by lactate production [81, 66].

$$L'(0) = L'(R) = L^*. \quad (3.6)$$

3.3 Non-dimensionalized model

Let R be the length of tumor tissue, $\widehat{C}$ be the characteristic oxygen concentration, and $\widehat{L}_0$ be the characteristic lactate concentration which is same as normal physiologic value of lactate in blood. We use the 'prime' symbol to designate the dimensionless variables and parameters. So, the dimensionless variables are defined as,

$$x = \frac{x'}{R}, \quad C = \frac{C'}{\widehat{C}} \text{ and } L = \frac{L'}{\widehat{L}_0} \quad (3.7)$$

The parameters and variables are made dimensionless in the following way: $C'_H = C_H \widehat{C}$, $C'_N = C_N \widehat{C}$, $d = \frac{d' D'_c}{\gamma'_c D'_l L_0}$, $C_l = \frac{C'_l}{\widehat{C}}$, $C_r = \frac{C'_r}{\widehat{C}}$ and $\phi = R \sqrt{\frac{\gamma'_c}{D'_c}}$, where ϕ is the Thiele modulus. The Thiele modulus is a quantification of the relationship between the intrinsic rates of oxygen consumption and diffusion. It is important to recognize that in the idealized tumor tissue, where constant physical properties are assumed, the only variable in the Thiele Modulus definition is R , representing the length of the avascular tumor tissue.

The oxygen diffusion Eq. (2.6) in dimensionless form becomes,

$$0 = \frac{d^2 C}{dx^2} - \phi^2 C H(C - C_N). \quad (3.8)$$

The non-dimensional forms of oxygen concentrations at both the boundaries are given as,

$$\begin{aligned} C &= C_l \text{ at } x = 0, \\ C &= C_r \text{ at } x = 1. \end{aligned} \quad (3.9)$$

It should be noted that $\widehat{C} = C_l$ in the current study.

The non-dimensional form of lactate diffusion equation is given as,

$$0 = \frac{d^2 L}{dx^2} + \phi^2 \alpha L H(C - C_N) H(C_H - C) - \phi^2 \beta L H(C - C_H) + d\phi^2 H(C - C_N), \quad (3.10)$$

where $\alpha = \frac{D_c k}{D_l \gamma_c}$, $\beta = \frac{D_c \gamma_l}{D_l \gamma_c}$ are two non-dimensional numbers. Here, it is considered that $\widehat{L}_0 = L^*$. Hence, the corresponding boundary conditions are given as

$$L(x=0) = 1, \quad \frac{dL}{dx} = 0 \text{ at } x=1 \quad (\text{in vivo case}), \quad (3.11)$$

$$L(x=0) = L(x=1) = 1 \quad (\text{in vitro case}). \quad (3.12)$$

Table 3.1: Parameter values.

Parameter	Physical description	Dimensional value	Reference
D'_c	Oxygen diffusivity	$1820 \mu m^2 s^{-1}$	[3]
D'_l	Lactate diffusivity	$1100 \mu m^2 s^{-1}$	[76]
C'_H	Hypoxic threshold value	$5 mmHg$	[39]
C'_N	Necrotic threshold value	$0.2 - 0.5 mmHg$	[39]
γ'_c	Oxygen consumption rate	$0.5685 s^{-1}$	Estimated
γ'_l	Lactate uptake rate	$1.3715 s^{-1}$	Estimated
k'	Rate of lactate production in anaerobic glycolysis	$0.06322 s^{-1}$	Estimated
d'	Lactate produce by other than anaerobic glycolysis	$0.015 mM s^{-1}$	[77]
C'_l	Oxygen concentration near the blood vessel	$1.43 \times 10^{-2} mM$	[39]
$\widehat{L}_0$	Lactate in blood vessel in normal physiologic condition	$2 mM$	[53]

3.4 Analytical Solution

The governing equations are solved with the help of analytical methods. Typically, when a tumor reaches a size beyond a few millimeters, it undergoes spatial differentiation into three distinct zones: a normoxic region characterized by sufficient oxygen levels ($C \geq C_H$), a hypoxic

region with moderate oxygen deficiency ($C_N < C < C_H$), and a necrotic region experiencing severe hypoxia ($C \leq C_N$). At this stage, the tumor is considered fully developed. Assuming that ϕ (i.e., the length of tumor tissue R) is sufficiently large, the tumor is considered to be fully developed. Suppose $x_{H,l}$ and $x_{H,r}$ are the interface points of hypoxic region and nor-

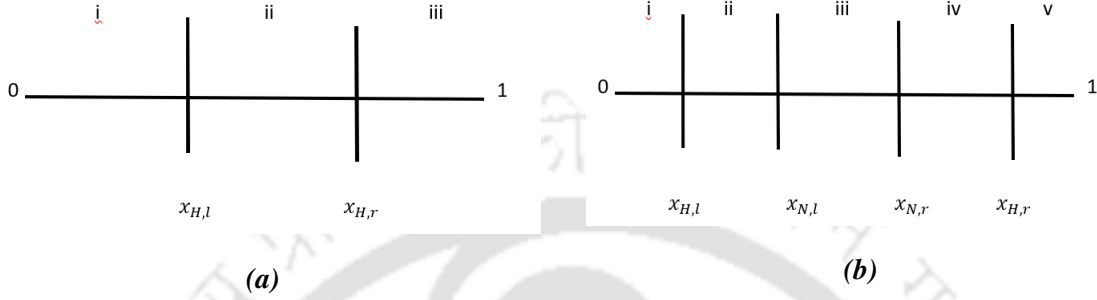


Figure 3.2: The schematic region of a tumor (a) having only hypoxic region, not necrotic core at center and (b) tumor have necrotic core and hypoxic region (fully developed tumor).

moxic region, and $x_{N,l}$ and $x_{N,r}$ are the interface points of necrotic region and hypoxic region respectively, where l denotes the left side and r denotes the right side point in Figure 3.2a-3.2b. The positions of these interface points (i.e., $x_{N,l}$, $x_{N,r}$, $x_{H,l}$, $x_{H,r}$) remain undetermined. It is assumed that oxygen is consumed by live tumor cells. The oxygen diffusion equation (3.8) for a fully developed tumor can be rewritten as,

$$\left. \begin{aligned} 0 &= \frac{d^2 C}{dx^2} - \phi^2 C, & x \in [0, x_{N,l}) \cup (x_{N,r}, 1], \\ 0 &= \frac{d^2 C}{dx^2}, & x \in [x_{N,l}, x_{N,r}]. \end{aligned} \right\} \quad (3.13)$$

Using boundary conditions $C(0) = C_l$, $C(1) = C_r$, $C(x_{N,l}) = C_N$ and $C(x_{N,r}) = C_N$, the solution of Eqs. (3.13) are given as,

$$C(x) = \begin{cases} \frac{1}{\sinh(\phi x_{N,l})} (C_l \sinh(\phi(x_{N,l} - x)) + C_N \sinh(\phi x)), & 0 \leq x < x_{N,l} \\ C_N, & x_{N,l} \leq x \leq x_{N,r} \\ \frac{1}{\sinh(\phi(1-x_{N,r}))} (C_r \sinh(\phi(x - x_{N,r})) + C_N \sinh(\phi(1-x))) & x_{N,r} < x \leq 1. \end{cases} \quad (3.14)$$

At the interface points $x_{N,l}, x_{N,r}$, it is assumed that C has continuous fluxes. Using this criterion in Eq. (3.14), we have

$$x_{N,l} = \frac{1}{\phi} \ln \left(\frac{C_l + \sqrt{C_l^2 - C_N^2}}{C_N} \right), \quad (3.15)$$

$$x_{N,r} = 1 - \frac{1}{\phi} \ln \left(\frac{C_r - \sqrt{C_r^2 - C_N^2}}{C_N} \right). \quad (3.16)$$

The question remains that what is the maximum value of ϕ for which tumor does not have any necrotic region i.e., oxygen concentration value attains C_N at a point in the tumor region. That means C has a minimum value C_N at a single point in $[0, 1]$.

Prior to the formation of necrotic region in the tumor, the corresponding oxygen concentration is given as,

$$C(x) = \frac{1}{\sinh(\phi)} (C_r \sinh(\phi x) + C_l \sinh(\phi(1-x))) \text{ in } x \in [0, 1]. \quad (3.17)$$

C in Eq. (3.17) has minimum value at

$$x_{min} = \frac{1}{2\phi} \ln \left(\frac{\frac{C_l}{C_r} e^{\phi} - 1}{1 - \frac{C_l}{C_r} e^{-\phi}} \right). \quad (3.18)$$

The minimum value of C can not exceed C_N . By solving $C(x_{min}) = C_N$, one can get the corresponding value of ϕ (says it is ϕ_{max}).

For an *in vivo* case ($C_l \neq C_r$), $C(x_{min}) = C_N$ becomes a transcendental equation of ϕ , which is given as,

$$\begin{aligned} AC_N^2 \cosh(3\phi) - (4C_l^2 + C_N^2(1 + A^2)) \cosh(2\phi) + (8C_l(C_r + 2AC_l) - AC_N^2) \cosh(\phi) = \\ 4C_l^2 + 2(C_r + 2AC_l)^2 - C_N^2(1 + A^2), \end{aligned} \quad (3.19)$$

where $A = \frac{C_l}{C_r}$. This equation is solved by Newton-Rapshon method to obtain the approximate values of ϕ_{max} .

For *in vitro* case ($C_l = C_r$), $x_{min} = \frac{1}{2}$ (from Eq. (3.18)) and the corresponding $\phi_{max} = 2 \cosh^{-1}(\frac{C_l}{C_N})$ (from Eq. (3.19)).

From these results, one can find that the maximum length R_{max} (from dimensionless Thiele modulus ϕ_{max}) of the tumor before formation of the necrotic core for both *in vivo* and *in vitro* cases if C_N, C_l and C_r are explicitly known.

The interface points $x_{H,l}$ and $x_{H,r}$ between normoxic and hypoxic regions can be easily obtained from Eqs. (3.14) and (3.17) by putting $C = C_H$ for a tumor before and after the formation of the necrotic core in an *in vivo* and *in vitro* situations.

Lactate dynamics: Here, lactate dynamics is examined within a tumor tissue for two situations (i) pre-necrotic and (ii) post-necrotic under *in vivo* and *in vitro* scenarios.

In the case (i), before tumor develops necrotic core (i.e., for $\phi \leq \phi_{max}$), tumor tissue can be categorized into three parts. The central part is occupied by hypoxic cells and it is surrounded by normoxic cells (Figure 3.2a). Hence, the governing equation (3.10) takes the form for this case as

$$\left. \begin{aligned} 0 &= \frac{d^2 L}{dx^2} - \phi^2 \beta L + d\phi^2, \quad \text{in } x \in [0, x_{H,l}) \cup (x_{H,r}, 1] \\ 0 &= \frac{d^2 L}{dx^2} + \phi^2 \alpha L + d\phi^2 \quad \text{in } x \in [x_{H,l}, x_{H,r}]. \end{aligned} \right\} \quad (3.20)$$

Its corresponding solutions are given as,

$$L(x) = \begin{cases} a_1 \cosh(\phi\sqrt{\beta}x) + a_2 \sinh(\phi\sqrt{\beta}x) + \frac{d}{\beta} & \text{in } x \in [0, x_{H,l}), \\ a_3 \cos(\phi\sqrt{\alpha}x) + a_4 \sin(\phi\sqrt{\alpha}x) - \frac{d}{\alpha} & \text{in } x \in [x_{H,l}, x_{H,r}], \\ a_5 \cosh(\phi\sqrt{\beta}x) + a_6 \sinh(\phi\sqrt{\beta}x) + \frac{d}{\beta} & \text{in } x \in (x_{H,r}, 1]. \end{cases} \quad (3.21)$$

The values of the coefficients a_i for $i = 1, \dots, 6$ are determined by ensuring the continuity of L and its fluxes at the interface points.

For the case (ii), when tumor is fully developed i.e., tumor has necrotic core at center and proliferative rim at the outer side and in between tumor has hypoxic cells ($\phi > \phi_{max}$) and lactate is generated by anaerobic glycolysis in regions ii,iv and it is consumed in regions i,v and not consumed and produced in region iii in Figure 3.2b. The lactate diffusion Eq. (3.10) is rewritten as

$$\left. \begin{aligned} 0 &= \frac{d^2 L}{dx^2} - \phi^2 \beta L + d\phi^2 \quad \text{in } x \in [0, x_{H,l}) \cup (x_{H,r}, 1], \\ 0 &= \frac{d^2 L}{dx^2} + \phi^2 \alpha L + d\phi^2 \quad \text{in } x \in [x_{H,l}, x_{N,l}) \cup (x_{N,r}, x_{H,r}], \\ 0 &= \frac{d^2 L}{dx^2} \quad \text{in } x \in [x_{N,l}, x_{N,r}]. \end{aligned} \right\} \quad (3.22)$$

The solutions of the Eqs. (3.22) are given by,

$$L(x) = \begin{cases} a_1 \cosh(\phi\sqrt{\beta}x) + a_2 \sinh(\phi\sqrt{\beta}x) + \frac{d}{\beta}, & \text{in } x \in [0, x_{H,l}) \\ a_3 \cos(\phi\sqrt{\alpha}x) + a_4 \sin(\phi\sqrt{\alpha}x) - \frac{d}{\alpha}, & \text{in } x \in [x_{H,l}, x_{N,l}) \\ a_5x + a_6, & \text{in } x \in [x_{N,l}, x_{N,r}] \\ a_7 \cos(\phi\sqrt{\alpha}x) + a_8 \sin(\phi\sqrt{\alpha}x) - \frac{d}{\alpha}, & \text{in } x \in (x_{N,r}, x_{H,r}] \\ a_9 \cosh(\phi\sqrt{\beta}x) + a_{10} \sinh(\phi\sqrt{\beta}x) + \frac{d}{\beta}, & \text{in } x \in (x_{H,r}, 1]. \end{cases} \quad (3.23)$$

At the interface points i.e., at $x = x_{H,l}, x_{H,r}, x_{N,l}, x_{N,r}$, L and its flux terms are continuous. Using this virtue and the boundary conditions ((3.4), (3.6)), the coefficients a_i 's (for $i = 1, 2, \dots, 10$) has been evaluated.

3.5 Results

3.5.1 Validation with experimental observations and parameter estimation

The parameters involved in the present model are not available in a single existing experimental study. Oxygen diffusivity, lactate diffusivity, consumption rates of nutrients and production rate of lactate may not be always constant rather these are dependent on cellular composition, temperature, tumor type, tumor location and time. For the sake of simplicity, we assume that these parameters are constant throughout the study and their values are taken from the existing literature listed in Table 3.1. These parameters are not available according to present model requirement, these parameters are estimated. Oxygen partial pressure near the blood vessel in *in vivo* situation remains constant and it equals to 13.5 mmHg [39]. According to Henry's law, oxygen partial pressure relates with oxygen concentration and its solubility coefficient can be expressed by a relation. The relation is oxygen concentration = oxygen partial pressure $\times$ solubility coefficient (here, solubility coefficient is 1.05×10^{-3} mM/mmHg). This gives an estimate of C_l' as 1.43×10^{-2} mM. To estimate the oxygen consumption rate of tumor cells, we compared the analytical solution in Eq. (3.17) with the spatial data of Helmlinger et al. [39] using the least squares fitting technique. Our model's results closely align with the experimental data (Figure 3.3). It gives Thiele modulus $\phi = 7.07$ and non-dimensional necrotic threshold value is 0.03488. It follows that $\gamma'_c = 0.5685$ s $^{-1}$ with $C'_N = 0.469$ mmHg. The

obtained C'_N is consistent with the experimental data provided in [39] for the necrotic threshold value of oxygen concentration.

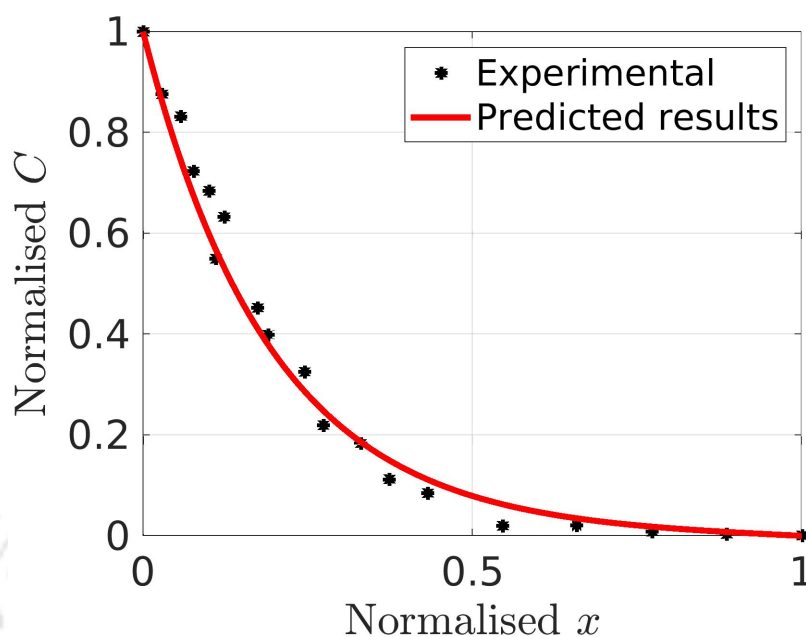


Figure 3.3: Comparison of present simulation results with the results of Helmlinger et al. [39].

Table 3.2: Non dimensional parameter values obtained using Table 3.1.

Parameter	value
C_H	0.37 – 0.74
C_N	0.0348
C_l	1
α	0.184
β	3.42
d	0.021
L^*	1

It may be noted that oxygen partial pressure in an *in vitro* situation is much higher than that in an *in vivo* situation. In an *in vitro* case, $C_l = C_r$ is maintained always. However, in an *in vivo* situation, C_r and C_l are not equal. Hence, C_r is treated as a model parameter in the current study to visualize the effects of oxygen gradient on lactate dynamics. Although the diffusion coefficient of oxygen and lactate and their consumption rates have distinct values in *in vivo* and *in vitro* settings, we keep the parameter values same for both *in vivo* and *in vitro* setups.

Because it will not impact the overall qualitative behaviour of the model, the non-dimensional values of parameters as shown in Table 3.2 are utilized for subsequent analysis.

3.5.2 Oxygen profiles of a tumor prior to the formation of a necrotic core in *in vitro* and *in vivo* settings

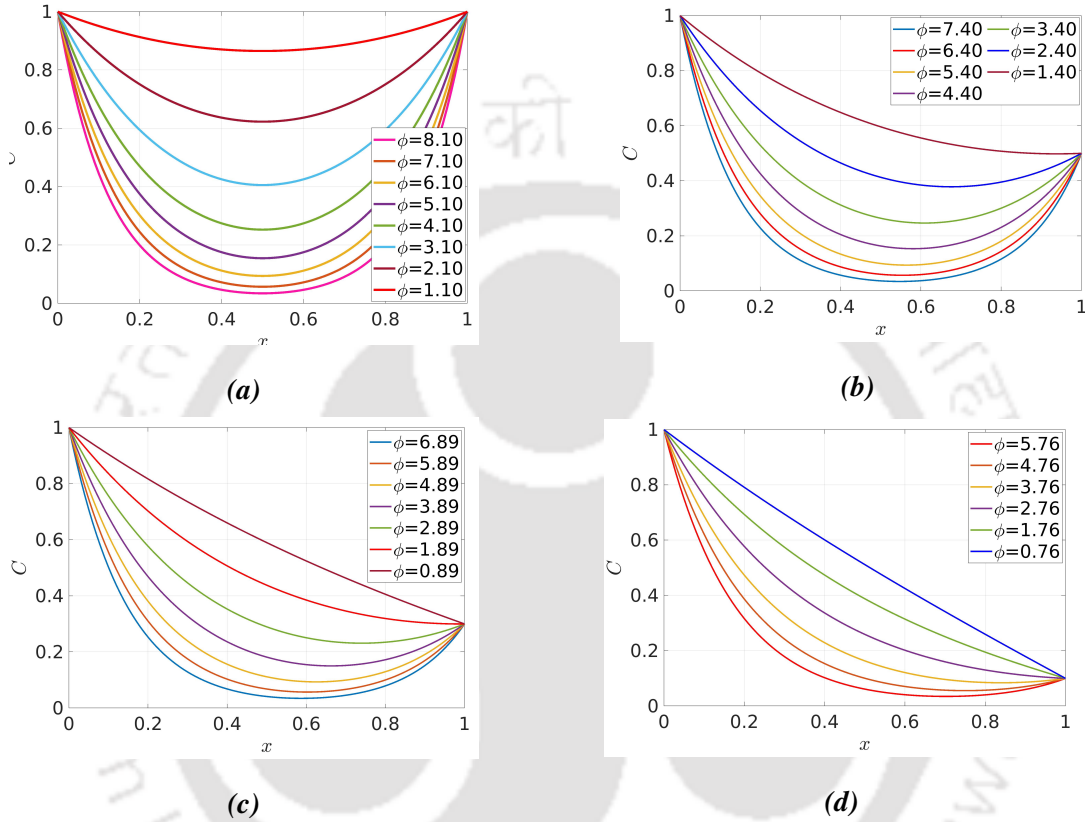


Figure 3.4: Oxygen concentration profile in pre-necrotic tumor for various values of ϕ ($\leq \phi_{max}$) with (a) $C_l = 1, C_r = 1$ and $\phi_{max} = 8.10$, (b) $C_l = 1, C_r = 0.5$ and $\phi_{max} = 7.40$, (c) $C_l = 1, C_r = 0.3$ and $\phi_{max} = 6.89$ and (d) $C_l = 1, C_r = 0.1$ and $\phi_{max} = 5.77$.

The tumor tissues' oxygen profiles prior to development of a necrotic core are depicted in Figure 3.4. It is seen that oxygen concentration falls with the increase in value of ϕ (i.e., tumor tissue length R). So, as the size of the tumor increases, the oxygen concentration starts to decrease inside the tumor. It happens because oxygen is consumed to maintain livelihood of tumor cells and mitotic cell division. It is observed that oxygen is distributed symmetrically about the center point $x = 0.5$ (Figure 3.4a). At this point, the oxygen concentration inside the tumor reaches the necrotic threshold value C_N for *in vitro* situation. However, for an *in vivo* situation, oxygen concentrations at the boundaries are not equal, and the corresponding oxygen

profiles are displayed in Figures 3.4b, 3.4c, and 3.4d. In these cases, the oxygen concentration profiles are no longer symmetric about $x = 0.5$. For an *in vivo* and *in vitro* setting, the tumor with entire viable cells, the corresponding Thiele Modulus (ϕ_{max} from Eq. (3.19)) and the points (x_{min} from Eq. (3.18)) where oxygen concentration attains the necrotic threshold value (C_N) are shown in the Table 3.3. From Table 3.3, one can conclude that the maximum size

Table 3.3: Thiele Modulus and the point where oxygen touches C_N .

Condition	Thiele Modulus (ϕ_{max})	x_{min}
$C_l = C_r = 1$	8.10	0.5
$C_l = 1, C_r = 0.5$	7.40	0.5468
$C_l = 1, C_r = 0.3$	6.89	0.5875
$C_l = 1, C_r = 0.1$	5.77	0.70

of the pre-necrotic tumor in an *in vitro* situation is larger than that in an *in vivo* situation. The point at which the oxygen concentration touches the necrotic threshold value shifts toward a lower oxygen edge.

3.5.3 Oxygen profiles after the formation of a necrotic core in tumor in *in vitro* and *in vivo* scenarios

In this section, our focus is on the effects of oxygen concentration at the tumor boundary on the generation of the necrotic core inside tumor. The tumor develops a necrotic core when Thiele Modulus (ϕ) crosses the value ϕ_{max} for the cases ($C_l = C_r = 1$; $C_l = 1, C_r = 0.5$, $C_l = 1, C_r = 0.3$ and $C_l = 1, C_r = 0.1$), which are available in Table 3.3. Figures 3.5a-3.5d portray the corresponding oxygen profiles. One can notice that the oxygen profile has a central plateau region in the tumor. It occurs because oxygen is not consumed at this plateau region as tumor cells die. The diameter of the necrotic core is found to be $|x_{N,r} - x_{N,l}|$ for the specific value of ϕ ($> \phi_{max}$), where $x_{N,l}$ and $x_{N,r}$ are the boundary points of the necrotic core, given by the Eqs. (3.15) and (3.16) respectively. The necrotic radius is calculated for the tumor under different oxygen concentrations at the right boundary to examine the effects of oxygen concentration on necrotic core formation which is shown in Table 3.4. It is concluded that the size of the necrotic core increases with the increase in the ratio of oxygen concentrations at the right and left boundaries (i.e., $\frac{C_r}{C_l}$). Hence, it hints that tumors have larger necrotic cores in an

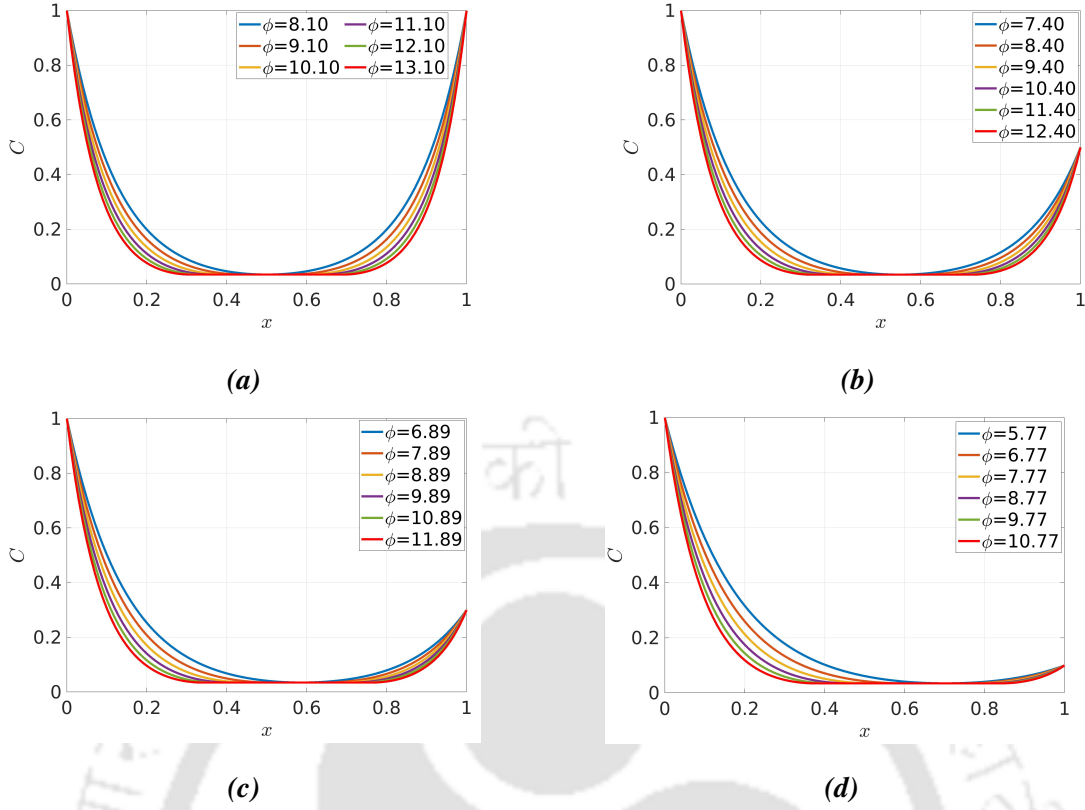


Figure 3.5: Oxygen concentration profile of tumor tissue having necrotic core for various values of ϕ ($\geq \phi_{max}$) with (a) $C_l = 1$ and $C_r = 1$, (b) $C_l = 1$ and $C_r = 0.5$, (c) $C_l = 1$, $C_r = 0.3$ and (d) $C_l = 1$, $C_r = 0.1$.

in vivo setting than that in an *in vitro* setting.

3.5.4 Lactate profile inside the tumor prior to the formation of a necrotic core

This section focuses on the investigation of lactate accumulation in tumors considering the effects of tumor size in situations like prior to the formation of a necrotic core and after the emergence of a hypoxic region. We calculate the minimum ϕ value at which tumor begins developing a hypoxic region as per the same evaluation process employed to obtain ϕ_{max} . By substituting C_H for C_N in Eq. (3.19), we determine the resulting value, denoted as ϕ_{min} . For the *in vitro* case ($C_l = C_r = 1$), $\phi_{min} = 3.3025$ and for a *in vivo* case ($C_l = 1$, $C_r = 0.5$), $\phi_{min} = 2.4667$. Hence, for $\phi_{min} < \phi < \phi_{max}$, tumor has no necrotic region. When the oxygen levels fall below the hypoxic threshold at the right boundary, tumor cells in the vicinity of right boundary no longer have access to a normoxic environment. This leads to the halt of lactate

Table 3.4: Diameter of necrotic core

For $C_l = C_r = 1$			
$\phi (> \phi_{max})$	$x_{N,l}$	$x_{N,r}$	$ x_{N,r} - x_{N,l} $
9	0.4501	0.5499	0.0998
10	0.4051	0.5949	0.1898
11	0.3683	0.6317	0.2635
12	0.3376	0.6624	0.3248
For $C_l = 1, C_r = 0.5$			
$\phi (> \phi_{max})$	$x_{N,l}$	$x_{N,r}$	$ x_{N,r} - x_{N,l} $
9	0.4501	0.6270	0.17689
10	0.40509	0.66431	0.25921
11	0.36827	0.69482	0.32655
12	0.33758	0.72026	0.38267
For $C_l = 1, C_r = 0.3$			
$\phi (> \phi_{max})$	$x_{N,l}$	$x_{N,r}$	$ x_{N,r} - x_{N,l} $
9	0.45011	0.68401	0.23389
10	0.40509	0.71560	0.31051
11	0.36827	0.74146	0.37319
12	0.33758	0.76301	0.42542
For $C_l = 1, C_r = 0.1$			
$\phi (> \phi_{max})$	$x_{N,l}$	$x_{N,r}$	$ x_{N,r} - x_{N,l} $
9	0.45011	0.80923	0.35911
10	0.40509	0.82830	0.42321
11	0.368271	0.843913	0.47564
12	0.33758	0.85692	0.51933

consumption and the onset of a site for lactate production. For the cases ($C_l = 1, C_r = 0.5$ and $C_l = 1, C_r = 0.1$), ϕ_{min} is zero. The lactate profile are displayed in Figure 3.6 for the *in vitro* and *in vivo* setups. It is observed that lactate is distributed symmetrically about the tumor center and it is consumed at both the ends of the tumor for an *in vitro* case (Figure 3.6a). Lactate starts to accumulate at central region with the increase in size of tumor i.e., size of hypoxic region. In Figures 3.6b, 3.6c, and 3.6d, lactate profiles are portrayed corresponding to *in vivo* situations of $C_l = 1, C_r = 0.5$; $C_l = 1, C_r = 0.3$ and $C_l = 1, C_r = 0.1$ respectively. Since, in *in*

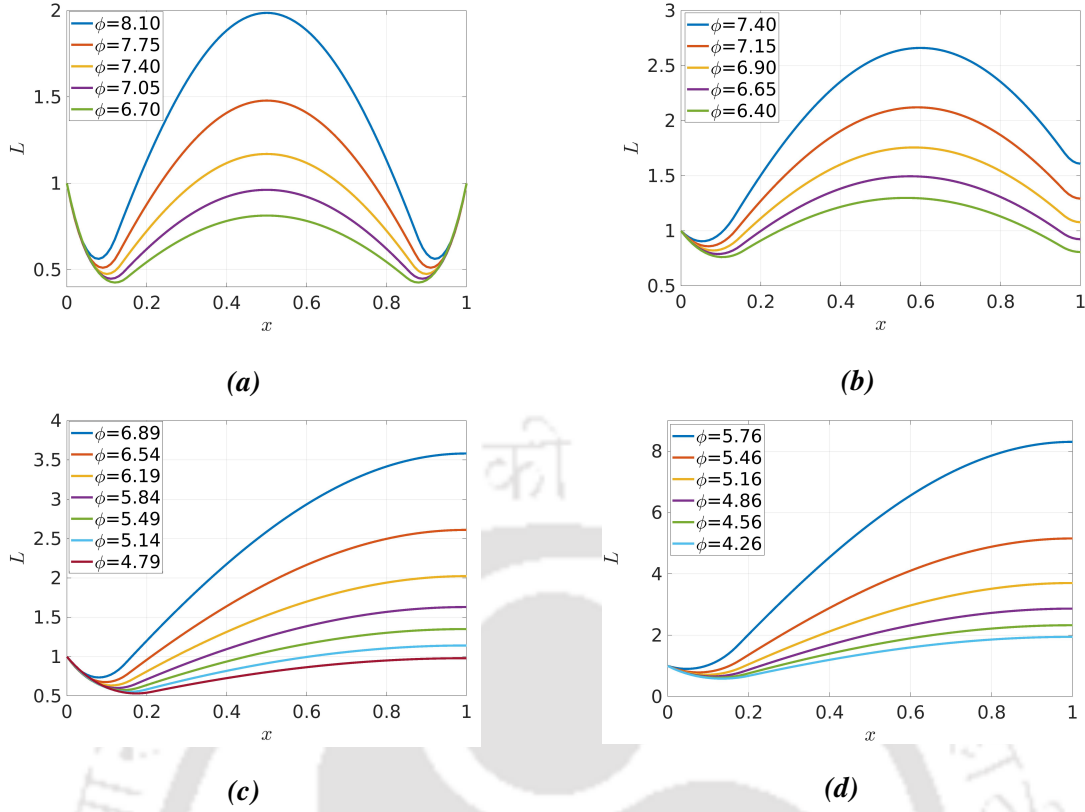


Figure 3.6: Lactate concentration profile of tumor tissue having necrotic core for various values of ϕ ($\leq \phi_{max}$) with (a) $C_l = 1$ and $C_r = 1$, (b) $C_l = 1$ and $C_r = 0.5$, (c) $C_l = 1$, $C_r = 0.3$ and (d) $C_l = 1$, $C_r = 0.1$.

in vivo situations, lactate is not flushed out at the end, which is situated far from blood vessel, lactate accumulation is observed at the boundary having low oxygen concentration compared with other end oxygen concentration.

From this section, one may conclude that lactate accumulation increases with the increase in oxygen gradient at the boundary.

3.5.5 Lactate profile after the formation of a necrotic core in tumor

In this section, we explore the lactate profile inside the tumor in the presence of a necrotic core. The tumor has a necrotic core when ϕ surpasses ϕ_{max} . After the initial formation of the necrotic core within the tumor, there is a noticeable decrease in lactate levels compared to the lactate profile observed when $\phi = \phi_{max}$. This is because the necrotic region does not produce lactate and lactate gets diffused in the necrotic region. When tumor size increases, hypoxic regions increase and produce enough lactate to refill the lactate level. As a result, the lactate

level rises. Lactate profiles are displayed in Figure 3.7. Interestingly, the impact of lactate consumption on lactate profile appears to be independent of the available lactate concentration in tumors characterized by oxygen concentrations of $C_l = 1$ and $C_r = 0.1$ at their boundaries (as illustrated in Figure 3.7d). This behavior is likely attributed to the substantial presence of lactate within the tumor microenvironment. But, for *in vitro* case ($C_l = C_r = 1$), the impact of lactate consumption by normoxic tumor cells on lactate profile inside the tumor is observed (Figure 3.7a). Also, for the *in vivo* cases ($C_l = 1$, $C_r = 0.5$ and $C_l = 1$, $C_r = 0.3$), lactate consumption effects is noticed (Figures 3.7b and 3.7c).

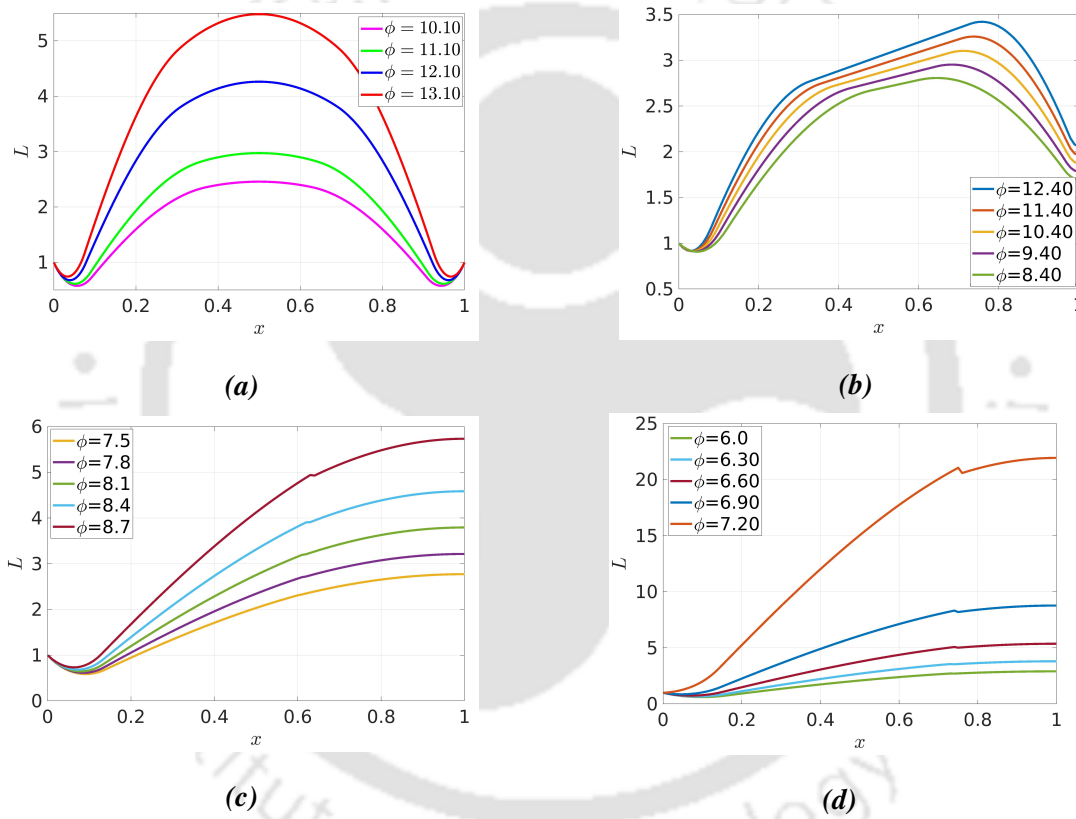


Figure 3.7: Lactate concentration profile of tumor tissue having necrotic core for various values of ϕ ($\geq \phi_{max}$) with (a) $C_l = 1$ and $C_r = 1$, (b) $C_l = 1$ and $C_r = 0.5$, (c) $C_l = 1$, $C_r = 0.3$ and (d) $C_l = 1$, $C_r = 0.1$.

From this section, it is concluded that during the initial stages of necrotic core formation, there is an influence of necrotic core on lactate accumulation within the tumor. As the process advances to later stages, the presence of necrotic core appears to have a diminished impact on the lactate status within the tumor.

3.6 Conclusions

In this chapter, we proposed a mathematical model to explore the lactate accumulation inside the tumor tissue. Probably for the first time, lactate that serves as an alternative fuel source in oxygen-rich environments is incorporated, encompassing both in *in vivo* and *in vitro* scenarios within a single mathematical model. The model is solved analytically. The diameter of necrotic core is also calculated analytically. The following conclusive remarks are drawn from the present study.

1. The size of necrotic core of a tumor in the *in vivo* scenario is smaller compared to that in the *in vitro* setup.
2. If the oxygen concentration at one boundary is less than that at the other boundary, it prompts the necrotic core to reposition itself towards the boundary with the lower oxygen concentration.
3. In *in vivo* environments, lactate accumulation constitutes a larger fraction compared to that in an *in vitro* setting.
4. In an *in vitro* setup, lactate accumulation is predominantly found at the tumor core, while in an *in vivo* environment, it shifts towards the boundary with lower oxygen concentration.
5. A sharp elevation in lactate concentration occurs at the right boundary where the oxygen concentration falls below the hypoxic threshold.
6. Some effects of presence of the necrotic core on lactate concentration is seen when the necrotic core starts to form. However, at a later stage, no significant impact is observed.

CHAPTER 4

IMPACT OF NONLINEAR OXYGEN-LACTATE INTERACTION ON TUMOR GROWTH IN CYCLIC HYPOXIA

In the previous chapter, oxygen and lactate consumptions were in a linear manner. However, scientific studies indicate that their consumptions are actually non-linear phenomena. Additionally, tumors may have complex initial shapes, and oxygen levels within the tumor microenvironment fluctuate over space and time. This chapter investigates the effects of various initial tumor shapes on lactate accumulation in response to the non-linear interaction between oxygen and lactate in a cyclic hypoxia environment. This aligns with the third objective of the thesis, as mentioned in Chapter 1.

4.1 Introduction

In the early, pre-angiogenesis stage, the tumor relies entirely on nearby blood vessels for oxygen and nutrients. At this stage, it is termed as an avascular tumor. Such a tumor is generally differentiated into three distinct layers: the necrotic layer, where cells die from lack of oxygen and nutrients; the normoxic layer, where cells receive sufficient resources to sustain rapid growth; and the hypoxic layer, situated between the necrotic and normoxic layers [49]. In the hypoxic layer, cells adapt by switching their metabolic pathways to favour a shorter energy-production process. Glucose primarily undergoes a series of chemical reactions to produce lactate and two units of adenosine triphosphate (ATP), enabling survival in low-oxygen condi-

tions [116]. This lactate is released from cells to the extracellular fluid portion of a tissue. As a result, the tumor microenvironment becomes acidic.

Many mathematical studies were devoted in exploring the tumor microenvironment (TME) impact on its growth. Casciari et al. [18] proposed a model to study the TME in one-dimensional framework on tumor progression. Webb et al. [2] elucidated the TME acidity using a dynamical system. Later, they also studied the impact of acidic TME on tumor growth. Venkatesubrahiam et al. [102] modeled energy production in tumor tissues using mathematical framework. Sonveaux et al. [92] experimentally showed that the tumor cells consume lactate as a metabolic fuel in normoxic areas. In Chapter 3, a mathematical model was proposed to understand how lactate status is influenced for *in vivo* and *in vitro* cases in an one-dimensional framework. In that work, the consumptions of lactate in normoxic areas and oxygen by live tumor cells were considered to be linear. However, these consumptions actually do not occur in a linear fashion [48].

Recent studies reported that multiple tumors may emerge in *in vivo* conditions [30, 43]. Dankbar et al. [23] investigated the effects of vascular endothelial growth factor and interleukin-6 on paracrine tumor-stromal cell interactions in the growth dynamics of multiple myeloma. Microenvironmental factors, such as hypoxia and acidification in the tumor microenvironment (TME), play important roles in tumor progression [31]. Therefore, understanding the growth patterns of multiple tumors that evolve over time in response to the non-linear interaction between lactate and oxygen is crucial.

Further, cyclic hypoxia is a common characteristic in solid tumor. It is the outcome of spatial and temporal oxygen fluctuations in a tumor site [64]. It has involvement in various events such as, vascular expansion and regression signaling loop, radiation therapy efficacy, and toxic cytokines production [22, 97]. Recently, Celora et al. [19] studied the effects of cyclic hypoxia on cell cycle progression using a mathematical modeling framework. The responses of cyclic hypoxia to characterising cancer cell type were also studied [20]. They found that same cell line can exhibit markedly different responses to cyclic hypoxia depending on the dynamics of the oxygen fluctuations. As oxygen level in a tumor plays a decisive role in the formation of a hypoxic region, lactate generation and cyclic hypoxia also have a significant role in lactate accumulation. Lactate is a key factor in tumor invasion, drug sensitivity, and immune suppression. Hence, the effects of cyclic hypoxia on lactate accumulation and on tumor volume are important events to be studied.

In this chapter, we propose a mathematical model to elucidate the tumor growth pattern in response to the non-linear interaction between oxygen and lactate under both steady and cyclic hypoxia environments. We use a system of coupled partial differential equations for oxygen, lactate concentration, and tumor cell density to describe the underlying biological phenomena. In this study, we examine the growth patterns of tumors having regular shapes like circular and elliptical, and also irregular shapes such as petal-like, under the non-linear interaction of oxygen and lactate. Additionally, we also investigate the growth patterns of multiple small tumors situated in a single tissue. We also decode the effects of cyclic hypoxia on tumor growth and on lactate status.

4.2 Mathematical model formulation

In this work, oxygen is the main element for cell respiration. The governing equation for oxygen is formulated using mass balance theory. The reaction-diffusion equation for oxygen is given as,

$$\frac{\partial C'}{\partial t'} = \nabla \cdot (D'_C \nabla C') - \mathcal{F}_C T', \quad (4.1)$$

where $C'(x', y'; t')$ represents concentration of oxygen (O_2), and D'_C is the corresponding diffusion coefficient. (x', y') and t' are the spatial position and time, respectively. $T'(x', y'; t')$ denotes the tumor's cellular density. The notation ∇ stands for $\hat{i} \frac{\partial}{\partial x'} + \hat{j} \frac{\partial}{\partial y'}$. $\mathcal{F}_C$ is the consumption term of oxygen, and it maintains Michaelis–Menten kinetics. It is given as,

$$\mathcal{F}_C = \gamma'_C \frac{C'}{K'_m + C'}, \quad (4.2)$$

where γ'_C is the maximal oxygen consumption rate, and K'_m is the oxygen concentration where half maximal consumption rate is attained.

Lactate is considered the only metabolic byproduct in this work. Lactate is produced by tumor cells in hypoxic areas and is consumed by normoxic tumor cells. The reaction-diffusion equation for lactate is given as,

$$\frac{\partial L'}{\partial t'} = \nabla \cdot (D'_L \nabla L') + \mathcal{F}_L T', \quad (4.3)$$

where $L'(x', y'; t')$ denotes concentration of lactate, and D'_L represents the diffusion coefficient for lactate. $\mathcal{F}_L$ denotes the lactate production and consumption. It is given as

$$\mathcal{F}_L = \mu'_L H(C' - C'_N) H(C'_H - C') - \gamma'_L \frac{L'}{K'_L + L'}, \quad (4.4)$$

where μ'_L is lactate production rate, and γ'_L is the maximum consumption rate of lactate. K'_L denotes the lactate concentration where half maximal consumption is achieved. C'_H and C'_N are the critical values of oxygen concentrations for cells in hypoxic and necrotic regions, respectively.

Tumor cells proliferate with the rate r'_T in logistic manner when the oxygen level is above the hypoxic threshold value (C'_H). Tumor cells die at lactate levels above a particular threshold value L'_N . Also, the tumor dies when the oxygen level falls below the critical level C'_N . Tumor cells may exhibit random diffusion with motility coefficient D'_T . These phenomena are assembled in mathematical form, and it is given as [28],

$$\begin{aligned} \frac{\partial T'}{\partial t'} = & D'_T \nabla^2 T' + r'_T T' \left(1 - \frac{T'}{K'}\right) H(C' - C'_H) \\ & - \lambda'_L L' T' H(L' - L'_N) - \lambda'_C T' H(C'_N - C'), \end{aligned} \quad (4.5)$$

where K' denotes the carrying capacity of the tumor.

Hence, the complete system of equations describing the interactions between the tumor cells, oxygen and lactate is:

$$\begin{aligned} \frac{\partial C'}{\partial t'} = & \nabla \cdot (D'_C \nabla C') - \gamma'_C \frac{C'}{K'_m + C'} T', \\ \frac{\partial L'}{\partial t'} = & \nabla \cdot (D'_L \nabla L') + (\mu'_L H(C' - C'_N) H(C'_H - C') - \gamma'_L \frac{L'}{K'_L + L'}) T', \\ \frac{\partial T'}{\partial t'} = & D'_T \nabla^2 T' + r'_T T' \left(1 - \frac{T'}{K'}\right) H(C' - C'_H) - \lambda'_L L' T' H(L' - L'_N) - \lambda'_C T' H(C'_N - C'). \end{aligned} \quad (4.6)$$

4.2.1 Initial and boundary conditions

To close the model, the appropriate initial and boundary conditions are posed without violating the physics of the problem. Here, we assume the domain of the problem as a square, say Ω . A tumor having initial density T'_0 is placed at the center of the domain with an initial shape of either circular, elliptical, or petal-like (Figure 4.1).

The initial conditions for oxygen and lactate concentrations are given as

$$C'(x', y'; 0) = C_\infty \text{ and } L'(x', y'; 0) = L_0, \text{ in } \Omega, \quad (4.7)$$

where C_∞ is the oxygen concentration, considered the normal physiological level, and L_0 is physiologic level of lactate concentration in normal tissue.

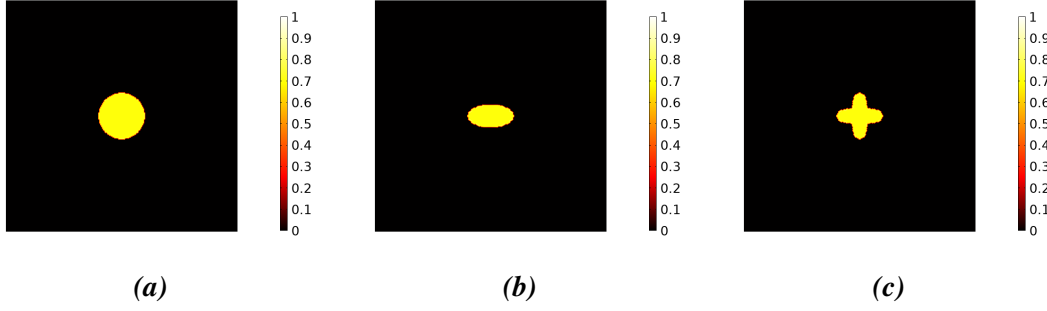


Figure 4.1: A tumor is situated at the center of the square domain. The initial shapes are displayed as (a) circular, (b) elliptic, and (c) petal with four wings.

The boundary conditions for the governing equations (4.1), (4.3), and (4.5) are, respectively,

$$C'(x', y'; t') = C_\infty, \nabla L' \cdot \hat{n} = 0, \text{ and } \nabla T' \cdot \hat{n} = 0 \text{ at } \delta\Omega, \quad (4.8)$$

where $\hat{n}$ denotes the unit outward normal to $\delta\Omega$, which is the boundary of the domain Ω .

4.2.2 Non-dimensional form

To convert the governing equations into a non-dimensional form, the characteristic values for the variables are chosen as follows: $R_m = 5 \text{ cm}$, representing the length equal to the tumor's maximal diameter in the laboratory setup; $C_\infty = 13.5 \text{ mmHg}$, representing the oxygen concentration; and L_0 , representing the lactate concentration at its normal physiological level [39]. The characteristic time is considered to be the reciprocal of the proliferation rate of tumor cells. Therefore, the non-dimensional variables are given as:

$$x = \frac{x'}{R_m}, y = \frac{y'}{R_m}, C = \frac{C'}{C_\infty}, L = \frac{L'}{L_0}, T = \frac{T'}{K'}, \text{ and } t = \frac{t'}{(1/r'_T)}. \quad (4.9)$$

The corresponding dimensionless parameters are obtained as

$$\begin{aligned} D_C &= \frac{D'_C}{r'_T R_m^2}, D_L = \frac{D'_L}{r'_T R_m^2}, D_T = \frac{D'_T}{r'_T R_m^2}, \gamma_C = \frac{\gamma'_C K'}{r'_T}, \gamma_L = \frac{\gamma'_L K'}{r'_T}, \\ \mu_L &= \frac{\mu'_L K'}{r'_T}, \lambda_L = \frac{\lambda'_L L_0}{r'_T}, \lambda_C = \frac{\lambda'_C}{r'_T}, K_m = \frac{K'_m}{C_\infty}, K_L = \frac{K'_L}{L_0}, C_H = \frac{C'_H}{C_\infty}, \\ C_N &= \frac{C'_N}{C_\infty}, L_N = \frac{L'_N}{L_0}, \text{ and } T_0 = \frac{T'_0}{K'}. \end{aligned} \quad (4.10)$$

Using these non-dimensional variables, the governing equations in (6.5) are given as

$$\frac{\partial C}{\partial t} = \nabla \cdot (D_C \nabla C) - \gamma_C \frac{C}{K_m + C} T, \quad (4.11)$$

$$\frac{\partial L}{\partial t} = \nabla \cdot (D_L \nabla L) + (\mu_L H(C - C_N) H(C_H - C) - \gamma_L \frac{L}{K_L + L}) T, \quad (4.12)$$

$$\frac{\partial T}{\partial t} = D_T \nabla^2 T + T(1 - T) H(C - C_H) - \lambda_L L T H(L - L_N) - \lambda_C T H(C_N - C). \quad (4.13)$$

The initial conditions are as follows:

$$C(x, y; 0) = 1, L(x, y; 0) = 1, \text{ and } T(x, y, 0) = \begin{cases} T_0, & \text{in } \mathcal{A} \\ 0, & \text{in } \Omega/\mathcal{A} \end{cases}, \quad (4.14)$$

where $\mathcal{A}$ denotes the space occupied by the tumor.

The boundary conditions are given as

$$C(x, y; t) = 1, \nabla L \cdot \hat{n} = 0, \text{ and } \nabla T \cdot \hat{n} = 0. \quad (4.15)$$

4.2.3 Parameter estimation

Before conducting the numerical simulations of the model, it is necessary to estimate the parameters involved in the model to represent the biological phenomena accurately. Oxygen diffusivity in the tumor tissue is $1820 \mu m^2 s^{-1}$ [18] and its consumption rate is $0.5685 s^{-1}$ [90]. The lactate diffusivity in tumor tissue is $1.77 \times 10^{-6} cm^2 s^{-1}$ [18]. Lactate consumption rate of tumor cells are estimated as the one third value of oxygen consumption rate, i.e., $\gamma_C = 3\gamma_L$ [62]. Lactate production rate (μ_L) was measured in the work of Papadogiorgaki et al. [71]. They predicted its value as $2.3 \times 10^{-11} mol cell^{-1} day^{-1}$. The other parameters values are taken from the article [90]. Using non-dimensional analysis, the non-dimensional parameters values are estimated and enlisted in the Table 4.1.

4.3 Results

In this section, the model is implemented to investigate the effects of non-linear interaction between oxygen and lactate on tumor growth in cyclic hypoxia environment. During the simulations, we considered the continuous approximation of the Heaviside function, which is given as [55]

$$H(x) = \frac{1}{2} \left(1 + \tanh\left(\frac{x}{\epsilon}\right) \right),$$

Table 4.1: Dimensionless parameter values

Parameter	Value	Source
D_c	0.864	Estimated
D_L	0.153	Estimated
D_T	4.32×10^{-5}	Estimated
C_H	0.45	[90]
C_N	0.1	[90]
K_m	0.5	[9]
γ_C	3.63	Estimated
γ_L	1.21	[71]
λ_L	0.125	[90]
λ_C	0.08	[90]
L_N	1.8	[90]
T_0	0.7	Assumed

where ϵ is sufficiently small. We choose $\epsilon = 10^{-2}$ in our study. The model is solved in the COMSOL Multiphysics 6.0 interface, which is based on the finite element methods. For simulation purposes, we consider a square domain of dimensions $[-l, l] \times [-l, l]$ with an unstructured mesh generated using the Delaunay Triangulation method, resulting in approximately 60,000 elements and around 1,000,000 degrees of freedom, with $l = 1$. Simulated results are carried out up to $t = 50$ using the parameters values in the Table 4.1. Note that for the sake of illustration, the results are presented in non-dimensional form that provide qualitative understanding of the inherent biology.

4.3.1 Experimental validation

The proposed model is validated against experimental data. The comparison is made for the oxygen concentration with the experimental data provided in Mueller-Klieser and Sutherland [68]. They measured oxygen status inside a large tumor using a non-invasive technique. The normalized C , which is the transversely averaged oxygen concentration, is defined as $\frac{1}{2l} \int_{-l}^l C(x, y; t) dy$, for the sake of comparison. The comparison is presented in the Figure 4.2a, demonstrating excellent agreement between the numerical results and the experimental data. We also compared the tumor volume obtained from the simulation with the experimentally ob-

served data from the study of Li et al. [56]. Experimental measurements of tumor volume were taken from two days after the initial day and continued up to twenty days. We calculated the tumor volume using the formula $\int \int_{\mathcal{R}} T(x, y, t) dx dy$, where $\mathcal{R}$ denotes the area of the tumor, which evolves with time. Figure 4.2b shows that the simulated results are in good agreement with the experimental data. These comparisons confirm the model's realism and reliability.

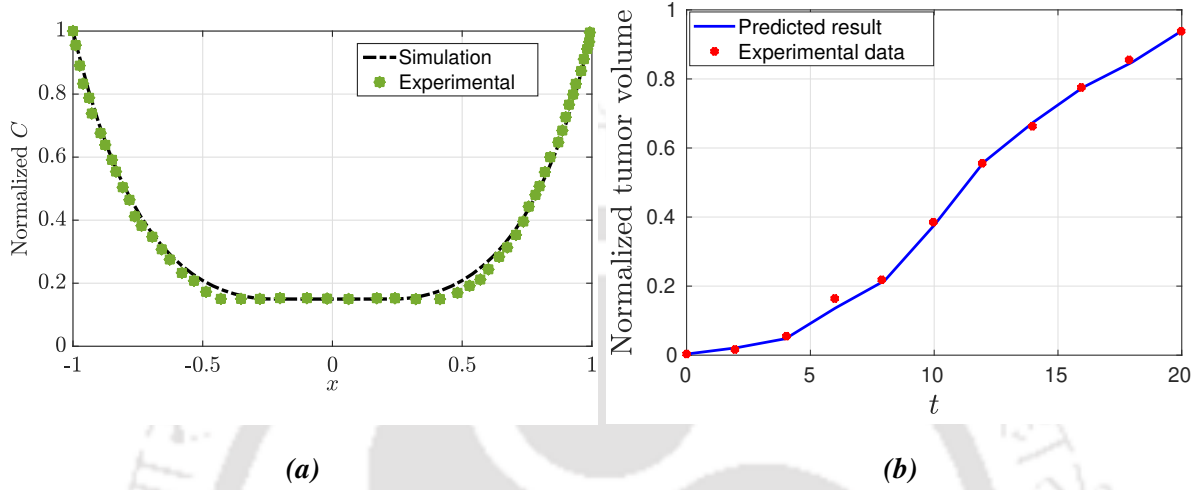


Figure 4.2: In (a), a comparison between the simulated oxygen concentration results and the experimental data obtained by Mueller-Klieser and Sutherland [68], whereas (b) shows a comparison between the simulated tumor volume and the experimental data from the study of Li et al. [56]. Both comparisons are based on normalized values, with the assumption that the tumor initially had a circular shape.

4.3.2 Pattern of tumor growth and lactate status when the initial shape of the tumor was circular

Here, the compact support of initial condition for tumor cell density is considered and is given as

$$T(x, y; 0) = \begin{cases} 0.7, & \text{in } \frac{x^2}{0.2^2} + \frac{y^2}{0.2^2} < 1 \subset \Omega \\ 0, & \text{otherwise} \end{cases}$$

Its pictorial representation is shown in the Figure 4.1a. In Figure 4.3, the oxygen concentration, lactate concentration, and tumor cell density are presented in the first, second, and third columns, respectively. The snapshots are taken at the time $t = 10, 20, 30, 40$ and 50 (correspond

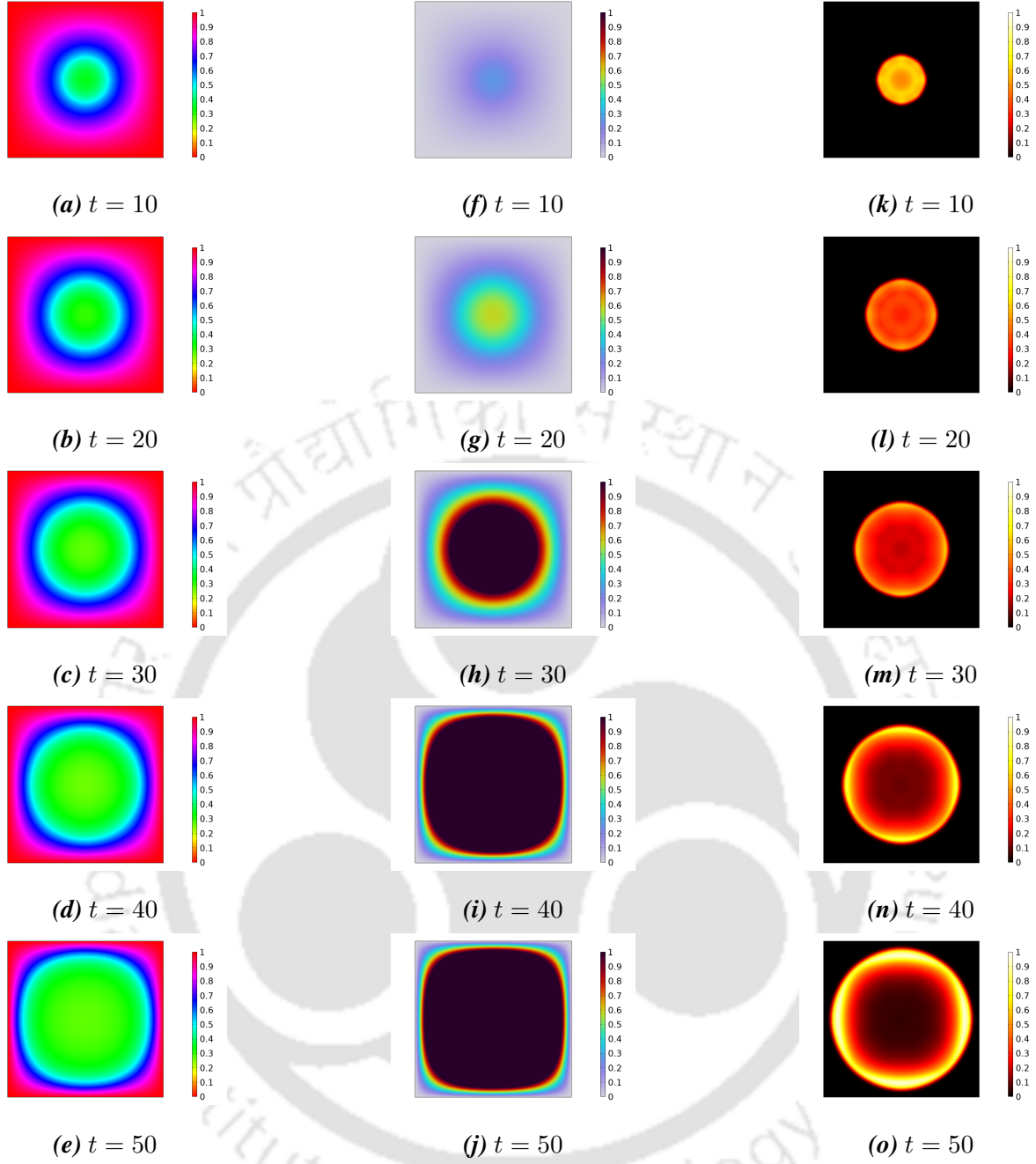


Figure 4.3: The snapshots are taken at the time $t = 10, 20, 30, 40$, and 50 for oxygen, lactate concentration, and tumor cell density for circular initial shaped tumor. The first column represents oxygen level, the second one for lactate levels, and the third column denotes the tumor cell density evolution. These simulation results are obtained using the parameters' values listed in the Table 4.1. Lactate concentration is normalized using its maximum value.

to dimensional time $t' = 180 \text{ hour}, 360 \text{ hour}, 540 \text{ hour}, 720 \text{ hour}$ and 900 hour). One may observe that oxygen concentration at the central part of the tumor is above the hypoxic threshold value C_H for $t = 10$ (Figure 4.3a). The corresponding tumor profile shows an increase in tumor

density as well as in size. However, as time progresses, oxygen level starts to decrease and falls below C_H (Figures 4.3b - 4.3e). As a result, lactate level upsurges in the tumor (Figures 4.3g - 4.3j). We observe that the area of lactate accumulation expands over time, reaching its maximum at $t = 50$. Tumor cell density spreads with time. Clearly, tumor cell density decreases in the time interval $10 \leq t \leq 20$ (Figures 4.3k - 4.3l). Subsequently, an increase in tumor cell density is observed, predominantly at the tumor's outer portion. It happens because tumor cells proliferate in well-nourished conditions, and lactate does not have enough levels, so they do not die due to lactate. This characteristic is also observed in the experimental setup and human tumors. One can note that the oxygen and lactate concentrations are not iso-concentric lines in shape (Figures 4.3e - 4.3j). It is because the organization of the proliferative layer inside the tumor is not in the iso-concentric circle, where oxygen is consumed at a higher rate. However, the overall tumor shape preserve its initial circular geometry (Figure 4.3o). Noted that, for a larger time, the tumor becomes a square shape, which is presumably the effect of the square boundary.

In this section, it is concluded that lactate status depends on oxygen heterogeneity inside the tumor and tumor cell density pattern is severely impacted by lactate concentration.

4.3.3 Pattern of tumor growth and lactate status when tumor had elliptical shape initially

In experimental design, tumor shapes are often chosen as common geometries, such as circular or elliptical, for growth studies. In this case, the tumor's initial shape is elliptical, and the corresponding initial condition is given as

$$T(x, y; 0) = \begin{cases} 0.7, & \text{in } \frac{x^2}{0.2^2} + \frac{y^2}{0.1^2} < 1 \subset \Omega \\ 0, & \text{otherwise.} \end{cases}$$

Its pictorial representation is displayed in Figure 4.1b. Here, we examine the growth dynamics of an initially elliptical-shaped tumor and its corresponding lactate distribution. The oxygen distribution, lactate status and tumor cell density pattern are portrayed in the first, second, and third columns of Figure 4.4. These snapshots are clicked at different time stages: $t = 10, 20, 30, 40$, and 50 . It is observed that the oxygenation status of the tumor did not change significantly compared to the previous case. In this case as well, oxygen levels fall below the hypoxic threshold, as shown in Figure 4.4b- 4.4e. However, the oxygen level is above the

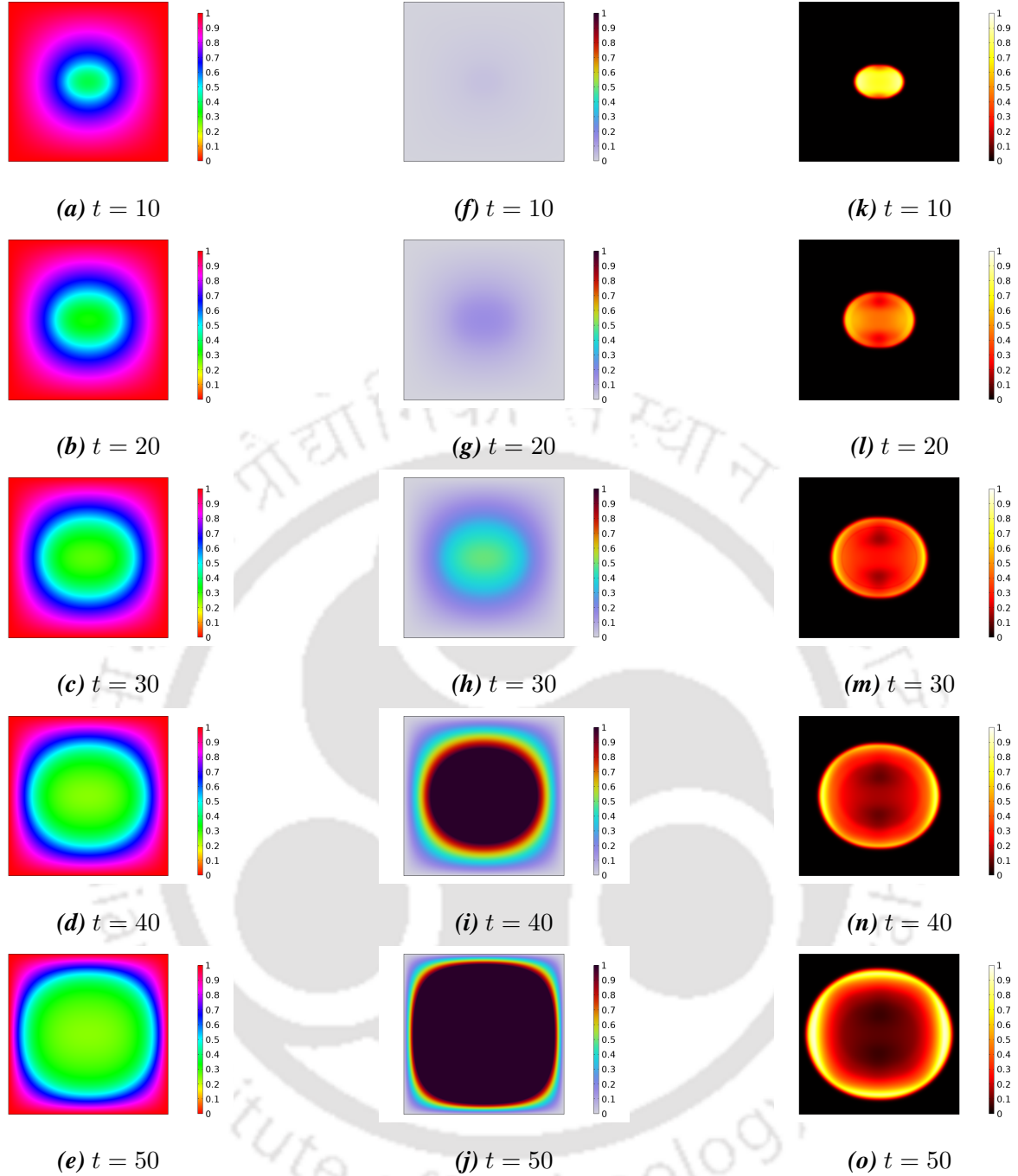


Figure 4.4: The snapshots are at the time $t = 10, 20, 30, 40$, and 50 for oxygen, lactate concentration and tumor cell density for elliptical initial shaped tumor. The first column represents oxygen level, the second column for lactate levels, and the third column denotes the tumor cell density evolution. These simulation results are obtained using the parameters' values listed in Table 4.1. Lactate concentration is normalized using its maximum value.

hypoxic threshold value at the time $t = 10$ (Figure 4.4a). It happens because the tumor size is small at that time. Oxygen levels are sufficient for cell proliferation throughout the entire tumor

(Figure 4.4k). Lactate concentration increases with time when $0 < t \leq 50$. A rapid increase in the area of lactate accumulation is observed in the time span $40 \leq t \leq 50$. The key qualitative difference between this case and the previous one is that, at $t=40$, the lactate accumulation area in the tumor with a circular initial shape is considerably larger than that in the tumor with an elliptical initial shape.

The evolution of tumor cell density with time is shown in Figures 4.4k - 4.4o. After the initial transient phase ($t > 10$), the proliferating rim is observed at the outer side of the tumor. The lactate level did not cross the critical value L_N up to $t = 30$. However, the lactate level crossed the critical value L_N for cells dead for acidification after $t = 40$ and 50 . As a result, tumor cells die. Hence, a large dead cell area is observed.

4.3.4 Impacts of petal shape tumor on its growth pattern and on lactate status

Tumor are not usually in regular shape, for example, tumors in breast, brain are irregular in shape. In this section, we have investigated the growth pattern of a petal-like initial shaped tumor. The initial distribution of tumor cell density is given as

$$T(x, y; 0) = \begin{cases} 0.7, & \text{in } \mathcal{A}, \\ 0, & \text{in } \Omega/\mathcal{A}, \end{cases}$$

where $\mathcal{A}$ denotes the petal and is governed by $r = a + b\cos(m\theta)$, here $(a + b)$ is the length of the petal and m is number of petal. In this simulation, we have considered $m = 4$, $a = 0.15$, and $b = 0.05$. The geometrical representation of initial shape of tumor is given in Figure 4.1c.

Figure 4.5 illustrates the tumor density, lactate levels and oxygen levels at $t = 10, 20, 30, 40$, and 50 . It is evident that tumor cell proliferation occurs at the petals during its growth, and eventually, these petals collide. Overall, the growth behaviours are similar to those observed in the last two cases; however, the morphological patterns are distinct, particularly for the necrotic core. Throughout the growth periods, lactate levels increase significantly, as the necrotic core is less prominent in the tumor's central region in this case. We believe that in a later stage, the necrotic core will become more pronounced and occupy a larger portion of the tumor.

Figure 4.6 displays the comparison of the time evolution of the total lactate concentration with tumor volume for initial circular, elliptic, and petal shapes. It is seen that total lactate concentration increases over time after the initial transition phase for every initial shape of

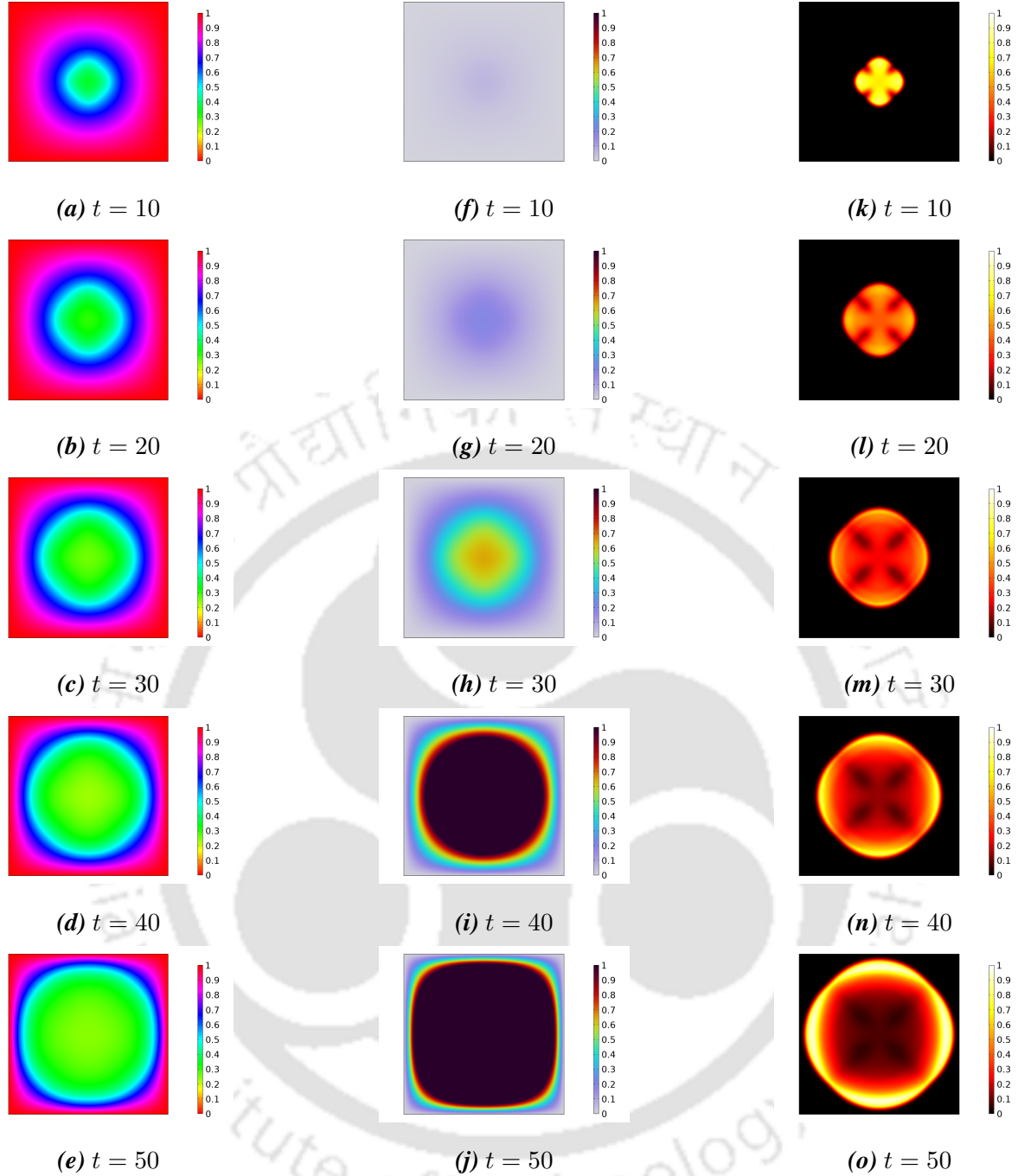


Figure 4.5: The snapshots are taken at the time $t = 10, 20, 30, 40$, and 50 for oxygen, lactate concentration, and tumor cell density for petal initial shaped tumor. The first column represents oxygen level, the second column for lactate levels, and the third column denotes the tumor cell density evolution. These simulation results are obtained using the parameter values listed in Table 4.1. Lactate concentration is normalized using its maximum value.

tumors (Figure 4.6a). Notably, the lactate levels increase in the following order: elliptic, petal, and circular shapes. It occurs because the initial circular-shaped tumor has a bigger surface

area compared to the areas of the other two tumors. Bigger surface area of a tumor allows the tumor to develop a large hypoxic region with time. The tumor microenvironment lactate level influences therapeutic efficacy, especially for chemotherapeutic drugs [93]. A high amount of lactate decreases the effectiveness of chemotherapeutic agents. Therefore, we expect that the tumor with an initial circular tumor shape may face more difficulty in achieving the optimum outcome of therapeutic drugs. In the context of tumor volume, one can see that tumor leads to

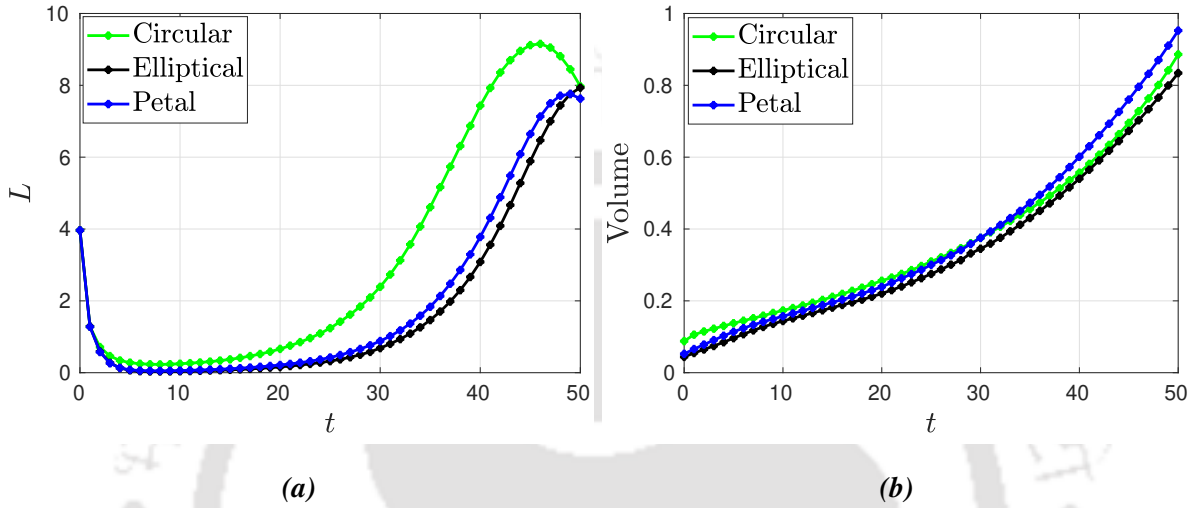


Figure 4.6: The time evolution of total lactate concentration (a) and tumor volume (b) for various initial tumor shapes, such as circular, elliptical, and petal-like.

a larger volume for initial petal-shaped tumor as it has a sustained proliferation.

4.3.5 Effects of cyclic hypoxia on tumor growth and on lactate status

Oxygen is not steadily delivered through tumor boundary to the *in vivo* tumor. Experimental investigations show that oxygen levels fluctuate over time due to alternating periods of deep hypoxia and moderate hypoxia, commonly associated with a hypoxia-reoxygenation pattern [64]. This phenomenon is termed as cyclic hypoxia. In this section, we investigated the growth patterns of tumors with circular, elliptical, and petal-like initial shapes under the cyclic hypoxia environment. Also, scientists noticed that the time scales of cyclic hypoxia have a wide range. In our simulation, we divide the total simulation time into sub-intervals of length t_p . During the first time sub-interval, oxygen concentration $C = 1$ is supplied through the boundaries, and in the next time sub-interval, the amount of supplied oxygen is reduced to $C = 0.4$. In the third and fourth time sub-intervals, the oxygen concentrations are again maintained at 1 and 0.4

respectively. This cycle continues, alternating the oxygen levels until the final time is reached. In this study, we choose $t_p = 5$ and 10. We observe that when oxygen switches from higher

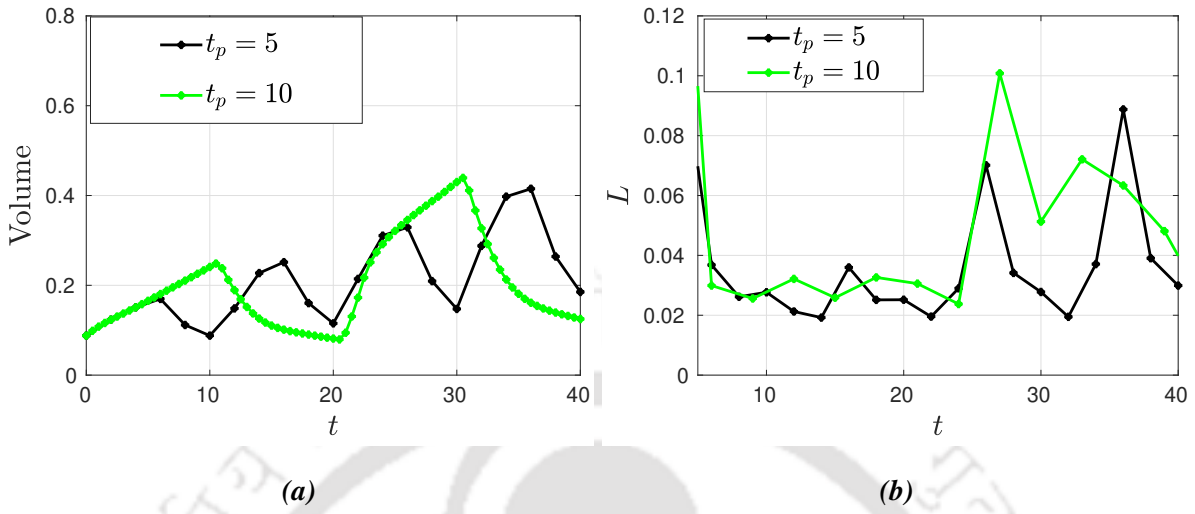


Figure 4.7: The time evolution of tumor volume (a) and total lactate concentration (b) under cyclic hypoxia. To obtain these results, we used a tumor with a circular initial shape in the simulation.

to lower level, tumor cells start to die, causing the tumor volume to shrink. However, when tumor is again reoxygenated, tumor regains its proliferate nature. It leads to increase in tumor volume when tumor shifts from hypoxic to reoxygenation phase (Figure 4.7a). Simulation results predict that lactate levels remain high when cyclic hypoxia is applied to a longer time periodicity (Figure 4.7b). It may occur when low oxygen levels are supplied to the tumor site, the entire tumor becomes hypoxic, leading to increased lactate production. Consequently, tumor cells die due to high lactate concentration. On the other hand, when supplied oxygen level is higher into the tumor site for a period of $t = 10$, the tumor also has a hypoxic region inside it, and a proliferation rim is also observed.

4.3.6 Pattern of tumor growth when multiple tumors are present in the tissue

Multiple tumors in a tissue are commonly seen in many carcinomas, such as liver, kidney, and many more. In this section, we model a case where five small tumors situated at the points $(0, 0)$, $(0.5, 0.5)$, $(-0.5, 0.5)$, $(-0.5, -0.5)$ and $(0.5, -0.5)$ are considered in the domain. The

corresponding initial distribution is given as

$$T(x, y; 0) = \begin{cases} 0.7, & \text{in } \mathcal{A}, \\ 0, & \text{in } \Omega/\mathcal{A}, \end{cases}$$

where $\mathcal{A} = \{(x, y) : \frac{x^2}{0.2^2} + \frac{y^2}{0.2^2} < 1\} \cup \{(x, y) : \frac{(x-0.5)^2}{0.2^2} + \frac{(y-0.5)^2}{0.2^2} < 1\} \cup \{(x, y) : \frac{(x+0.5)^2}{0.2^2} + \frac{(y-0.5)^2}{0.2^2} < 1\} \cup \{(x, y) : \frac{(x-0.5)^2}{0.2^2} + \frac{(y+0.5)^2}{0.2^2} < 1\} \cup \{(x, y) : \frac{(x+0.5)^2}{0.2^2} + \frac{(y+0.5)^2}{0.2^2} < 1\}$. It is

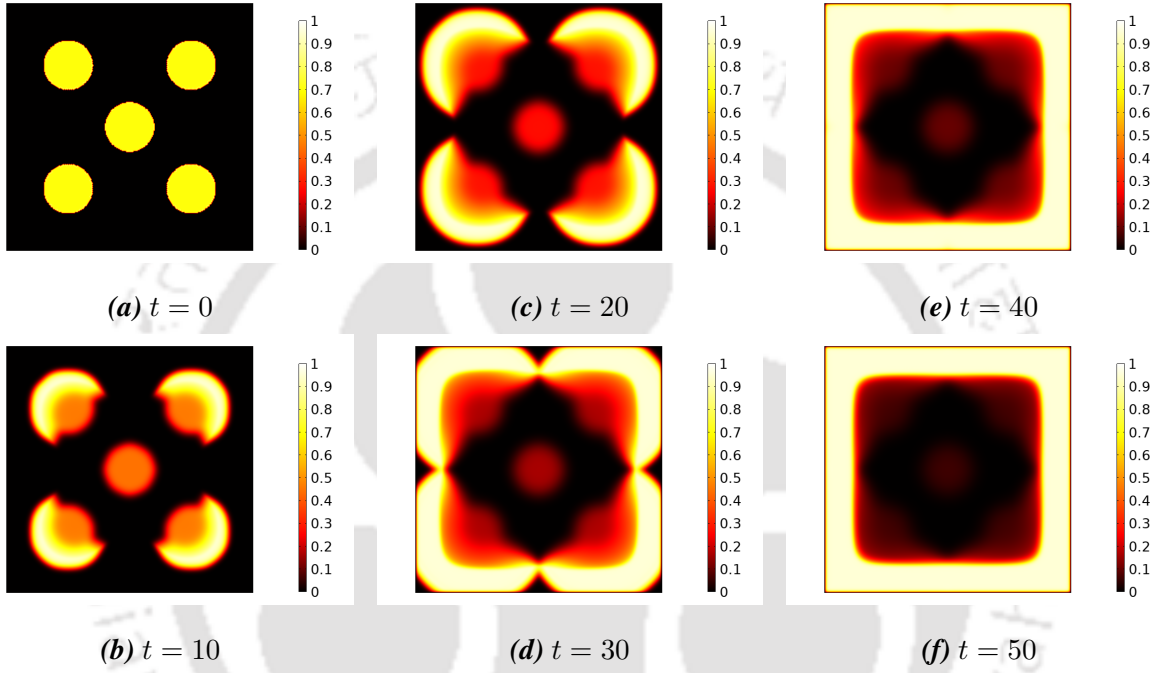


Figure 4.8: Snapshots were taken at times $t = 0, 10, 20, 30, 40$, and 50 to illustrate the evolution of tumor cell density for multiple tumors with an initially circular shape within a domain, under a constant oxygen supply from the boundary.

depicted in Figure 4.8a. The pattern of multi tumor growth is portrayed at $t = 10, 20, 30, 40$, and 50 (Figures 4.8b-4.8f). The shape of the outer tumors deviates from the original circular shape, and their size is larger than the central tumor. At $t = 10$ (Figure 4.8b), the outer tumors do not collide with each other, and the similar patterns are observed at $t = 20$. At $t = 20$, the tumor cell density in the central tumor decreases due to a reduction in oxygen levels caused by the consumption of oxygen by the outer tumors, which leads to cell death in the central tumor (Figure 4.8c). At a later stage, the outer tumors collide with each other and form a petal-like structure at $t = 30$ (Figure 4.8d). Over the time, the proliferation rim is observed only at the outer part of the domain, eventually merging into a single tumor (Figures 4.8e and 4.8f).

4.4 Conclusions

In this chapter, we proposed a mathematical model to study the tumor growth pattern for three different initial shapes, such as circular, elliptical and petal, under non-linear interaction between oxygen and lactate in a cyclic hypoxia environment. Additionally, we investigated the multiple tumors growth pattern in the same environment. In order to understand the underlying biological phenomena, we used a continuum mathematical modeling framework and simulated the proposed model using the finite element-based software COMSOL multiphysics 6.0. Simulation results indicate that the lactate level increases when the tumor has an initial circular shape, compared to tumors with other initial shapes. However, investigations show that the initial shape of the tumor does not affect the qualitative behaviour of tumor volume evolution. In the case of multiple circular tumors within the tissue, the growth pattern is significantly different from that of a single circular, elliptical or petal-shaped initial tumor. In the initial periods, the shapes of the multiple circular tumors do not remain intact, except for the central tumor. However, the central tumor maintains its initial shape through out its journey. It is noted that the multiple tumors are eventually merge into a single tumor. Additionally, we investigated the effects of cyclic hypoxia on tumor growth dynamics and lactate status in the tumor. Simulation results indicate that shorter cyclic hypoxia periodicity leads to an increase in tumor volume. However, for lactate concentration, numerical experiments reveal that higher cyclic hypoxia periodicity increases the tumor's lactate concentration. We also simulated the model for different shapes of initial tumors under a cyclic hypoxia environment. We observed similar qualitative behaviour for all different initial shaped tumors.



CHAPTER 5

EFFECTS OF EXOSOME ON TUMOR PROGRESSION IN ACIDIC ENVIRONMENT

So far, we have assumed that tumor cells proliferate based on the locally available oxygen concentration. However, tumor cells also secrete various growth factors to promote their growth, one of which is exosomes, a bag of lipid, protein and mRNAs. Exosomes increase the tumor cell proliferation rate. Experimental studies show that the rate of exosome secretion increases in an acidic tumor microenvironment. In this work, lactate is considered to be the main contributor to acidification in the tumor microenvironment. This chapter focuses on how exosomes affect tumor growth dynamics in an acidic tumor microenvironment and fulfills the fourth objective mentioned in Chapter 1.

5.1 Introduction

Lactate not only contributes to the acidic environment in the tumor, but it also serves as a growth factor, particularly promoting disease progression in terms of invasion and drug resistance [31]. Researchers have found that cancer cells secrete various soluble mediators in the tumor microenvironment. Exosome is one of them [113]. The exosome is a cargo of lipids, nucleic acids, and proteins, and it is released by all types of human cells, both in healthy and diseased conditions. The exosomes not only transport lipid nucleic acids, proteins, mRNAs, and microRNAs (miRs) but also activate many growth factors. It stimulates many regulatory signals of tumor growth, chemo-resistance, and the immune system failure. Recent evidence

reveals that tumor-derived exosomes have the ability to convert mesenchymal stem cells into tumor-like cells. Qu et al. [82] showed that exosomes participate in tumor cell proliferation. Moreover, exosomes initiate the reduction of apoptosis in cancer cells. [104, 51]. Also, exosomes can suppress the human body's immune system [32, 107]. Logozzi et al. [59] demonstrated a significant upsurge in exosome release under acidic conditions. Moreover, in an *in vitro* experiment, they found that buffering the medium substantially reduced exosome release across various cancer types. So, the tumor microenvironment controls the secretion of exosomes from tumor cells.

Recently, Lai and Friedman [51] investigated the roles of exosomes in tumor progression through a mathematical model that described the involvement of exosomal miRNAs (miRs) in early-stage non-small-cell lung cancer (NSCLC). Their research quantified the relationship between tumor volume and the total mass of miRs within the tumor. In follow-up studies, they further expanded their analysis to include colorectal cancer (CRC) [52]. Utilizing computational methods, Koomullil et al. [50] examined exosome transport within the tumor microenvironment (TME). It is well-established that low pH is a characteristic feature of the TME, resulting from the oxygen gradient present inside the tumor. Moreover, an acidic TME promotes the secretion of exosomes from tumor cells [59, 73]. However, the intrinsic relationship between exosomes and tumor growth is not fully understood.

In this chapter, we propose a mathematical model to examine the effects of acidic tumor microenvironment (TME)-driven exosome secretion and oxygen pressure on tumor growth. In this analysis, lactate is considered as a primary agent responsible for TME acidification. A coupled system of partial differential equations (PDEs) is employed to describe the concentrations of oxygen, lactate and exosomes as well as tumor cell density. This model decisively investigates the critical crosstalk between oxygen and exosomes, highlighting their significant role in driving tumor progression.

5.2 Mathematical model

We propose a mathematical model in a one-dimensional regime and consider a domain is $[0, R']$ along the x -axis, where $x' = 0$ is the center of the tumor. The spatial-temporal changes of oxygen ($C'(x', t')$), lactate ($L'(x', t')$), exosomes ($E'(x', t')$), tumor cell density ($T'(x', t')$) and dead cell density ($n'(x', t')$) are modeled using mass balance equation in $[0, R']$, where x'

denotes the spatial position and t' represents time.

5.2.1 Governing equations

Oxygen diffusion equation: The oxygen transport equation is given as,

$$\frac{\partial C'}{\partial t'} = D'_c \frac{\partial^2 C'}{\partial x'^2} - \gamma'_c T' \frac{C'}{K'_c + C'}, \quad (5.1)$$

where D'_c denotes the diffusion coefficient. The last term on the right-hand side (RHS) of Eq. (5.1) represents oxygen consumed by tumor cells in Michaelis-Menten (MM) manner with a rate γ'_c .

Lactate diffusion equation: The lactate is produced by those tumor cells that are present in the hypoxic region. The lactate transport equation is given as,

$$\frac{\partial L'}{\partial t'} = D'_l \frac{\partial^2 L'}{\partial x'^2} + k' T' L' H(C' - C'_N) H(C'_H - C') - \gamma'_l T' L' H(C' - C'_H), \quad (5.2)$$

where D'_l represents the diffusion coefficient of lactate, and C'_N is the necrotic threshold of oxygen concentration of tumor cells; below this concentration, the tumor cells will die. Tumor cells in the region where oxygen concentration lies in $C'_N < C' \leq C'_H$ produce lactate acid with rate k' , and it consumes by normoxic ($C' > C'_H$) tumor cells with a rate γ'_l . Where C'_H is the hypoxic threshold value, tumor cells sense oxygen shortage below this value.

Exosomes diffusion equation: Exosomes are soluble mediators and these are secreted by tumor cells in the tumor microenvironment. Here, we assume that exosome secretion increases with an increase in lactate in the tumor microenvironment (TME). It is modeled using MM kinetic formulation. Exosomes may degrade in many ways, such as phagocytosis and clearance through circulatory systems. However, we assume exosomes are decaying linearly irrespective of the manner of clearance. The following reaction diffusion equation governs the exosomes dynamics,

$$\frac{\partial E'}{\partial t'} = D'_e \frac{\partial^2 E'}{\partial x'^2} + \left(\lambda'_{En} + \delta'_E \frac{L'}{L'_{max} + L'} \right) T' - \mu'_e E', \quad (5.3)$$

where D'_e represents the diffusivity of exosomes. In the second term on the RHS, λ'_{En} denotes the constant rate of exosome secretion by tumor cells, and δ'_E represents the rate of exosome secretion that depends on lactate concentration in tumor microenvironment. The last term on the RHS presents the decay of exosome with a rate μ'_e .

The cancer cells dynamics equation: The tumor cell density dynamics is given as,

$$\frac{\partial T'}{\partial t'} = D'_T \frac{\partial^2 T'}{\partial x'^2} + \mathcal{S}(T', C', L', E'), \quad (5.4)$$

where T' represents the tumor cell density, D'_T denotes the diffusion coefficient of tumor cells, and $\mathcal{S}(T', C', L', E')$ characterizes the tumor cells' birth and death depend on oxygen (C'), lactate (L') and exosomes (E') concentrations. With an increased value of C' , cell proliferation increases. Cells start to die with an increased value of L' after the critical value L'_N . Also, exosomes contribute to the enhancement of tumor cell proliferation. Hence the function $\mathcal{S}(T', C', L', E', n')$ is defined as

$$\begin{aligned} \mathcal{S}(T', C', L', E', n') = & \left(\lambda'_c \frac{C'}{K'_c + C'} + \lambda'_e \frac{E'}{K'_e + E'} \right) T' \left(1 - \frac{T' + n'}{T'_m} \right) H(C' - C'_H) \\ & - \lambda'_l L' T' H(L' - L'_N) - \lambda'_d T' H(C'_N - C'), \end{aligned} \quad (5.5)$$

where we assume a logistic growth that depends on both oxygen and exosomes with rates λ'_c and λ'_e respectively, which is shown in the first term on RHS of Eq. (5.5). The second term on the RHS of Eq. (5.5) represents the tumor cells die due to lactate. The last term denotes the cells die when tumor cells face a heavy hypoxic environment, i.e., when $C' < C'_N$.

The dead cells dynamics equation: The dead material is commonly observed in the central part of the tumor. In this study, we consider that the dead material generated through lactate drives death and deaths due to oxygen shortage. We account that dead materials degrade regularly. The governing equation for dead cells density is given as,

$$\frac{\partial n'}{\partial t'} = \lambda'_l L' T' H(L' - L'_N) + \lambda'_d T' H(C'_N - C') - \mu'_d n'. \quad (5.6)$$

Hence, the complete system of equations describing the interaction between oxygen, lactate, exosome, tumor cells, and necrotic core is

$$\frac{\partial C'}{\partial t'} = D'_c \frac{\partial^2 C'}{\partial x'^2} - \gamma'_c T' \frac{C'}{K'_c + C'}, \quad (5.7)$$

$$\frac{\partial L'}{\partial t'} = D'_l \frac{\partial^2 L'}{\partial x'^2} + k' T' L' H(C' - C'_N) H(C'_H - C') - \gamma'_l T' L' H(C' - C'_H), \quad (5.8)$$

$$\frac{\partial E'}{\partial t'} = D'_e \frac{\partial^2 E'}{\partial x'^2} + \left(\lambda'_{En} + \delta'_E \frac{L'}{L'_{max} + L'} \right) T' - \mu'_e E', \quad (5.9)$$

$$\begin{aligned} \frac{\partial T'}{\partial t'} = & D'_T \frac{\partial^2 T'}{\partial x'^2} + \left(\lambda'_c \frac{C'}{K'_c + C} + \lambda'_e \frac{E'}{K'_e + E'} \right) T' \left(1 - \frac{T' + n'}{T'_m} \right) H(C' - C'_H) \\ & - \lambda'_l L' T' H(L' - L'_N) - \lambda'_d T' H(C'_N - C'), \end{aligned} \quad (5.10)$$

$$\frac{\partial n'}{\partial t'} = \lambda'_l L' T' H(L' - L'_N) + \lambda'_d T' H(C'_N - C') - \mu'_d n'. \quad (5.11)$$

5.2.2 Boundary and initial conditions

Eqs. (5.7)-(5.11) govern the dynamics of proposed model. To close the model, appropriate boundary and initial conditions need to be imposed. We assume symmetry in growing tumor and define coordinates such that $x' = 0$ is at the center of the tumor, where we impose symmetry conditions for oxygen concentration (C'), lactate concentration (L'), exosome concentration (E'), and tumor cell density (T'):

$$\frac{\partial T'(0, t')}{\partial x'} = \frac{\partial C'(0, t')}{\partial x'} = \frac{\partial L'(0, t')}{\partial x'} = \frac{\partial E'(0, t')}{\partial x'} = 0 \text{ for all } t' > 0, \quad (5.12)$$

We assume that lactate, exosome, and tumor cells are not leaving the domain $[0, R_m]$, whereas the oxygen concentration at $x = R'$ is equal to its value near the blood vessel..

$$\frac{\partial T'(R', t')}{\partial x'} = \frac{\partial L'(R', t')}{\partial x'} = \frac{\partial E'(R', t')}{\partial x'} = 0 \text{ and } C'(R', t') = C_\infty \text{ for all } t' > 0. \quad (5.13)$$

Initially, we assume that the oxygen level is the same as the physiological level of oxygen concentration at the blood vessel in the tumor region $[0, R']$. Hence, the initial condition is given as,

$$C'(x', 0) = C_\infty \text{ for } 0 \leq x' \leq R'. \quad (5.14)$$

A tumor emerges from genetically flawed cells for the *in vivo* scenario. On the contrary, some tumor cells are cultured in a petri dish for *in vitro* scenario to study the growth dynamics. Without loss of generality, tumor cells are uniformly distributed and considered in a small portion at the center of the domain. This type of initial condition is known as compact support initial condition, and it is given as

$$T'(x', 0) = \begin{cases} T'_0 & \text{in } [0, \alpha'], \\ 0 & \text{in } (\alpha, R']. \end{cases} \quad (5.15)$$

Initially, a very small fraction of cells are produced lactate in this tumor region and given as

$$L'(x', 0) = \begin{cases} L'_0 & \text{in } [0, \alpha'], \\ 0 & \text{in } (\alpha, R']. \end{cases} \quad (5.16)$$

The initial distribution of exosome is given as

$$E'(x', 0) = \begin{cases} E'_0 & \text{in } [0, \alpha'], \\ 0 & \text{in } (\alpha, R']. \end{cases} \quad (5.17)$$

where E_0 is the concentration of exosomes in the tumor tissue.

We assume initially, no dead cells were present in the tumor. It is given as

$$n'(x', 0) = 0. \quad (5.18)$$

5.2.3 Non-dimensionalization

We initially non-dimensionalized the equations in order to solve the problem numerically. Using the following reference parameters, the variables and parameters in the governing equations and the corresponding boundary conditions are non-dimensionalized.

- Reference time unit, $\hat{t}$ is the time which is required for mitotic cell division, i.e., $\hat{t} = \frac{1}{\lambda'_c}$. In literature, the estimated proliferation rate of tumor (λ'_c) is $4.95 \times 10^{-2} \text{ hour}^{-1}$ in well nourished condition [102, 103].
- The reference length scale is taken as $\hat{X} = \sqrt{\hat{t}D'_c}$, where the value of D'_c is $2 \times 10^{-9} \text{ m}^2\text{s}^{-1}$ [34, 68]. It leads to $\hat{X} = 1.2 \times 10^{-2}$.
- Reference tumor cell density T'_m , oxygen concentration C_∞ , lactate concentration L'_0 and exosome concentration E'_0 (where T'_m is maximum tumor cells capacity and its value $5 \times 10^8 \text{ cells cm}^{-3}$, C_∞ is the oxygen concentration is supplied at boundary. $L'_0 = 2 \text{ mM}$ and $E'_0 = 0.36 \times 10^{-3} \text{ mM}$ are the concentration in physiological condition).

Therefore, the non-dimensional variables are defined as,

$$x = \frac{x'}{\hat{X}}, C = \frac{C'}{C_\infty}, L = \frac{L'}{L'_0}, E = \frac{E'}{E'_0}, T = \frac{T'}{T'_m}, n = \frac{n'}{T'_m}, \text{ and } t = \frac{t'}{\hat{t}}. \quad (5.19)$$

The parameters and variables are made dimensionless in the following way: $D_l = \frac{D'_l}{D'_c}$, $D_e = \frac{D'_e}{D'_c}$, $D_T = \frac{D'_T}{D'_c}$, $R = \frac{R'}{\hat{X}}$, $C'_H = C_H C_\infty$, $C'_N = C_N C_\infty$, $\gamma_c = \frac{\gamma'_c T'_m}{C_\infty}$, $k = k' \hat{t} T'_m$, $\gamma_l = \gamma'_l \hat{t} T'_m$, $\lambda_{En} = \frac{\lambda'_{En} T'_m \hat{t}}{E'_0}$, $\delta_E = \frac{\delta'_E T'_m \hat{t}}{E'_0}$, $\mu_e = \mu'_e \hat{t}$, $\lambda_e = \lambda'_e \hat{t}$, $\lambda_l = \lambda'_l L'_0 \hat{t}$ and $\lambda_d = \lambda'_d \hat{t}$.

The dimensionless governing equations are given as,

$$\frac{\partial C}{\partial t} = \frac{\partial^2 C}{\partial x^2} - \gamma_c T \frac{C}{K_c + C}, \quad (5.20)$$

$$\frac{\partial L}{\partial t} = D_l \frac{\partial^2 L}{\partial x^2} + kTLH(C - C_N)H(C_H - C) - \gamma_l TLH(C - C_H), \quad (5.21)$$

$$\frac{\partial E}{\partial t} = D_e \frac{\partial^2 E}{\partial x^2} + \left(\lambda_{En} + \delta_E \frac{L}{L_{max} + L} \right) T - \mu_e E, \quad (5.22)$$

$$\begin{aligned} \frac{\partial T}{\partial t} = D_T \frac{\partial^2 T}{\partial x^2} + \left(\frac{C}{K_c + C} + \lambda_e \frac{E}{K_e + E} \right) T (1 - n - T) H(C - C_H) \\ - \lambda_l LTH(L - L_N) - \lambda_d TH(C_N - C), \end{aligned} \quad (5.23)$$

$$\frac{\partial n}{\partial t} = \lambda_l LTH(L - L_N) + \lambda_d TH(C_N - C) - \mu_d n. \quad (5.24)$$

5.2.4 Non-dimensional boundary and initial conditions

Boundary conditions:

$$\frac{\partial T(0, t)}{\partial x} = \frac{\partial C(0, t)}{\partial x} = \frac{\partial L(0, t)}{\partial x} = \frac{\partial E(0, t)}{\partial x} = 0 \text{ for all } t > 0 \quad (5.25)$$

and

$$\frac{\partial T(R, t)}{\partial x} = \frac{\partial L(R, t)}{\partial x} = \frac{\partial E(R, t)}{\partial x} = 0 \text{ and } C(R, t) = 1 \text{ for all } t > 0. \quad (5.26)$$

Initial conditions:

$$C(x, 0) = 1, \quad L(x, 0) = \begin{cases} 1 & \text{in } [0, \alpha], \\ 0 & \text{in } (\alpha, R] \end{cases}, \quad E(x, 0) = \begin{cases} 1 & \text{in } [0, \alpha], \\ 0 & \text{in } (\alpha, R] \end{cases}, \quad (5.27)$$

$$T(x, 0) = \begin{cases} T_0 & \text{in } [0, \alpha], \\ 0 & \text{in } (\alpha, R] \end{cases}, \text{ and } n(x, 0) = 0 \text{ in } [0, 1]. \quad (5.28)$$

5.2.5 Estimation of the parameters

Parameters' values in our model are primarily estimated from available experimental data. However, as a large number of parameters are involved for exosomes in a lactate environment, some remain unquantified. In cases, where experimental data are lacking, we prioritize the qualitative reproduction of observed system behaviors. For these parameters, values are chosen to match best the qualitative trends of the experimental results.

Estimation of diffusion coefficients D_l , D_e and D_T

For lactate diffusion coefficient D'_l , Casciari et al. [18] took values $1.77 \times 10^{-10} \text{ m}^2\text{s}^{-1}$. Whereas, Gatenby et al. [31] chose its value $5 \times 10^{-10} \text{ m}^2\text{s}^{-1}$. By dimensional analysis, the non-dimensional value of D_l lies between $0.0885 - 0.25$.

Lai and Friedman [51] took $1.23 \times 10^{-4} \text{ cm}^2\text{day}^{-1}$ as the value of exosome diffusion coefficient. So, in this study non-dimensional value for exosome diffusion (D_e) is 0.00007.

For diffusion coefficient of tumor cells, $8.64 \times 10^{-7} \text{ cm}^2\text{day}^{-1}$ is taken by Lai and Friedman [52] in their study. Bray [10] measured the animal cells random motility coefficient $5 \times 10^{-10} \text{ cm}^2\text{s}^{-1}$. In our study, the non-dimensional value of cells random motility coefficient (D_T) lies in the range $0.5 \times 10^{-6} - 2.5 \times 10^{-5}$.

Oxygen consumption (γ_c), lactate production (k) and consumption rate (γ_l)

Oxygen consumption rate (γ'_c) may depend on the cell line of the body parts. Various experimental studies reported its value in the range $2.5 \times 10^{-18} - 1 \times 10^{-15} \text{ mol cell}^{-1}\text{s}^{-1}$ [114, 102].

Lactate production rate (k') is measured in the work of Papadogiorgaki et al. [72]. They predicted that the value of it is $2.3 \times 10^{-11} \text{ mol cell}^{-1}\text{day}^{-1}$.

Lactate consumption rate of tumor cells estimated as the one third value of oxygen consumption rate i.e., $\gamma'_c = 3\gamma'_l$ [62].

Hence, by non-dimensional analysis, the dimensionless value of the parameters γ_c , k and γ_l are 3×10^3 , 2×10^2 and 10^3 , respectively.

Exosome secretions and degradation parameters

Riches et al. [86] measured that 10^6 cancer cells release 5×10^8 exosomes in 1 day in normal physiologic conditions. Therefore, $\frac{5 \times 10^2}{A} \text{ mol cell}^{-1}\text{day}^{-1}$ where $A = 6.022 \times 10^{23}$ is Avogadro number. Assuming one cell volume as 10^{-9} cm^3 . So, exosome secretion rate by tumor cells (λ'_{En}) is estimated as $8.3 \times 10^{-13} \text{ mol cell}^{-1}\text{cm}^3\text{day}^{-1}$. By non-dimensional analysis, the dimensionless λ_{En} is found to be 16.14.

Logozzi et al. [59] experimentally observed that the lactate environment uplifted the exosome secretion even by 100 folds of the secretion of tumor cells in normal physiologic conditions. Here, we take δ'_E is in the range $[2, 100]\lambda_{En}$.

Exosomes can be degraded via cancer cell uptake, loss through flow through the circulatory system, or normally eradicated. However, in our study, we assumed exosome degraded with

the constant rate (μ'_e) in an irrespective manner. Also, our model accounted for exosome-driven cell proliferation. So, its value can be chosen as a greater equal value of λ_e .

Oxygen critical values for hypoxic and necrotic

Oxygen concentration outside the domain is considered to be equal to the oxygen level at the blood vessel. So, its value (C_∞) is $1.43 \times 10^{-2} \text{ mM}$ [90]. The critical oxygen concentration value for hypoxia (C'_H) is 5 mmHg [92]. The necrotic threshold value (C'_N) lies between $0.2 - 0.5 \text{ mmHg}$ [90]. Hence, the non-dimensional of parameters C_H and C_N are 0.74 and 0.0348, respectively.

Tumor cells proliferation and death rate

Scientific studies showed that exosome enhances the proliferation rate of tumor cells [83]. To the best of our knowledge, the cell proliferation rate in the presence of tumor-derived exosome (λ_e) is not estimated.

For tumor cells death rate due to oxygen shortage (λ'_d), its value was estimated as $\frac{1}{10} \text{ day}^{-1}$ in the article [88].

Hence, by non-dimensional analysis, the dimensional less value of λ_c , λ_e and λ_d are found to be 1, $1.5 - 2$ and $\frac{1}{10}$, respectively.

Parameters K_c , K_e and L_{max}

The value of K'_c is given the value $0.28 \times 10^{-9} \text{ mol mm}^{-3}$ in [72]. Its non-dimensional value is 0.1 [106]. Values of the parameters L_{max} and K_e are not found in the literature. We choose the values of these parameters based on the right physics of the problem.

5.3 Numerical method

The computational tissue domain $[0, 1]$ was divided into $(N - 1)$ equal sub intervals $0 = x_0 < x_1 < x_2 < \dots < x_{i-1} < x_i < x_{i+1} < \dots < x_{N-2} < x_{N-1} = 1$ with uniform step length $\Delta x = \frac{1}{(N-1)}$. Δt was considered as the uniform time step length. Computation was carried out up to final time T_f with $n\Delta t = T_f$, where n was the number of time steps.

Table 5.1: Dimensionless parameter values.

Parameter	Value	Range values
D_l	0.15	0.0885 – 0.25
D_e	0.00007	—
D_n	0.5×10^{-6}	$0.5 \times 10^{-6} - 2.5 \times 10^{-5}$
γ_c	3	—
k	20.384	—
γ_l	10	—
λ_{En}	16.14	—
δ_E	$100\lambda_{En}$	$[2 - 120]\lambda_{En}$
μ_e	0.0001	—
C_H	0.5	—
C_N	0.0348	—
λ_e	0.2	0 – 0.5
λ_d	0.03	—
λ_d	0.02	—
K_c	0.1	—
L_{max}	10	—
K_e	5	—

Discretization of oxygen diffusion equation

Oxygen diffusion Eq. (5.20) was discretized using the central difference for spatial derivative and forward difference for temporal derivative. The discretized equation was obtained as,

$$-D_1 C_{i-1}^{n+1} + (1 + 2D_1) C_i^{n+1} - D_1 C_{i+1}^{n+1} = C_i^n - \gamma_c \Delta t C_i^n T_i^n, \quad (5.29)$$

where $i = 1, 2, 3, \dots, (N - 3)$ and $D_1 = \frac{\Delta t D_e}{\Delta x^2}$.

At the points $i = 1, (N - 2)$, Eq. (5.29) became,

$$(1 + 2D_1) C_0^{n+1} - 2D_1 C_1^{n+1} = C_0^n - \gamma_c \Delta t T_0^n C_0^n, \quad (5.30)$$

$$-D_1 C_{N-3}^{n+1} + (1 + 2D_1) C_{N-2}^{n+1} = D_1 + C_{N-2}^n - \gamma_c \Delta t T_{N-2}^n C_{N-2}^n. \quad (5.31)$$

Discretization of lactate diffusion equation

The lactate diffusion equation (5.21) was discretized as,

$$-D_2 L_{i-1}^{n+1} + (1 + 2D_2) L_i^{n+1} - D_2 L_{i+1}^{n+1} = L_i^n (1 + k \Delta t T_i^n H(C_i^n - C_H) H(C_N - C_i^n) - \gamma_l \Delta t T_i^n H(C_i^n - C_H)), \quad (5.32)$$

where $D_2 = \frac{D_l \Delta t}{\Delta x^2}$. Since, flux boundary conditions available at the boundary, i.e., at $i = 0$, using ghost point criteria, Eq. (5.21) became,

$$(1 + 2D_2) L_0^{n+1} - 2D_2 L_1^{n+1} = L_0^n (1 + k \Delta t T_0^n H(C_0^n - C_H) H(C_N - C_0^n) - \gamma_l \Delta t T_0^n H(C_0^n - C_H)). \quad (5.33)$$

Also, at the boundary $i = N - 1$, no flux condition is imposed. Using ghost point criteria, Eq. (5.21) became,

$$\begin{aligned} -2D_2 L_{N-2}^{n+1} + (1 + 2D_2) L_{N-1}^{n+1} &= L_{N-1}^n (1 + k \Delta t T_{N-1}^n H((C_{N-1}^n - C_H)(C_N - C_{N-1}^n)) \\ &\quad - \gamma_l \Delta t T_{N-1}^n H(C_{N-1}^n - C_H)). \end{aligned} \quad (5.34)$$

Discretization of exosome diffusion equation

The exosome diffusion equation (5.22) is discretized as follow,

$$-D_3 E_{i-1}^{n+1} + (1 + 2D_3) E_i^{n+1} - D_3 E_{i+1}^{n+1} = E_i^n + \Delta t \left(\lambda_{En} + \delta_e \frac{L_i^n}{L_{max} + L_i^n} \right) T_i^n - \Delta t \mu_e E_i^n, \quad (5.35)$$

where $D_3 = \frac{D_e \Delta t}{\Delta x^2}$ and $i = 1, 2, \dots, N - 2$. At the boundary nodes i.e., at $i = 0, N - 1$, Eq. (5.22) gave,

$$(1 + 2D_3) E_0^{n+1} - 2D_3 E_1^{n+1} = E_0^n + \Delta t \left(\lambda_{En} + \delta_e \frac{L_0^n}{L_{max} + L_0^n} \right) T_0^n - \Delta t \mu_e E_0^n, \quad (5.36)$$

$$-D_3 E_{N-2}^{n+1} + (1 + 2D_3) E_{N-1}^{n+1} = E_{N-1}^n + \Delta t \left(\lambda_{En} + \delta_e \frac{L_{N-1}^n}{L_{max} + L_{N-1}^n} \right) T_{N-1}^n - \Delta t \mu_e E_{N-1}^n, \quad (5.37)$$

Discretization of tumor cells density equation

The governing equation for the tumor cell density Eq. (5.23) was discretized as follows,

$$\begin{aligned}
& -D_4 T_{i-1}^{n+1} + (1 + 2D_4) T_i^{n+1} - D_4 T_{i+1}^{n+1} = \\
& T_i^n + \Delta t \left(\lambda_c \frac{C_i^n}{K_c + C_i^n} + \lambda_e \frac{E_i^n}{K_e + E_i^n} \right) T_i^n (1 - T_i^n - n_i^n) H(C_i^n - C_H) \\
& - \Delta t \lambda_l L_i^n T_i^n - \Delta t \lambda_d T_i^n H(C_N - C_i^n),
\end{aligned} \tag{5.38}$$

where $D_4 = \frac{D_T \Delta t}{\Delta x^2}$ and $i = 1, 2, \dots, N-2$.

At the boundary nodes i.e., $i = 0, N-1$, the discretized form of Eq. 5.23 was given as,

$$\begin{aligned}
(1 + 2D_4) T_0^{n+1} - 2D_4 T_1^{n+1} = & T_0^n + \Delta t \left(\lambda_c \frac{C_0^n}{K_c + C_0^n} + \lambda_e \frac{E_0^n}{K_e + E_0^n} \right) T_0^n (1 - T_0^n - n_0^n) H(C_0^n - C_H) \\
& - \Delta t \lambda_l L_0^n T_0^n - \Delta t \lambda_d T_0^n H(C_N - C_0^n),
\end{aligned} \tag{5.39}$$

$$\begin{aligned}
& -2D_4 T_{N-2}^{n+1} + (1 + 2D_4) T_{N-1}^{n+1} = T_{N-1}^n \\
& + \Delta t \left(\lambda_c \frac{C_{N-1}^n}{K_c + C_{N-1}^n} + \lambda_e \frac{E_{N-1}^n}{K_e + E_{N-1}^n} \right) T_{N-1}^n (1 - T_{N-1}^n - n_{N-1}^n) H(C_{N-1}^n - C_H) \\
& - \Delta t \lambda_l L_{N-1}^n T_{N-1}^n - \Delta t \lambda_d T_{N-1}^n H(C_N - C_{N-1}^n),
\end{aligned} \tag{5.40}$$

All the discretization equations led to a system of linear equations, which we solved using a tri-diagonal matrix algorithm (TDMA). Note that all spatial derivatives in the model are treated implicitly in time, which leads to an unconditionally stable scheme [78].

5.4 Results

To carried out the simulation, the parameters' values in Table 5.1 were used. The simulations carried out up to $T_f = 180$. We also compute the total tumor volume (TV), and the total exosome concentration (Exo) in the tumor medium using the following formulas:

$$TV(t) = \frac{1}{\xi} \int_0^\xi (T + n)(x, t) dx, \tag{5.41}$$

$$Exo(t) = \frac{1}{\xi} \int_0^\xi E(x, t) dx, \tag{5.42}$$

where ξ represents the tumor front, which varies with time t and satisfies the condition $T(\xi, t) + n(\xi, t) = \epsilon$. Here, ϵ is a sufficiently small quantity, and in this study, we take $\epsilon = 10^{-7}$.

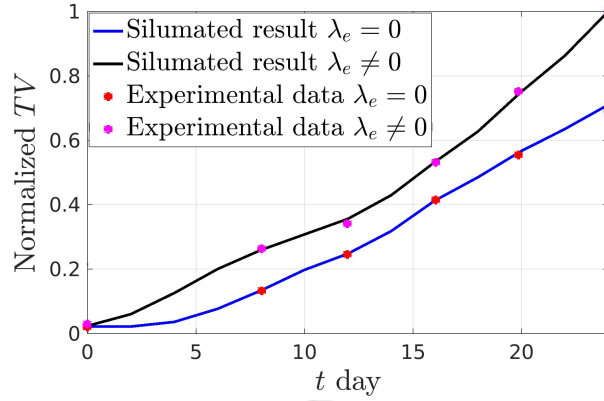


Figure 5.1: A comparison is presented between the computational results and experimental data from Luo et al. [60]. Black solid and blue solid lines represent the normalized TV for the cases with and without exosome effects, i.e., $\lambda_e \neq 0$ and $\lambda_e = 0$, respectively.

5.4.1 Experimental validation

The proposed model was validated against experimental data the study of Luo et al. [60]. In their research, they performed an *in vivo* experiment on mice to investigate the effects of exosomes on tumor progression. To conduct this study, they injected B16-F10 melanoma cell line into the mice. The tumor volume was measured from the eighth day after tumor seeding in the mice, continuing until 24 days, with measurements taken every 4 days. They observed that exosome rises tumor volume. Figure 5.1 shows the comparison between the experimental data and simulated data for tumor volume. The simulated results demonstrate a strong alignment with the experimental data, underscoring the model's validity and ensuring its practical relevance.

5.4.2 The crosstalk between oxygen and exosome in tumor microenvironment

Oxygen gradients within tumor regions influence the metabolic pathways of tumor cells. In hypoxic conditions, tumors favor anaerobic metabolism over aerobic metabolism to generate energy through glucose fermentation. This process leads to lactate production by tumor cells. Lactate, in turn, acts as an inflammatory factor, stimulating exosome secretion.

In this section, we explore the interplay between oxygen and exosome dynamics. A series of simulations was conducted to examine the impact of oxygen consumption rate (γ_c) on tumor volume and the corresponding exosome levels within the tumor. Figure 5.2a depicts the time evolution of total tumor volume for $\gamma_c = 1.5, 3, 6$ and 12 . The qualitative behaviors of tumor

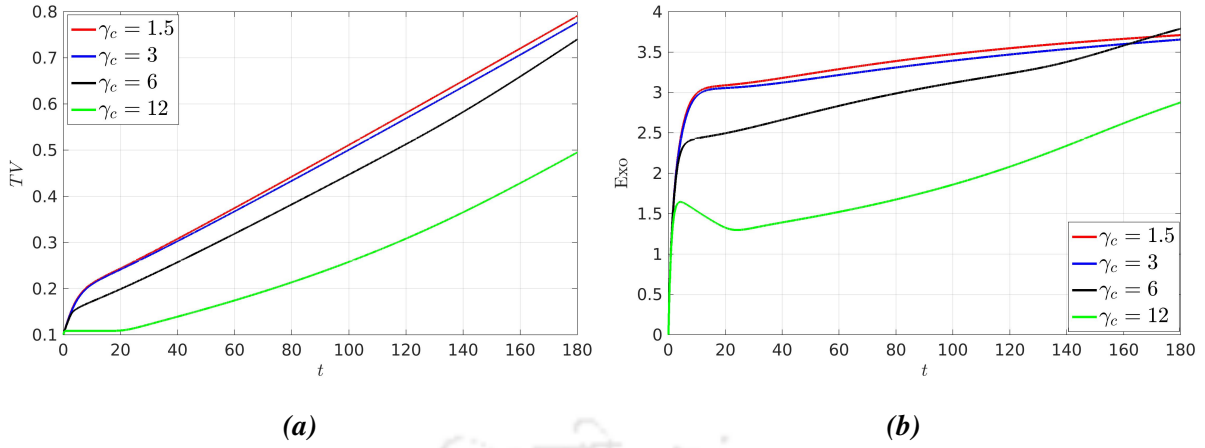


Figure 5.2: The time evolution of (a) tumor volume dynamics and (b) exosome levels in the TME are portrayed for different values of γ_c .

volume dynamics are similar for $\gamma_c = 1.5, 3$ and 6 . However, for $\gamma_c = 12$, a significant impact on tumor volume evolution is observed, with its behavior differing from the lower values of γ_c . Notably, the total tumor volume decreases as γ_c increases. A higher γ_c corresponds to greater oxygen consumption by the tumor, leading to oxygen depletion in the local environment. As a result, insufficient oxygen is available at the tumor site, reducing the proliferation rate. Figure 5.2b illustrates the time evolution of exosome levels in a tumor for different values of γ_c . Here, we observe that exosome level increases as γ_c decreases. This trend is similar to the tumor volume evolution observed for varying γ_c values. Significantly low amount of exosome gathers for high value of $\gamma_c = 12$. This may be due to the higher oxygen consumption rate, which slows tumor growth and results in a smaller tumor size. In a smaller tumor, the hypoxic region is also reduced, leading to lower lactate accumulation in the tumor microenvironment. Since lactate enhances exosome secretion, a smaller tumor size may result in lower exosome levels.

5.4.3 Effects of exosomes on the tumor growth

Exosomes act as a growth factor for tumors, enhancing their proliferation. In this section, the effects of exosomes on tumor growth dynamics are discussed. Figures 5.3 and 5.4 illustrate the spatial distributions of tumor cell density, necrotic cell density, and oxygen concentration at different time points ($t = 1, 10, 30, 60, 90, 120, 150$ and 180). Figures 5.3 represents the scenario where exosomes are not considered as a growth factor, and figure 5.4 depicts the results when exosomes are accounted in the model as a contributing factor to tumor cell proliferation. It is observed that the necrotic core begins to form in the tumor center and expands over time.

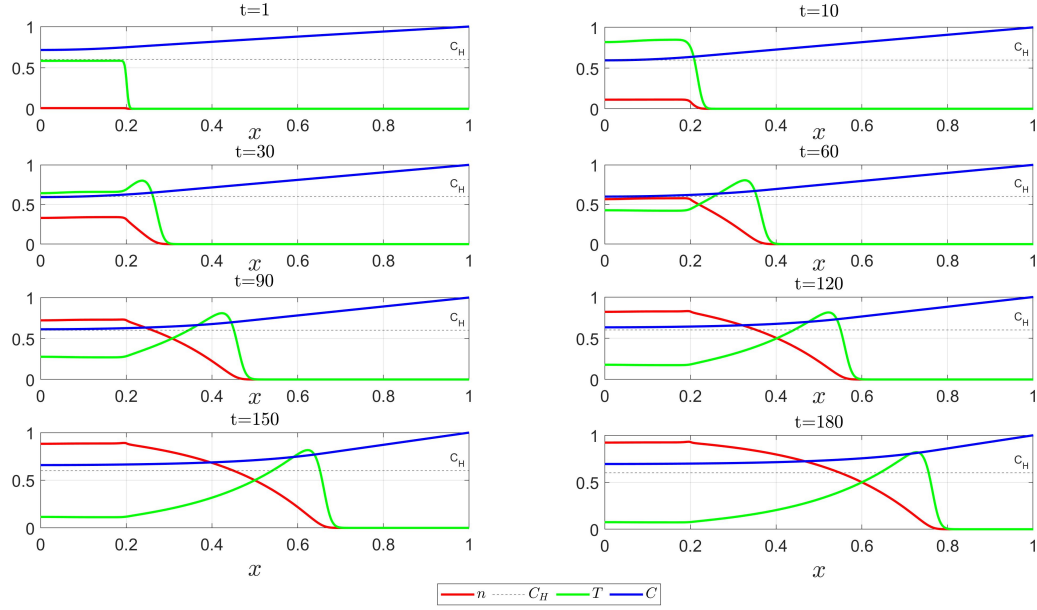


Figure 5.3: The spatial distribution of tumor cells, necrotic core densities, and oxygen concentration at $t = 1, 10, 30, 60, 90, 120, 150$, and 180 when exosomes are not considered as a growth factor

This necrosis develops due to a combined effects of cell death caused by high lactate levels and oxygen deprivation. Lactate accumulation increases as oxygen levels drop below the threshold value C_H , as shown in Figures 5.3 and 5.4. The spatial distribution of tumor cell density indicates that tumors are larger when the influence of exosomes is considered compared to the case where impact is ignored. This suggests that exosomes play a role in enhancing the aggressiveness of tumor growth. The propagation velocities of the tumor front over time, with ($\lambda_e \neq 0$) and without ($\lambda_e = 0$) exosome effects, are displayed in Figure 5.5. Here, v is estimated using the formula $v(t) \approx \frac{\xi(t+\tau) - \xi(t)}{\tau}$, where τ is the time gap between two successive measurements of the position ξ . It is observed that the tumor propagates rapidly for the case $\lambda_e \neq 0$ compared to the case $\lambda_e = 0$. During the initial transit period, the tumor grows almost at the same speed for both the cases. In this transit period, exosome levels in the tumor medium are insufficient to enhance tumor cell proliferation.

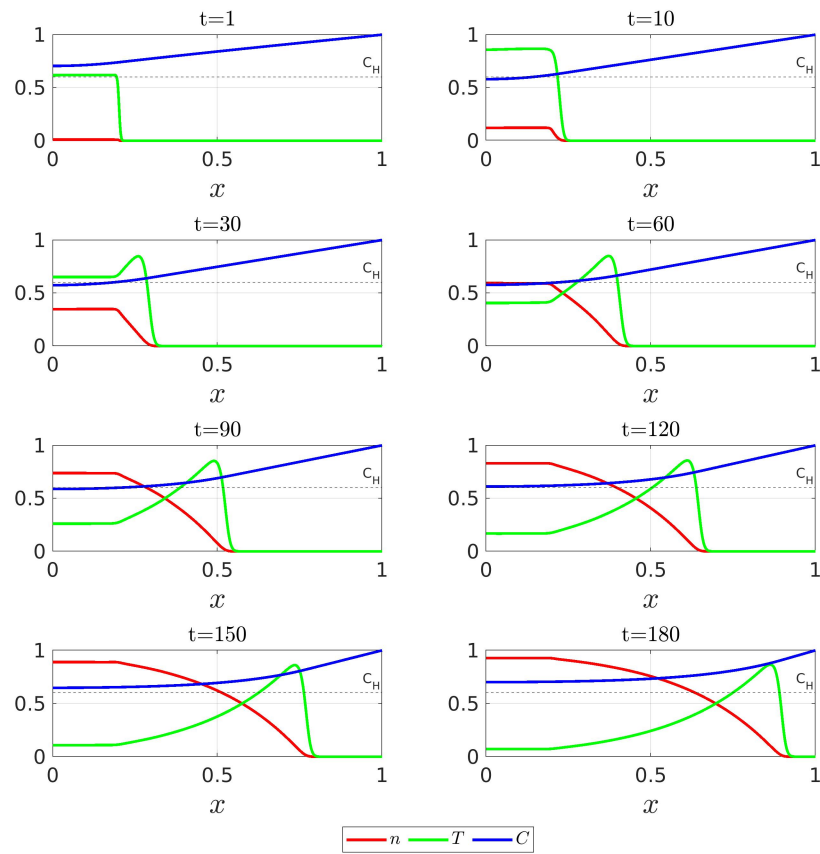


Figure 5.4: The spatial distribution of tumor cells, necrotic core densities, and oxygen concentration at $t = 1, 10, 30, 60, 90, 120, 150$, and 180 when exosomes are considered as a growth factor.

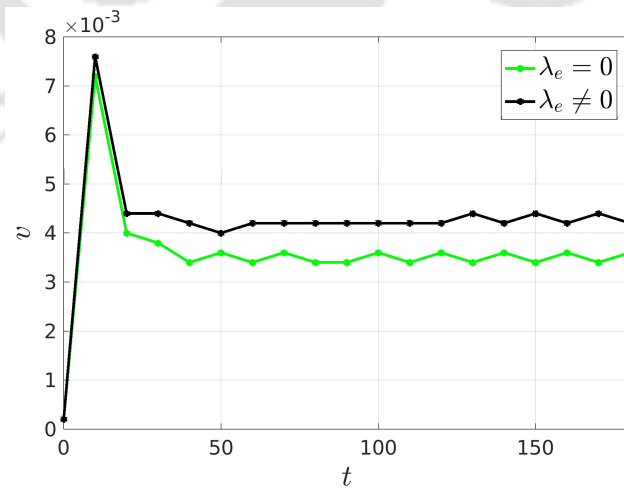


Figure 5.5: Propagation of the tumor front velocity (v) over time for both cases: with and without exosome effects.

5.4.4 Effects of lactate on exosome level and effects of exosome on the tumor volume

Many experimental studies showed that lactate enhances exosome secretion and tumor derived exosomes participate in many events in tumor biology, such as proliferation, angiogenesis, invasion and drug resistance [60, 52]. In this section, the effects of exosomes on tumor volume and the effects of lactate on exosome levels in the tumor are explored. Figure 5.6a shows tumor volume evolution over time for both the cases—whether exosomes are considered or not. No qualitative behavioral changes in tumor volume evolution are observed. However, a small increase in tumor volume is seen over time when exosomes are included as a growth-promoting factor. Figure 5.6b portrays the effects of lactate on exosome levels in the tumor. It is noted that

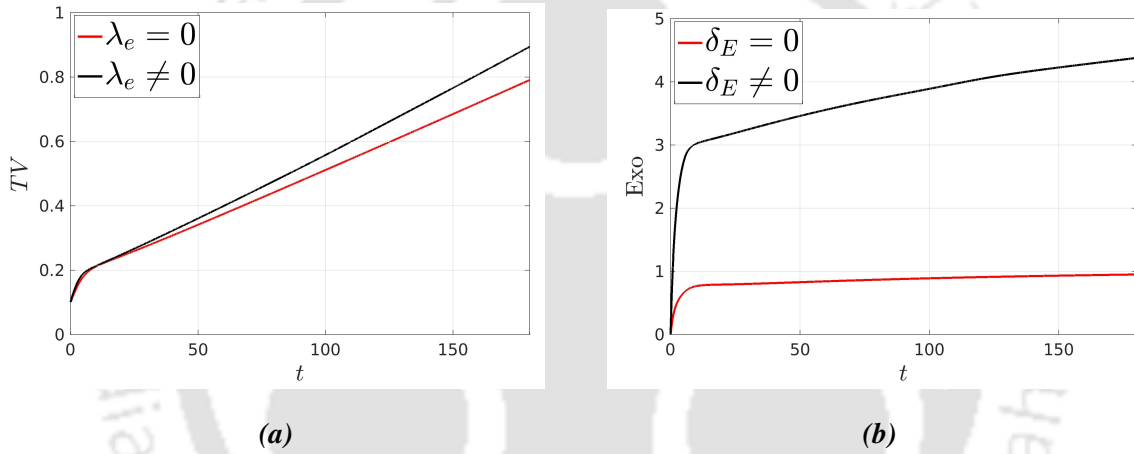


Figure 5.6: The time evolution of tumor volume dynamics and exosome levels in the TME is portrayed. (a) shows the effect of exosomes on tumor volume evolution, while (b) shows lactate effect on exosome levels in the tumor.

exosome levels reach a steady state after an initial transit period when lactate is not considered as an influencing parameter for exosome secretion. However, exosome levels significantly increase when lactate is considered as a promoter of exosome secretion. Additionally, it is observed that exosome levels do not attain a steady state in this case.

5.4.5 Sensitivity analysis

To assess sensitivity, we employ the normalized sensitivity coefficient (NSC), defined as [108],

$$\text{NSC} = \frac{X}{\text{mean}(U)} \frac{\partial U}{\partial X}, \quad (5.43)$$

where X is the input parameter and $\text{mean}(U)$ denotes the mean value of U over the spatial domain. The normalized coefficient allows for more accurate comparison of parameter sensitivity, which is not achievable with simple derivatives due to the wide range of individual parameter values. The nominal value of the parameters are taken from Table 5.1. The NSC is calculated by perturbing the nominal values by 10%. Since, the objective of this chapter is to check the effects of exosome on tumor growth in lactate environment. To enhance result interpretation, the sensitivity analysis is conducted by isolating distinct processes (such as exosome production and proliferation rates) within the model for variable exosome and tumor cell only. This sensitivity analysis is performed on the results at $t = 100$. The NSC values are shown in Figure 5.7. Figure 5.7a displays the NSC values of the exosome concentration for the

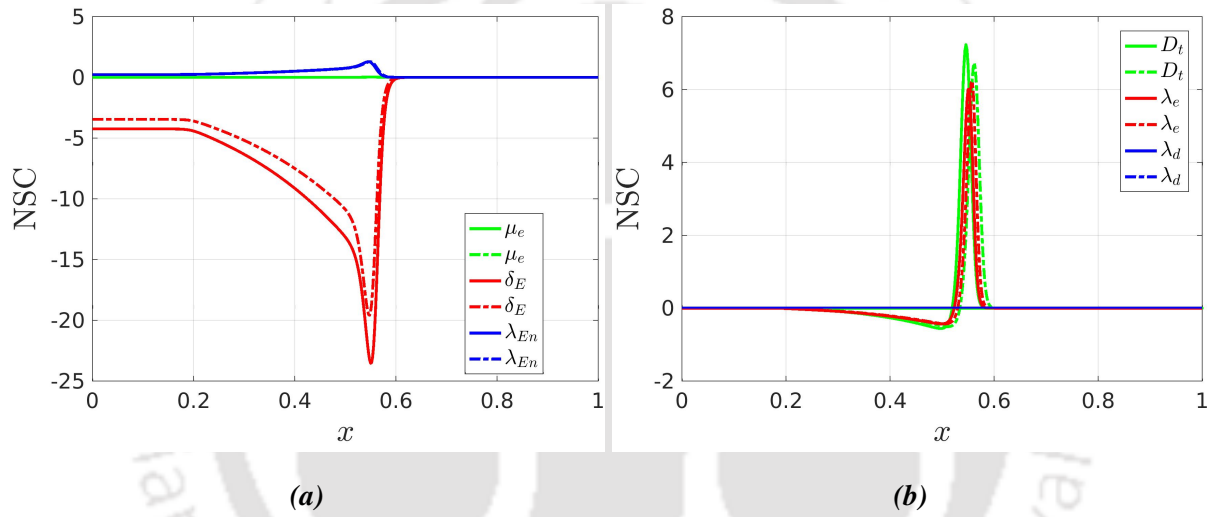


Figure 5.7: This figure shows the sensitivity analysis of parameters on (a) exosome and (b) on tumor growth. The ‘solid lines’ display the results for the 10% reduction in the nominal values while the ‘dashed lines’ show the results for 10% increase.

parameters μ_e , δ_e , and λ_{En} . The values are positive for μ_e , λ_{En} and negative for δ_e . It is noticed that the δ_E is the most sensitive parameter while μ_e is the least one. The order of importance in sensitivity decreases in the order δ_e , λ_{En} and μ_e . The pattern is similar for each curve, and each NSC curve retains the same sign throughout the spatial domain. The NSC curves approach zero nearly $x = 0.6$, which accounts for the exosome concentration that reaches zero. The numerical experiments for sensitivity are performed for tumor cell distribution, and the results are shown in Figure 5.7b. It is observed that the order of importance in sensitivity decreases in the order D_t , λ_e and λ_d . Also, one can be noticed that D_t is more sensitive in the context of tumor growth.

5.5 Conclusions

In this chapter, a mathematical model is proposed to study the effects of exosomes on tumor growth in an acidic tumor microenvironment. In this work, lactate is considered as the only contributor to TME acidification. To elucidate the inherent biological laws, we use a continuum mathematical modeling framework. The model comprises a system of partial differential equations for the variables oxygen, lactate, exosome concentrations, and tumor cell density. The model accurately captures the interplay between oxygen and lactate, as well as between lactate and exosomes, providing insight into the crosstalk between oxygen and exosomes and its impacts on tumor growth dynamics. The model is simulated using a finite difference method framework. The following findings are observed from the simulated results:

1. Exosome levels decrease with an increase in oxygen consumption rate of tumor cells.
2. Exosomes are gathered in greater amount in the presence of lactate compared to that in the absence of lactate in the tumor medium.
3. Exosome leads to increase the tumor volume.
4. Presence of exosomes helps tumor to spread rapidly.
5. The qualitative behavior of tumor growth dynamics remains unaltered when exosome is considered as a growth factor for tumor.



CHAPTER 6

EFFECTS OF OXYGEN HETEROGENEITY ON EMT DYNAMICS

In this chapter, tumor cells are classified into two subpopulations: epithelial and mesenchymal cells. Not all tumor subpopulations exhibit motile characteristics. Experimental evidence suggests that when epithelial cells acquire mesenchymal traits, they gain motile properties. Oxygen depletion in the tumor microenvironment may trigger epithelial-to-mesenchymal transition (EMT). Considering the objectives outlined in Chapter 1, this study aims to thoroughly investigate how oxygen heterogeneity influences EMT dynamics.

6.1 Introduction

The epithelial-mesenchymal transition (EMT) is a cellular process that occurs when epithelial cells achieve mesenchymal characteristics. EMT is a crucial event for various biological processes, including embryogenesis, wound healing, and cancer [100]. Epithelial tissue is well structured in cobblestone patterns with the help of cell-cell adhesive properties and a complex, tight chemical gene regulatory system. This epithelial phenotype is maintained by E-cadherin, which helps to stick epithelial cells together [63]. During EMT, cells lose their epithelial trait and adopt the spindle-shaped mesenchymal trait, helping to acquire an invasive phenotype [42].

In the context of cancer, EMT of tumor cells is the first move to achieve tumor migration and leads to destructive metastasis for cancer-related death. There are two main key features that are commonly manifested in the EMT process. First, the loss of proteins that characterize

an epithelial phenotype, along with the acquisition of mesenchymal proteins, is regarded as the foundation of EMT [7]. Second, signals from the tumor microenvironment (TME) drive downregulation of epithelial genes and upregulation of mesenchymal genes by EMT-relevant transcription factors (e.g., SNAIL, SLUG, TWIST), leading to reorganization of the cell cytoskeleton [17]. These signatures have been described for many different types of carcinomas, such as lung, liver, breast, prostate, colorectal, head and neck, and ovarian. Scientific communities are hunting the cause of EMT in tumors. Many publications indicate that some defective genetic regulation is the reason behind EMT [67, 26, 89, 69]. Katz et al. [47] identified a 9-gene signature characteristic of EMT in breast cancer. They showed that these signatures demonstrate the hallmark of EMT, including loss of the E-cadherin protein and acquisition of the expression of N-cadherin and vimentin. Various investigations reveal that the EMT process can be reversible by silencing the signaling pathway involved in EMT. It is known as the mesenchymal-epithelial transition (MET) process. The processes EMT and MET are not binary. To complete EMT, epithelial cells first transit into hybrid epithelial/mesenchymal states and then switch to mesenchymal cells. The number of these hybrid states may be more than one in some tumors. However, under which circumstances this genetic malfunction is triggered, in order to initiate the EMT or MET processes, are not fully understood.

A well-established characteristic of the solid tumor is hypoxia [35]. Depending on the oxygen supply from the primary blood vessel near the tumor, a hypoxic region is found even in a tiny tumor. The oxygen gradient develops inside the tumor, which leads to oxygen heterogeneity in tumors. This variation in oxygen levels triggers several biological events, such as metabolic adaptation, angiogenesis, immunomodulation, and many more. Hypoxia is also a purported driver of EMT in tumors [15, 111]. In the hypoxic region, hypoxia-inducible factors (e.g., HIF-1 α) are secreted by tumor cells. It has been proved that HIF-1 α is the major mediator of hypoxia-driven EMT [96]. Zhang et al. [112] experimentally showed that hypoxia stabilized HIF-1 α promoted EMT by increasing SNAIL transcription. The hypoxic tumor alters its metabolic process from oxidative phosphorylation to anaerobic glycolysis [116]. During the glycolysis process, hypoxic tumor cells secrete lactate and acidify TME [2]. Meanwhile, the secretion of lactate from cells elevates the intracellular pH, which activates Wnt signaling, potentially leading to EMT in order to help the metastasis cascade [57]. As hypoxia depends on the oxygen level within the tumor, a correlation exists between the oxygen levels in the tumor and the EMT process. Many experimental studies are devoted to decoding the mysteries of

the interplay between oxygen levels and EMT dynamics [96]. Depending on the oxygen level, epithelial cells acquire the mesenchymal phenotype and vice versa. However, how oxygen heterogeneity inside the tumor impacts the EMT dynamics and tumor growth remains to be studied. In addition, experimental work indicates that when epithelial tumor cells convert to the mesenchymal type, the mesenchymal cells develop aggressive motile characteristics. The effects of mesenchymal cell motility on EMT dynamics have not yet been explored. In *in vivo* tumor, cyclic hypoxia is a well-established characteristic in solid tumors. This characteristic can be closely mimicked by the precious control of oxygen supply to the tumor culture medium. The intricate relationship between cyclic hypoxia and EMT is not yet fully understood, and further research is required to uncover the underlying mechanisms and their potential implications for tumor growth dynamics.

The mathematical modeling framework offers an alternative approach to study complex, time-consuming biological phenomena. In EMT program, a large number of EMT transcription factors are involved. He et al. [38] proposed a minimal dynamical model to investigate EMT and MET processes of tumor cells based on the transcriptional regulatory mechanisms between microRNA-200 and ZEB transcription factor in an individual tumor cell. Recently, Tripathi et al. [99] explored the mechanism for epithelial-mesenchymal heterogeneity in a population of cancer cells using ODE-based mathematical modeling. Their study highlights how factors known to promote a hybrid epithelial-mesenchymal phenotype can alter the phenotypic composition of a population. EMT and MET processes are not readily captured by an ODE-based model, as these processes are linked to spatial interactions with the tumor's surrounding medium, nutrient gradients, and oxygen heterogeneity. Several spatio-temporal models are adopted to describe EMT-driven tumor invasion [79, 29]. The role of the surrounding medium of the tumor, for example, the ECM, and the interaction between the ECM and cancer cells play a major role in the tumor invasion and metastasis cascade. Prasanna et al. [79] studied to know how tumor invasiveness on a multicellular scale is influenced by the spatial heterogeneity of cell-cell and cell-ECM adhesion. They classified tumor cell populations into epithelial and mesenchymal based on the value of cell-cell and cell-ECM adhesion strengths. Experimental studies show that low-level oxygen concentration encourages epithelial cells to induce the mesenchymal characteristic, and when mesenchymal trait cells are exposed to the normoxic oxygen level, cells again go back to the epithelial phenotype. Oxygen heterogeneity is a well-established feature in solid tumors. Hence, it would be wrathful to study how oxygen

heterogeneity impacts the EMT dynamic in the solid tumor.

In this chapter, we propose a continuum mathematical model to unfold the missing link between oxygen heterogeneity and the epithelial-mesenchymal transition in the solid tumor. Here, we consider a system of coupled PDEs for the variables mesenchymal, epithelial, dead cell densities, and oxygen concentration. Since mesenchymal cells acquire that ability to move, the proposed model allows mesenchymal cells to be motile. As epithelial cells are tightly connected with each other, we assume that epithelial cells are unable to move.

6.2 Model formulation

In this section, we describe how spatio-temporal changes of the mesenchymal cell density $m'(x', t')$, epithelial cell density $e'(x', t')$, dead cells density $n'(x', t')$ and oxygen concentration $C'(x', t')$ involved in EMT dynamics and derive partial differential equations governing the evolution of each variable, where x' denotes the spatial position and t' represents time. The schematic diagram is presented in Figure 6.1.

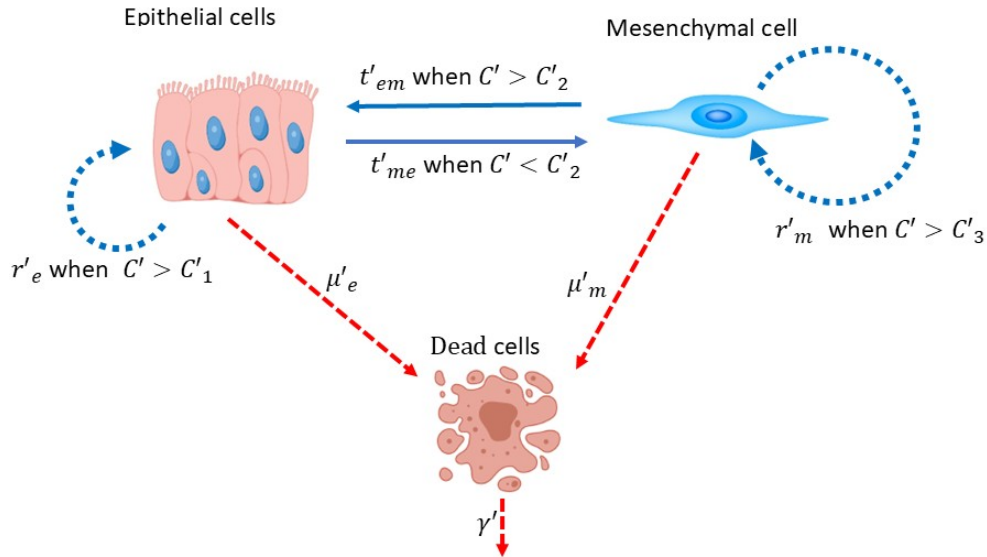


Figure 6.1: Schematic showing the interactions between epithelial, mesenchymal, and dead cells, e' , m' , n' , respectively, in response to oxygen concentration C' during the EMT process.

(a) Mesenchymal cells:

We assume that the density of mesenchymal cells changes due to the dispersion that results from random locomotion, and we choose D'_m as the random cell motility coefficient, characterizing how the cells would disperse from higher to lower densities. Mesenchymal cells proliferate at the rate r'_m when the oxygen concentration is above the critical value of C'_1 . Epithelial cells are transferred to the mesenchymal phenotype at a rate t'_{em} when oxygen concentration falls below the critical value C'_2 . However, mesenchymal cells turn into epithelial cells again when the oxygen level is raised above C'_2 . Mesenchymal cells die at the rate μ'_m .

To summarize, the spatio-temporal evolution of mesenchymal cell density leads to the differential equation (using mass conservation law),

$$\begin{aligned} \frac{\partial m'}{\partial t'} = & \underbrace{D'_m \frac{\partial^2 m'}{\partial x'^2}}_{\text{mortality}} + \underbrace{r'_m m' \left(1 - \frac{m' + e' + n'}{K'}\right) H(C' - C'_1)}_{\text{proliferation}} + \underbrace{t'_{em} \left(1 + \theta \frac{m'}{K'}\right) e' H(C'_2 - C') H(C' - C'_4)}_{\text{E to M under influence of M}} \\ & - \underbrace{t'_{me} \left(1 - \delta \frac{m'}{K'}\right) m' H(C' - C'_2)}_{\text{M to E under influence of M}} - \underbrace{\mu'_m m'}_{\text{apoptosis}} - \underbrace{\mu'_{d1} m' H(C'_4 - C')}_{\text{necrotic death}}, \end{aligned} \quad (6.1)$$

where K' is the carrying capacity of tumor. The second term on the right-hand side of Eq. (6.1) represents the mesenchymal cells proliferate in the logistic manner with the rate r'_m in the environment where oxygen concentration above a threshold value C'_1 . The third term on the RHS denotes the change of epithelial cell to mesenchymal cell with a rate t'_{em} when epithelial cells are exposed to the oxygen environment; oxygen concentration falls below C'_2 , i.e., $C' < C'_2$, with the influence of mesenchymal cells. θ is the influence parameter. The fourth term on the RHS denotes mesenchymal cells back to epithelial with a rate r'_{me} when oxygen concentration is above the critical value C'_2 . The parameter δ represents the extent to which mesenchymal cells inhibit MET. The fifth term represents that the mesenchymal cells die at a rate μ'_m . The last term on the RHS indicates that the mesenchymal cells die at a rate μ'_{d1} when C' falls below the necrotic threshold value C'_4 .

(b) Epithelial cells:

Experimental evidence shows that epithelial cells are attached through highly adhesive molecules. So, we assume that epithelial cells are not motile. Epithelial cells proliferate when oxygen concentration is above the threshold value C'_3 . When oxygen concentration $C' > C'_2$, mesenchymal cells regain epithelial phenotype. Therefore, the third term in Eq. (6.1) acts as a source term for epithelial cell density. Epithelial cells transit into mesenchymal cells under the influence of mesenchymal cells. Hence, the second term of Eq. (6.1) acts as a sink term. To summarize, the

mass conservation for epithelial cells is applied, which gives

$$\begin{aligned} \frac{\partial e'}{\partial t'} = & \underbrace{r'_e e' \left(1 - \frac{m' + e' + n'}{K'}\right) H(C' - C'_3)}_{\text{proliferation}} + \underbrace{t'_{me} \left(1 - \delta \frac{m'}{K'}\right) m H(C' - C'_2)}_{\text{M to E under influence of M}} \\ & - \underbrace{t'_{em} \left(1 + \theta \frac{m'}{K'}\right) e H(C'_2 - C')}_{\text{E to M under influence of M}} - \underbrace{\mu'_e e'}_{\text{apoptosis}} - \underbrace{\mu'_{d2} e' H(C'_4 - C')}_{\text{necrotic death}}, \end{aligned} \quad (6.2)$$

where r'_e denotes the proliferation rate of epithelial cells and μ'_e represents the death rate of epithelial cells.

(c) Dead cells:

Dead cells are generated in various ways. The most common ways are cellular program death and cellular death due to a shortage of oxygen. Also, dead cells undergo natural degradation into cellular fragments and soluble components through autolysis and enzymatic breakdown, occurring at a rate γ'_1 .

$$\frac{\partial n'}{\partial t'} = \mu'_m m' + \mu'_e e' + \mu'_{d1} m' H(C'_4 - c') + \mu'_{d2} e' H(C'_4 - C') - \underbrace{\gamma'_1 n'}_{\text{decay}}, \quad (6.3)$$

(d) Oxygen concentration:

Cells need nutrients and oxygen to sustain life. In our study, oxygen is taken as a pivotal element for a cell's metabolic element. Oxygen is consumed by epithelial and mesenchymal cells. These consumption terms can be non-linear. However, we assume that oxygen is consumed linearly by epithelial and mesenchymal cells. The oxygen reaction-diffusion equation is given as,

$$\frac{\partial C'}{\partial t'} = D'_c \frac{\partial^2 C'}{\partial x'^2} - \underbrace{\lambda'_e C' e'}_{\text{consumption by E}} - \underbrace{\lambda'_m C' m'}_{\text{consumption by M}}, \quad (6.4)$$

where λ'_e and λ'_m represent the oxygen consumption rates for epithelial and mesenchymal cells, respectively. D'_c denotes the diffusion coefficient of oxygen.

Hence, the complete system of equations describing the interactions between the mesenchymal cell density, epithelial cell density, dead cell density, and oxygen concentration are given

as:

$$\begin{aligned} \frac{\partial m'}{\partial t'} = D'_m \frac{\partial^2 m'}{\partial x'^2} + r'_m m' \left(1 - \frac{m' + e' + n'}{K'}\right) H(C' - C'_1) + t'_{em} \left(1 + \theta \frac{m'}{K'}\right) e' H(C'_2 - C') H(C' - C'_4) \\ - t'_{me} \left(1 - \delta \frac{m'}{K'}\right) m' H(C' - C'_2) - \mu'_m m' - \mu'_{d_1} m' H(C'_4 - C'), \end{aligned} \quad (6.5)$$

$$\begin{aligned} \frac{\partial e'}{\partial t'} = r'_e e' \left(1 - \frac{m' + e' + n'}{K'}\right) H(C' - C'_3) + t'_{me} \left(1 - \delta \frac{m'}{K'}\right) m' H(C' - C'_2) \\ - t'_{em} \left(1 + \theta \frac{m'}{K'}\right) e' H(C'_2 - C') - \mu'_e e' - \mu'_{d_2} e' H(C'_4 - C'), \end{aligned}$$

$$\frac{\partial n'}{\partial t'} = \mu'_m m' + \mu'_e e' + \mu'_{d_1} m' H(C'_4 - C') + \mu'_{d_2} e' H(C'_4 - C') - \gamma' n,$$

$$\frac{\partial C'}{\partial t'} = D'_c \frac{\partial^2 C'}{\partial x'^2} - \lambda'_e C' e' - \lambda'_m C' m'.$$

Table 6.1: Parameter description.

Parameter	Description
D'_m	Diffusion coefficients of mesenchymal cells
r'_m	Proliferation rate of mesenchymal cells
r'_e	Proliferation rate of epithelial cells
t'_{em}	Transition rate from E to M
t'_{me}	Transition rate from M to E
μ'_m	Death rate of mesenchymal cells
μ'_e	Death rate of epithelial cells
θ	Influence of mesenchymal cells on E to M transition
δ	Influence of mesenchymal cells to reduce M-E transition
D'_c	Diffusion coefficients of oxygen
λ'_e	Oxygen consumption rate of epithelial cells
λ'_m	Oxygen consumption rate of mesenchymal cells
C'_1	Critical value of oxygen concentration is required for mesenchymal cell proliferation
C'_2	Critical value of oxygen concentration required for M to E transition
C'_3	Critical value of oxygen concentration is required for epithelial cell proliferation
K'	Carrying capacity

6.2.1 Initial and boundary conditions

To close the system, the initial and boundary conditions are prescribed for m' , e' , n' , and C' .

Boundary conditions: We assume “no flux” boundary condition for mesenchymal cells at both the boundaries, namely $x' = 0$ and $x' = R'$. Oxygen concentration at $x' = 0$ is assumed to be a symmetric boundary condition, and it is assumed that oxygen is supplied to the system through the boundary $x' = R'$ with constant rate c_∞ . Hence, these conditions are given as,

$$\frac{\partial m'}{\partial x'} = \frac{\partial C'}{\partial x'} = 0 \text{ at } x' = 0 \forall t' > 0, \quad (6.6)$$

$$\frac{\partial m'}{\partial x'} = 0, C' = C_\infty \text{ at } x' = R' \forall t' > 0. \quad (6.7)$$

Initial conditions: We assume that oxygen initially is available at a sufficient amount throughout the domain. It is considered that the oxygen concentration is same as that at the boundary ($x' = R'$). In *in vitro* study, the experiment is conducted with fewer mesenchymal cells than epithelial cells. Hence, the initial conditions are posed as

$$m'(x', 0) = A' \exp\left(-\frac{x'^2}{\epsilon}\right), e'(x', 0) = B' \exp\left(-\frac{x'^2}{\epsilon}\right), \quad (6.8)$$

$$n'(x', 0) = 0 \text{ and } C'(x', 0) = C_\infty, \text{ with } \epsilon = 0.01, \quad (6.9)$$

where A' and B' denote the initial density of mesenchymal and epithelial cells, respectively, with $A' < B'$.

6.3 Non-dimensionalization

To put the model in a non-dimensional form, we follow the standard methodology. The reference time scale (τ) is the time value for mitotic cell division of mesenchymal cells. Therefore, $\tau = \frac{1}{r'_m}$ day. The characteristic length (X) is taken to be equal to $\sqrt{D'_c \tau}$. The characteristic value of oxygen concentration is considered to be the same as that of oxygen supplied at the boundary. We set

$$t = \frac{t'}{\tau}, x = \frac{x'}{X}, e = \frac{e'}{K'}, m = \frac{m'}{K'} \text{ and } C = \frac{C'}{C_\infty}. \quad (6.10)$$

The non-dimensional model is given as

$$\begin{aligned} \frac{\partial m}{\partial t} = D_m \frac{\partial^2 m}{\partial x^2} + m(1 - m - e - n)H(C - C_1) + t_{em}(1 + \theta m)eH(C_2 - C)H(C - C_4) \\ - t_{me}(1 - \delta m)mH(C - C_2) - \mu_m m - \mu_{d_1} m H(C_4 - C), \end{aligned} \quad (6.11)$$

$$\begin{aligned} \frac{\partial e}{\partial t} = r_e e(1 - m - e - n)H(C - C_3) - t_{em}(1 + \theta m)eH(C_2 - C)H(C - C_4) \\ + t_{me}(1 - \delta m)mH(C - C_2) - \mu_e e - \mu_{d_2} e H(C_4 - C), \end{aligned} \quad (6.12)$$

$$\frac{\partial n}{\partial t} = \mu_m m + \mu_e e + \mu_{d_1} m H(C_4 - C) + \mu_{d_2} e H(C_4 - C) - \gamma_1 n, \quad (6.13)$$

$$\frac{\partial C}{\partial t} = \frac{\partial^2 C}{\partial x^2} - \lambda_e C e - \lambda_m C m. \quad (6.14)$$

The boundary conditions for the governing equations are given as

$$\frac{\partial C}{\partial x} = 0 \text{ at } x = 0, C(R, t) = 1, \quad (6.15)$$

$$\frac{\partial m}{\partial x} = 0 \text{ at } x = 0, R \forall t > 0. \quad (6.16)$$

The initial conditions are considered as

$$m(x, 0) = A \exp\left(-\frac{x^2}{\epsilon}\right), e(x, 0) = B \exp\left(-\frac{x^2}{\epsilon}\right), n(x, 0) = 0 \text{ and } C(x, 0) = 1 \text{ in } [0, R]. \quad (6.17)$$

The non-dimensional parameter groups introduced into Eqs. (6.11)-(6.14) are defined below as

$$\begin{aligned} D_m = \frac{D'_m}{D'_c}, r_e = \tau r'_e, t_{em} = \tau t'_{em}, t_{me} = \tau t'_{me}, \mu_m = \tau \mu'_m, \mu_e = \tau \mu'_e K', \lambda_m = \tau \lambda'_m K', \\ \lambda_e = \tau \lambda'_e, R = \frac{R'}{X}, \gamma = \gamma' \tau, A = \frac{A'}{K'} \text{ and } B = \frac{B'}{K'}. \end{aligned}$$

6.3.1 Parameter estimation

Since no single experiment estimated all the necessary parameters values, we collect the parameters' values from the existing literature. The value of oxygen diffusivity inside the tumor is taken as $2 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ [34]. The diffusivity of mesenchymal tumor cells lies in the range $0.75 \times 10^{-11} - 0.01 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$ [95, 13]. Various experimental studies suggest that the proliferation rate of epithelial cells is faster than that of mesenchymal cells [61]. Suzuki et al. [94] experimentally showed that epithelial cells proliferation rate is up to 3.6-fold in *in vitro* study. Other parameters are as follows $r'_m = 0.44 \text{ day}^{-1}$, $r'_e = 1.38 \text{ day}^{-1}$, $t'_{em} = 0.36 - 0.45 \text{ day}^{-1}$, $t'_{me} = 0.156 - 0.2 \text{ day}^{-1}$ [44, 110, 8, 61]. The value of characteristic length (X) is 1.97 cm .

The value of C_4' is 1.7 mmHg , $C_\infty = 10 - 20 \text{ mmHg}$ [45]. Mesenchymal cells consume oxygen at a maximum rate that varies in the range $0.1 - 3 \text{ pmol h}^{-1} \text{ cell}^{-1}$ [54, 27]. The apoptotic death rates for epithelial cells (μ_e') and mesenchymal cells (μ_m') are taken as 0.054 day^{-1} and 0.054 day^{-1} , respectively, which belong to the ranges given in [58]. Table 6.2 shows values of the dimensionless parameters.

Table 6.2: Dimensionless parameter values.

Parameter	Base value	Range
D_m	0.0000375	0.0000005 – 0.00375
r_m	1	
r_e	3.5	
t_{em}	0.75	0.674 – 0.9675
t_{me}	0.43	0.335 – 0.43
μ_m	0.065	
μ_e	0.095	
δ	0.5	0 – 1
θ	3	1 – 4
λ_e	2.5	
λ_m	2.5	
C_1	0.6	
C_2	0.5	
C_3	0.6	
C_4	0.17	
γ	0.02	
μ_{d_1}	0.022	
μ_{d_2}	0.022	
A	0.2	
B	0.8	
R	2.54	–

6.3.2 Numerical simulation:

In order to solve numerically, the governing equations (6.11)-(6.14) subject to the initial conditions (6.17) and boundary conditions (6.15)-(6.16) over the domain $[0, R]$ are discretized in finite difference framework using forward time and central space (FTCS) scheme. The domain $[0, R]$ is partitioned into $(N - 1)$ number sub-interval into equal width Δx . Therefore, $\Delta x = \frac{R}{(N-1)}$. We carry out simulation up to T_{final} . To march the final time T_{final} , we choose Δt as the time step length for P times. So, $\Delta t = \frac{T_{final}}{P}$. We denote $m(i\Delta x, k\Delta t) = m_i^k$, $e(i\Delta x, k\Delta t) = e_i^k$, $n(i\Delta x, k\Delta t) = n_i^k$, and $C(i\Delta x, k\Delta t) = C_i^k$, where $i = 0, \dots, N - 1$ and $k = 0, \dots, P$.

The difference equation for Eq. (6.11) is obtained as,

$$\begin{aligned} -D_1 m_{i-1}^{k+1} + (1 + 2D_1) m_i^{k+1} - D_1 m_{i+1}^{k+1} = & m_i^k + \Delta t (m_i^k (1 - m_i^k - e_i^k - n_i^k) H(C_i^k - C_1) \\ & + t_{em} (1 + \theta m_i^k) e_i^k H(C_2 - C_i^k) H(C_i^k - C_4) - t_{me} (1 - \delta m_i^k) m_i^k H(C_i^k - C_2) \\ & - \mu_m m_i^k - \mu_{d1} m_i^k H(C_4 - C_i^k)), \end{aligned} \quad (6.18)$$

which is valid for $i = 1, \dots, N - 2$ and $k = 0, 1, \dots, P$. Here $D_1 = \frac{\Delta t D_m}{\Delta x^2}$.

At boundary nodes (i.e., $i = 0, N - 1$), by employing no-flux boundary condition, the difference equations for Eq. (6.11) are obtained as,

$$\begin{aligned} (1 + 2D_1) m_0^{k+1} - 2D_1 m_1^{k+1} = & m_0^k + \Delta t (m_0^k (1 - m_0^k - e_0^k - n_0^k) H(C_0^k - C_1) \\ & + t_{em} (1 + \theta m_0^k) e_0^k H(C_2 - C_0^k) H(C_0^k - C_4) - t_{me} (1 - \delta m_0^k) m_0^k H(C_0^k - C_2) \\ & - \mu_m m_0^k - \mu_{d1} m_0^k H(C_4 - C_0^k)), \end{aligned} \quad (6.19)$$

$$\begin{aligned} -2D_1 m_{N-2}^{k+1} + (1 + 2D_1) m_{N-1}^{k+1} = & m_{N-1}^k + \Delta t (m_{N-1}^k (1 - m_{N-1}^k - e_{N-1}^k - n_{N-1}^k) H(C_{N-1}^k - C_1) \\ & + t_{em} (1 + \theta m_{N-1}^k) e_{N-1}^k H(C_2 - C_{N-1}^k) H(C_{N-1}^k - C_4) - t_{me} (1 - \delta m_{N-1}^k) m_{N-1}^k H(C_{N-1}^k - C_2) \\ & - \mu_m m_{N-1}^k - \mu_{d1} m_{N-1}^k H(C_4 - C_{N-1}^k)). \end{aligned} \quad (6.20)$$

Eqs. (6.12) and (6.13) are discretized respectively as,

$$\begin{aligned} e_i^{k+1} = & e_i^k + \Delta t (r_e e_i^k (1 - m_i^k - e_i^k - n_i^k) H(C_i^k - C_3) - t_{em} (1 + \theta m_i^k) e_i^k H(C_2 - C_i^k) H(C_i^k - C_4) \\ & + t_{me} (1 - \delta m_i^k) m_i^k H(C_i^k - C_2) - \mu_e e_i^k - \mu_{d2} e_i^k H(C_4 - C_i^k)), \end{aligned} \quad (6.21)$$

$$n_i^{k+1} = \mu_m m_i^k + \mu_e e_i^k + \mu_{d1} m_i^k H(C_4 - C_i^k) + \mu_{d2} e_i^k H(C_4 - C_i^k) - \gamma n_i^k, \quad (6.22)$$

which is valid for all spatial grid points.

The oxygen diffusion equation (6.14) is discretized as,

$$-D_2 C_{i-1}^{k+1} + (1 + 2D_2) C_i^{k+1} - D_2 C_{i+1}^{k+1} = C_i^k - \Delta t C_i^k (\lambda_e e_i^k + \lambda_m m_i^k), \quad (6.23)$$

which is applicable for the grid points $i = 1, \dots, N - 3$ with $D_2 = \frac{\Delta t}{\Delta x^2}$.

At the boundary node $i = 0$, by deploying no-flux boundary condition, the discretized form of Eq. (6.14) is given as,

$$(1 + 2D_2)C_0^{k+1} - 2D_2C_1^{k+1} = C_0^k - \Delta t C_0^k (\lambda_e e_0^k + \lambda_m m_0^k). \quad (6.24)$$

At the node $i = N - 2$, the finite difference equation of Eq. (6.14) takes from as follows,

$$-D_2C_{N-3}^{k+1} + (1 + 2D_2)C_{N-2}^{k+1} = C_{N-2}^k - \Delta t C_{N-2}^k (\lambda_e e_{N-2}^k + \lambda_m m_{N-2}^k). \quad (6.25)$$

Note that all spatial derivatives in the model are treated implicitly in time, which leads to an unconditionally stable scheme [78].

6.4 Numerical results

Here, we simulate the proposed model up to dimensionless time $T_{final} = 120$. We have used the dimensionless parameter values, shown in Table 6.2. To calculate the total volume ($V_{tot}(t)$) of the tumor, the total volume is defined as,

$$V_{tot}(t) = \frac{1}{L_{max}} \int_0^{L_{max}} (m(x, t) + e(x, t) + n(x, t)) dx, \quad (6.26)$$

where L_{max} is the length between the tumor center and the tumor tip point at each time step. $L_{max}(t)$ is calculated using $m(L_{max}(t), t) + e(L_{max}(t), t) + n(L_{max}(t), t) = \eta$ at each time point. Here, we fix $\eta = 10^{-6}$ arbitrarily sufficiently small.

The total volume of epithelial ($E(t)$) and mesenchymal cells ($M(t)$) are defined as,

$$E(t) = \frac{1}{L_{max}} \int_0^{L_{max}} e(x, t) dx \text{ and } M(t) = \frac{1}{L_{max}} \int_0^{L_{max}} m(x, t) dx. \quad (6.27)$$

Also, the fraction of total live cells is defined as,

$$(\text{fraction of live cells}) = \frac{E(t) + M(t)}{V_{tot}(t)}. \quad (6.28)$$

The volume fraction of mesenchymal (M_{frac}) and epithelial cell (E_{frac}) are defined as,

$$M_{frac}^{live}(t) = \frac{M(t)}{E(t) + M(t)}, \quad E_{frac}^{live}(t) = \frac{E(t)}{E(t) + M(t)} \quad (6.29)$$

$$M_{frac}^{total}(t) = \frac{M(t)}{V_{tot}(t)}, \quad E_{frac}^{total}(t) = \frac{E(t)}{V_{tot}(t)}, \quad (6.30)$$

where superscripts *live* and *total* denote the fraction of epithelial and mesenchymal are calculated corresponding to total live cell volume and total tumor volume, respectively.

To estimate the velocity of the tumor tip point, we use the following formula,

$$v(t, t + \delta t) = \frac{L_{max}(t + \delta t) - L_{max}(t)}{\delta t},$$

where δt is the time between two subsequent measurements of the position of the tumor front point (L_{max}).

6.4.1 In the case where oxygen is uniformly distributed within the tumor domain

In the *in vitro* protocol, tumor cultures are maintained with fixed oxygen levels in the growth medium to study the impact of oxygenation on various biological phenomena, which rely on specific critical thresholds. In this study, we consider that when the oxygen concentration (C) falls below C_2 , the epithelial-mesenchymal transition begins and when the oxygen level is above C_2 , the reverse epithelial-mesenchymal transition begins. In addition, fluctuation in oxygen is a common feature in the microenvironment of a real tumor. This biological characteristic can be mimicked in the *in vitro* setup by controlling oxygen supply in tumor culture medium [5]. Experimental studies show that necrotic materials are released from the tumor region and appear on the surface of the culture medium. Therefore, the competition for space between epithelial and mesenchymal cells is considered. Hence, the governing equations are redefined as,

$$\begin{aligned} \frac{\partial m}{\partial t} = & D_m \frac{\partial^2 m}{\partial x^2} + m(1 - m - e)H(C - C_1) + t_{em}(1 + \theta m)eH(C_2 - C)H(C - C_4) \\ & - t_{me}(1 - \delta m)mH(C - C_2) - \mu_m m - \mu_{d1} mH(C_4 - C), \end{aligned} \quad (6.31)$$

$$\begin{aligned} \frac{\partial e}{\partial t} = & r_e e(1 - m - e)H(C - C_3) - t_{em}(1 + \theta m)eH(C_2 - C)H(C - C_4) \\ & + t_{me}(1 - \delta m)mH(C - C_2) - \mu_e e - \mu_{d2} eH(C_4 - C), \end{aligned} \quad (6.32)$$

where the oxygen levels ($C = C(t)$) (where t is time) are assumed to be spatially homogeneous. Figure 6.2 illustrates four different protocols based on oxygen levels in the culture medium. In each protocol, the oxygen concentration level is kept at fixed value. In the first protocol, the oxygen concentration in the culture medium is greater than C_2 . In the second protocol, the oxygen level is maintained at a concentration below C_2 . The third protocol involves changing

the oxygen levels, where it is above C_2 for the first half of the simulation and then drops to below C_2 for the second half. In the final protocol, the oxygen level remains fixed below C_2 during the first half of the simulation, and then it is increased to above C_2 for the second half. The effects of these four protocols on EMT dynamics are discussed in the following.

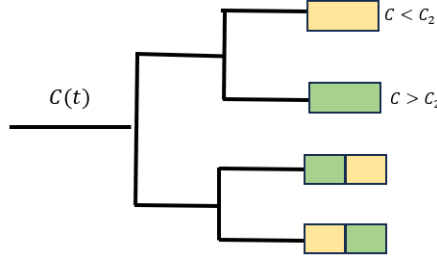


Figure 6.2: Schematic representation of oxygen levels distribute spatially homogeneous in the tumor growth medium. The green colour denotes $C > C_2$, and the yellow colour denotes $C < C_2$. The branches are split into two categories, the culture medium is either greater than or less than C_2 through the culture time, and the other one is at the first half time of the course of the simulation, $C(t) > C_2$ and on the last half of it, $C(t) < C_2$, and vice-versa.

Protocol 1: When the oxygen concentration in the growth medium has a higher value than critical oxygen concentration of the mesenchymal-to-epithelial transition i.e., $C > C_2$ uniformly

We consider that the oxygen level is fixed at $C = 0.7 (> C_2)$ in the tumor culture medium. Figure 6.3a reveals that after the initial transition period, the tumor consisting of the epithelial and mesenchymal cell population converts into an epithelial tumor. As per the culture medium's oxygen level, mesenchymal cells immediately turn into epithelial phenotype. Epithelial cells do not have motile characteristics. Hence, no spatial advancement of the tumor is observed. It highlights the significant impact of oxygen levels on the phenotypic switch of tumor populations. In this case, the entire tumor turns into an epithelial kind.

Protocol 2: When oxygen concentration has a lower value than critical oxygen concentration of the mesenchymal-to-epithelial transition i.e., $C < C_2$ in the tumor medium

In this case, we mimic the condition in which tumor cultures are in fixed oxygen medium lower than the critical oxygen level to switch the epithelial to mesenchymal transition. In Figure 6.3b,

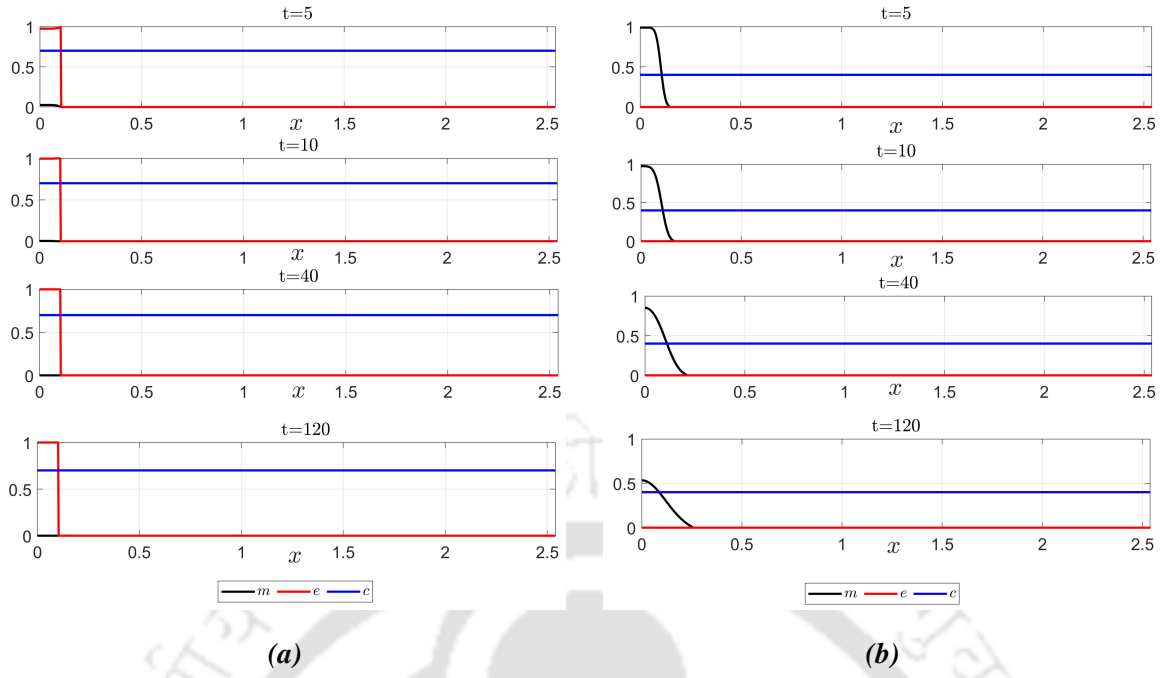


Figure 6.3: The spatial distribution of epithelial and mesenchymal cell densities at times $t = 10, 40, 80$, and 120 when the tumor growth in a medium with a spatially uniform oxygen level (a) $C = 0.7$ and (b) $C = 0.4$.

it can be seen that the tumor only has a mesenchymal cell population after the initial transition time. Since the oxygen level is below the oxygen proliferation threshold value, it is evident that mesenchymal cells cannot proliferate. As a result, the density of mesenchymal cells decreases. Moreover, it is observed that the tumor spatially expands as the mesenchymal cells have a motility feature.

Protocol 3: When tumor exposed in $C > C_2$ up to $t = 60$ initially, later on tumor grows in the environment $C < C_2$

The oxygen level is maintained at the above of C_2 up to $t = 60$. After this time, the oxygen level is fixed at a lower value of C_2 . This, in silico condition, mimics the situation in which the tumor is cultured at a high oxygen level, and after a certain time period, the tumor shifts to a growth environment of a lower oxygen level. Figure 6.4a illustrates that when the tumor grows in $C = 0.7 (> C_2)$, the tumor consists only of epithelial cells except for the initial transient periods of growth. However, in the growth circumstance $C < C_2$, that is, after $t = 60$ onward, the tumor changes all its epithelial population into a mesenchymal phenotype.

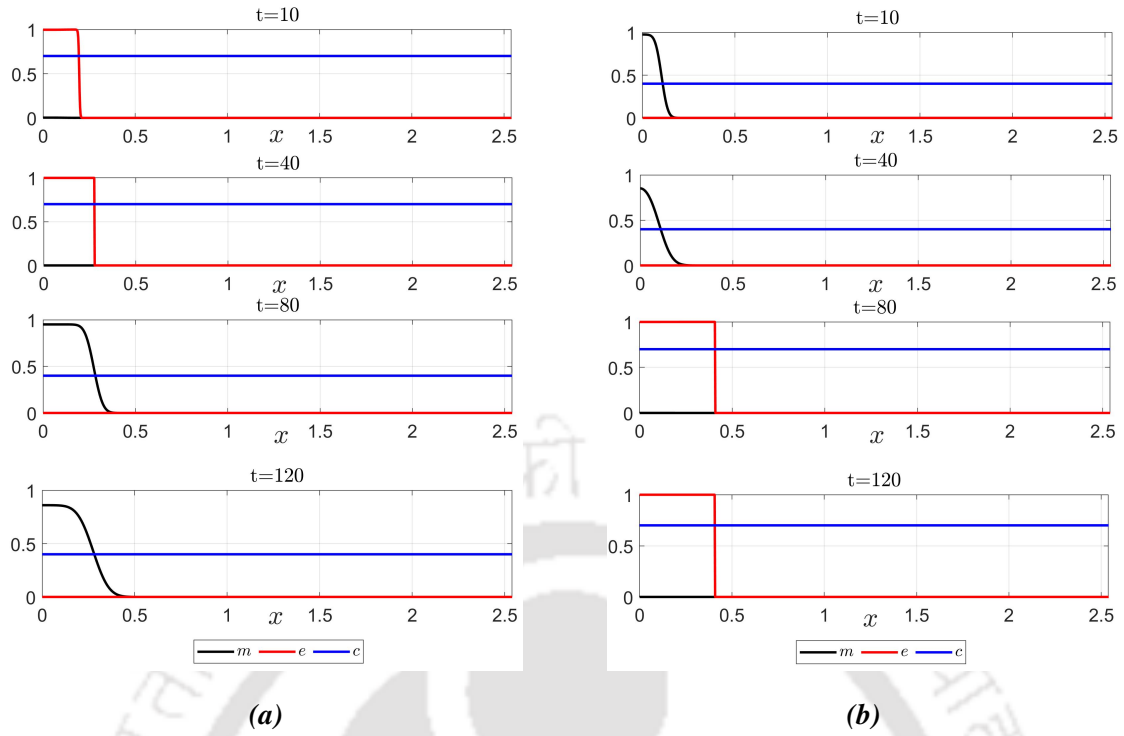


Figure 6.4: The spatial distribution of epithelial and mesenchymal cell densities at times $t = 10, 40, 80$, and 120 when the oxygen concentration is kept at $C = 0.7$ from the initial time up to $t = 60$, after which it remains at $c = 0.4$ for the rest of the simulation in (a) and when $C = 0.4$ for initial $t = 60$ and later stage oxygen levels set at $C = 0.7$ in (b).

Protocol 4: When tumor exposed in $C < C_2$ up to $t = 60$ initially, later on tumor grows in the environment $C > C_2$

In this case, we let the tumor grow in a medium with $C < C_2$ for the first half of the growth period, and then the tumor is placed into a medium with an oxygen level of $C > C_2$ for the remaining duration.

Figure 6.4b displays the spatial distribution of mesenchymal and epithelial cells for various time stages $t = 10, 40, 80$ and 120 . One can observe that when the growth medium has an oxygen level $C < C_2$, the tumor consists only of cells of the mesenchymal phenotype before the culture medium shifts into a higher oxygen level, that is $C > C_2$. The qualitative behavior is similar to that observed in Protocol 3. However, in this case, the tumor appears as an epithelial kind.

From all of the above cases, it is concluded that tumor populations switch into a single type sub-population depending on the oxygen level in the growth medium. However, it is interesting to observe that the tumor volume in culture protocol 4 is higher than that in culture protocol 3.

In addition, it is also observed that when the culture medium changes at the time point $t = 60$, the tumor takes more time to convert all mesenchymal populations into a complete epithelial phenotype when the growth medium changes from $C < C_2$ to $C > C_2$ compared to the time it takes for the epithelial population tumor to switch to a complete mesenchymal phenotype when the growth environment changes from $C > C_2$ to $C < C_2$ (Figure 6.5b).

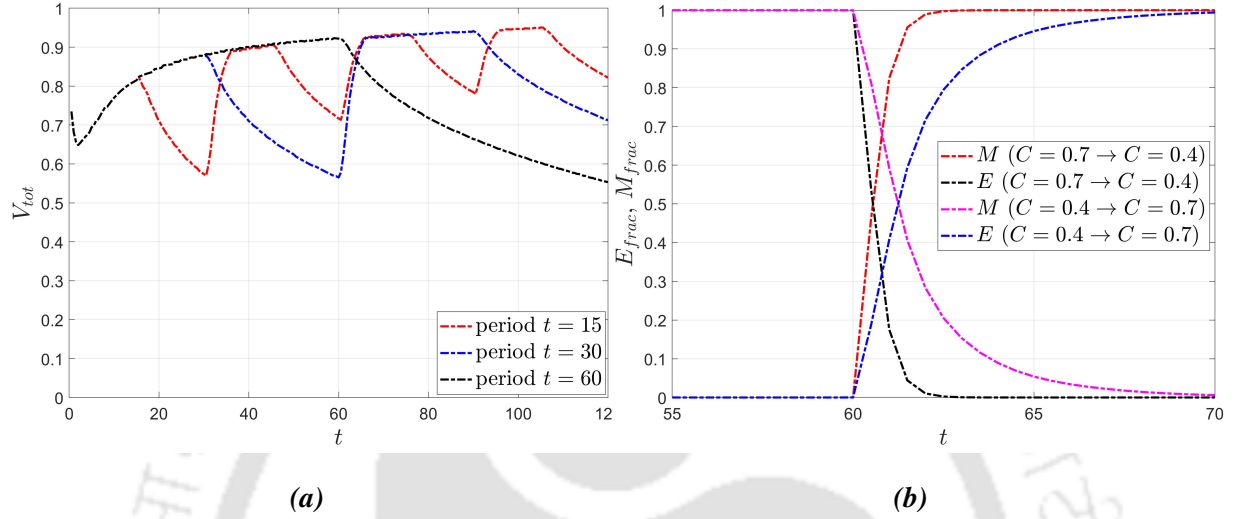


Figure 6.5: (a) Total tumor volume evolution over time in the case when oxygen concentration $C > C_2$ changes to $C < C_2$ periodically at different periods $t = 15, 30$, and 60 . (b) Time evolution of the volume fractions of epithelial and mesenchymal cells, for two cases: (i) the oxygen concentration is kept at $C = 0.7$ from the initial time to $t = 60$, after which it remains at $C = 0.4$ for the rest of the time; and (ii) the oxygen concentration is kept at $C = 0.4$ from the initial time to $t = 60$, after which it remains at $C = 0.7$ for the rest of the time.

Experimental studies showed that the time scale of oxygen fluctuation at the tumor site has a wide range [65]. Inspired by this observation, we further investigated to understand the impacts of the more frequent switch from a high-oxygen environment to a low-oxygen environment on the tumor volume and tumor dynamics. We performed simulations for different values of the oxygen environment switching time periods, e.g., $t = 15, 30$, and 60 . Figure 6.5a shows the evolution of tumor volume over time when the tumor is initially exposed to a culture medium of higher oxygen ($C > C_2$) and then transferred to a medium of lower oxygen ($C < C_2$) in a periodic manner with time periods of $t = 15, 30$ and 60 . During a specific time period, the tumor is cultured in a high-oxygen medium, leading it to transform into an epithelial type. However, in the subsequent time period, when the tumor is placed in a low-oxygen medium, it changes into a mesenchymal type and exhibits spatial characteristics (Figure 6.4). However,

one can see that the total volume increases in rapid oxygen fluctuation. In the rapid oxygen fluctuation case, the tumor is exposed to oxygen levels greater than C_2 , not for a long time at once. So, the tumor is unable to sustain rapid proliferation (Figure 6.5a). In the case of an oxygen-fluctuating environment, starting with a low-oxygen field ($C < C_2$) and time periods of $t = 15, 30$, and 60 , the time evolution of tumor volume exhibits a qualitative behavior similar to that observed in the previous case. However, by the end of the final simulation time, the tumor volume is higher than in that the previous case.

6.4.2 Effects of spatial oxygen heterogeneity on EMT dynamics

Oxygen heterogeneity is a well-established feature in the *in vivo* solid tumor [101]. Oxygen is delivered from the existing blood vessel to the tumor site. As the tumor grows in size, the oxygen gradient starts to develop inside the tumor. As a result, oxygen concentration decreases with increasing distance from the periphery to the center of the tumor. Oxygen level falls to such a level that cells are no longer live. Therefore, the necrotic region is formed. This heterogeneity of oxygen in the tumor triggers various events, such as angiogenesis, metabolic adaptation, and alterations in cell phenotype. Here, we investigated how the mesenchymal and epithelial cells and necrotic core distribution over the domain are impacted by oxygen heterogeneity. Figure 6.6 displays the distribution of epithelial, mesenchymal, necrotic cell density and oxygen concentration in the spatial domain of the tumor at different time levels such as $t = 1, 5, 10, 40, 80$, and 120 . One may observe that at $t = 1$, the presence of epithelial cell density is higher compared to mesenchymal cell density (Figure 6.6a). As time progresses gradually, the density of mesenchymal cells increases rapidly. It happens because the oxygen concentration falls below the threshold value of the EMT transition in the tumor domain. The striking thing is that the mesenchymal cells gain the tumor front seat (Figure 6.6b). However, at subsequent times, epithelial cells appear on the tumor front (Figures 6.6c-6.6f). This development is due to the tumor being exposed to a higher oxygen environment where the mesenchymal phenotype population is switched into the epithelial phenotype. Also, it is noticed that a necrotic core is observed to form in the center of the tumor over the course of tumor growth. By $t = 5$, this necrotic core emerges as the dominant feature within the tumor.

The results are shown in Figure 6.7a, which represents the volume fraction of epithelial cells and mesenchymal cells. It is observed that the volume fraction of epithelial cells with respect to the total volume of the live population decreases rapidly during the initial transient time. In

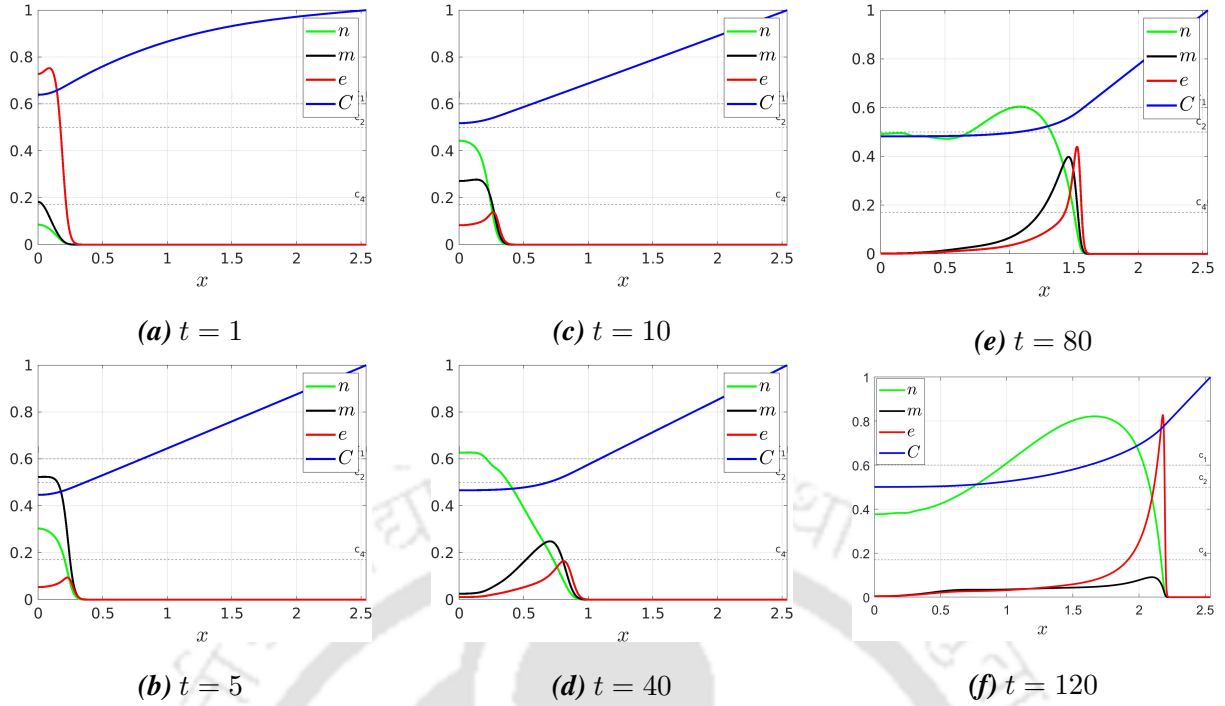


Figure 6.6: Spatial density profiles for epithelial, mesenchymal, necrotic cells, and oxygen concentration at $t=1, 5, 10, 40, 80$, and 120 . The results are obtained by solving model Eqs (6.11) -(6.17) using the parameter values in Table 6.2.

this phase, the oxygen level drops below a critical threshold, which facilitates the transition from the epithelial phenotype to the mesenchymal phenotype. However, E_{frac}^{live} increases with time in the subsequent time. It happens because when a tumor increases in size, the periphery of the tumor is exposed to a oxygen level that is above the cell proliferation threshold. As a result, rapid proliferations of epithelial and mesenchymal cells occurs. Additionally, reverse EMT, i.e., the MET process is activated in this region. All of these collective events lead to an increase in the epithelial volume fraction with respect to the total volume of live cells. The complete mirror results are observed for M_{frac}^{live} . One may note that E_{frac}^{total} and M_{frac}^{total} have qualitative behaviors similar to E_{frac}^{live} and M_{frac}^{live} , respectively. Figure 6.7b shows the velocity of the tumor front point. It shows that the tumor grows approximately linearly except for the initial transient periods.

6.4.3 Effects of diffusivity of mesenchymal cells on EMT process

In this study, live tumor cells are categorized into two cell phenotypes: epithelial and mesenchymal. Experimental studies established that when epithelial cells acquire a mesenchymal pheno-

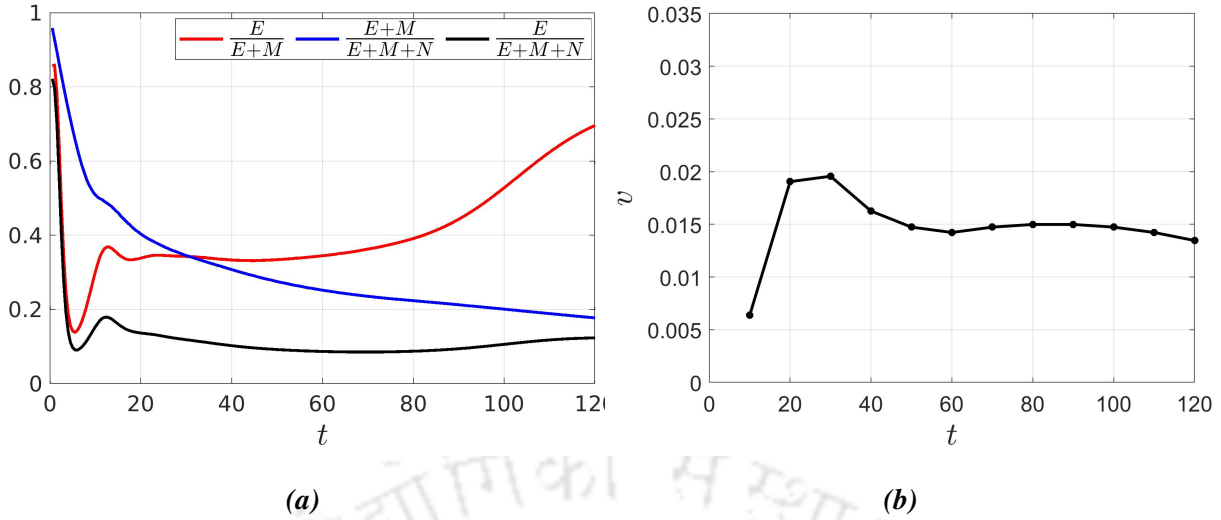


Figure 6.7: (a) The volume fraction of epithelial cell and mesenchymal cell corresponding to total live cell volume and total volume (using the formula Eqs. (6.29)) and (b) the velocity of the front point of tumor evolution with respect to time.

type, mesenchymal cells are exhibited as motile [87]. In this section, we investigated the effects of mesenchymal motility on EMT dynamics and tumor growth in an oxygen-heterogeneous environment. First, we simulate the model, ignoring the effects of mesenchymal cell motility in the simulation. The spatial distributions are given in Figure 6.8. It is seen that the distributions are spatially restricted in the initialization domain. However, heterogeneous populations are observed due to the heterogeneous oxygen environment. The effects of different non-zero values of mesenchymal cell motility on mesenchymal, epithelial, and necrotic core distribution are shown in Figure 6.9. It is noted that we have depicted the results up to $t = 100$. This is due to the emergence of boundary effects at higher value of the motility coefficient, such as $D_m = 0.000115$. The results show that the peak for epithelial cells is achieved for a higher value of mesenchymal cells' motility coefficient. The density of mesenchymal cells increases with time; however, it begins to decay when the tumor size increases. Moreover, one can see that the high value of D_m facilitates the dispersion of epithelial cells. It occurs as the value of D_m increases, the mesenchymal cells migrate to areas where sufficient oxygen is available. In these areas, oxygen levels are adequate to trigger the mesenchymal-epithelial transition (MET) process, allowing mesenchymal cells to adopt epithelial traits. It also leads to the fact that the speed of tumor propagation also increases with the increase in D_m (separate figure for tumor propagation velocity is not shown).

Figure 6.10 demonstrates the time evolution of M_{frac}^{live} for cases $D_m = 0, 0.000005, 0.0000375$,

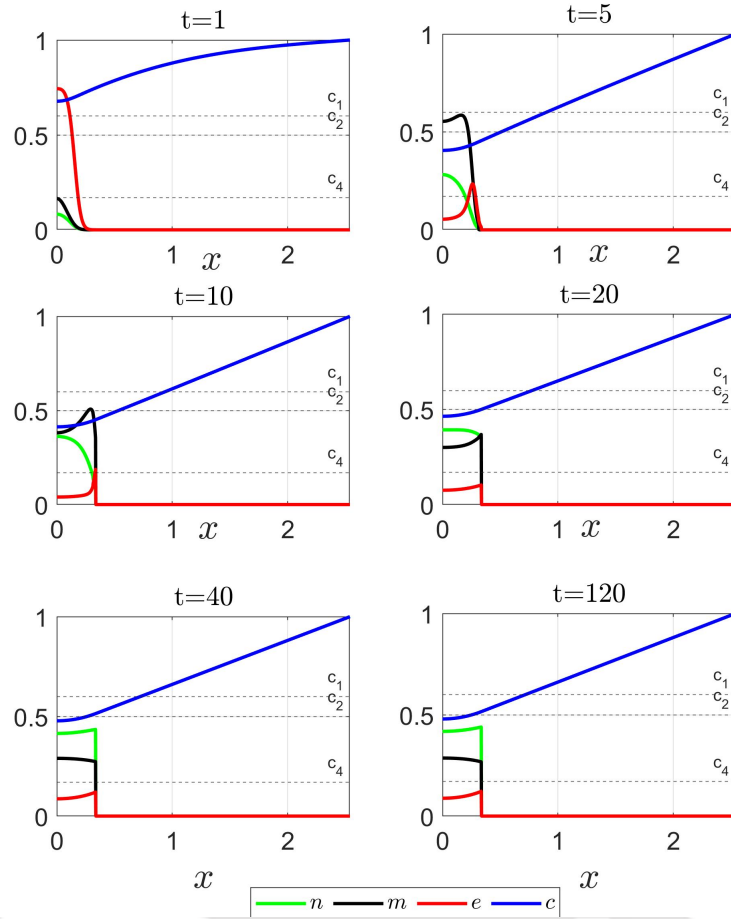


Figure 6.8: The distribution of epithelial, mesenchymal, necrotic core, and oxygen levels when the diffusivity of mesenchymal cells is neglected (i.e., $D_m = 0$). All other parameters are as in Table 6.2.

and 0.000115. It is observed that the volume fraction of mesenchymal cells (M_{frac}^{live}) decreases with time t (when $t > 75$) with the increase in mesenchymal diffusivity D_m . Moreover, M_{frac}^{live} is a rapidly decreasing function for a higher value of D_m . This happens because mesenchymal cells quickly reach the oxygen source site for a higher value of D_m and they are switched into epithelial type immediately. However, for the case of zero mesenchymal motility, M_{frac}^{live} reaches a steady state after the initial transient time. E_{frac}^{live} is the complement of M_{frac}^{live} . Therefore, E_{frac}^{live} exhibits characteristics that are the complete inverse of those of M_{frac}^{live} . It implies that E_{frac}^{live} increases rapidly with the time for higher values of D_m .

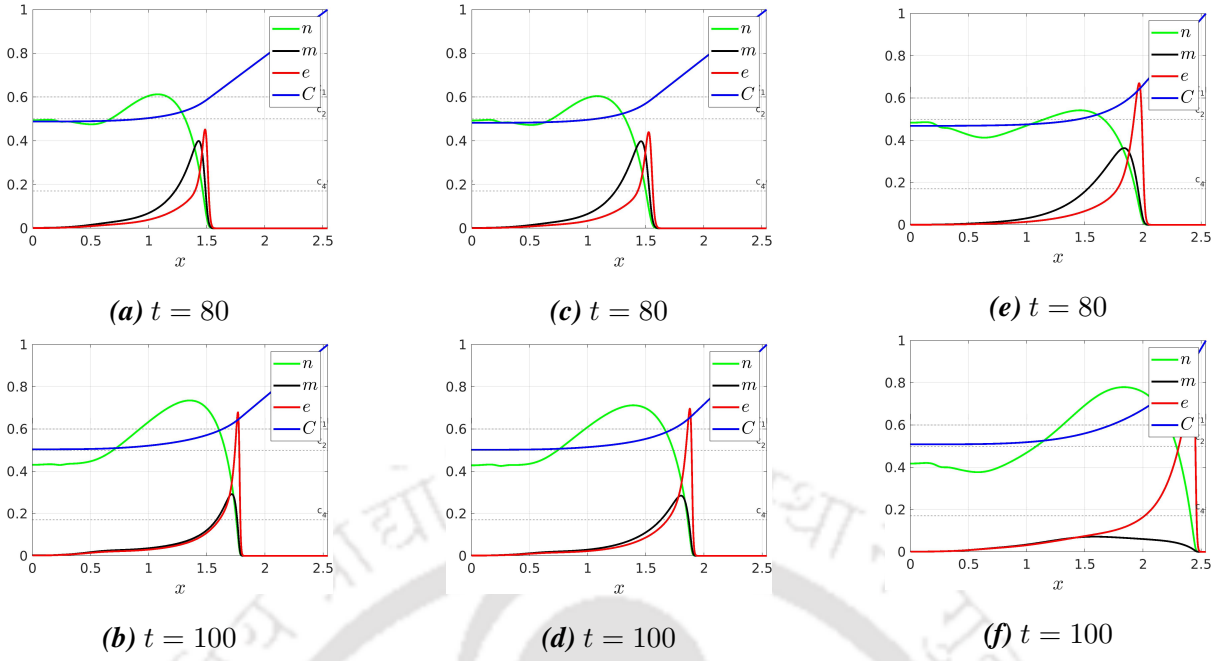


Figure 6.9: First column for $D_m = 0.000005$, second column for $D_m = 0.0000375$, and third column for $D_m = 0.000115$

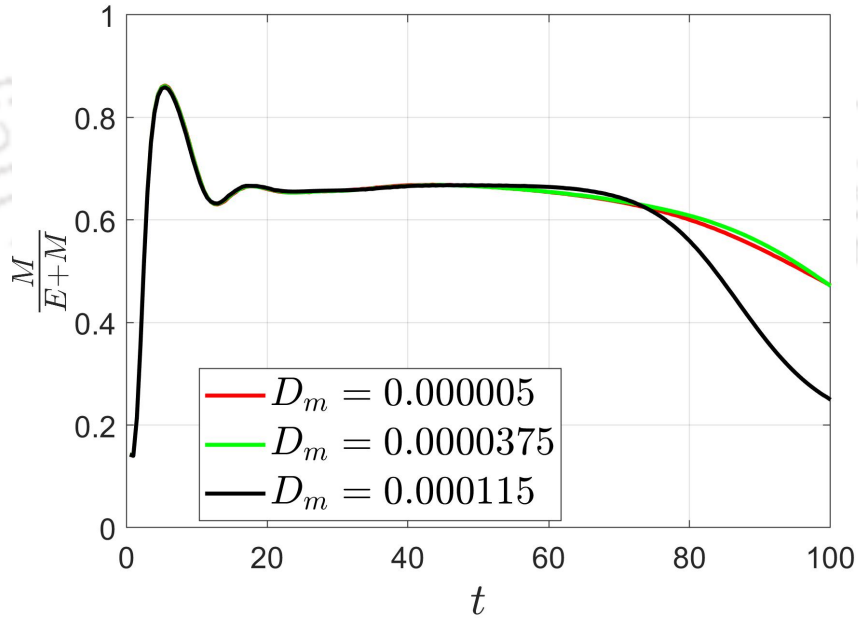


Figure 6.10: For different values of mesenchymal cell diffusivity, the time evolution of M_{frac}^{live} is displayed.

6.4.4 Effects of critical oxygen concentration on the proliferation of epithelial and mesenchymal cells in EMT dynamics

Experimental studies showed that epithelial cells might require a higher oxygen concentration to proliferate compared to mesenchymal cells [cite]. In this section, we discuss the impact of the threshold oxygen concentration on the proliferation of mesenchymal and epithelial cells in EMT dynamics. Here, we consider two cases:

Case 1. The critical oxygen concentration for epithelial cell proliferation is higher than that for mesenchymal cells, i.e., $C_3 > C_1$.

Case 2. Both cell types share the same critical oxygen concentration for proliferation, i.e., $C_3 = C_1$.

Figure 6.11a demonstrates the temporal evolution of the volume fraction of epithelial cells. It is observed that epithelial cells are present in the tumor in higher amounts for case 1 compared to case 2, and completely mirror image results are observed for the mesenchymal cellular volume fraction. It signifies that the total volume of live cells decreases in case 2 compared to case 1. Because in case 1, live cells need more oxygen to increase their population. Figure 6.11b

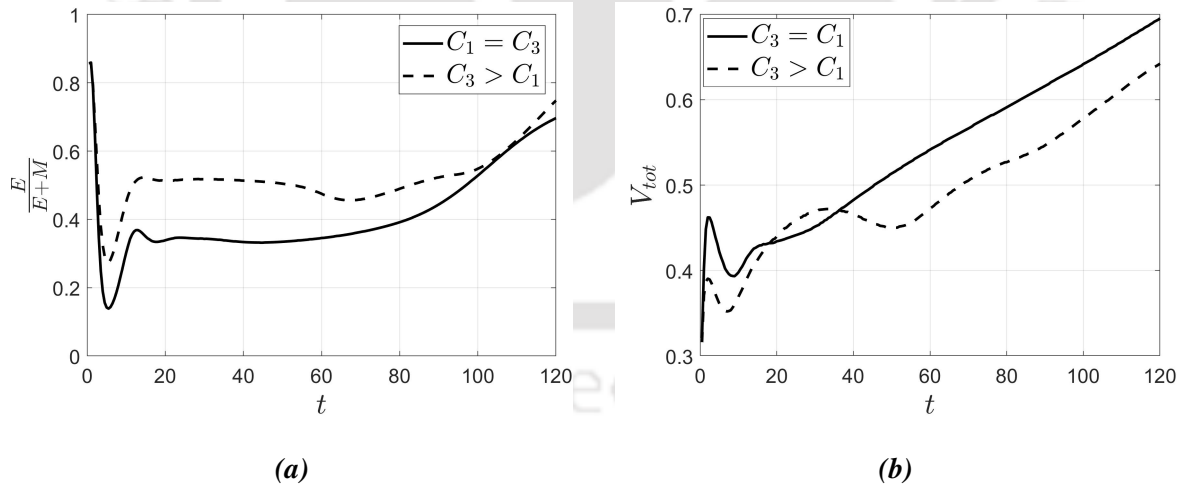


Figure 6.11: The effects of the oxygen threshold value for the proliferation of epithelial and mesenchymal cells are examined (a) for epithelial cell volume fraction and (b) for total volume. Here, we consider for Case 1: $C_1 = 0.6, C_3 = 0.7$ and for Case 2: $C_1 = C_3 = 0.6$.

indicates that the total tumor volume in Case 2 initially increases with time and then maintains an approximately linear growth profile. However, in Case 2, the tumor volume exhibits a

sudden increase after the initial growth period. In Case 1, the tumor volume increases between the durations of $10 < t < 35$, after which it gradually decreases and starts to grow linearly. A key difference between the tumor growth in Case 1 and Case 2 is that the tumor in Case 2 reaches linear growth more quickly than the one in Case 1. Additionally, it is noted that the tumor volume in Case 1 is significantly lower than that in Case 2.

6.4.5 Influence of mesenchymal cells on EMT dynamics

Many studies report that mesenchymal cells influence the transition from epithelial to mesenchymal and vice versa [44, 74]. In this section, we focus on how the mesenchymal population influences EMT and MET when the oxygen distribution is in a spatially heterogeneous environment. Recall from Eq (6.11), where the parameter δ represents the extent to which mesenchymal cells inhibit MET, and the parameter θ represents the strength with which mesenchymal cells promote EMT.

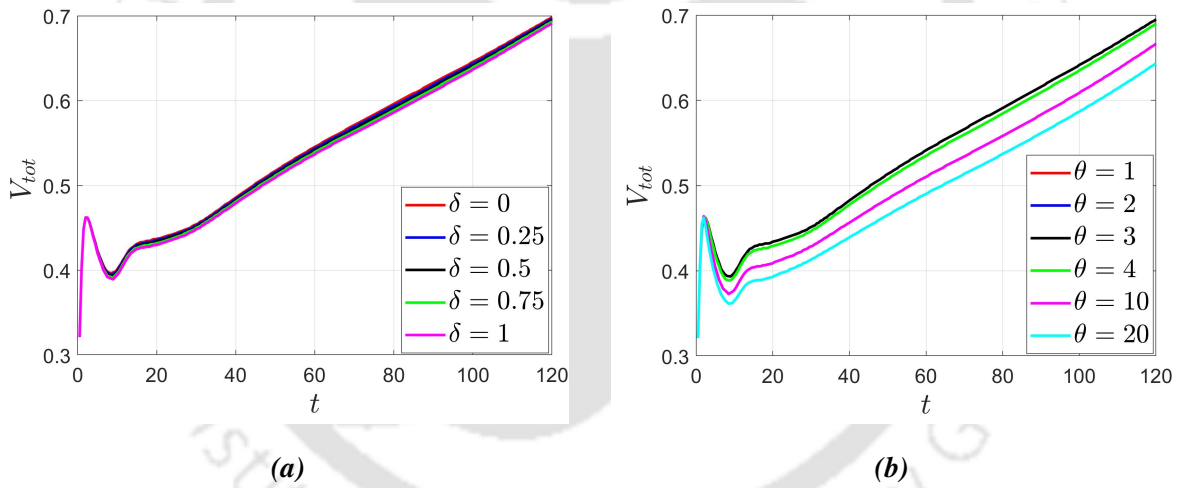


Figure 6.12: Effects of δ and θ on total volume of the tumor.

We conducted a series of simulations varying δ ($\delta = 0, 0.25, 0.5, 0.75$ and 1) and θ ($\theta = 1, 2, 3, 4, 10$ and 20). We observed that the volume fraction of mesenchymal cells increases over time as δ increases. This increase occurs because mesenchymal cells resist acquiring epithelial traits during the mesenchymal-epithelial transition (MET) process in a heterogeneous environment. Consequently, the volume fraction of epithelial cells decreases with higher values of δ . Change in δ does not affect the qualitative dynamics of the total tumor volume. As expected, we observed a small decrease in the total tumor volume with the increase in δ (Figure 6.12a).

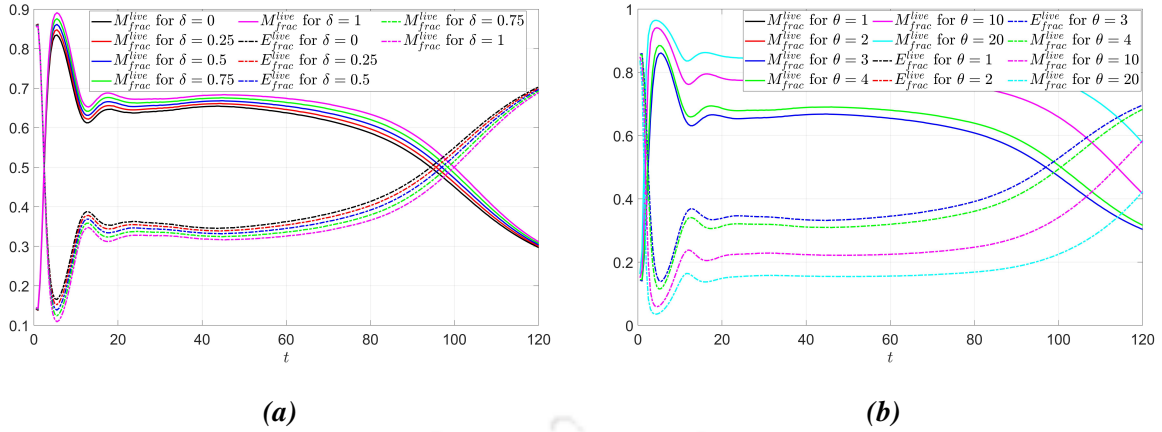


Figure 6.13: Effects of δ and θ on volume fraction of epithelial and mesenchymal cells.

Varying the parameter θ has a more significant effect on the dynamics of mesenchymal cells than varying δ (Figure 6.13). This phenomenon occurs because mesenchymal cells influence epithelial cells to adopt mesenchymal characteristics, which also leads to a decrease in the fraction of epithelial cell volume. Epithelial cells proliferate rapidly, but their number decreases as θ increases. For a large value of θ we observed a decrease in total tumor volume (Figure 6.12b). In summary, we did not see any change in the tumor propagation speed or the dynamics of the total live volume fraction inside the tumor as θ and δ vary.

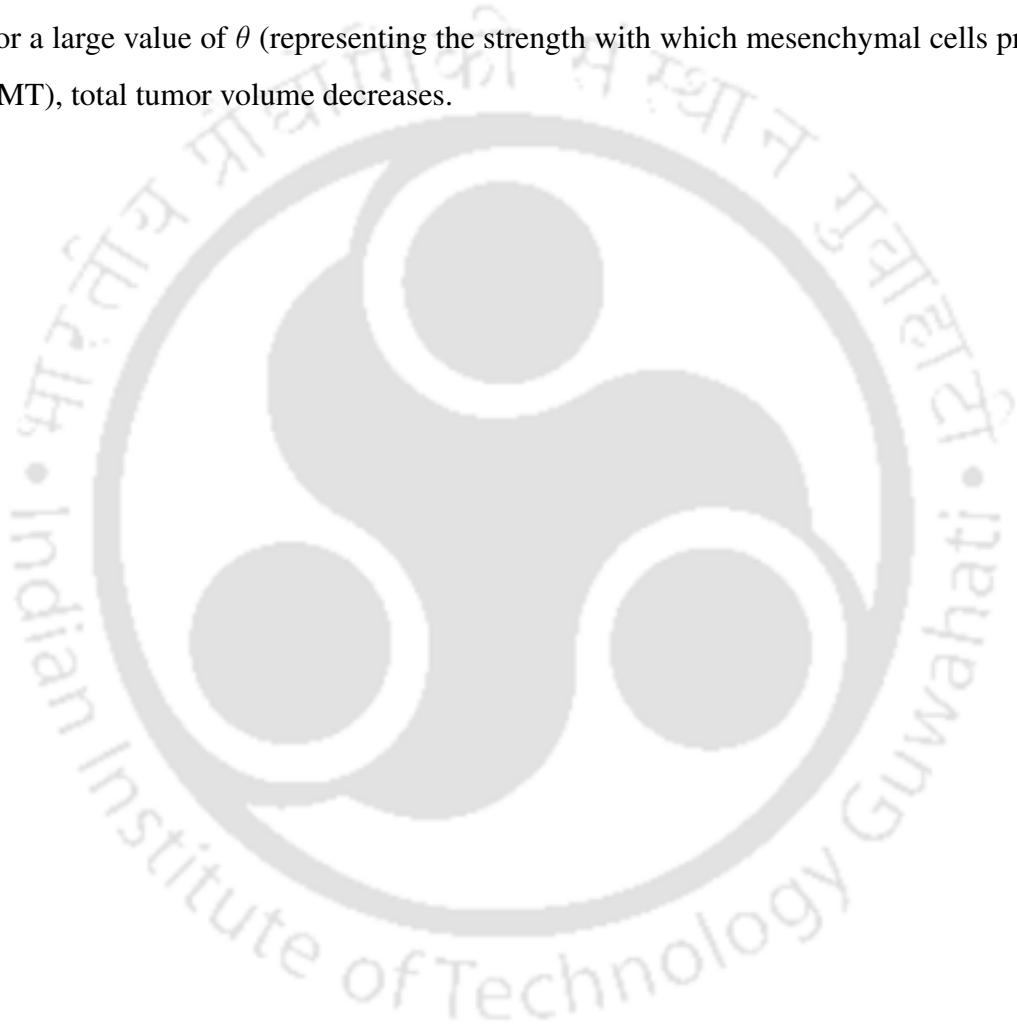
6.5 Conclusions

The model is simulated to understand the effects of oxygen heterogeneity and mesenchymal cell motility on EMT dynamics. The effects of cyclic hypoxia on EMT process are also analyzed. This study of EMT dynamics and tumor progression leads to the following findings.

1. Numerical simulations indicate that the tumor cells adopt a single phenotype based on the oxygen levels: under normoxic conditions, the cells exhibit an epithelial phenotype, while under hypoxic conditions, they transition to a mesenchymal phenotype.
2. It is observed that temporal changes in oxygen levels affect the tumor's overall growth rate.
3. Investigations reveal that as the timescale of the oxygen fluctuations decreases, the tumor's growth rate increases.
4. Simulation results indicate that spatial oxygen heterogeneity generates a heterogeneous

population within the tumor, where epithelial cells are localized on the outer rim of the tumor and mesenchymal cells are concentrated at the tumor center.

5. Numerical experiments show that an increase in mesenchymal diffusivity increases the density of the epithelial cells and also the tumor's volume.
6. Change in δ (which represents the inhibitory influence of mesenchymal cells on MET), does not affect the qualitative dynamics of the total tumor volume.
7. For a large value of θ (representing the strength with which mesenchymal cells promote EMT), total tumor volume decreases.



CHAPTER 7

CONCLUSIONS AND FUTURE SCOPE

7.1 Conclusions

In this thesis, a continuum mathematical modeling framework is employed to describe the underlying biological phenomena of tumor growth. In an *in vivo* scenario, the oxygen concentration at the tumor boundary near a blood vessel is different from that at the boundary far from the blood vessel. In Chapter 2, the effects of unequal oxygen concentrations on tumor growth are modeled using a multiphase framework. Oxygen tension inside the tumor forces tumor cells to switch their metabolic process from aerobic to anaerobic glycolysis. As a result, lactate is generated as a metabolic byproduct, which also contributes to the energy production cycle in normoxic tumor regions. In Chapter 3, the effects of unequal oxygen concentrations at tumor boundary on lactate accumulation are investigated. In this work, oxygen and lactate utilization are considered as linear phenomena. However, scientific literature indicates that oxygen and lactate consumptions are nonlinear processes. Additionally, it has been observed that tumor shapes are geometrically complex. In Chapter 4, a mathematical model is proposed to study whether the initial shape of a tumor impacts lactate accumulation and tumor growth dynamics in response to nonlinear interactions between oxygen and lactate. Additionally, the effects of cyclic hypoxia on tumor growth dynamics are explored. Lactate is also considered as a promoter of exosome secretion in the tumor microenvironment. Exosomes, which are cargo carriers of lipids, proteins and mRNAs, act not only as messengers to nearby cells but also to those distant from the tumor site. Exosomes also contribute to the increased proliferation of tumor cells. In Chapter 5, the effects of exosomes on tumor growth dynamics in an acidic

microenvironment are explored. Thus far, in this thesis, tumor cells are treated as a single cell population. In reality, they consist of several subpopulations, such as epithelial and mesenchymal cells. Epithelial cells acquire mesenchymal phenotype to initiate invasion, a process known as epithelial-mesenchymal transition (EMT). Experimental evidence showed that oxygen heterogeneity plays a key role in EMT. In Chapter 6, the impact of oxygen heterogeneity on EMT dynamics is investigated. All the proposed models in this thesis are simulated numerically. The following key findings are observed through numerical investigations of the proposed models.

- If the oxygen concentration at one boundary is lower than that at the other, both the tumor and its necrotic core grow asymmetrically around the tumor center. Additionally, the proliferating rims become of different widths at the tumor's edges.
- Unequal oxygen concentrations at the tumor boundary result in variations in the magnitude of the cellular phase velocity. Conversely, when oxygen concentrations are equal at the boundary, the velocity remains the same.
- It is observed that a tumor with a larger necrotic core grows more slowly compared to a tumor with a smaller necrotic core.
- In an *in vitro* setup, lactate accumulation is predominantly found at the tumor core, whereas in an *in vivo* environment, it shifts toward the boundary having lower oxygen concentration.
- The effects of necrotic core on lactate concentration are noticeable when the core begins to form. However, at a later stage, no significant impact is observed.
- Experiments conducted with tumors of different initial shapes reveal that the initial shape influences lactate accumulation in the tumor. However, its effect on tumor volume dynamics is negligible.
- The simulation results suggest that exosomes promote an increase in tumor volume.
- It is found that the presence of exosomes accelerates the spread of the tumor.
- Experiments with varying time periodicities of cyclic hypoxia show that a shorter periodicity results in an increased tumor growth rate.

- Simulation results indicate that spatial oxygen heterogeneity generates a heterogeneous population of cells within the tumor, with epithelial cells localized on the outer rim of the tumor and mesenchymal cells concentrated at the tumor center.

From the findings of this thesis, it is concluded that tumor growth dynamics are strongly impacted by oxygen heterogeneity within the tumor. Consequently, EMT dynamics are affected by oxygen levels in the tumor microenvironment. The experiment shows that cyclic hypoxia influences both tumor growth dynamics and lactate accumulation.

7.2 Future scopes

Recent advancements in biomedical research have been made possible by the availability of an unprecedented and rapidly expanding data across molecular, physiological, and clinical platforms from in vitro, in vivo and biomedical investigations. However, experimental biomedical research is expensive and time-consuming. Mathematical studies serve as excellent alternatives to experimental work. As discussed earlier, mathematical modeling plays an indispensable role in generating predictive hypotheses and providing significant insights into the mechanisms of complex physiological activities, multi-etiological disease, such as cancer.

This dissertation primarily focuses on integrating mathematical and physiological insights to investigate tumor growth dynamics. It presents the development and advancement of mathematical models, evolving from basic frameworks to more complex and biologically realistic representations of tumor progression. The proposed model can serve as a foundational framework for future investigations, with further developments potentially contributing to mathematical oncology research. The outcomes of these models will aid in the evolution of future models by incorporating more biological complexity and exploring different tumor growth mechanisms, depending on how closely cancer progression aligns with real physiological conditions. These investigations provide a foundation for further research, with several possible future directions outlined below.

- **Mathematical modeling of interactions between tumor and its microenvironment**

So far in this thesis, it is considered that oxygen plays a decisive role in various processes, including generation of necrotic cores, production of lactate, secretion of exosomes and epithelial-mesenchymal transition, all of which significantly influence tumor growth. However, within the tumor microenvironment, tumors consist of different

subpopulations of cells, including hypoxic, quiescent and proliferating cells, each responding differently to oxygen levels. Therefore, incorporating these subpopulations into mathematical models for studying tumor growth may provide a more appropriate representation of the reality.

- **Mathematical modeling of immune-tumor interaction**

In the tumor microenvironment, immune cells are present, but their effectiveness is reduced by tumor cells. However, genetically engineered T cells kill these tumor cells. Scientific studies reveal that the effectiveness of T cells depends on site oxygen level. These physical phenomena may be modeled mathematically.

- **Mathematical model of chemo/radiation therapies effects on tumor evolution**

To eradicate tumors or halt their growth, most patients receive chemotherapy, radiation, or a combination of both therapies. However, understanding the tumor growth during treatment is challenging. Mathematical studies can provide insights into the underlying phenomena.

- **Multi-scale mathematical model on tumor growth**

Tumor progression is a complex process that occurs at multiple scales, typically ranging from the molecular, cellular, and tissue levels to the organ or even the whole-body level. A robust multi-scale mathematical model of tumor growth effectively integrates molecular, cellular, and tissue dynamics to deliver a comprehensive understanding of tumor progression, interactions within the microenvironment, and responses to therapeutic interventions. This sophisticated approach not only enhances our understanding of tumor biology but also paves the way for more effective therapeutic solutions.

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The following research articles are drawn from this thesis work:

1. **Gopinath Sadhu**, K S Yadav, Siddhartha Sankar Ghosh and D C Dalal. On impact of oxygen distribution on tumor necrotic region: a multiphase model (Revised version submitted in *Acta Biotheoretica*).
2. **Gopinath Sadhu** and D C Dalal. A mathematical study of the interaction between oxygen and lactate in an *in vitro* and *in vivo* tumor, *International Journal of Biomathematics*, 2024. doi: 10.1142/S1793524524501389
3. **Gopinath Sadhu** and D C Dalal. Effects of non-linear interaction between oxygen and lactate on solid tumor growth under cyclic hypoxia, *Bulletin of Mathematical Biology*, **87**(3), 41, 2025. doi: 10.1007/s11538-025-01420-6
4. **Gopinath Sadhu**, D C Dalal and Helen M. Byrne “Micro-environmental acidity driven exosomes role on tumor progression: a mathematical approach” (Communicated).
5. **Gopinath Sadhu**, Helen M. Byrne and D C Dalal “The impact of oxygen heterogeneity on epithelial-to-mesenchymal transitions: a theoretical study” (Communicated).