MATHEMATICAL MODELING OF MMPS IN CANCER

by

Şirin Yonucu

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ABSTRACT

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New approaches like mathematical modeling appeared in biology to explain the accumulation of extensive data about complex biological systems like tumors. Mathematical models of tumors which have been studied for over 50 years, aimed to explore the dynamics and characteristics of tumor progression and to make predictions by combining the elementary principles of biology to clinical and experimental data. During cancer progression, genetical and microenvironmental factors mutually affect each other in molecular scales and, this results in a behavior in tissue scale which threatens the patients' life. What happens in molecular and tissue scales, are mostly known, but the relation between these two is impossible to discover with linear thinking. The discovery of how these events are linked, helps us to see the potential targets for treatments and to experiment on current treatments. With this motivation, we build a mathematical model of tumor growth in this thesis. We used continuous modeling approach and model the tumor progression within the framework of mixture theory. We focused on the activity of matrix degrading enzyme, MMP and its inhibition. In order to study delivery of MMP inhibitors, we set up a structure for a dynamic vessel web which also can help us to simulate activities of other substances and chemicals delivered by blood.

ÖZET

KANSERDEKİ MMP'LERİN MATEMATİKSEL MODELLENMESİ

Biyolojide, matematiksel modelleme gibi yeni yaklaşımlar, tümör gibi karmaşık biyolojik sistemler hakkındaki kapsamlı veri birikimini açıklamak için ortaya çıkmıştır. Yaklaşık elli yıldır araştırılan matematiksel tümör modelleri, temel biyoloji prensiplerini klinik ve deneysel verilerle birleştirerek, tümör gelişiminin dinamiklerini ve karakteristiklerini incelemeyi ve öngörüde bulunmayı amaçlamıştır. Kanser gelişimi esnasında, genetik ve mikro-ortamsal faktörler, karşılıklı olarak birbirini moleküler ölçekte etkilerler ve bu, doku ölçeğinde hastanın hayatını tehlikeye sokan bir davranışa neden olur. Moleküler ve doku ölçeğinde gerçekleşen olaylar çoğunlukla bilinmekle beraber, bu ikisinin arasındaki ilişkiyi, lineer mantıkla keşfetmek imkânsızdır. Bu olayların birbiriyle nasıl ilintilendiğinin keşfi, tedaviler için potansiyel hedefler görmemize ve mevcut tedavilerle deney yapmamıza yardımcı olur. Bu motivasyon sayesinde, bu tezde tümör gelişiminin matematiksel bir modelini oluşturduk. Sürekli modelleme yaklaşımını kullanarak tümör gelişimini, karışım teorisi çerçevesinde modelledik. Ekstra-selüler matris parçalayıcı enzimin aktivitesine, MMP'ye ve MMP'nin inhibisyonuna odaklandık. MMP inhibitörlerinin taşınmasını incelemek amacıyla, kanla taşınan kimyasalların ve diğer maddelerin aktivitesini simüle etmemize de yardımcı olabilecek bir dinamik damar ağı oluşturduk.

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LIST OF SYMBOLS

a	TAF concentration
A	acceleration
dS	change of entropy of living systems
d_eS	change of entropy by import
d_iS	entropy change by irreversible process
f	force per unit volume
f_{crit}	critical force
F	force acting on the volume element dV of the fluid
$F_{long-range}$	long-range forces
$F_{short-range}$	short- range forces
g	gravitational acceleration
I	identity matrix
L	typical length scale
k_{cT}	chemotactic motility coefficient of tumor cells
k_E	ECM degradation coefficient
k_h	hypoxia induced death rate coefficient of tumor cells
k_{hT}	haptotactic motility coefficient of tumor cells
k_{ij}	coefficient of drag forces between phases i and j $\in \{T, E, L\}$
k_{n1}	diffusion coefficient of nutrients
k_{n2}	nutrient uptake rate coefficient by tumor cells
k_{n3}	nutrient uptake rate coefficient by host tissue
k_{n4}	nutrient supply rate by the pre-existent host capillary
k_{m1}	diffusion coefficient of MMP
k_{m2}	MMP production rate coefficient
k_{m3}	decay rate of MMP
k_p	proliferation rate coefficient of tumor cells
k_{rT}	random motility coefficient (diffusion constant) of tumor cells
m	concentration of MMP
M	mass

$\mathbf{M}_k$	average interfacial momentum source
n	nutrient concentration
n_p	threshold value of concentration of nutrients for proliferation
n_h	threshold value of concentration of nutrients for hypoxia
p	pressure
P	interstitial fluid pressure
P_i	the rate of production or destruction of Q_i
p_k	pressure for phase k
Q_i	measure of the i-the element of an open system
R	Reynolds number
T_i	velocity of transport of Q_i
$\mathbb{T}_k$	total stress of phase k
$\mathbf{u}_{Fk}$	fluctuating component of the velocity
$\mathbf{u}_k$	average velocity of phase k
$\overline{\mathbf{u}_{lk}}$	local instant velocity of phase k
v	velocity of the fluid
V	average velocity of object relative to liquid
η	viscosity
ρ	density of the fluid
ρ_k	averaged density of phase k
ρ_{lk}	local density of phase k
ρ_m	mass density
μ	viscosity
μ_T	viscosity of tumor
σ	total stress tensor
τ	viscous stress tensor
τ_k	average viscous stress tensor
τ_{Dm}	diffusion stress
τ_T	viscous stress of tumor cells
τ_{Tk}	average turbulent stress tensor
φ_k	volume fraction of the phase k

φ_T^*	maximum volume fraction of tumor without compression
φ_{T_MIN}	volume fraction after which there is no adhesive interaction
Σ	field that accounts for compression and adhesion
Σ_T	deviatoric stress of tumor cells
Γ_k	the rate of mass generation
Ψ	scalar field

LIST OF ACRONYMS/ABBREVIATIONS

BM	Basement membrane
CT	Computed tomography
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
IF	Interstitial fluid
IFP	Interstitial flow pressure
MMP	Matrix metalloproteinase
MMPi	Matrix metalloproteinase inhibitor
MRI	Magnetic resonance imaging
SEM	Scanning electron micrograph
TAF	Tumor angiogenesis factor
TIMP	Tissue inhibitor of metalloproteinase
TIMP-1	Metallopeptidase inhibitor 1
TIMP-2	Metallopeptidase inhibitor 2
VEGF	Vascular endothelial growth factor

1. INTRODUCTION

"Biology today is where physics was at the beginning of the twentieth century; it is faced with a lot of facts that need an explanation." This observation is made by José Onuchic, who is the co-director of the Center for Theoretical Biological Physics at the University of California, San Diego [1]. This is why mathematicians, physicists and computer scientists begin to share the burden of handling these unexplained facts of biology, with novel interdisciplinary approaches. Now, we have the very detailed pictures of the smallest biological structures and molecular pathways; but in order to make sense out of these data, we need to find out how these small parts are combined and they function together. This kind of information is crucial, especially in dealing with diseases like cancer, which is a biological system with great complexity. Understanding the biology of cancer, by resolving the functional relations between its different components, is strongly needed for designing new treatments and improving the current ones.

1.1. Why Biology Needs Physics?

Up until recent years, biological research was directed by reductionist approach which included dividing biological systems to their small components and then trying to explain the small parts. This approach took its roots from the idea that all biological complex systems in their smallest parts are ruled by chemistry and understanding of these small parts will lead to understanding the big picture.

Nowadays, reductionist approach has reached to its limits [2]. With the advances in technology, we have a great deal of very detailed information about biological systems that we do not yet know how to interpret. We see that collecting information about diverse small biological phenomena is not enough to give an explanation about the whole system that they make up. Constituents of complex systems connect in various ways. Hence, without knowing the ways of connections, the knowledge of individual parts does not make us understand how the whole system works.

Understanding how biological systems work holds more promise than just describing them, it leads the way to controlling or even manipulating them. This holistic approach to the biology will certainly result in clinical benefits. There is a steady decline in the number of new drugs that is approved by US Food and Drug Administration even though there are significant amount of research which costs around 30 billion US Dollars [2]. This situation is associated with the infertile information gained by reductionist approach and it clearly shows the need of new methods in biology and clinical studies which will improve our comprehension about functions of the parts while they are interacting with each other. This will bring about new explorations and enhance our understanding about biological systems. When we really begin to understand these systems, we will be able to control and manipulate them. This will open us so many doors, the most important one is to design new therapies for various diseases and invent new drugs.

Reductionist information gathering may be a small first step in understanding the biological systems. Once we figured out how each individual part functions, we need to figure out how components are connected and controlled and how they function together [3]. This is a new approach for biology which has been a descriptive and empirical science. This is where physics enters the scene. Physicists are already used to defining the nature by using mathematics. Putting the pieces together by the help of simplifications and trying to grasp the collective behavior is a very well-known procedure for physicists. The excessive data piled up by reductionist approach can be handled using the holistic approach of physics.

In principle, physics should have the ability of explaining the living part of the nature, as well as the non-living part. It has already been used for understanding mechanics and dynamics of living parts so far. The mechanical characteristics of cells are resolved by describing them as viscoelastic matter by using soft condensed matter physics [4]. Bodily fluids and extracellular fluids are described with fluid mechanics. In addition to describing the mechanical behavior of biological structures, physics also has the potential of describing the complex biological systems. It has the ability of combining dynamics of phenomena happen in different scales together and finding out the characteristics of the whole system.

Biological systems are generally ruled by phenomena happen in different spatial and temporal scales. Gene expression and molecular composition of environment affect the individual cells in subcellular scales. The cells respond to these, and decide which metabolic activity they will perform, whether they will proliferate or be digested by programmed cell death. Then, these individual responses of cells are combined to make up regularly functioning colonies or living tissues. A change appears in one scale triggers other changes in other scales. The connections between the different scales are mostly far from being simple. Physics searches some patterns in this complexity to derive theories about the living matter.

However, understanding the living matter is harder than understanding the non-living matter. A physicist can perfectly describe the movement of a ball using Newtonian Dynamics without individually considering near 10^{30} atoms forming it [4]. Physics has the ability of considering 10^{30} particles as one body because of the fact that we can approximate each atom combining it as practically the same. By geometrical symmetry, we can find a center of mass representing these 10^{30} particles and we can describe the system with one equation of motion. However, that is not the case for biology. The identity of a cell is given by its nuclear genetic code. If we consider the numbers of available combinations of different DNA chains, we see that the cells can have huge variety in their composition and their biological properties. Even within the same tissue, differences in microenvironmental factors will lead to the activation of different parts of DNA, which will then result in a variety in the cells. Enormous numbers of chemical events change the composition of the cell environment, resulting in heterogeneous distributions of substrates and solutes, hence, erasing the geometrical symmetries. Even though this picture seems too complex to capture, there are some examples, which show that with practical approximations, characteristics of biological systems can be estimated successfully. As an example, Gompertz curve successfully describes growth in biology, from avascular tumors [5,6] to the bacterium populations.

Before deciding the right kinds of approximations and simplifications, a physicist needs to find biologically relevant questions. Not every feature of a biological system has to have functional importance because of the fact that evolution has chosen the best

available option rather than the best option. Physicists should collaborate with biologists to decide what is important in order not to drawn in the sea of vast biological data [1].

1.2. Physics of Biological Systems

We said that there is no reason for not explaining living matter by using physics and we talked about the difficulties involved in this process. However, there is one main issue left to be resolved at this point, why biological matter seems so unphysical?

We know that total entropy increases in each physical process from the second law of thermodynamics. Equilibrium is reached when the systems attain their maximum entropy. Hence, systems tend to go towards the states, having higher entropy and disorder. However, when we look at the living nature, we see a very high organization. From the building blocks of living nature, proteins which are molecules of great complexity to the living organism, which are composed of nearly 10^{14} cells, at each step of organization, the order is building up. We say that nature is ruled by physical laws, so how can we explain this increase in order of living nature?

When we analyze a physical system by thermodynamics, we are used to handling closed systems. Closed systems are the ones that prohibit material exchange between the boundaries of the system. A closed system eventually reaches equilibrium in maximum entropy and minimum free energy, and the ratio of phases stays constant after reaching the equilibrium. On the other hand, biological systems are open systems with a continuous flow of materials in and out. Inside the system, there is a building up and breaking down of the components continuously.

When closed systems reach the state of equilibrium, the energy transaction is finalized. A system in equilibrium cannot perform work anymore. The continuous capacity of doing work is needed for biological systems in order to sustain life. Thus, they necessarily have to be composed of open systems [7].

Open systems never reach the equilibrium, rather they reach a steady state where the system remains constant as a whole, and in its phases even though there is a flow of

materials. That is why this steady state is also named as dynamic equilibrium. Let Q_i be a measurement of the i-th element of an open system then the time dependent behavior of the system can be written as

$$\frac{dQ_i}{dt} = T_i + P_i \tag{1.1}$$

where P_i is the rate of production or destruction and T_i represents the velocity of transport of Q_i. In steady state,

$$\frac{dQ_i}{dt} = 0, \tag{1.2}$$

$$T_i + P_i = 0. \tag{1.3}$$

There are some results of having steady state for systems [7] are given below.

(i) The composition of system remains constant.

(ii) The ratio of components in steady state only depends on the system constants, not on environmental conditions.

These characteristic exactly match those of the living systems. Homeostasis requires constant flow of materials while trying to preserve a certain composition. The second result means that the system creates forces against disturbances of its steady state, which means that the system adapts to the new state and even if the amounts of imported materials changes, the composition remains constant. Hence, we can say that metabolism is the maintenance of steady state in living systems [7].

Since we have stated that the living organisms are composed of open systems, we can look back to the original question of validity of second law in the biological systems. The entropy of living systems can be represented by Equation 1.4

$$dS = d_eS + d_iS \tag{1.4}$$

where d_eS is the change of entropy by import which can be negative or positive and d_iS is the entropy change by irreversible process which is always positive. Hence, the total entropy change can be both positive and negative. When it is negative, the change of the

entropy of the environment that surrounds the system increases, to compensate this negative entropy. Entropy of the universe increases, obeying the second law [7].

Living systems use useful energy to create order and give out heat to the environment at the end of the process. Plants use sun light to make photosynthesis and build up energy rich simple sugars which can be broken up in the plant cells to provide the energy for metabolic processes such as synthesis of complex molecules like proteins. Animals use the chemical bond energy of organic molecules to sustain their metabolic activity. Energy flows from sun to the earth leaving behind increasing order and radiation of heat [8].

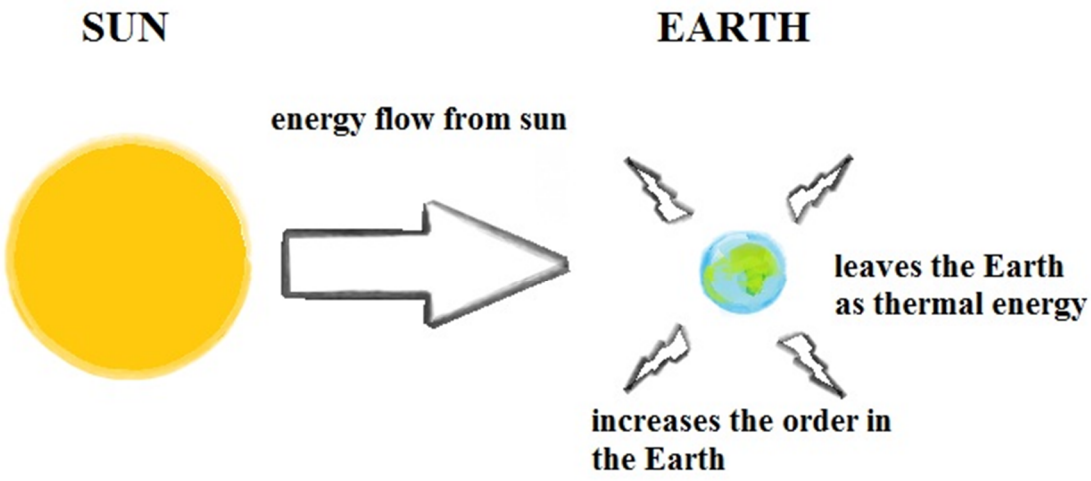


Figure 1.1. Energy flow to the earth.

1.2.1. Physics of the cell world

When we investigate a system in physics, we first consider the size of the system. When we know the size, we can have an idea about which physical equations can be applied to the system. World of small particles is ruled by quantum mechanics, while Newtonian dynamics applies in the regular sizes. When we have a system composed of many similar particles, statistical approaches are used to solve the dynamics of the system.

Now, if we look at biological systems, we see that they are all composed of the smallest units of life, the cells. So in order to capture the behavioral patterns of biological systems, we should first acknowledge the physical properties of the cells. Typical cell size is 10 μm and a typical cell mass is 1 ng. In animal cells, a full cell cycle takes about 24 hours [9]. Each cell is filled with cytoplasm, which is a jelly like material composed of 80% of water with salts and organic material inside. Cytoskeleton is stretched between the cell membranes, nucleus, and various organelles like a spider web to provide the cell with structural integrity.

Cells can be modeled as discrete or continuous structures. The right approach can be chosen by considering the relevant biological issues and the involved scales of these issues [10]. In continuous picture, physical responses of the cells can be understood by means of soft condensed matter physics. Cells can be modeled as viscoelastic fluids, porous medium etc. with different characteristics [11].

Cells are surrounded by other cells, extracellular matrix and interstitial fluid. Interstitial fluid is basically the water outside the cells. The composition is like that of the cytoplasm but the concentrations of components are different from the inside of the cell. Therefore, the cell and its surroundings can easily be approximated as water based solutions with different characteristics. We can have an idea about cells and their environment by studying fluid motion.

1.2.1.1. Fluid Motion in the Cell World. There are two kinds of flow in fluids. Laminar flow is seen when adjacent layers of fluid slide one by one. This motion stops, as soon as the forces that make them slide stop. Applying the exact opposite force will reverse the process and make the system return to its initial state. Frictional forces are dominant in this type of motion. The other kind of motion, which is turbulent flow, represents a more complex irreversible motion. This time, inertial effects will dominate over frictional effects. The type of flow is determined by the nature of the fluid and scale of the process. In order to determine which type of motion a fluid will execute, we will define critical force, f_{crit} as

$$f_{crit} = \eta^2/\rho_m \tag{1.5}$$

where η is viscosity and ρ_m is mass density. The type of motion with the influence of force f will be determined by the ratio f/f_{crit}. If the ratio f/f_{crit} is large then turbulent flow will appear. We will neglect the effects of frictional forces. If it is small, then the inertial effects can be neglected. The motion will be ruled by friction causing laminar flow [8].

Now, if we return to the cells which can be simplified as being composed of only water, we can calculate f_{crit} for water. At 20^0C, viscosity of the water is $10^{-3}\,kg/sm$ and mass density is $998kg/m^3$, f_{crit} is found to be $10^{-9}\,kgm/s^2$. We know that the typical forces in cells are in piconewton range which is equal to $10^{-12}\,kgm/s^2$. Then $f/f_{crit} \approx 10^{-3}$ in cell world, which shows that the determinant of the motion in the cell world is the frictional forces.

There is a dimensionless quantity called Reynolds number, to quantify the relative importance of frictional effects and inertial effects on motion. Reynolds number is given by the Equation 1.6

$$R = \rho_m VL/\eta \tag{1.6}$$

where V gives average velocity of object relative to liquid and L gives the typical length scale [8]. Taking $L \approx 10\mu m$ which is the size of the cell and $V \approx 10\mu m/s$, we can find $R \approx 10^{-5}$. The emergence of turbulent flow happens when $R \geq 1000$. We see that only laminar motion is seen on the cell scale. Hence, we can ignore inertial effects while determining the cell motion.

1.2.1.2. Forces and Conservation Relations. We stated that biological systems are open systems which allow material and energy flow with the environment. Nutrients flow to the tissue, and converted to mass inside, then, waste products flow out. This incident can be represented by mathematical conservation relations. When we consider a fixed cubic volume, the rate of accumulation of mass in this volume will be equal to the difference of the flow rate of mass into this volume, and out of this volume.

$$\frac{\partial \rho}{\partial t} = -\nabla.(\rho v) \tag{1.7}$$

where ρ represents the density of the fluid and v is the velocity of the fluid. If we expand the right hand side of Equation 1.7, we get

$$\frac{\partial \rho}{\partial t} + v.\nabla\rho = -\rho\nabla.v \cdot \tag{1.8}$$

The left hand side represents the accumulation of mass. The first term is the change in density with time (instantaneous change) which can account for the effects like density change due to temperature change. The second term is the change in density due to the flow of mass across the volume (convective change). The two terms can be organized in a single derivative called material derivative.

$$\frac{D\rho}{Dt} = -\rho\nabla.v \ . \tag{1.9}$$

Momentum is another conserved quantity that can help us understand the physical characteristics of the tissue. The rate of momentum accumulation is equal to difference between the rate of momentum that flow in and flow out, and the summation of all forces acting on the volume dV.

$$dV\left[\rho\frac{\partial v}{\partial t} + \rho v\nabla.v\right] = \sum F \tag{1.10}$$

or using the material derivative representation, we get

$$\rho dV\frac{Dv}{Dt} = \sum F \tag{1.11}$$

which is actually Newton's second law of motion,

$$\sum F = MA. \tag{1.12}$$

Now, we can take a closer look to the forces acting on this volume element dV. We can organize all forces in two groups as short-range or long-range forces.

$$\sum F = \sum F_{short-range} + \sum F_{long-range}. \quad (1.13)$$

Short-range forces decrease rapidly with the distance. They are also called the surface forces because they exist only on boundary of the fluid elements that is in contact. Surface force per unit area is called the stress, σ, which is a tensor. Stress includes pressure and viscous stress. Pressure represents the stress of fluid element which is at rest. It is always normal to the surface and it is the same in all directions. Viscous stress emerges as a result of motion of the fluid element and the velocity gradients inside the fluid. It has components in both normal and tangential directions. The stress tensor can be written as

$$\sigma = \tau - Ip \quad (1.13)$$

where τ is the viscous stress tensor, p is the pressure (normal stress), and I is the identity matrix. Since the stress is equal to the force per area, we can equate the force on the surface of the infinitesimal volume element dV as,

$$F_{short-range} = \sigma da\ . \quad (1.14)$$

By using Gauss' Law, we can rewrite the Equation 1.14 as

$$F_{short-range} = \nabla\sigma dV = (\nabla\tau - \nabla p)dV\ . \quad (1.15)$$

Long-range forces are called the body forces. The origin of force is far away from the body. The variation of the forces on the body of the fluid is negligible. Hence, they can be considered as homogeneous across the body. Gravity and electromagnetic forces are examples of body forces. Total body force is proportional to the volume and can be written as

$$F_{long-range} = fdV$$

where f is the force per unit volume. If we combine all forces, we can write momentum conservation equation as

$$\rho dV \frac{Dv}{Dt} = \sum F = (\nabla\tau - \nabla p)dV + fdV\,, \tag{1.16}$$

$$\rho \frac{Dv}{Dt} = \nabla\tau - \nabla p + f\,. \tag{1.17}$$

Now, if we study more about viscous stress, we see that it is connected to the fluid velocity. Shear force is the force stem from the different velocities between the planes of liquid. This velocity difference between the planes stretches the volume element. Shear force per unit area is equal to viscous stress.

$$\tau_x = \mu \frac{dv_x}{dy} \tag{1.18}$$

where μ is the viscosity. Considering viscous stress in three-dimensions, we can write this as

$$\tau_{ij} = \mu\left(\frac{\partial v_i}{\partial x_j} + \frac{\partial v_j}{\partial x_i}\right). \tag{1.19}$$

In vector form, Equation 1.19 becomes

$$\tau = \mu(\nabla.v + (\nabla.v)^T)\,. \tag{1.20}$$

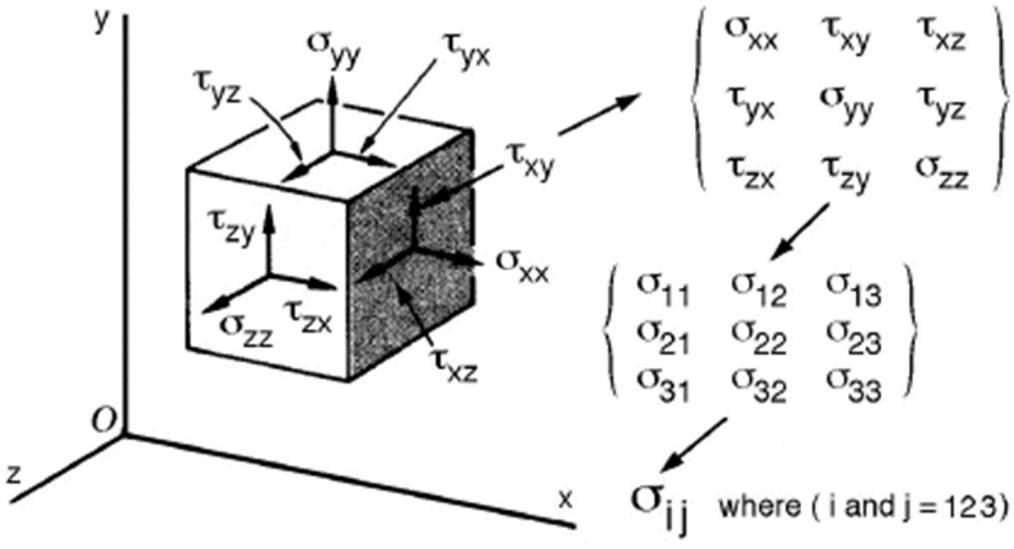


Figure 1.2. Stress tensor components [12].

Now, let us return to the original momentum conservation relation, and manipulate the viscous stress term as

$$\rho \frac{Dv}{Dt} = \nabla\tau - \nabla p + f, \tag{1.21}$$

$$\rho \frac{Dv}{Dt} = \mu\nabla^2 v - \nabla p + f. \tag{1.22}$$

This form of momentum conservation is called the *Navier-Stokes* equation.

We examined the types of motion in the low Reynolds Number case and stated that all motions in the low Reynolds Number world is laminar. Hence, inertial effects are negligible, compared to viscous effects. Now, if we consider Navier-Stokes equation again with this statement in mind, we can approximate the inertial term in the left hand side as zero. Rewriting the Equation 1.22 gives the *Stokes equation,*

$$\mu\nabla^2 v = \nabla p - f. \tag{1.23}$$

Since the body force density is very unlikely to change by its nature, the change in viscous stress balances the pressure change. At low Reynolds numbers the flow responds to the change in conditions, such flow is called as quasi-steady flow or Stokes flow. Low Reynolds number flow is valid for values less than 0.1. We calculated the Reynolds number for the cell world as $R \approx 10^{-5}$, so we can say that Stokes flow happens in cell world.

2. BIOLOGICAL BACKGROUND

In order to model the tumor growth, we need to understand the underlying biology. With its complex nature, cancer is a subject of the computational systems biology. There are various interactions among tumor cells and their environment; these interactions can be understood by systems biology approach. In this thesis, tumor biology is explained through the tumor cells, their environment and its progression with close connections to microenvironment. In this part, we tried to approach to the biology of cancer like a physicist, with quantitative features is always in mind, while explaining the necessary biology without extensive chemical details.

2.1. Tumor Cells

Cancer is the uncontrolled growth of abnormal cells in the body. A chain of mutations causes cancer to arise. Even though the cancer is one of the scariest diseases of our century, the initialization of cancer is enabled by mutations that are very rare. Only one per gene out of $2x10^7$ division will result in mutations [13]. Once they appear, these mutations will proceed in a way that analogous to Darwinian evolution. Successive changes of genetic material which brings growth advantages will accumulate to provide the change from healthy cells to malignant kind [14].

The differences in sequence and types of mutations, combined with effects of varying microenvironmental factors, cause the creation of the large family of cancers. During tumor progression, microenvironment changes in a way that supports tumor growth. Immune response to neoplasm triggers the release of the chemicals like growth factors, angiogenic factors and ECM degrading enzymes to tumor microenvironment. These conditions enhance the growth of the neoplasm into a tumor. Microenvironment shapes the evolutionary trajectories by applying selective pressures. Hence, different microenvironments impose their own unique constraints during tumor progression [15]. When we look at the tumors from this perspective, we can say that each cancer is unique due to the difference in genome and microenvironmental variables. Since tumor cells

emerge from normal cells by mutations, in order to understand how a tumor cell works, first we need to understand how a normal cell works.

2.1.1. Structure of the Normal Cells

Being a part of the very complex living organisms, the smallest units of life, cells have their own very complex inner machinery. Metabolic activities that cells need to conduct to function are divided between organelles and specialized cell structures.

Cells contain a nucleus that surrounds their DNA, which also acts as the control center of cells. Gene expression is conducted in nucleus, except the translation part, which takes place in cytoplasm. Confinement of genetic material in nucleus enables a higher regulation; it helps the gene expression to be controlled at the level of transcription [9].

Cells have a bilipid cell membrane separating them from environment. It allows passive diffusion of small molecules. There are pumps that actively transport materials from the membrane against concentration gradients. By controlling which materials are in and which are out, the membrane decides the composition of the cell [9]. There are proteins on membrane for the selective traffic of ions and biological molecules. Other proteins act as sensors that receive signals from the environment. As a result, the membrane provides the information flow between cell and environment.

Cytoplasm fills the gap between nucleus and bilipid membrane. Organelles that are responsible for metabolic activities are present in cytoplasm. The protein synthesis takes place in ribosomes. The endoplasmic reticulum, Golgi apparatus, endosomes, and lysosomes are involved in protein processing and delivery by vesicular transport. Mitochondria provide energy for the metabolic events of the cells.

Mechanical stability and cell movements are presented to cells by cytoskeleton which consists of microtubules and actin polymer fibers. These fibers are stretched between the nucleus and the cell membrane and they act like a highway to handle the transportation of organelles and other structures. They also help to maintain the communication inside the cell.

2.1.2. Cell Cycle

The cells are reproduced by the division of parent cell by mitosis. The cell cycle is the series of events involved in cell division. At the end of the cell cycle, parent replicates itself and divides into two daughter cells. Cell division is controlled in both cell growth and DNA replication to ensure that daughter cells have intact genomes. Cell cycle is regulated with growth factors in higher eukaryotes, which enables single cells to proliferate according to the needs of the whole organism [9]. When defects occur in this machinery, abnormal proliferation begins, which may lead to cancer.

2.1.3. Apoptosis and Necrosis

Cells need to function regularly in order to form fine functioning tissues. That is why tissue homeostasis is maintained with numerous regulation mechanisms. The regulation of the balance between the proliferation and cell death is crucial for tissues. Cells have an intrinsic control mechanism called apoptosis providing programmed cell death [16]. Apoptosis is also used for elimination of defective cells. Hence, when apoptosis is evaded, defective cells can accumulate to form neoplasmic growths.

Other mode of the cell death is necrosis. Cell damage can appear in two ways; either a shrinkage or a swelling of cytoplasm which initiates the process which then accompanied by dysfunction of membranes and organelles, dissolution of chromatin and finally interruption of membrane integrity. The dead cell leaves behind necrotic debris, which is cleaned up by phagocytic cells [17].

Apoptosis and necrosis can easily be separated with visual inspection. The morphology of the two modes of death can be used to differentiate one from another. During apoptosis rapid condensation and budding of the cell is followed by formation of membrane enclosed bags which includes the organelles. Nearby resident cells phagocytose and digest these bags. This process does not induce inflammation [18].

2.1.4. Hypoxia

Hypoxia refers to the reduced O_2 availability, which leads to a reduction or elimination of functions of cells. Hypoxia is associated with irregular blood circulation within the tumor. Thus, the presence of hypoxia is discovered in tumors while studying the distribution of necrosis relative to the blood vessels [19]. Lack of blood delivered to tumor may make tumor cells hypoxic and hypoxic cells die if this deprivation is continued to a limit that cannot sustain life. The emergence of the necrotic core stems from the death of cells by hypoxia, which is a common property of avascular cells. Hypoxia is an area of active research, because of the fact that it has more important implications than the emergency of the necrotic core. It is known to enhance tumor progression and increase the malignancy by applying selective pressure and speeding up the evolution. The cells that have the ability to migrate to other sites are the ones that can survive the hypoxia. Hypoxia also causes therapeutic problems by reducing treatment efficiency. Hypoxic cells are known to be more resistant to radiotherapy and chemotherapy [20].

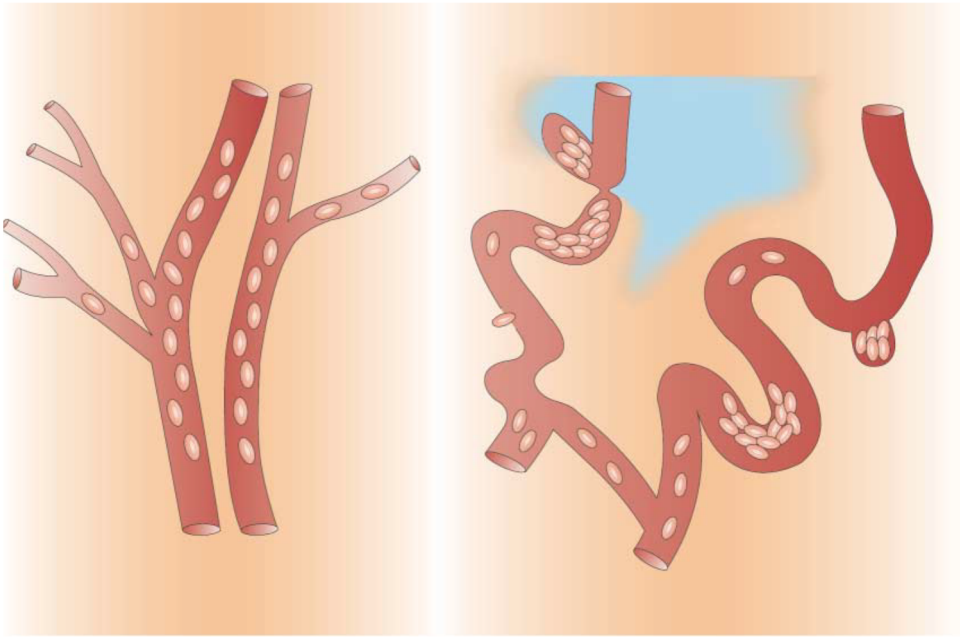


Figure 2.1. Vessels of healthy and cancerous tissues [19]. It shows the difference between host vessels and tumor vessel to emphasize the abnormalities in the tumor vessels which result in inadequate circulation.

2.1.5. Cancer Genes

There are special genes which play important role in the control of cell population by regulating gene expression, proliferation, growth and death. When a dysfunction of these genes occur, cancer is very likely to arise. The three main groups of cancer genes are composed of oncogenes, tumor suppressor genes and DNA repair cells.

Oncogenes encode the proteins that regulate proliferation and apoptosis. These include transcription factors, chromatin remodelers, growth factors, growth factor receptors, signal transducers, and apoptosis regulator [21]. Tumor suppressor genes are also called anti-oncogenes, which suppress proliferation. Their loss of functioning eliminates the control mechanism against abnormal proliferation. The third group involves DNA-repair mechanisms that provide accurate gene expression. The failure of these mechanisms results in chromosomal abnormalities [22].

2.1.6. Transformation of Healthy Cells to Malignancy

Although cancer is a very complex disease that includes nonlinear relations among tumor parameters and diverse microenvironmental variables, Hannahan and Weinberg say that the complexities of the disease will became understandable in terms of small numbers of underlying principles [14]. They explain the principles of transformation of healthy cells into malignant cell in their famous article “Hallmarks of Cancer”. Cancer cells have malfunctions in the regulatory circuits that govern cell proliferation and homeostasis. They group these malfunctions in six essential alterations which collectively result in malignant growth.

(i) *Self-sufficiency in growth signals:* Normal cells cannot divide in the absence of growth signals. Experimental and clinical results showed that tumor cells divide invariably indifferent to presence of growth signals, which derives the conclusion that they can generate their own growth signals.

(ii) *Insensitivity to growth inhibitory signals:* Cells decide to divide on the basis of external signal they sense from the environment. Both growth promoter and inhibitor

signals coexist in cell environment. If there are more growth promoters than inhibitors, cells divide and vice versa. Cellular quiescence and tissue homeostasis are maintained by growth inhibitory signals. Malignant cells establish insensitivity to these anti-growth signals to sustain abnormal division and growth.

(iii) *Evasion of programmed cell death (apoptosis):* The tumor population expands in number not, only by excessive division but also by evading programmed cell death. Experimental and clinical studies revealed that evading apoptosis is a common trait of nearly all cancer types.

(iv) *Limitless replicative potential:* The above three acquired capabilities ensures that tumor divides independently from the conditions in the environment. However, studies showed that they are not enough to sustain expansive tumor growth. Nearly all of the mammalian cells have an intrinsic mechanism to limit cell division which must be disactivated in order to provide malignant growth.

(v) *Sustained angiogenesis:* Even though cells need to be close to vessels for survival, they lack an intrinsic angiogenic ability which provides development of new blood vessels around tissue. Angiogenesis is triggered by the signaling mechanism. Tumors use that mechanism by changing the balance of angiogenesis inducers and inhibitors. When inducers outnumber inhibitors, angiogenesis begins and tumor provides itself with the nutrient source for further growth.

(vi) *Tissue invasion and metastasis:* Tumor attains its malignant character when it begins to metastasize and spread to other tissues, which leads to the invasion of the body [14]. The underlying mechanisms of invasion are still vastly unknown. Lack of cell to cell, cell to matrix attachment proteins is related to the invasive phenotype.

Evading immune destruction is another common property of tumor cells, and seems to be an important hallmark for certain kinds of tumors; but there is much evidence needed to generalize it [23].

2.1.7. Morphological and Functional Characteristics of Tumor Cells

Cancer cells do not only work differently from normal cells but they also look different. They have a large nucleus, irregular in size and shape. Surface, volume, density and homogeneity of tumor cells' nucleus distinguish them from that of a normal cell's. Nuclear membrane pores get larger, and intra-nuclear canals are formed between nuclear membrane and the nucleolus. These morphologic changes reflect the changes in metabolism. Structures involved in cell division expands, while structures relate to other activities diminish. As a result, gigantic nuclei and multinucleate cells are seen [24].

Cytoplasm is scarce in tumor cells, and it is either intensely colored or pale. The composition of cytoplasm changes, increased accumulation of RNA makes it basophilic. Numbers and sizes of organelles change to support high proliferation rate. Mitochondria gets smaller and high glycolysis occurs in tumor cells, which is known as "Warburg phenomenon" [24].

Changes in cell membrane are able to influence the evolution of the tumor and host response to the tumor. Changes in the cell surface receptors can change sensitivity of regulatory mechanisms provided by the host. These changes can be in the amount or shape of the receptors. In the latter, surface receptors cannot bind the corresponding ligand. New receptors can also appear which were previously hidden. The membrane supports the increased transport of nutritious substances, like sugars and amino acids [24].

Differences among cells inside the tumor mass can also be seen. Cells in the interior regions of the tumor mass have their normal intercellular connections; while for the ones at the rims, these connections are decreased [24]. For highly invasive tumors, they are gone completely.

Basal membrane, which is present in benign tumors, begins to disappear during the transition to malignancy. Tumor cells need to step out of all confinements in order to spread around the body.

High rate of proliferation is a characteristic of tumor cells. In order to develop and sustain this characteristic, they need to evade the growth control mechanisms of the body. They acquire the ability to proliferate in environments that do not support proliferation chemically. As an example, they can proliferate in the absence of growth factors.

There are also physical control mechanisms that regulate proliferation. If a cell is already surrounded by other cells, it does not divide. This behavior is called contact inhibition. However, tumor cells do not obey this rule; they keep on dividing even though they are densely surrounded by other cells. The opposite of this situation is also forbidden for normal cells. Cells that have no contact with other biological structures, are not supposed to divide. Cells are unable to proliferate in the absence of adhesion to extracellular matrix. Tumor cells break this rule by acquiring anchorage independence. This gives tumor cells an opportunity to survive and grow in inappropriate locations in the body. This is a characteristic that distinguishes malignant from benign tumors [25].

Changes that enhance cell proliferation are supported by the changes in the energy metabolism. Cells get their energy from glucose in aerobic conditions, and they turn to glycolysis in anaerobic conditions. It is observed that cancer cells uses glycolysis even when there is oxygen, and they also use it as the main source energy. Glycolysis produces less energy than procession of glucose, but it is observed that switching to glycolysis in turn enhances the uptake of the glucose [23]. Cancerous cells use massive amounts of glucose, nearly 5 or 10 folds compared to normal cells and they convert glucose to lactic acid [24].

Tumor cells have a reduced number of cell organelles, especially in the case of the endoplasmic reticulum and the Golgi apparatus. Malignant cells morphologically and functionally become similar to the fetal cells of the host tissue by the loss of differentiation. However, the main characteristic is not the loss of differentiation and the simplification of malignant cell structures, but structural and functional reorientation [24].

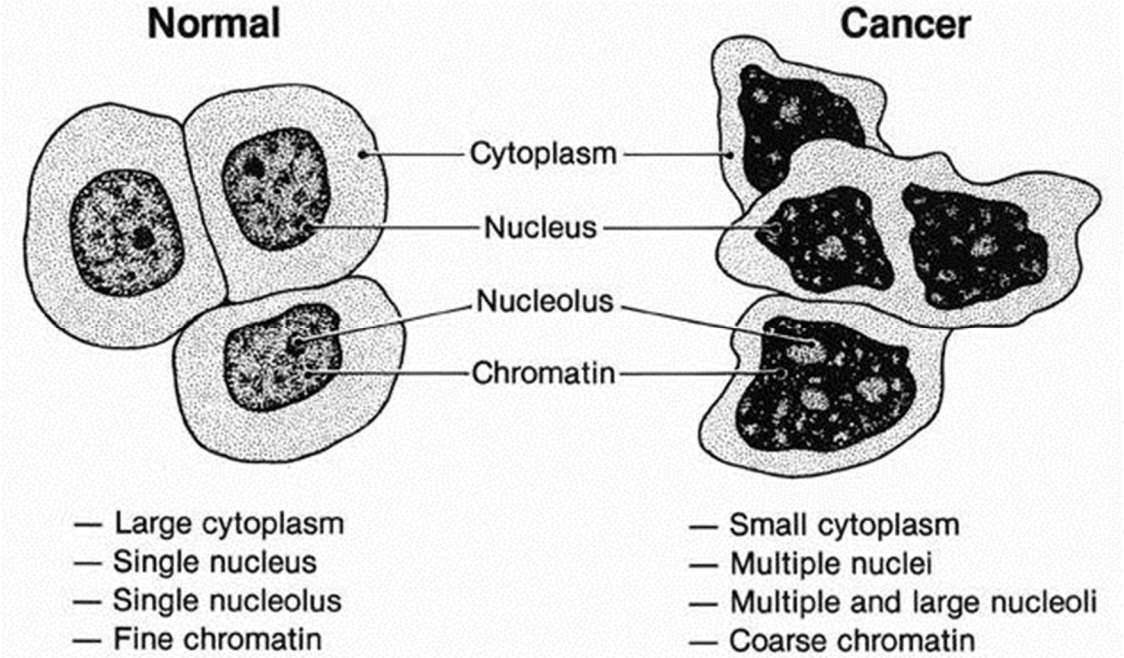


Figure 2.2. Main differences between cancerous and healthy cells [26].



Figure 2.3. Colored scanning electron micrograph (SEM) of a small cancerous tumor (pink) filling an alveolus of the human lung [27].



Figure 2.4. Colored SEM of a single breast cancer cell. The cell has an uneven surface with blebs (blue) and cytoplasmic projections (red, at left) [28].

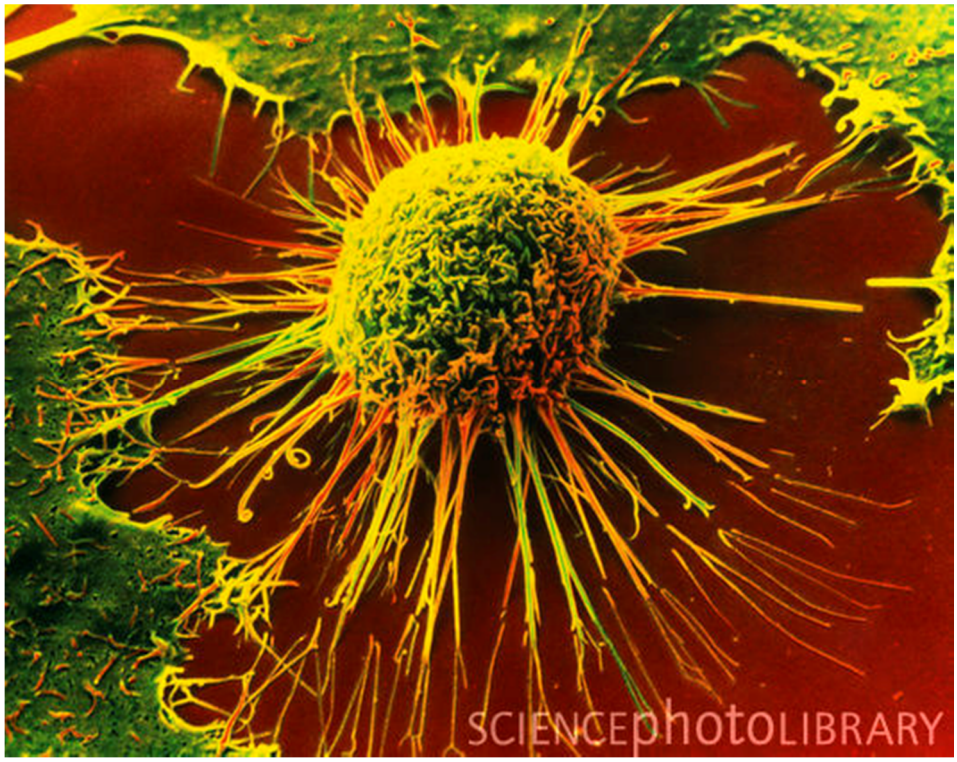


Figure 2.5. Colored SEM of a cell of a cervical carcinoma [29].

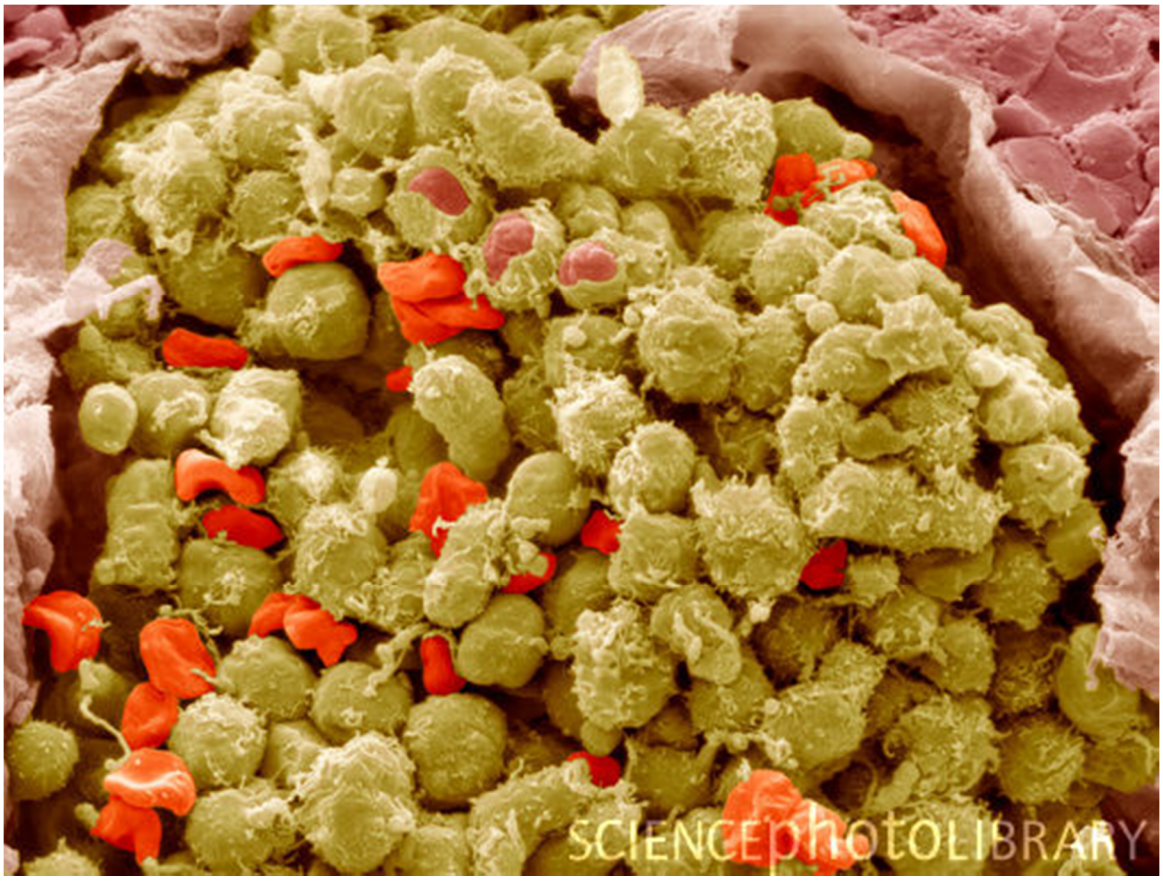


Figure 2.6. SEM of lymphoma cancer cells (yellow) in a lymph node [30]. Some red blood cells are also seen. The cells are surrounded by a connective tissue capsule (purple). Lymphoma is a cancerous growth of white blood cells.

2.1.8. Cell Motility in Tumor Cells

Tumor cells proliferate fast and fill all the available space. In order to grow further, they need to degrade basement membranes and extracellular matrix that is blocking their way. This degradation causes the release of water inside the cells and edema begins to appear. The edema is explained by high permeability of blood vessels and insufficient draining, caused by lack of lymphatic inside and around the tumor. The interstitial fluid volume facilitates cell locomotion [24].

Tumor cells need to acquire motile ability to move through the tissue. In order to move along, cells need to form new structures like lamellipodia; and focal adhesions are

created, in order to provide traction. Anchorage independency enables them to survive without attaching to ECM. They lose cell to cell junctions; hence, they are free to move.

The migration of cells can be induced by chemical and physical factors in the tumor microenvironment. Random movement, chemokinesis, occurs when a chemical factor induces movement without any preferable direction. Directed movement, chemotaxis, occurs when a chemical factor is applied heterogeneously in space. In this case, tumor cells migrate to the regions where the chemical factor is available [31]. Another form of motion, haptotaxis is the migration of cells towards the adhesion force of ECM [32].

2.2. Tumor Microenvironment

Tumor microenvironment refers to the total functional and structural material which accommodates the tumor [33]. The nutrients like oxygen and glucose, which are necessary for cell survival, are supplied by vessels to the microenvironment. Proteins like growth factors, chemokines or matrix degrading enzymes, which are already present in the microenvironment in the absence of neoplastic growth, may be induced further with the emergence of the tumor, according to the needs of the developing tumor. They are either secreted by stromal cells or the tumor itself. The latter is more plausible since the tumor cells lose their organelles to mainly focus on the proliferation. The proteins, which are not matrix bound, can be transported with the flow of interstitial fluid and with diffusion [34].

The tumor microenvironment is very different from the microenvironment of normal tissue. This difference emerges by various ways. The introduction of tumor cells to the microenvironment triggers the host tissues response. This response may be in the form of the activation of immune system or tissue remodeling mechanisms, and it changes the composition of biochemicals in the tumor microenvironment. Another group of changes include the direct results of metabolic activity and continuous proliferation of tumor cells. Tumor metabolism needs a lot of energy to sustain their continuous proliferation. So they act like nutrient sinks that reduce the concentration of nutrients in the tumor microenvironment. Tumor cells release chemicals to activate proteins that are already in the tumor microenvironment or they make host cell produce and release special proteins that are responsible for various tasks. As an example, tumors start the chain of events that

will lead to the production of MMP by the host cells. Then this MMP can be used to degrade the membranes or ECM to make room for growth of tumor cell population. The vessels of tumors have high anomalies that result in unbalanced blood supply. There are a lot of hypoxic regions that have inadequate nutrient supply which causes tumor cells to produce acids, as a result of their energy metabolism. Therefore, tumor microenvironments are highly acidic [35]. Even though there are a lot of unresolved relations between tumor and its microenvironment, we can say that microenvironment is manipulated by tumor cells, in a way that it supports excessive tumor proliferation and tissue invasion.

2.2.1. Structures in Tumor Microenvironment

Tumor microenvironment generally consists of tumor cells, tumor stroma, blood vessels, infiltrating inflammatory cells and host cells which vary according to the location of the tumor. The host tissues are healthy tissues which can be summarized in four groups, supporting tissues, mesenchyme, tissue specific cells; epithelium, defense cells; the haematolymphoid system, and the nervous system [36].

Organ surfaces are covered in epithelial cells that perform specialized functions. These cells compose the epithelium. Epithelium is separated from the supporting tissue stroma with basement membrane, BM.

2.2.1.1. Basement Membrane. The basement membrane is the thin sheet of fibers that separates epithelium. Benign tumors have basement membranes. In the transition to malignancy, basement membrane disappears. In addition to its own, basement membranes of different tissues or vessels also act as a barrier for tumor cells that try to spread. Hence, tumor cells need to degrade them to keep going.

2.2.1.2. Stroma. Stroma is composed of collagen fibres and fibroblasts which produce collagen fibres. The stroma is made up of extracellular matrix, ECM. Between the fibres of ECM; blood vessels, lymphatic vessels and nerves pass through. They bring the nutrients and other substances needed for metabolic activities of cells.

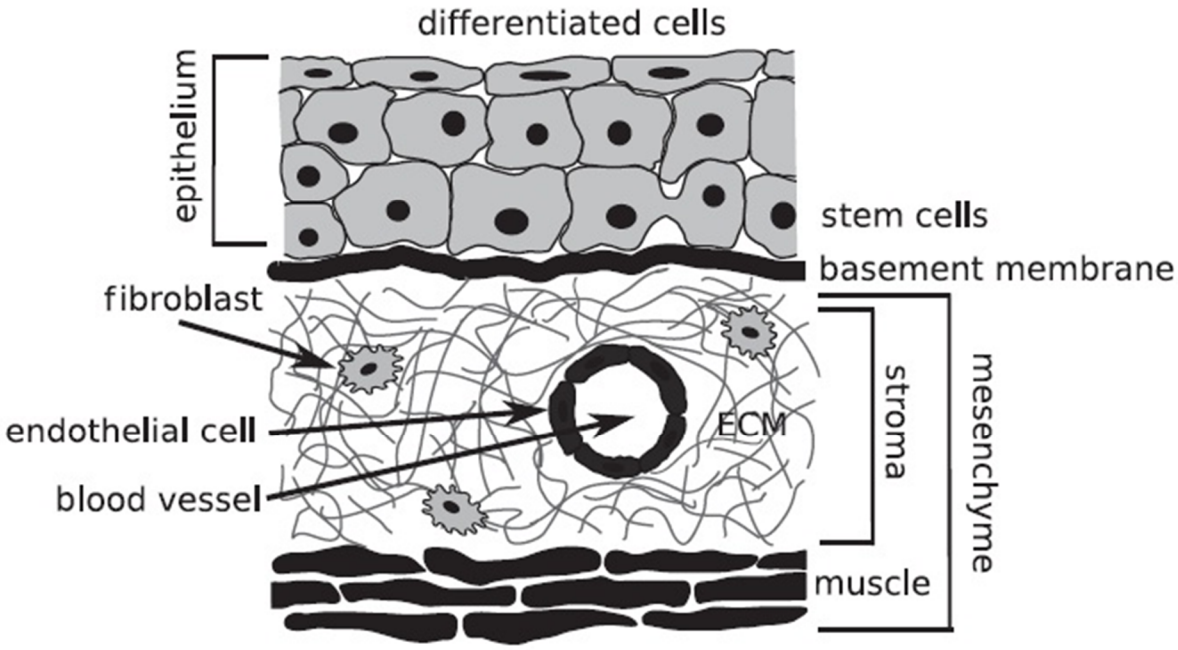


Figure 2.7. Microenvironment of epithelium [37]. Epithelium is the tissue that covers the organs; hence, it experiences neoplastic occurrences.

2.2.1.3. Extracellular Matrix. Most cells in the tissues are embedded in the extracellular matrix, ECM which fills the spaces between the cells, and also binds the cells and tissues together. ECM has elastic fibres which provide structural support to cells. There are many types of ECM according to the difference in the composition and amount of components which includes variety of proteins and polysaccharides. Structural fibrous proteins reside inside in a gel-like polysaccharide ground substance. In addition to them, there are proteins responsible for the adhesion between the matrix components and cells [9].

ECM has an important role in cells' life for supplying the materials. Vessels are present in ECM which carries nutrients to cell. Biochemicals like growth factors are also found in ECM, which regulates cells metabolism and survival by the help of signaling.

ECM transmits physical stress and pressure to cells. Density and porosity of ECM varies around the body, which changes the mechanic behavior of the ECM. Fibers provide a path for cells to migrate and for some cases; the web structure of ECM will become an obstacle to cells which try to move. Thus, tumor cells induce the release of matrix

degrading enzymes to degrade ECM to create space for growth. ECM degradation and remodeling are also needed for angiogenesis.

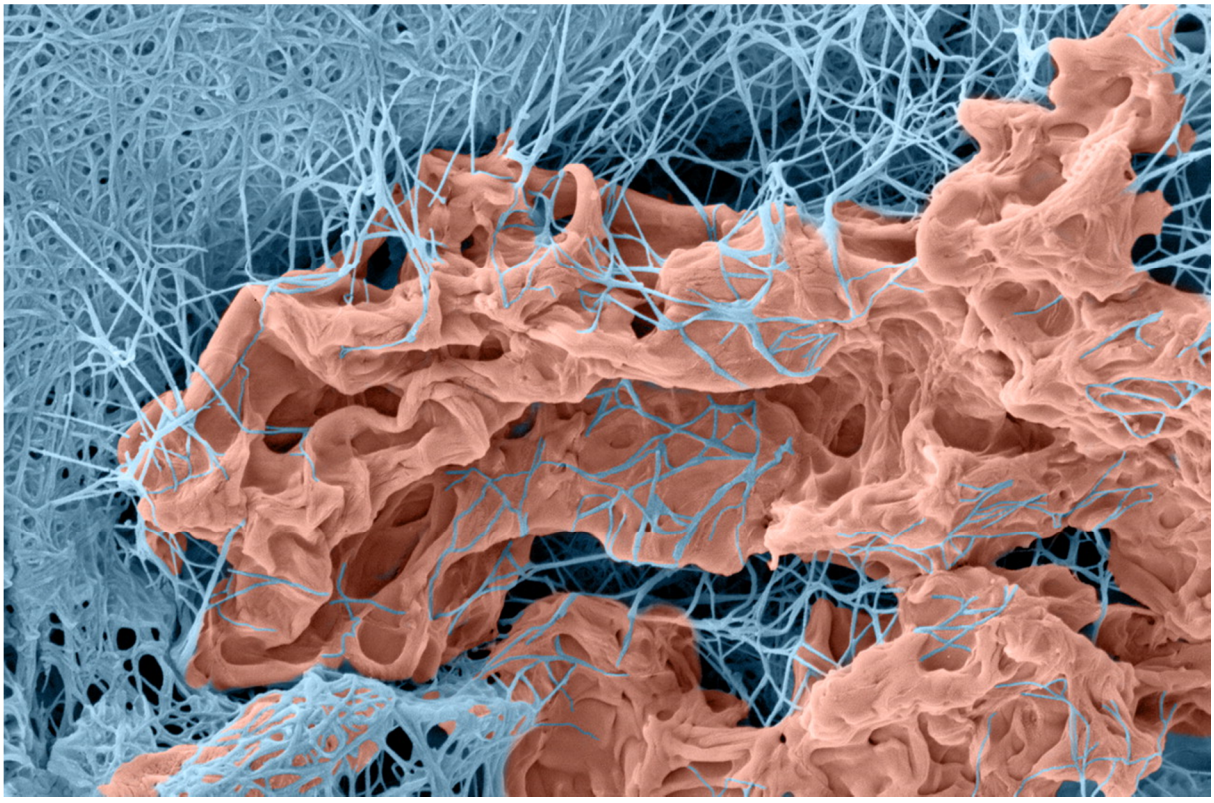


Figure 2.8. Colored SEM of ECM (blue) and infiltrating tumor cells (pink)[38].

2.2.1.4. Interstitial fluid. Interstitial fluid, IF, flows inside the fibers in ECM and surrounds the cells. Chemicals and nutrients which are solved in this fluid are taken inside the cells; while the waste from the cells is transferred from the cells into this fluid [34]. The rate of passage of fluid between capillaries and interstitial space is governed by differences in hydrostatic and osmotic pressure differences between these two regions and relative permeabilities of each region [39].

The interstitial fluid can affect cells in four ways. It can apply shear stress and normal force on cell surfaces. It can compel the adhesive bonds between cells and ECM, causing detachment of cells from ECM. Lastly, it can create chemical gradients in the direction of flow [40]. It is also necessary to transport nutrients and in cell-cell signaling when diffusion is not enough.

The flow is low compared to blood flow in normal conditions. It is experimentally measured between 0.1 μms^{-1} and 4.0 μms^{-1} . During inflammation, abnormal interstitial flow rates occur which triggers ECM remodeling. Migration of endothelial cells can be activated by shear stress caused by IF flow [41].

The interstitial fluid pressure, IFP, is known to be higher on the tumors. Normally, it is less than 10 mmHg; in tumors, it varies between 10 mmHg and 20 mmHg, with extreme measurements like 90 mmHg. Hence, in tumors, fluid cannot pass from the blood to the stroma as easily as in normal tissues. The reasons of high IFP include lack of functional lymphatics within the tumor. Since tumor has higher IFP compared to the healthy tissue surrounding it, there is a net flow out from the tumor into the healthy tissue [41].

According to other studies, another reason for high IFP is explained as a result of tumor induced angiogenesis. The blood vessels around the tumor formed by angiogenesis have structural abnormalities that make them leaky. Hence, they have higher permeability to both water and solutes; and this condition causes high IFP. In addition to that, the remodeled ECM is stiffer. This helps to maintain the high IFP within the tumor.

IFP is involved in the drug delivery problem that scientists try to overcome in order to increase the effectiveness of the treatments. High IFP brings about a higher diffuse permeability, however; it lowers the convective driving force.

2.2.2. Interactions with Microenvironment

2.2.2.1. Signaling. Cells sense environment by receiving signals with proteins called receptors which resides on the surface of the cell. Each cell has a variety of receptors for various specific duties. The signals that cell receives from outside world regulates metabolism, movement, proliferation, survival and differentiation.

Only certain types of molecules, which are called ligands, attach to receptors; which can be a peptide or other small molecule, such as a neurotransmitter, a hormone, a pharmaceutical drug, or a toxin. The shape of ligands must fit to the receptor for binding to

occur. After ligand is bind, a biochemical pathway is opened. This way, the cell can learn about [42]

(i) physical and chemical conditions outside the cell,
(ii) contact signals stem from adhesion of other cells or ECM,
(iii) signals sent by cells to regulate gene expression and other activities of other cells,
(iv) signals regarding homeostasis of the cell, including physical stress and internal balances of the cell.

Signaling is closely connected to cancer. The breakdown in signaling pathways that impose normal cell proliferation and survival can cause cancer.

2.2.2.2. Transportation of molecules. Cells need constant delivery of nutrients and growth regulators to survive. Concentrations of these chemicals need to be controlled. Transportations of these molecules are provided with diffusion, convection and binding interactions. Diffusion and convection make up the passive transportation mechanism. Molecules move along the concentration gradients with diffusion. Moving fluids in tissue microenvironment, like interstitial fluid, carry molecules to cells by convection.

Cells have receptors on their surface that connects them to the outside world by receiving signals. Weak intermolecular interactions between molecules produce a net stable force between specific molecules. Only a limited number of molecules can bind to a particular receptor. Binding molecules are used for selective transportation of molecules binding into the cell. When this transportation is done against a concentration gradient, cell needs to use some energy to conduct it and it is called the active transport [43].

2.2.2.3. Cell adhesion. Adhesion forces are produced by intermolecular receptor-ligand interactions and responsible for binding the cell to other surfaces like BM, ECM or another cell. They are named cell-to-cell, cell-to-BM and cell-to-ECM adhesions according to the surfaces that they are formed in. All adhesion forces act collectively to create the tissue geometry. Adhesion forces also play a role in cell motility. Especially, cell-BM and cell-ECM adhesion forces are crucial in providing the traction in cell motility [37]. Cells sense

other cells by the help of their adhesion forces. When epithelial cells are in contact with many other cells, the cell cycle stops and proliferation ceases.

2.3. Cancer Progression

Tumor development arises from oncogenic transformations happening in many cells simultaneously. However, only one cell will survive and multiply to form the tumor. The evolution will continue during growth; hence, tumor will have a high genotypic and phenotypic heterogeneity among the cell population, which will result in creation of subclones from the original neoplastic clone [24].

A tumor is called benign if it is confined to its original location without invading the surrounding normal tissue and spreading to distant sites. Benign tumors are mostly harmless and they can be removed surgically from the body [9]. That is why the name cancer is only used for the malignant tumors. Malignant tumors can both invade the surrounding normal tissue and spread throughout the body using circulatory or lyphatic systems to start other colonies in the other parts of the body. The classification of tumors is made according to the cell type they originate from. Hence, they are named carcinomas, if they arise in epithelial cells; sarcomas, in connective tissues like muscle or bone; lymphomas, in blood-forming cells and the cells of the immune system. The full description of the tumor also includes the tissue of origin combined with classification from the cell type, and tumors get names like breast or lung carcinomas.

2.3.1. Diffusion Limited Stage

The earliest detectable tumors which are only a few millimeters in size (10^6 cells, 1 mg mass) are called in situ cancers. In that stage, tumor is also called avascular, which means that tumor has not build its own network of blood vessels to access oxygen and other nutrients. Nutrients are delivered to tumor cells by diffusion. Diffusion of nutrients is the limiting factor in growth. In this stage, tumor may remain dormant for several years [44].

At this stage, growth of the tumor population resembles a typical Gompertzian growth curve for cells growing in culture. It starts off with a slow rate of proliferation due to the small number of population at the beginning; followed by a logarithmic growth phase, and entering slow phase again as the limiting effect of diffusion takes its toll. Eventually, the rates of population generation and population loss reach a steady state when tumor reaches its maximum size in avascular stage.

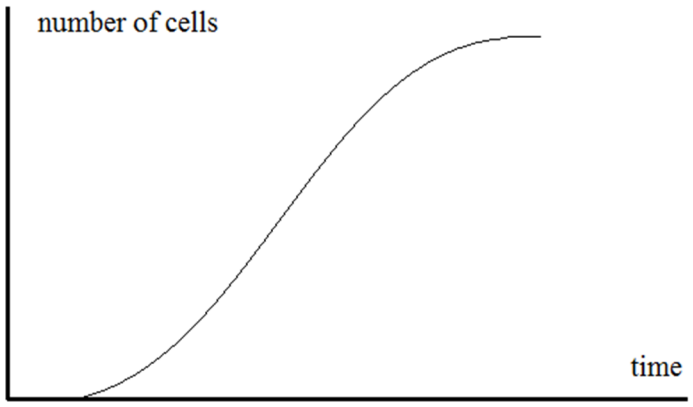


Figure 2.9. Tumor cell population which fits to Gompertz curve in avascular state.

Since the nutrients are consumed inside the tumor mass, the concentration of nutrients must decrease towards the center. After some point, it falls below a value that can no longer sustain life of the tumor cells [45].

At this point, a necrotic core begins to appear at the center of tumor. The growth rate decreases significantly after the formation of necrotic core. Tumor cannot find enough nutrients to proliferate, and waste disposal by diffusion becomes inadequate for tumor cells. Spherical tumor mass is divided into three concentering shells, according to states of cells. The necrotic core is in the innermost part, followed by quiescent cells in the middle, which continue to live but do not divide. Proliferating rim constitutes the outermost shell. Cells can change state according to the environmental and genetical factors; for example, quiescent cells that suffer from hypoxia, can proliferate when they are supplied with nutrients.

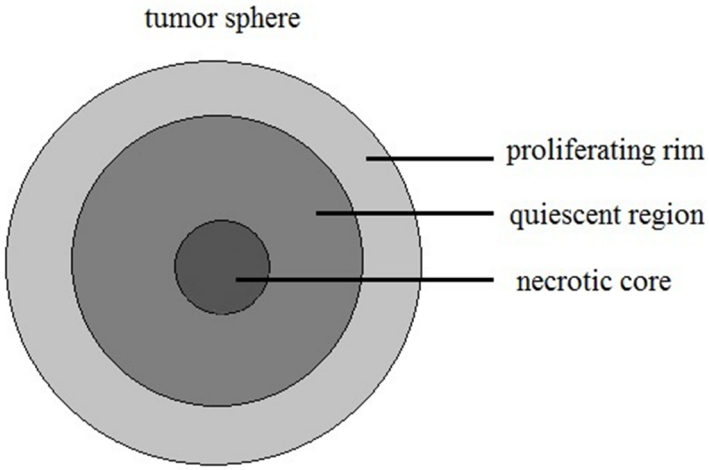


Figure 2.10. Avascular tumor spheroid which is mainly composed of proliferating rim, quiescent middle region and a necrotic core inside.

A tumor formed in epithelium that is separated from vessels by the basement membrane can be stay dormant until it changes into a more invasive phenotype that can pass through the membrane. The exact machinery that causes avascular tumors to transform to rapidly growing invasive tumors is not yet found. However, this transformation is thought to be linked to the vascularization process [44].

2.3.2. Angiogenesis

Angiogenesis refers to the growth of new vessels from pre-existent vasculature. It happens during wound healing, reproductive cycle, or tumor growth. This event is triggered by special proteins called tumor angiogenesis factors, TAFs. Tumors can shift the balance of angiogenesis inducers and inhibitors by altering gene expression and, for example, increasing expression of vascular endothelial growth factor, VEGF, a common TAF [24].

Angiogenesis occurs in a couple of steps [44]:

(i) Angiogenesis activators must outnumber the inhibitors around the subendothelial basement membrane of the existing vessels.

(ii) Endothelial cell must proliferate.

(iii) The newly formed endothelial cells must migrate towards the angiogenic stimulus.

(iv) They begin to lay down a basal membrane around the nascent capillary.

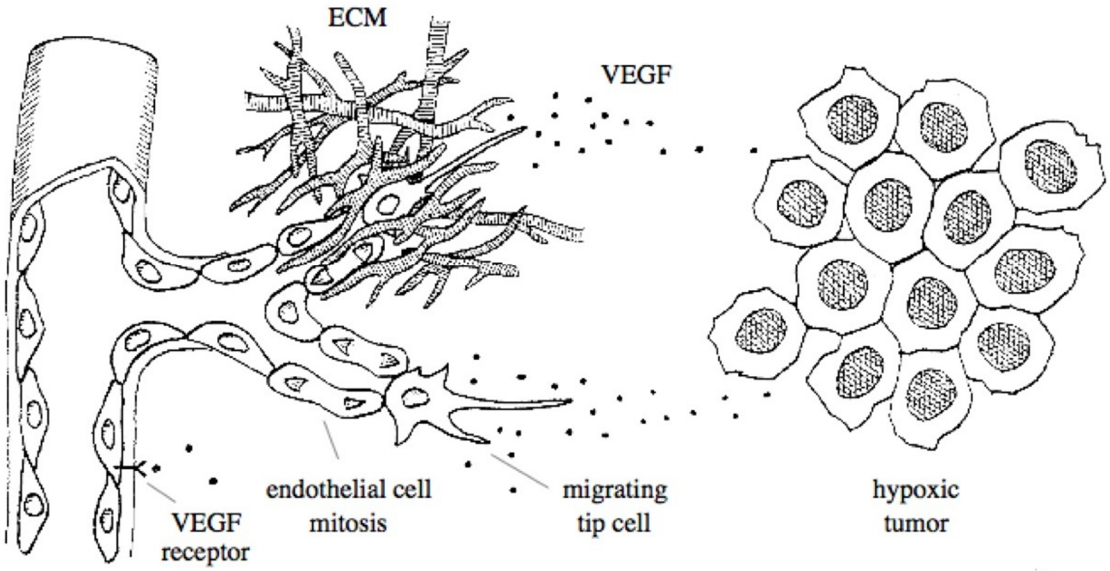


Figure 2.11. Angiogenesis [46].

Most cancer treatments aim to kill the tumor cells. However, with the comprehension that tumors need their own blood supply, a new therapeutic approach appeared targeting the blood supply of the tumor. When you target tumor cells, they may evolve and became resistant to the drug. However, by eliminating the blood supply, tumor cell can be killed even if they are drug resistant [22].

2.3.3. Vascularized Tumor

There are two different populations of vessels in a vascularized tumor; the preexisting host vessels, and tumor micro-vessels arising from the activity of tumor angiogenesis factors. The preexisting vessels can sustain the tumor cells in avascular state, during this time, some morphologic changes occur in these vessels. They become tortuous, elongated and dilated. As tumor mass grows, the preexisting vessel web stays constant; hence, the diffusion surface per unit tumor mass decreases. Some of these vessels collapse as a result of the pressure built by the excessive proliferation.

Neovascularization occurs in the capillaries within the tumor mass, or from the capillaries of the host tissue. Micro-vessels of highly differentiated tumors can have a mature architecture. On the other hand, hastily formed new vessels can have a lot of structural abnormalities. These irregular growths result in a microcirculation, which is heterogeneous spatially and temporally within the tumor [47].



Figure 2.12. SEM of a freeze-fractured section through the colon wall of a patient suffering from colon cancer [48]. This is the sub-mucosa, the layer that lies beneath the lining of the colon. Red blood cells (red) are seen in the blood vessels that infiltrate the sub-mucosa.

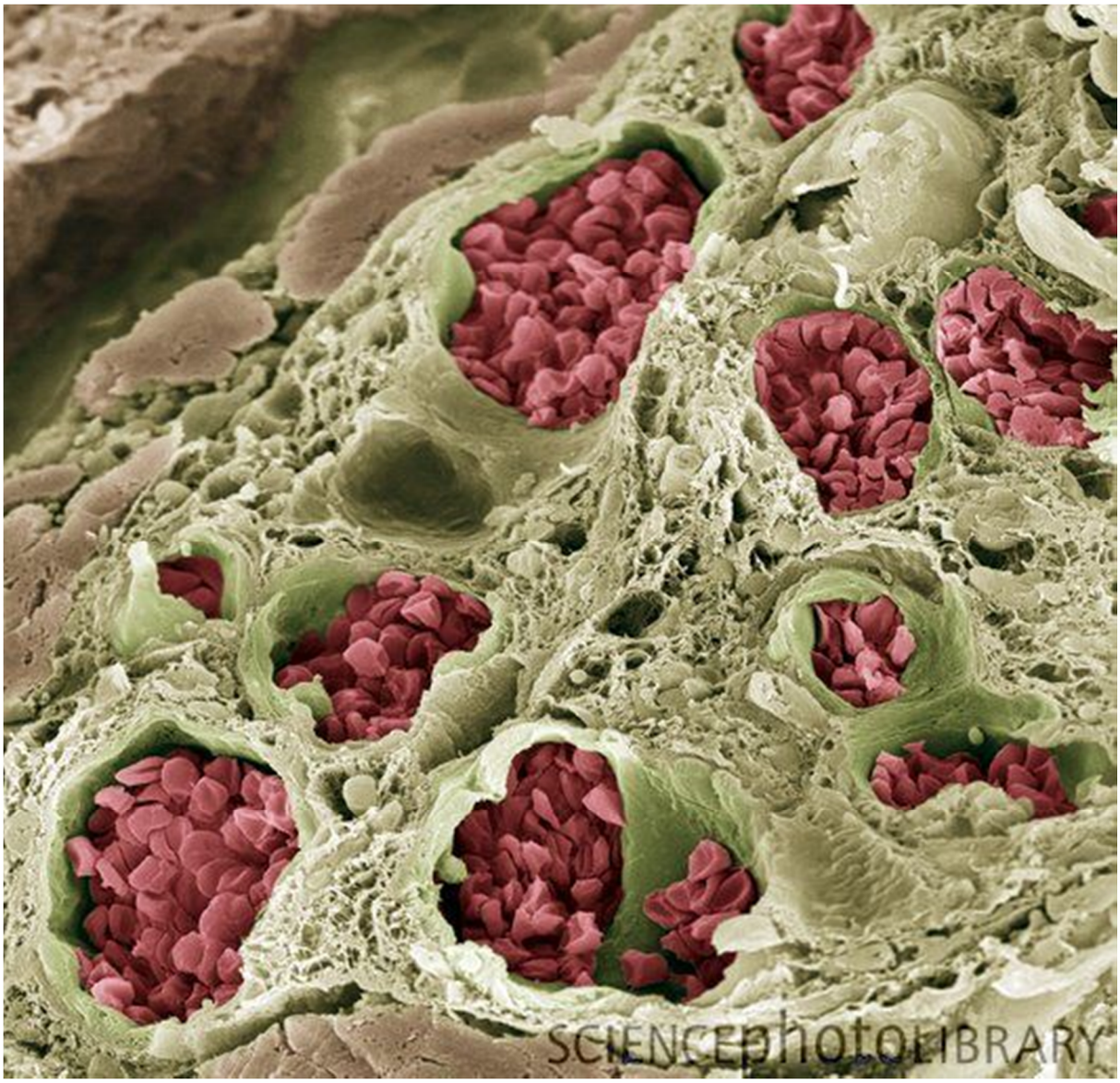


Figure 2.13. Intestinal blood vessels [49]. Colored SEM of a freeze fracture section through the lining of the colon, showing red blood cells (red) inside the blood vessels of the lamina propria (green). This tissue layer lies beneath the epithelium of the intestines.

The colored SEM that shows healthy and cancerous intestines exhibits the differences induced in the tissue due to appearance of cancer. The healthy tissues have more intact vessels compared to the cancerous one.

Vascularization brings about a rapid growth. Tumor mass grows to 1 cm and 1 g in a few months or years depending on the cell type. A tumor of this size can barely be detected by sensitive diagnostic methods such as CT and MRI scan. When it reaches to detectable

size, tumor reaches a population of 10^9cells, which means roughly 30 population doublings. If it is not detected after this point, only in five more doublings, it will become about 4 cm and 32 g [44].

Clinical studies showed that angiogenesis is an important step for metastasis. Highly vascular tumors can proliferate in very high rates. This presents the opportunity to produce more mutations, which will help to metastase, as a result of many divisions. In other words, tumor evolution becomes faster. Heterogeneity of tumor mass increases which leads to metastasis. However, the most important contribution comes from the supplement of efficient route of exit for tumor cells to leave the tumor mass and enter the blood stream. Since angiogenesis increases the density of blood vessels which have also high permeability, tumor cells have a higher rate of entrance to the blood stream [50].

2.3.4. Metastasis

Metastasis occurs when a secondary colony of tumor cells develop at a distance from primary tumor by migration from the primary tumor colony and hold on to a completely different tissue. Metastases can occur early, simultaneously with the primary tumor or they can be developed later.

Metastasis rate is higher in highly vascularized tumors, but it is not a compulsory condition. All vascularized tissues can be the target of secondary growths while filter organs like lymph nodes and capillary-rich organs, such as the lungs, liver, kidneys, bone marrow have a higher frequency of metastases. Experiments showed that for some cancer cells, there might be an affinity for a certain organ or tissue. Receptors on the tumor cell surface are responsible for this kind of selective migration [24]. Poor adhesion of cells helps tumor cells to detach as well as histolytic enzymes. Metastatic cells are much more independent from the growth factor, compared to their non-metastatic or less metastatic partners.

In order to produce metastasis, the cells of the primary tumor must pass some obstacles and go through the stages given below [24].

(i) The cell or the cell group must detach from the primary tumor,

(ii) enter a blood or lymphatic vessel,

(iii) migrate into the blood or lymph flow,

(iv) through lymphatic circulation, neoplastic cells can stop in a lymph node, multiply and disseminate,

(v) through blood circulation, cells can stop in the lung or the liver, they form growth centers and subsequently disseminate,

(vi) some migrating cells reach and stop in other organs such as brain, bone and kidney.

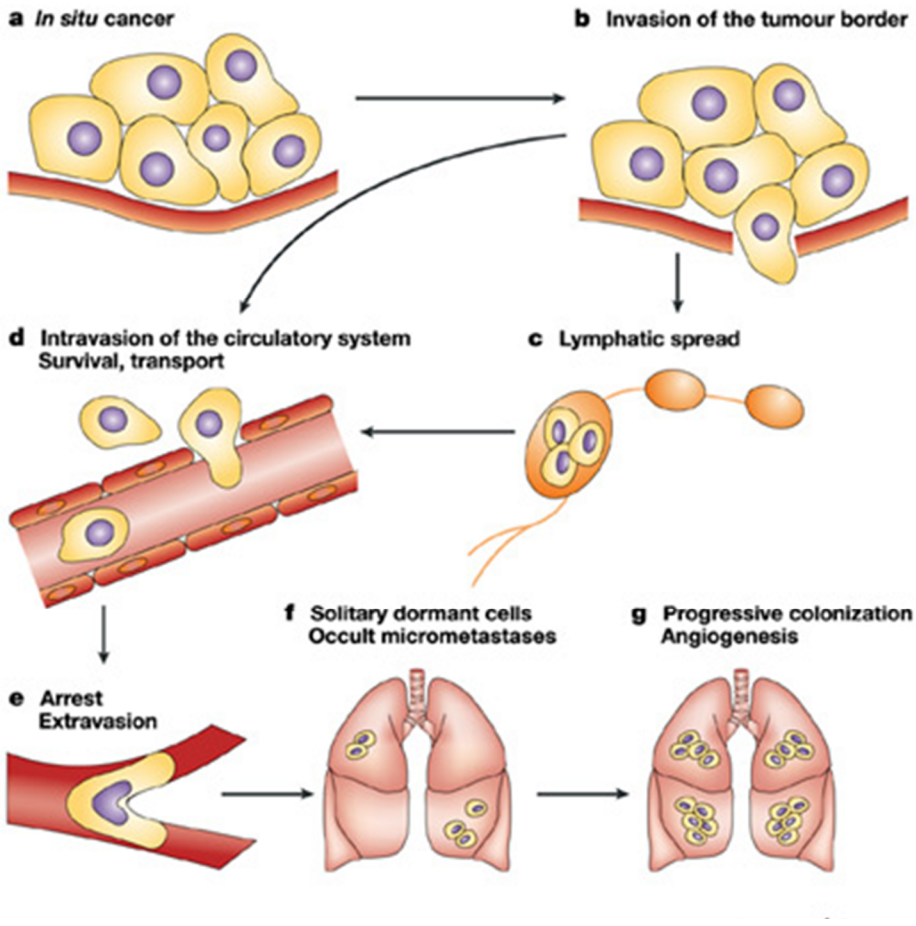


Figure 2.14. The invasion of in situ tumors by circulatory and lymphatic systems [51]. The tumor cells then reach to lungs to form secondary colonies.

It is experimentally demonstrated that metastasizing is a selective process, in which only the cells with special characteristics are able to overcome this obstacles and

form colonies in distant sites. It is also demonstrated that, the selection among primary cell clone is also correlated with its metastatic potential. Metastasizing cell clones, have a higher growth autonomy, which means that they are less affected by the environment during proliferation [24]. Thus, metastatic cells can suppress the non-metastatic ones in the presence of environmental pressure.

2.4. Cancer Treatment

The therapies for tumor are mostly focused on killing the tumor cells. Whether it is radiotherapy or anti-cancer drugs, they aim to kill the highly proliferating tumor cells. However, while doing this; they also killed the healthy cells. Hence, we need to find ways to selectively kill the tumor cells. This brings the necessity of finding aspects of tumor cells that is different from healthy cells and design treatments by using this contrast [52]. As a result, we can spare the healthy cells while killing the malignant ones.

Tumor microenvironment highly differs from the microenvironment of a healthy tissue. Thus, tumor cells go through some changes to adapt these different conditions. These changes can be used to create new targets for drugs [52].

Activities of anti-cancer drugs are limited to the insufficient carrying capacity of the abnormal tumor vessel web. That is why the hypoxic regions are one of the main problems in cancer treatment. Since they are beyond the diffusion length of chemicals, delivery of the medicine to these regions is especially hard.

The leakiness of tumor vessels provides another opportunity for anti-cancer drugs. Since pores in the tumor vessels are bigger, big molecules that normally cannot pass through the vessels can be delivered only to the tumors; preventing healthy tissues from getting harmed. Special drugs that will serve this purpose are designed; however, the heterogeneous tumor vessels prevented the drug from reaching to some regions inside the tumor. Hence, once again a treatment has failed to yield net success due to the bad circulation of blood.

These results stimulated the search for different methods. Angiogenesis is important for tumors to the transition into malignancy. In the absence of blood supply, a tumor can only grow to the sizes of a few millimeters. Because of the fact that angiogenesis, is a tumor induced event, anti-angiogenic drugs are designed. Hence, these drugs will not disturb the healthy cells while preventing the tumors from accessing to blood supply. Anti-angiogenic medicine is a promising field in cancer research that hopefully gives net benefits to patients.

A significant increase in amount of matrix degrading enzymes, which are normally very low on the tissues, points towards another target for therapy. Yet MMP treatments also resulted inconclusive and questions have remained behind.

The realization of importance of the tumor microenvironment for the progression of the disease, strengthen the idea of each cancer is unique. That is because of the reason that different microenvironmental factors lead to different tumors and each microenvironment is unique. As a result, patient-specific treatment approaches became a research subject. Patient-specific medicine includes processing of data; which is composed of tumor type, size, location, patient age, conventional grading and staging of the disease, molecular fingerprint and growth kinetics of the tumor. Due to the fact that every tumor is unique, patient specific treatments are thought to be needed to cope with the disease [53].

2.5. MMP in Tumor Growth

The understanding that tumor microenvironment is as important as genetic mutations makes scientists to take a closer look at the tumor microenvironment. Biochemicals and their activity, interactions and signaling have been researched extensively to decipher the relations with tumor growth. MMP family also got its share from this microenvironment exploration trend. High levels of MMP that have been seen nearly in the all kinds of malignant tissues, suggested a strong relationship between malignancy and MMP activity. Thus, for 30 years MMPs have been studied as candidates for cancer therapy [54].

MMP family with nearly over 20 members, are classified as zinc dependent proteinases that capable of degrading nearly all kinds of matrix structures as well as

basement membrane. The large group of MMP substrates includes other proteinases, proteinase inhibitors, clotting factors, chemotactic molecules, growth factors, growth factor binding proteins, cell surface receptors and adhesion molecules. Each MMP is able to degrade a particular subset of the matrix proteins [55]. They are important regulators of tissue microenvironment with their role in tissue remodeling and organ development [56].

2.5.1. Regulation of MMP

The activity of MMP is highly regulated by four different mechanisms. They can be regulated in gene expression or with compartmentalization, activation of enzyme and its inhibitors [55]. The regulation in the gene expression level corresponds to the regulation in the production. MMP production is induced or inhibited, according to the needs of the tissue. Normally, MMPs are expressed in very low levels, expression is induced when there is active tissue remodeling in the tissue.

MMPs are produced as inactive enzymes. Their activation serves as another control mechanism. In some cases, activation of one MMP initiates the activation of other MMPs [55]. Their activity depends on the local balance between MMPs and their physiological inhibitor. The major physiological inhibitors are the TIMPs which can inhibit the active forms of MMP [57].

2.5.2. Functions of MMP in Tumor Development and Growth

In general, MMP levels are low in healthy tissues and go up only if there is a challenge to the system like wound repair or a disease state. However, MMPs are up-regulated in nearly all kinds of tumors; this is why we link them to tumor progression [54]. MMP levels serve as a prognostic indicator of clinical outcome in multiple tumor types since MMP levels and malignancy is closely related [55].

MMPs are largely produced by stromal cells present in the tumor microenvironment which suggested that increased amount of MMP is a result of host tissue response to the tumor. It is thought that host tissue reacts to the tumor like it is a wound and initiates wound healing response which includes MMP [55].

According to a surprising result, MMPs are up regulated and present in active form even before destruction of basement membrane and emergence of malignancy; which suggested a more complicated involvement in tumor growth rather than simply degrading matrix and BM that is blocking the way.

The activities that MMP can mediate involve cell proliferation, cell differentiation, tumor associated angiogenesis and malignant conversion. It is likely that, effects of MMP in non-matrix substrates, like chemokines, growth factors, adhesion molecules and apoptotic mediators, induce rapid and critical responses required for tumor growth [54]. Hence, MMP does not just clear the way for tumor cells to move, by reacting with non-matrix substrates, it creates an environment conductive to tumor growth.

MMP can change the balance of growth and anti-growth signals in microenvironment by changing the bioavailability or functionality of multiple important factors that regulate growth [56]. Some MMPs attach to the receptors which are responsible of apoptosis, inhibiting induction of apoptosis. They also affect multiple signaling pathways that modulate the biological events in cell. During matrix degradation, growth factor embedded in matrix is released. MMPs can also directly target some growth factors, causing their release and activation [55]. Hence, increased levels of MMP are linked to tumor progression. Studies also showed that over expression of TIMPs, which are the natural inhibitors of MMPs, reduced metastasis [54].

2.5.3. MMP Treatments

For the reasons stated above, inhibiting MMP seemed a good way to prevent tumor growth to scientists. Therapies designed to inhibit MMP. Preclinical experiments on animal models showed promising results. However, clinical trials on humans were disappointing.

First candidate for inhibition of MMPs were their natural inhibitors TIMP-1 and TIMP-2. They were found to be unsuitable for that job due to their short half-lives. Hence, synthetic MMP inhibitors were produced [57]. Phase-I trials revealed side effects like musculoskeletal pain and inflammation. Phase-II trials were designed to test the efficiency

but they were problematic on execution also. Because of the fact that inhibiting MMP aims to arrest growth, rather than killing the tumor cells, conventional measures of efficiency could not be used to monitor drug activity. Results from phase-III trials were also disappointing. That led the scientists to conclude that MMP inhibitors had no therapeutic benefit in human cancers [54].

The unsuccessful results were thought to stem from the differences in preclinical and clinical trials. In mouse models, MMP inhibition was applied at the early stages of disease and continued through the progression. In clinical trials, MMP inhibitors were given to the patients who had been in the advanced state of the cancer and efficiency of the treatment was evaluated by the survival or progression of the disease. Considering the functions of MMP in developing cancers, the decrease in the effectiveness of the treatment in late stages of the disease is predictable [54].

Several treatments were terminated because of lack of efficiency. One case among them raised concern. Trials with a synthetic MMPi, Tanomastat, on pancreatic and lung cancers lowered the survival significantly, compared to placebo. There is also preclinical evidence that inhibiting MMPs can stimulate disease progression. It is also shown that in some cases elevation of MMP levels may help to prevent angiogenesis. In another series of trials, MMP inhibition has been shown to increase metastasis to liver cells from the breast and lymph cancers.

Even though MMP activity is regarded as a promoter of tumor growth, there are cases where MMP acts like a tumor suppressor. So, drug must conduct a selective inhibition in order to eliminate the MMPs that are responsible for tumor progression. This suggested that there is a more complicated picture of MMP activity which needs to be resolved, in order to design effective therapies. To design a treatment that can function in preventing tumor growth, there are questions that must be answered beforehand [55].

(i) Which particular activities of MMP should be inhibited for optimal therapeutic effects and minimum side effects?
(ii) What are the important substrates that contribute to tumor growth?
(iii) How MMPs and other proteinases work together to enhance tumor progression?

However, the future of MMP inhibiting drugs does not have to be all bad. It is now possible with the development of higher resolution probes to track the MMP activity. Tracking MMP molecules in vivo will help to refine MMP treatments by enabling us to obtain the spatial and temporal windows of MMP activity, in which the applied treatments will be effective [56].

This is also the motivation of our research group. We have the ability to track MMP in vivo along with other biochemicals and biological structures. We will use our mathematical model to simulate tumor growth with coupling it to the imaging data. This will enable us to make in silico experiments on activities of MMP and test the therapies of MMP inhibition.

3. COMPUTATIONAL SYSTEMS BIOLOGY APPROACH IN CANCER

Systems biology approach is developed as a response to the reductionist approach in biology. It handles biology as systems of interconnected components. It is particularly useful in explaining phenomena that cannot be accounted for in reductionist methods. Biological structures have some common properties that enable them to exhibit complex behaviors like emergence, modularity and robustness that can only be studied with systems biology approaches [2].

Even if we fully understand two different molecules; when they come together, they may work in a different way than when they are isolated. Theoretical biologists call these situations as emergence. When different small parts come together, they may create a property that was not present in the constituent parts. These emergent properties are the ones that are impossible to predict or deduce by calculation, or any other means.

Robustness is another property of biological systems that cannot be understood by means of reductionism. Systems that are persistent against perturbations in environment are called robust systems. These kinds of systems adapt to changes or generate additional parts to do some particular task as back-up if one part fails to work.

Biological systems are built as modular structures. Different parts are insulated to prevent the spreading of the failure of one module to others. This kind of organization prevents lethal damage in case of minor failures [2].

System level understanding of biological structure comes from the connection of key properties of the systems [58], which are

(i) system structure that includes biochemical pathways, information about cells and other structures of the system,
(ii) system dynamics, how systems evolve in time under various conditions,
(iii) the control method; mechanisms that systematically control the state of the system,

(iv) design method; the strategies to modify and construct biological systems to acquire the desired properties [58].

Computational systems biology is a sub-discipline of systems biology which aims to process the huge amount of data accumulation of reductionist biology by using modeling [59]. The models can either make predictions about biological behavior; they may unveil the unknown principles or they may be used to conduct in silico experiments.

Cancer is a very complex disease which makes it very eligible for system biology approach. Cancerous tissues are generated from healthy tissues with microenvironmental and genetic alterations. These numerous alterations are triggered one by one or simultaneously during cancer development, with close connection to each other. These connections need to be resolved in order to understand the tumor development and interrupt it with treatments opposing tumor growth. While the clinical and experimental studies have been going on by biologists and oncologist, mathematicians and physicist tried a new way, to produce information about cancer biology. They used the accumulation of biological data, and integrated them into quantitative dynamic models, that mimic the tumor behavior [59].

A successful computational model needs to satisfy some conditions to be useful.

(i) It must make successful predictions that match the experimental results.
(ii) It must provide data that cannot be generated with present day experimental capabilities.
(iii) It can be used as a platform to conduct in silico experiments which save time and money.
(iv) It must unveil non-intuitive insights about inner workings of the system and help to identify missing components.
(v) It must make the complex system better understood and visualized.
(vi) Model must use the quantitative data and derive useful results.

In order to produce useful models, we need to make a decision about from where we should begin modeling? Copying the exact biological structure from the individual molecules is obviously impossible. The models are partial representations that try to show

what is essential, which features are necessary or sufficient to understand the system that is modeled [60]. The amount of detail a model contains is directly connected to the aim of the model and it must be wisely chosen; because in order to add more detail to the model, we need to give up some features of the model.

There are some constraints that restrict all the computational models. A trade-off between realism, precision and generality features must be made in each model. Realism gives the amount of detail a model can have, precision refers to the accuracy of the output taken from the model, and generality determines the number of systems the model can be applied to. In order to increase one of these three features, the other two must be decreased [61]. So we need to make a decision about which features are important in order to see the biological picture that we want.

3.1. Cancer Models

Modeling cancer growth is not a new subject; cancer models are existent for 50 years now. Earliest attempts of modeling went back to 1966, when Burton modeled the distribution of oxygen in a spherical tumor and resulting radius of tumor limited by diffusion of oxygen. The model showed that the tumor growth can be represented with Gompertz curve, which is a function that is used to define the growth of populations.

The transition from simple Gompertz curve to multi-scale imaging data-integrated hybrid models is provided by the emergence of the high technology needed for experimentation and imaging, and most importantly, the increase in the computational power. Large amount of data about cancer is accumulated by the works of oncologists and tumor biologists, but they have no comprehensive model that can be used as a framework in understanding and applying these data [62]. This vast body of knowledge must be organized in some way to extract some general relations of biological systems. Modeling cancer helps understanding the complex relations between biological events and genetic and microenvironmental conditions. Modeling can also handle the problem of connecting the events happen in different scales, which will govern the tumor progression [63].

3.1.1. Importance of Microenvironment

The main focus of cancer studies was genetic mutations and their functional consequences in the past 25 years but these studies ignored the dynamic interaction of cancer cells and microenvironment [64]. Today, we know that tumor growth cannot be conceived of without its interactions with microenvironment. Biological phenomena affecting tumor growth have strong relationships with the microenvironment. Signaling helps the cells to understand the concentration gradients of nutrients, growth factors and other chemicals; in addition to that, it gives information to cells about mechanical stresses. Microenvironmental conditions not only affect the metabolic functions of cells, but also they have a control over the genome of the cells. Chemicals in the microenvironment may change the active parts in DNA [65].

In order to quantify the effect of tumor microenvironment to the tumor growth, we need computational models. There are large numbers of variables in the tumor microenvironment, which have nonlinear connections with each other and the resultant tumor morphology [64]. Therefore, the cumulative effect of tumor microenvironment on tumor growth is impossible to handle without help of modeling. Mathematical modeling has the potential to provide insight into these interactions through a systematic study of fundamental constituent processes [66]. Hence, in tumor models, linking tumor development to microenvironment is an important part.

3.1.2. Tumor Growth as a Multiscale Problem

When we look at the biological phenomena governing tumor growth, three natural scales appear; molecular, cellular and tissue scales [63]. Tumor development begins in molecular scale with genetic alterations, abnormal activation of signal transduction pathways that control cell growth and survival. Genetic factors are combined with environmental factors during the initialization of tumor. Molecular events will induce changes in cell scale which will then reveal itself in the tissue scale. The biological phenomena that happens in molecular scale includes the absorption of nutrients and other chemicals, signaling processes, genetic changes, mutations activation and suppression of different parts of DNA.

Cell scale is used to depict the main activities of the cells and their place in the cell cycle. Proliferation, quiescence, hypoxia, apoptosis happen, due to environmental factors in each cell. Immune response is another cell scale phenomena which involves recognition and elimination of the abnormal cells.

We see the collective behavior of cells in the tissue scale. Cells with similar characteristics form layers and clusters as a result of genetical and environmental conditions. Nutrient gradients appear at the tissue scale. We can divide tumor growth into two parts due to having access to nutrients as before and after vascularization. In avascular state, tumor cells accumulate in three layers, proliferative cells in the rim, quiescent cells in the intermediate region and necrotic core in center. Geometric organization of cells changes after new blood supplies are added with vascularization. Cell morphology adapts to the new situation by moving cells towards the nutrient gradients.

Events that happen in these scales are closely connected to each other. Resolving and quantifying these connections require modeling. The model calculates the result of these connections and sometimes it reveals previously unknown relations; hence, provides important information to scientists.

3.1.3. Functions of Tumor Models

In general, a model is technically defined as a description of a system which is a collection of interrelated objects. Scientific models have three technical uses [61] which are given below.

(i) They may be used to *understand* a physical system or a system of logic. The type of the problem in this case is synthesis where the input and the output is known, the system object is the one to reveal which will give the system characteristics.

(ii) They may be used to make *predictions* of future or of some state that is unknown. This time the problem type is analysis with input and system object is known and output is tried to be found.

(iii) They may be used to gain *control* over the system. The problem is instrumentation, designing a system that a specified output is the result of an input. The system object and output are given to find the input [61].

These three aspects of modeling can also guide us about the functions of cancer models. Cancer models are used to predict the tumor behavior, revealing underlying dynamics between variables related to tissues or microenvironmental factors, and, to conduct in silico experiments which can yield both predictive results or results that help us understand the inner workings of the tumor biology.

Tumor models can be grouped roughly as descriptive and mechanistic models [67]. Descriptive models try to imitate the gross characteristics of tumors like cell number or size. Without extensive biological detail, these models try to grasp the behavior of the population dynamics. Predictive models mostly contain descriptive forms. On the other hand, mechanistic models focus on specific aspects of tumor growth and try to reveal the underlying dynamics of that aspect. They are suitable for in silico experimenting and treatment design. These two approaches can both be used at the same time but we need to keep in mind that there are some constraints about having the prediction power and the amount of detail at the same time.

Predictive results given by models can be used in designating proper treatment strategies to patients. Determination of tumor boundaries is especially important in brain tumors since it can help to plan surgical action.

3.1.4. In Silico Experimentation and Treatment Design

Mathematical models of tumor can be constructed in order to conduct in silico experimentation. These experiments can be simply aimed at quantifying coefficients of cellular and microenvironmental variables included in the model. Another category of in silico experimentation includes analysis of model outcomes to make predictions and prepare testable hypothesis on this predictions. The hypothesis must then be tested in a conclusive experiment with the model [67].

Even though these experimentations cannot replace in vivo and in vitro counterparts, they may produce valuable information by costing less money and very little time compared to others. It is not easy to conduct an experiment in real life, since it needs a lot of time, effort, and money. But using in silico experimentation, researchers have an opportunity to try different experiment scenarios without actually doing them and decide on one to conduct in reality. Hence, in silico experiments may guide the real life counterparts. In silico experimentation can also be used to test cases that cannot be tested in reality. For example, testing therapies for gliomas is very hard due to short survival time of the patients [68]. What would happen to the tumor if the therapy could continue can be tested by help of the models? They may also give insight about unexpected results seen in experiments and clinical cases.

Models can also contribute to the development of new therapies. They help identifying the underlying mechanism of tumor growth which will help to create new targets for cancer treatment. They can also help us see the probable result of a designed treatment by producing simulations. They can help to optimize the treatment by calculating the dosage or time periods of the medication.

3.2. Types of Models

According to the aim of the model, we need to decide how we will represent the biological structures. The two main approaches of cancer modeling include the discrete and continuous models. In discrete models, we are able to see the individual cells but in continuous models, we are looking at the system from far away enough to lose details on the individual cells and see them as a continuous tissue. We cannot reach the information about individual cells from continuous models, but a good discrete model should exhibit the same behavior of a continuous model in the continuum limit. In some cases, a continuum and a discrete model will be prepared for comparison [69]. A novel approach called hybrid modeling makes it possible to benefit from the advantages of these two former approaches. Smartly designed hybrid models seem to level up the state of the models in the computational oncology world.

3.2.1. Discrete Models

Discrete modeling of tumor growth is based on the tracking of the individual cells in time and space, while the cells are evolving under the influence of the biophysical laws determined by the model. They are needed when we try to observe small scale phenomena in which tissue, cell and environmental properties vary in scales comparable to that of a single cell. It is used for studying phenomena happen in cell scale or the cases where the properties of the cells vary over a distance comparable to the size of a cell [70]. In discrete models, continuum fields are also used for tracking the compositions of environmental variables; such as nutrients, oxygen and chemicals involved in tumor growth. Even though they are the most detailed models spatially, their high detail comes with a price. There are approximately 10^6 cells in small tumors, computational cost of tracking single cells makes model cumbersome. There are two main approaches to discrete modeling; lattice based and lattice free models.

3.2.1.1. Lattice Based Models. In lattice based approach, cells are situated in two or three dimensional lattices. Each point on the lattice is updated according to the rules of the model. Each cell can either occupy one lattice site or more than one. Regular structures of lattice based models relieve the computation burden created by interactions between discrete cells since it only takes into account the interactions between only the immediate neighbors. It is also easy to couple the discrete cells to microenvironment by attaining the values of the environmental continuum variables to each point [37].

Methods borrowed from physics are used in the construction of the models. Ising and Potts models provide suitable structure for modeling tumor cells, since they allow local interactions on the lattice structure. Models that use Potts model [71,72], compute the Hamiltonians for cells, then using Boltzmann probability, cells decide to move on the empty sites of the lattice.

Predefined lattice geometry violates the isotropy of space [73]. The uniform spacing of lattice unifies the distance a cell can go in each time step. Cells on the lattice are only allowed to move on the nearest lattice sites so there are fewer available options for cells to

move than in reality. In some cases, these conditions prevent simulating the non-lattice paternings that can be seen in real tissue.

Cell Automata models are vastly used form of lattice based models. Characteristics of CA can be summarized as [74],

(i) each lattice site is assigned to a cell,
(ii) each site can be in the finite number of cell states,
(iii) each time step every site updates according to the transition rules,
(iv) the transition of a site depends on the spatial neighborhood configuration in a deterministic or probabilistic way,
(v) each site is equivalent and transition rules are the same for the whole.

3.2.1.2. Lattice Free Models. Lattice free models track individual cells which are free to move in space without restricting them in an artificial lattice. Cells are free objects that are allowed to move, divide and die according to the biophysical rules set by the model. In a lot of models, free body diagrams are applied to each cell to determine the movement [37]. Thus, the interactions between cells and other cells or ECM are defined mechanically. Dynamics of cells can be described by equations of motions for each cell or by Monte-Carlo approaches which yields comparable results to former in simulations [75]. Lattice free models differ in the morphological description of how detailed the cell will be. The complexity of the model varies from the cells that are represented simply by points in space to spheres that can change shape and be deformed [37]. However, it was demonstrated that cell shape, division mechanisms or assumptions about precise shape of the interaction force affects the growth behavior in long time scales [75]. Since lattice free models present free movement to cells, they are ideal for biological processes where cells free movement is important such as angiogenesis and metastasis.

Lattice free objects treat cells as free agents in space; hence, they are very suitable object- oriented programming languages like C++ and Java [37]. Computational cost of lattice free models is high which makes this approach practical only for small number of cells. Calculation of cell-cell interaction brings a heavy computational burden. In worst case, for a tumor composed of n cells, each cell interacts with other $n-1$ cells, then

$\mathcal{O}(n^2)$ cell-cell interaction have to be calculated in each time step [37]. Tumor growth is simulated as the process of growth from a single cell to $\mathcal{O}(10^{11})$ in vivo. It is very large for using the lattice free models. In studying tumor growth, it is sometimes unnecessary to track the fate of every cell [70].

It was demonstrated that with supplying the model with experimentally available cell properties and involve growth control mechanisms, these models are able to produce complex spatial growth and pattern formation [75].

3.2.2. Continuous Models

Continuous models are used when we are studying large scale phenomena where the tissue, cell and environmental properties vary in a scale larger than dimensions of a single cell. We consider tissues and other biological structures as continuous matter obeying continuum mechanics. Coupled partial differential and integro-differential equations that denote volume fractions or densities of biological structures are solved in order to simulate the tumor growth. Concentrations of chemicals involved in tumor growth can be added to the model. Interactions between different biological structures can be defined mechanically and phenomenally.

Different kinds of populations or structures can be defined as the different phases in the tumor models. Approximations are made in order to deal with different distributions of these phases in computational domain.

Continuum models can be divided into two subgroups as sharp interface and mixture models which reflect two different distributions. The two different approaches have both their applications on tumor growth modeling.

3.2.2.1. Sharp interface models. They mainly divide the computational domain two or more parts representing different biological structures. Thus, in one space coordinate, only one kind of structure is available. We can compare the sharp interface model by an oil and liquid mixture. In that mixture, components do not blend into each other so that we can see the boundaries of oil clearly. Water and oil are cannot coexist in the same space

coordinate. Tumor cells make up at least one part in the sharp interface model, the other parts might be composed of different combinations of extracellular matrix, extracellular liquid and normal cells. Tumor cells can also be divided into different populations and represented as different cell groups according to their genetic composition or their reproductive potential as quiescent, proliferative or necrotic cells. These models are especially useful when we need to track the interfaces related to tumor growth. They can enable us to track different interfaces, most important one being the tumor boundary or some other boundaries like the one that separates the necrotic core of tumor. Sharp interface approach can be used in modeling gliomas, a special kind of brain tumor, because of the clinical importance of determining the tumor boundary. Sharp interface models allow physical or biological interactions between different phases only on the interface of the components. Forces between different phases only act on the surface of boundary whereas in mixture models, different phases can interact whenever they coexist in the same coordinate.

3.2.2.2. Mixture models. They do not reserve a coordinate to only a single phase. Different phases coexist in the same coordinate. Hence, phases are defined by continuous distributions of mass on the space without sharp borders. Different phases interact all over the space where they come together, which is biologically more realistic. A huge variety of mechanical or phenomenological interactions can be added easily to the model with this approach. Thus, it is a widely used approach in tumor modeling scene, used by models that aim to explain various biological phenomena.

The main framework of the models is similar [76-79]. They all start with mass balance equations for phases. The choice of phases and included biophysical and biochemical interactions make these models differ. Mechanical responses of tumor cells [80], invasive characteristics [81], and interactions among cells [82] and microenvironment [83, 84] are studied with this approach.

In the scale where we see the tumor tissue as continuum, we actually zoom out from the system to a degree that one space coordinate of our computational domain consists of mixture of cells and other different structures. Hence, it seems unrealistic to attain one coordinate will have only one kind of population.

3.2.3. Hybrid Models

Hybrid modeling is the newest approach in the modeling scene. It aims to combine the advantages of both discrete and continuous models. Biological processes in cellular and subcellular scale, come together to create the tissue scale tumor morphology. Hybrid modeling includes single cells and aims to capture the emergent tumor behavior at the tissue scale.

Hybrid models are divided to two classes according to the state of the discrete part as composite and adaptive models. In composite hybrid models, all cells are discrete objects and they are coupled to continuum fields like concentrations of chemicals. In adaptive models, discrete parts are chosen dynamically and adaptively where they are needed. Hence, adaptive models aim to increase resolution of the model at the points of interest without overly increasing the computational burden [85].

In a good example of adaptive model by Kim [86], only the proliferating region is taken as discrete while the rest of the tumor and the microenvironment are modeled as continuous matter. The activities of proliferating cells determine the behavior of the tumor that is why model track them individually.

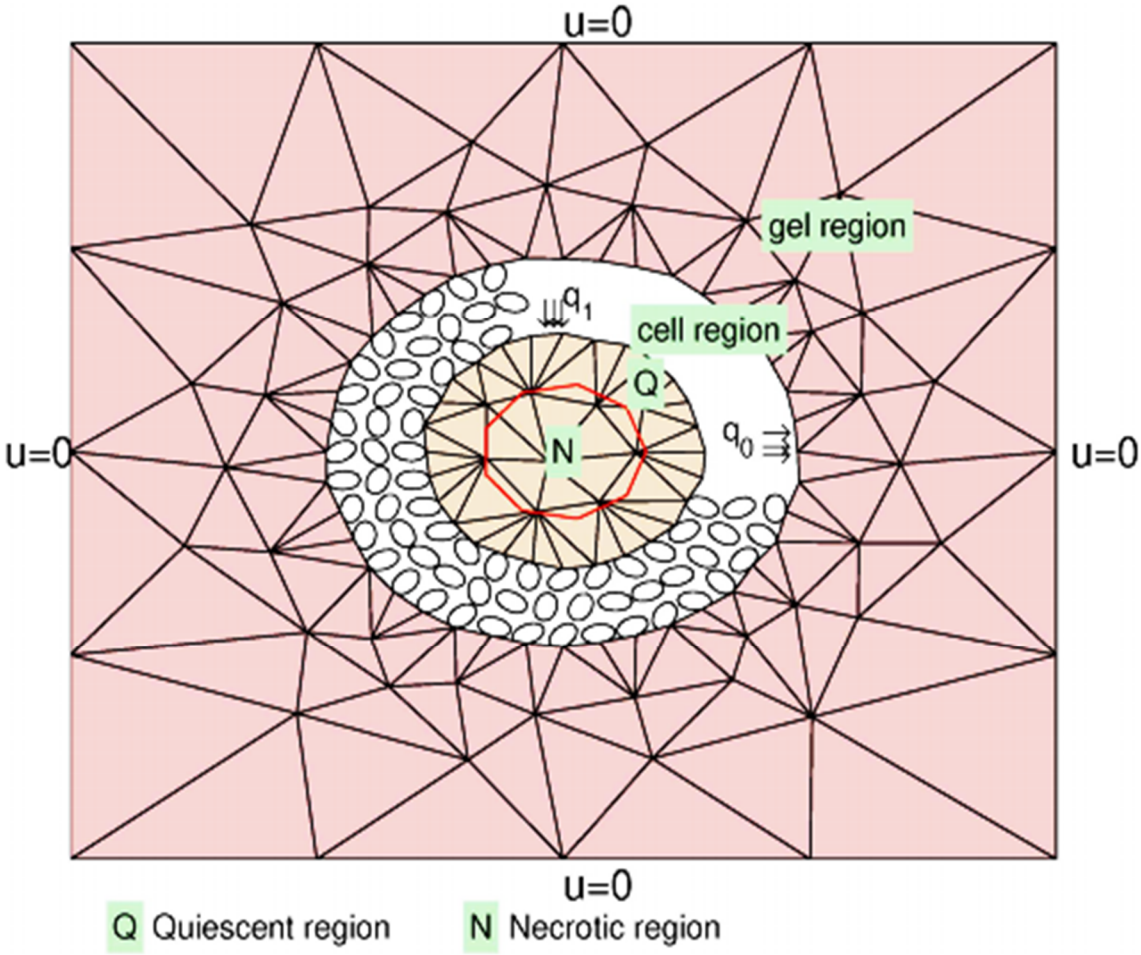


Figure 3.1. Computational domain of the adaptive model by Kim [86]. Figure 3.1 shows the discrete cells in the proliferating rim, other parts of the computational domain are continuous.

The discrete part of the hybrid model can either be lattice based or lattice free. With the ability to combine process in the different scales, hybrid models have the capacity to capture a realistic picture of tumor.

4. A MIXTURE THEORY APPROACH IN CONTINUUM MODELS

4.1. Mixture Theory

Continuum theory of mixtures is a mature theory for describing the non-biological growth phenomena. Its application for biology to describe tissue growth began to appear in 1990's and shortly after it was also applied to tumor growth [89]. Mixture theory claims that the single body of interest is composed of different components that mix together to fill up the whole space.

Continuity and momentum equations are written for all phases. Mass dissipation or generation is enabled by exchange in the continuity equation. Forces, stresses, and interactions can be included in momentum equation. In momentum equation, velocity field of phases can be linked to force balances which lead to mechanical analysis of the system. Average velocity of the components of multiphase flow can be denoted with $\mathbf{u}_k$which is actually the Favre-average of the local instant velocity of phase k, $\overline{\mathbf{u}_{lk}}$. Average velocity of phase k can be defined as

$$\mathbf{u}_k = \frac{\overline{\rho_{lk}\mathbf{u}_{lk}}}{\overline{\rho_{lk}}} = \frac{\overline{\rho_{lk}\mathbf{u}_{lk}}}{\rho_k} \tag{4.1}$$

where ρ_{lk} is the local density and ρ_k is the average density of phase k. The framework of modeling theory is explained below [90].

For each phase, we can write mass conservation using this averaged velocity $\mathbf{u}_k$

$$\frac{\partial}{\partial t}(\varphi_k\rho_k) + \nabla.(\varphi_k\rho_k\mathbf{u}_k) = \rho_k\Gamma_k \tag{4.2}$$

where φ_k represents the volume fraction of the phase k and Γ_k represents the rate of mass generation. Likewise, momentum equation is also written for each phase as

$$\frac{\partial}{\partial t}(\varphi_k\rho_k\mathbf{u}_k) + \nabla.(\varphi_k\rho_k\mathbf{u}_k\mathbf{u}_k) = -\varphi_k\nabla p_k + \nabla[\varphi_k(\tau_k + \tau_{Tk})] + \varphi_k\rho_k\mathbf{g} + \mathbf{M}_k \tag{4.3}$$

where $\mathbf{M}_k$ is the average interfacial momentum source and p_k is the pressure for phase k, τ_k and τ_{Tk} are average viscous and turbulent stress tensors respectively and g is the gravitational acceleration. Turbulent stress tensor is produced by the fluctuating component of the velocity, $\mathbf{u}_{Fk} = \mathbf{u}_{lk} - \mathbf{u}_k$. It is of the form

$$\tau_{Tk} = -\overline{\rho_{lk}\mathbf{u}_{Fk}.\mathbf{u}_{Fk}}\,. \tag{4.4}$$

In order to solve the momentum equations, interaction forces and stress terms need to be specified by constitutive equations. In some cases, solid stress is divided into a compressive normal stress, in other words, solid pressure and shear stress. Then the shear stress is connected to the viscosity by proper equations.

By using the equations constructed for each phase, we can write continuity and momentum equation for the whole mixture. For a mixture having n phases, we get the following equation

$$\frac{\partial}{\partial t}\sum_{k=1}^{n}(\varphi_k\rho_k) + \boldsymbol{\nabla}.\sum_{k=1}^{n}(\varphi_k\rho_k\mathbf{u}_k) = \sum_{k=1}^{n}\rho_k\Gamma_k\,. \tag{4.5}$$

Since total mass is conserved, the right hand side of Equation 4.5 will be equal to zero, ensuring the continuity of the mixture.

$$\sum_{k=1}^{n}\rho_k\Gamma_k = 0, \tag{4.6}$$

$$\frac{\partial\rho_m}{\partial t} + \boldsymbol{\nabla}.(\rho_m\mathbf{u}_m) = 0 \tag{4.7}$$

where mixture density and mixture velocity defined as

$$\rho_m = \sum_{k=1}^{n}\varphi_k\rho_k\,, \tag{4.8}$$

$$\mathbf{u}_m = \frac{1}{\rho_m}\sum_{k=1}^{n}\varphi_k\rho_k\,\mathbf{u}_k\,. \tag{4.9}$$

By making a similar summation to the momentum equations for each phase, we have

$$\frac{\partial}{\partial t}\sum_{k=1}^{n}(\varphi_k\rho_k\mathbf{u}_k) + \boldsymbol{\nabla}.\sum_{k=1}^{n}(\varphi_k\rho_k\mathbf{u}_k\mathbf{u}_k) = -\sum_{k=1}^{n}\varphi_k\boldsymbol{\nabla}p_k + \boldsymbol{\nabla}\sum_{k=1}^{n}\varphi_k(\tau_k + \tau_{Tk}) + \sum_{k=1}^{n}\varphi_k\rho_k\mathbf{g} + \sum_{k=1}^{n}\varphi_k\rho_k\mathbf{g} + \sum_{k=1}^{n}\mathbf{M}_k \,. \quad (4.10)$$

All terms except the second one on the left hand side can be easily transformed into mixture terms. In order to transform the second term, we are introducing $\mathbf{u}_{mk}$ as

$$\mathbf{u}_{mk} = \mathbf{u}_k - \mathbf{u}_m \quad (4.11)$$

and writing $\mathbf{u}_{mk}$ into Equation 4.10, we get

$$\boldsymbol{\nabla}.\sum_{k=1}^{n}(\varphi_k\rho_k\mathbf{u}_k\mathbf{u}_k) = \boldsymbol{\nabla}.(\rho_m\mathbf{u}_m\mathbf{u}_m) + \boldsymbol{\nabla}.\sum_{k=1}^{n}(\varphi_k\rho_k\mathbf{u}_{mk}\mathbf{u}_{mk}) \,. \quad (4.12)$$

Now, we can write the momentum for the whole mixture as

$$\frac{\partial}{\partial t}(\rho_m\mathbf{u}_m) + \boldsymbol{\nabla}.(\rho_m\mathbf{u}_m\mathbf{u}_m) + \boldsymbol{\nabla}.\sum_{k=1}^{n}(\varphi_k\rho_k\mathbf{u}_{mk}\mathbf{u}_{mk}) = -\boldsymbol{\nabla}p_m + \boldsymbol{\nabla}(\tau_m + \tau_{Tm}) + \rho_m\mathbf{g} + \mathbf{M}_m. \quad (4.13)$$

Defining the third term, diffusion stress τ_{Dm} which is

$$\tau_{Dm} = \sum_{k=1}^{n}(\varphi_k\rho_k\mathbf{u}_{mk}.\mathbf{u}_{mk}), \quad (4.14)$$

Equation 4.13 becomes

$$\frac{\partial}{\partial t}(\rho_m\mathbf{u}_m) + \boldsymbol{\nabla}.(\rho_m\mathbf{u}_m\mathbf{u}_m) = -\boldsymbol{\nabla}p_m + \boldsymbol{\nabla}(\tau_m + \tau_{Tm}) + \boldsymbol{\nabla}\tau_{Dm} + \rho_m\mathbf{g} + \mathbf{M}_m \quad (4.15)$$

where other stress tensors of mixture $\boldsymbol{\tau}_m$ and $\boldsymbol{\tau}_{Tm}$ are defined as

$$\tau_m = \sum_{k=1}^{n}\varphi_k\tau_k, \quad (4.16)$$

$$\tau_{Tm} = -\sum_{k=1}^{n}\varphi_k\,\overline{\rho_{lk}\mathbf{u}_{Fk}.\mathbf{u}_{Fk}} \,. \quad (4.17)$$

The pressure of the mixture, p_m, is defined as

$$\nabla p_m = \sum_{k=1}^{n} \varphi_k \nabla p_k \tag{4.18}$$

and $\mathbf{M}_m$ gives the whole surface tension force of the mixture

$$\mathbf{M}_m = \sum_{k=1}^{n} \mathbf{M}_k. \tag{4.19}$$

This framework will be adapted to reflect mechanics of biological matter. We will change and reduce the equations in order to simulate tumor growth.

4.2. Multiphase Mixture Model of Tumor Growth

We build a mixture model for cancer growth. In our model, we used mixture theory approach for the reasons given below.

(i) We did not need keep sharp interfaces separating the tumor and the environment during the simulation. Even if we need that, we can find it with the mixture models. As an example, after we run the model, we can draw an artificial tumor boundary by lining the coordinates where tumor volume fraction is equal to a specific value, $\varphi_{T_Boundary}$.

(ii) Mixture model seemed more reasonable to us because, in our continuous picture, we use length scales much greater than the size of a single cell. In reality, we will have different structures, tumor cells, ECM, host cells and IF in a box, which have the size of our unit volume, so it is natural we see the different phases in the same unit volume as a continuous mixture.

(iii) The mixture model provides a framework for mechanical analysis of components. As physicists this is important for us to combine the knowledge and experience in physics to model biology and use mechanical analyses rather than using heuristic approaches.

(iv) We wanted to be able to include more than two phases in our model. This was easier to do with mixture modeling.

We build a model that can provide framework for studies in the future. We are interested in the role MMP and interstitial fluid plays in the tumor growth. We have imaging data that will enable us to conduct experiments with our model in the future.

4.2.1. Phases in the Model

We try to replicate the variety of tumor microenvironment by representing different cell populations or structures with different phases. The decision of how detailed picture of the tumor microenvironment we would like to capture, depends directly on the purpose of the model. It is never possible to write a model that includes all biological processes because of the fact that there are too many and there are always connections among biological components or biochemicals that we have not explored yet. Luckily, that does not mean that we cannot produce useful models. Because according to what we hope to learn from the model, some biological details may just be computational burden, unnecessary to compute. Even when some details are necessary to create a realistic model, we intentionally take them out from the model in order to see the isolated effect of the parts that we are really interested in.

In our model, tumor cell population is considered as a one homogeneous body and represented with one phase. The host environment is divided into two phases as ECM and extracellular liquid. By this way, we blend the host cells inside the ECM and take them as one structure. In other models in the literature, the host environment may be composed of one phase either of ECM, host cells or sole liquid. In addition, the different combinations of these three phases are also available in the literature. However, very little number of models divides the host environment to three phases to contain ECM, host cells and liquid altogether. The models usually use a saturation condition by either setting the total volume fraction to a constant or to one. In our model, volume fractions of phases will add up to one.

4.2.1.1. Tumor Cells Phase. The common purpose of the computational tumor growth models is basically to replicate the behavior of the tumor in space and time. In order to see the time evolution of the tumor tissue, we obviously need to represent tumor in a model with at least one phase. The model may contain more than one tumor phase to introduce heterogeneity into tumor cells.

The most common examples of models with multiple tumor cell phases include the avascular tumor models. They have tumor cells that belong to proliferating, quiescent, and necrotic cell populations composing three tumor cell phases. In addition to that, tumor masses that include tumor cell populations having different genotypes, which may result from being in the different stages of evolution or being subject to different mutations can be modeled using multiple tumor phases. In this case, a subpopulation sharing the same genotype can be classified as one phase, and all phases with different genotypes are combined to compose the whole tumor body. This type of modeling is used when the effects of mutations during tumor progression are of special interest. Otherwise, we try to maintain the number of phases as low as possible in order not to make the model impossible to solve.

In our model, tumor cells are accepted to share the same genotype. All cells have the same chemical, biological, and mechanical response to the same chemical, biological, and mechanical effects. Therefore, we represent all the tumor cells as the tumor phase. Differences in tumor responses stem from only dynamically changed microenvironmental conditions like concentration of nutrients and the differences of pressure affecting the cell, no inherent variations are present through the tumor phase.

Changes in tumor mass at a certain position will be governed by mass conservation terms plus the mass increase due to exchange with other phases. The exact form will be given in the model equations part. However, we can simply say that the mass increase will be connected to available nutrient concentration and the movement of tumor phase will be induced by the formation of pressure gradients through the tissue. As tumor cells proliferate, they begin to push each other due to lack of space. They begin to feel pressure. Then they will try to move to coordinates with less pressure. However, host structures will

sometimes block their way and tumor phase will evade this blockage by degrading the host structures.

4.2.1.1. Extracellular Matrix (ECM) Phase**.** In this phase, we will combine the ECM fibers and embedded host cells as one phase. In the absence of the tumor, the computational domain will be composed of only ECM and extracellular liquid. ECM volume ratio per unit volume can be specified initially in order to represent different tissues in the different regions of the body. Hence, the model can be made applicable to different tissue types by this way. The characteristics of ECM are same everywhere. The only variation is the volume fraction of ECM in a space coordinate.

The ECM phase is composed of a static structure in normal conditions in our model. We can rationalize this claim by stating that ECM density is constant without the presence of disturbances to the system. ECM changes when tissue needs remodeling. Hence, we claim that ECM does not move but only gets degraded during encounters with tumor cells. Growth of tumor phase will be prevented physically by the ECM during growth. Tumor phase will try to replace ECM to sustain its growth. The replacement process will include degradation of ECM with matrix degrading enzymes. Then the degraded ECM fraction will turn into liquid phase and tumor cells will take in this liquid to expand in volume and proliferate.

4.2.1.2. Interstitial Fluid (IF) Phase. The IF phase will fill up the space that which is not occupied by ECM and tumor cells. Liquids distribute pressure evenly so IF phase helps to dispel the proliferation pressure of tumor phase, thereby its presence results in a decrease in pressure gradients of the tumor phase.

Another significant duty of the IF phase is to help the movements of biochemicals by convection. In our model, nutrients and matrix degrading enzymes are spread by both diffusion and advection with the IF.

4.2.2. Biochemicals and Nutrients in Our Model

Nutrients play an important role in our model as they are the only determinants of tumor proliferation rate. The equation that governs the nutrient concentration will be coupled to the volumetric tumor growth equation of our model.

The nutrients in our model are supplied by the pre-existing host vessels. Hence, we need to insert a capillary network to the microenvironment of the tumor as nutrient source. The diameter of a capillary varies between $5x10^{-6}m$ and $10x10^{-6}m$. In continuous picture, we cannot see the capillaries discretely. We rather see continuous cloud of capillaries that supply blood to the tissue. When we ignore the deformation caused by tumor body, we can approximate this capillary web as a homogeneous source of nutrient which supplies the same amount to each unit volume. The nutrients then will be delivered to tumor cells by means of diffusion and advection with IF. The depletion of nutrients by cells will be described by a sink term, which depends on the tumor cell fraction in a unit volume.

Biochemicals other than nutrients can be added to the model with a similar procedure. Once the transportation mechanism, source and sink of a biochemical are known, it can be added to the model so that it conducts the biological activity it is supposed to perform. Some models include growth factors that enhance growth and proliferation while others add chemoattractants which affect the cells motility. The range of biochemicals that can be added to the models is very wide. Their concentration equations are given by diffusion-advection-reaction type of equations. They are generally easy to solve when you take the computational domain isotropic for parameters like diffusion constant.

In our model, we study the activity of protein MMP which degrades the BM and ECM to increase tumor motility and help the invasion. MMP is produced by the host cells in ECM. The production is triggered when tumor cells and ECM come into contact. The production process is taken into account phenomenologically. The first form that comes into the mind is the production rate in chemical reactions; the rate must be proportional to the multiplication of concentrations of the reactants. In the model, we have the volume

fractions of ECM and tumor cells that will participate the production; hence, we can use a form like a constant times the multiplication of these two volume fractions.

Produced MMPs' transportation mechanisms are the same as the nutrients'; they diffuse through the tissue and they are advected with the flow of IF. There will be a decay term that will depend on the reaction rate of MMP degradation of ECM, which represent the usage and digestion of these MMPs. MMP concentration will have an impact on the ECM volume fraction by degrading it into IF.

4.2.3. Closure of the Model: Velocity Fields of Phases

In order to solve the mass conservation equations of the phases, we need the velocity of the phases. The determination of the governing equations for velocity fields will close the model. The closure can be made in three ways. The simplest way would be setting the motions of the cells as a potential flow [91]. In this approach, tumor cells have a constant volume fraction, $\bar{\varphi}_T$ and the density of the tumor cells is constant. The mass conservation equation for tumor phase which is given below, will reduce in this way

$$\frac{\partial}{\partial t}(\varphi_T \rho_T) + \nabla.(\varphi_T \rho_T \mathbf{u}_T) = \rho_T \Gamma_T, \tag{4.20}$$

$$\nabla.\mathbf{u}_T = \frac{\Gamma_T}{\bar{\varphi}_T}. \tag{4.21}$$

Then setting the velocity field equal to the gradient of the scalar field, we get

$$\mathbf{u}_T = \nabla\psi \tag{4.22}$$

and the mass conservation reaches its final form [91],

$$\nabla^2\psi = \frac{\Gamma_T}{\bar{\varphi}_T}. \tag{4.23}$$

Another widely used approach is using phenomenological closure. In this type, equations defining biophysics of the tumor growth are tried to be constituted by heuristic approaches [91]. One example is letting the tumor cells to move the areas where tumor cell

volume fraction is less than the departure point. This scenario can be easily obtained by setting tumor velocity proportional to the gradient of the volume fraction [92].

$$\mathbf{u}_T = -D_T \boldsymbol{\nabla} \varphi_T. \tag{4.24}$$

The minus sign comes from the fact that velocity of the tumor will be in the direction of the decrease in the tumor volume fraction. Hence, a linear diffusion equation will be obtained from the mass conservation equation of the tumor.

Another important example would be setting velocity to gradient of a field which both accounts for compression of cells and adhesion forces between tumor cells [77].

$$\mathbf{u}_T = K \boldsymbol{\nabla} \Sigma(\varphi_T). \tag{4.25}$$

By using chain rule Equation 4.25 becomes

$$\mathbf{u}_T = K \Sigma'(\varphi_T) \boldsymbol{\nabla} \varphi_T. \tag{4.26}$$

Here, $\Sigma(\varphi_T)$ is a function that includes the effects of compression of tumor cells and cell to cell adhesion.

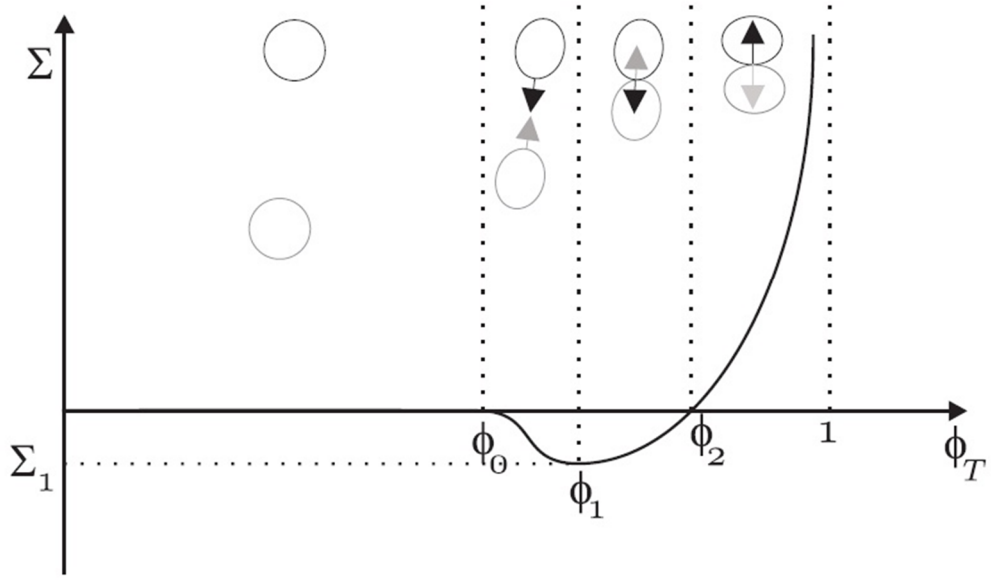


Figure 4.1. The field that describes the behavior of the tumor cells [77]. This field has some properties reflecting the physical interaction of the cells.

(i) They have no interaction when they are far apart.
(ii) After threshold value of distance, cells begin to attract each other.
(iii) If cells are in close contact, they experience repulsion and this repulsion goes to infinity at the maximum compression that cells can have.

The last approach is closing equations mechanically by analyzing all forces and momentum sources and mechanical responses of the tissues. By using these mechanical features, momentum conservation equations are written for each phase. These equations will give change in the momentum by the net force. The equation on the mixture theory section has the following form

$$\frac{\partial}{\partial t}(\varphi_k \rho_k \mathbf{u}_k) + \nabla.(\varphi_k \rho_k \mathbf{u}_k \mathbf{u}_k) = -\varphi_k \nabla p_k + \nabla[\varphi_k(\tau_k + \tau_{Tk})] + \varphi_k \rho_k \mathbf{g} + \mathbf{M}_k. \quad (4.27)$$

Now, we will adapt this equation to momentum change in biological structures in our model. In this form, there are some unnecessary terms on the right hand side. The turbulent stress, $\boldsymbol{\tau}_{Tk}$ will be eliminated due to the low Reynolds number. As we explained before, in

the low Reynolds world, all the flows are laminar. We will also eliminate the effect of the gravitational force. In order to simplify the model further, we can collect the terms in the left hand side as material derivative. Hence, Equation 4.27 will turn into the following form

$$\frac{D}{Dt}(\varphi_k \rho_k \mathbf{u}_k) = -\varphi_k \nabla p_k + \nabla(\varphi_k \tau_k) + \mathbf{M}_k. \tag{4.28}$$

Now, we have an equation in our hands of type, F = ma, with the left hand side being the ma part. There is more reduction to do at this point. In low Reynolds world, all inertial effects are very small when compared to frictional effects, hence; they can be ignored. By using this fact, we set ma part to zero. Thus, the net force in the unit volume will be zero.

$$-\varphi_k \nabla p_k + \nabla(\varphi_k \tau_k) + \mathbf{M}_k = 0. \tag{4.29}$$

Now, we have a force balance in our hands with a momentum source, pressure and viscous stress. By combining the pressure and viscous stress into total stress of phase k, $\mathbb{T}_k$, Equation 4.29 can be rewritten as

$$\nabla(\varphi_k \mathbb{T}_k) + \mathbf{M}_k = 0. \tag{4.30}$$

$\mathbf{M}_k$ includes the term $P\nabla\varphi_k$ for satisfying the saturation condition.

4.2.4. Stress Terms and Momentum Sources of Phases

Stress gives the internal forces acting within a deformable body. It measures average force per unit area of a surface within the body. Stress has components in there mutually orthogonal directions. The component which is normal to the surface gives direct stress and the other two give the shear stress. Direct stresses tend to change the volume of the material while shear stresses change the shape without changing the volume. The direct stresses can be divided into a pressure which is equal in all directions and deviatoric stress which is the unbalanced part of the normal stress which causes the deformation [93].

Stress terms will account for the mechanical response of the different structures in our model. The stress terms include to pressure, deviatoric stress and viscous stress parts according to tissue type. ECM and IF have no viscous stress terms because ECM is taken as a rigid web and IF is approximated simply as water with no response to stress. They both have only pressure in our model. Viscous and deviatoric stress terms, hence; will only be designated to tumor cells.

The gradient of the stresses will balance the momentum sources, $\mathbf{M}_k$, which are given by force applied to the unit volume. Forces in our model are friction forces as a result of relative motion of the phases. The velocity differences between phases will create friction forces at the points of contact. Biological phenomena such as haptotaxis, chemotaxis or random motion can be added to the momentum equation by the help of artificial forces. Adhesion can be directly added as a force or can be buried in stress terms to copy the biological event. We will explore the equations in depth in the model equations section.

4.3. Model Equations

During composition of model, our motivation was that we deal with a complex biological system by using physics, due to the fact that like all other systems it should also be subject to physical laws. In order to handle the system, we deal with the mechanics of the system. We first apply mass conservation to our model.

4.3.1. Mass Conservation

We state that for the whole mixture, mass is conserved. Mass exchange is allowed between phases. In our model, biological events like proliferation of normal cells and degradation of ECM are represented by mass exchanges. Degraded ECM turns into IF phase while tumor phase take up IF, to proliferate.

For each phase, we can write mass conservation using the averaged velocity field, $\mathbf{u}_k$

$$\frac{\partial}{\partial t}(\varphi_k \rho_k) + \nabla.(\varphi_k \rho_k \mathbf{u}_k) = \rho_k \Gamma_k \tag{4.31}$$

where φ_k represents the volume fraction and ρ_k is the density of the phase k and Γ_k represents the mass exchange between phases. Γ_k includes terms that depend on the concentrations of biochemicals and mechanical forces in the microenvironment. The velocity field of phases, $\mathbf{u}_k$, need to be determined in order to close the equations. We will close equations with mechanical analysis of forces.

We will make a simplification when we write the mass conservation equations for the phases of the model. The density of the phases, ρ_k, will be set as a constant for the whole space and all three of these phases will have the same constant ρ_k. Hence, we can drop the subscript of ρ_k and write it as ρ. The density will be cancelled out from the Equation 4.31 and the result is

$$\frac{\partial}{\partial t}\varphi_k + \nabla.(\varphi_k \mathbf{u}_k) = \Gamma_k. \tag{4.32}$$

We set the subscripts T, E, L are to the phases tumor, ECM and IF, respectively. The phase space is represented as $N = \{T, E, L\}$. Using the form above, we will write mass conservation for the tumor phase as

$$\frac{\partial}{\partial t}\varphi_T + \nabla.(\varphi_T \mathbf{u}_T) = \Gamma_T. \tag{4.33}$$

The mass conservation of ECM will be given as

$$\frac{\partial}{\partial t}\varphi_E + \nabla.(\varphi_E \mathbf{u}_E) = \Gamma_E. \tag{4.34}$$

We model ECM as a rigid structure that does not move in space. The phase only changes by degradation which is executed by the enzyme MMP. Hence, the velocity field of the phase is

$$\mathbf{u}_E = 0. \tag{4.35}$$

Then the Equation 4.34 reduces to

$$\frac{\partial}{\partial t}\varphi_E = \Gamma_E. \tag{4.36}$$

Finally, mass of IF will also be conserved according to

$$\frac{\partial}{\partial t}\varphi_L + \boldsymbol{\nabla}.(\varphi_L \mathbf{u}_L) = \Gamma_L. \tag{4.37}$$

The velocity field of IF, $\mathbf{u}_L$, will have a special importance because the chemicals will be carried by this flow, along with diffusion.

The total volume fraction of tumor cells, ECM and IF will always add up to one. In other words, the mixture is saturated.

$$\sum_k^N \varphi_k = 1. \tag{4.38}$$

4.3.2. The Mass Interchange Terms

Since mass of the whole mixture will be conserved, the mass interchange among phases must add up to zero.

$$\sum_k^N \Gamma_k = 0. \tag{4.39}$$

The change in the volume fraction of tumor phase will be given by Γ_T. In our model, it will only depend on nutrient concentration. It can be either positive or negative indicating increase in the tumor volume fraction in that unit volume by proliferation or cell migration or decrease in the volume by death. Γ_T is given by

$$\Gamma_T = \begin{cases} k_p(n - n_p) & n \geq n_p, \\ 0 & n_p > n > n_h, \\ -k_h(n_h - n) & n_h \geq n, \end{cases} \tag{4.40}$$

where n is the dynamic concentration of nutrients in the unit volume n_p and n_h are threshold values for proliferation and hypoxia induced death. The first one indicates the

minimum concentration of nutrients needed for tumor proliferation. The second one is maximum concentration, after which hypoxia begins. Hypoxic cells die in our model and result in a negative volume fraction. Between these two thresholds, volume fraction of cells remains constant. Stable cells enter the quiescency and migrating cells create a constant volume flux. The coefficients k_p and k_h can be extracted from data of in vivo experiments. The rate of proliferation with a constant nutrient concentration can be measured easily in vivo conditions.

The ECM is rigid and it does not move. Hence, the only process that changes its volume is degradation by MMP enzymes. This change is given by

$$\Gamma_E = -k_E m \varphi_E \tag{4.41}$$

where m is the dynamic concentration of MMP at that unit volume.

IF will be used up by proliferating tumor cells, it can be moved in order to make space for migrating tumor cells or it can increase in volume as a result of degradation of ECM. Hence, it is given by

$$\Gamma_L = -(\Gamma_T + \Gamma_E). \tag{4.42}$$

Thus, it satisfies the mass conservation condition for the whole mixture.

4.3.3. The Concentration of Biochemicals

The concentrations of nutrients, n, are given by advection-diffusion equations. The equation governing the nutrient concentration is given by,

$$\frac{\partial n}{\partial t} + \mathbf{u}_L . \boldsymbol{\nabla} n = k_{n1} \boldsymbol{\nabla}^2 n - k_{n2} \varphi_T n - k_{n3} \varphi_E n + k_{n4} \tag{4.43}$$

where the rightmost term is the nutrient supply rate by the pre-existent host capillary web which is homogeneous. We set this source term to a constant because of the assumption of

the homogeneous capillary web but it can be a function of space to describe different vessel web structures. We know from the clinical evidence that the vessels of tumors are highly irregular. They are leaky and some vessels are collapsed due to the high pressure within the tumor. Hence, in order to study these cases, this term can be set as a function of pressure in a way that it gets smaller as pressure gets higher. The third term on the right hand side accounts for the nutrient consumption rate by the host. Without the presence of the tumor, the nutrient supply should be adequate to nurture the host tissue. The second term on the right hand side is the uptake rate of the nutrients by the tumor cells. This is by far greater than the third and the last terms. The nutrient uptake rate of tumor is very large due to excessive proliferation. Hence, in our model, we ignore the third and the last terms since they nearly cancel each other and the rate of uptake in tumor is significantly higher than them. The first term in the right hand side is the diffusion and the second term on the left hand side the advection of the nutrients. After mentioned modifications, the Equation 4.43 becomes,

$$\frac{\partial \mathrm{n}}{\partial \mathrm{t}} + \mathbf{u}_{\mathrm{L}}.\boldsymbol{\nabla}\mathrm{n} = \mathrm{k}_{\mathrm{n1}}\boldsymbol{\nabla}^{\mathbf{2}}\mathrm{n} - \mathrm{k}_{\mathrm{n2}}\varphi_{\mathrm{T}}\mathrm{n}. \tag{4.44}$$

The second chemical in the model is MMP enzyme, denoted by m, which degrades the ECM. The change in the concentration of MMP is given by

$$\frac{\partial \mathrm{m}}{\partial \mathrm{t}} + \mathbf{u}_{\mathrm{L}}.\boldsymbol{\nabla}\mathrm{m} = \mathrm{k}_{\mathrm{m1}}\boldsymbol{\nabla}^{\mathbf{2}}\mathrm{m} + \mathrm{k}_{\mathrm{m2}}\varphi_{\mathrm{T}}\varphi_{\mathrm{E}} - \mathrm{k}_{\mathrm{m3}}. \tag{4.45}$$

The last term represents decay of MMP. The second term on left hand side gives the advection with IF flow and the first term on the right hand side gives the diffusion. The MMP production rate is given in the second term on the right hand side, which is a function of the volume fractions of ECM and tumor cells. Hence, MMP will not be produced when either of these phases will not be present.

4.3.4. Momentum Conservation

Momentum is conserved for the whole mixture. In addition to that, the net force for each phase needs to be zero as a result of elimination of inertial terms. Hence, the momentum conservation will turned into a simple force balance,

$$\boldsymbol{\nabla}(\varphi_k \mathbb{T}_k) + \mathbf{M}_k = 0 \tag{4.46}$$

where $\mathbb{T}_k$ is total stress of phase k and $\mathbf{M}_k$ is the force applied to the unit volume, in other words, it is the momentum source of phase k.

In order to ensure the net force on the mixture will be zero, the momentum sources need to satisfy the condition

$$\sum_k^N \mathbf{M}_k = 0. \tag{4.47}$$

Force balances are guaranteed by the absence of the inertial terms. We can write force balances of the phases as

$$\boldsymbol{\nabla}(\varphi_T \mathbb{T}_T) + \mathbf{M}_T = 0, \tag{4.48}$$
$$\boldsymbol{\nabla}(\varphi_E \mathbb{T}_E) + \mathbf{M}_E = 0, \tag{4.49}$$
$$\boldsymbol{\nabla}(\varphi_L \mathbb{T}_L) + \mathbf{M}_L = 0, \tag{4.50}$$

for the phase tumor, ECM and liquid respectively.

No deformation is allowed in liquid and ECM phases. Hence, their stress tensors are simply composed of pressure, P. Tumor is a deformable viscous fluid in our model. Its stress tensor has three parts, pressure, viscous and deviatoric stresses

$$\mathbb{T}_E = \mathbb{I}P, \tag{4.51}$$
$$\mathbb{T}_L = \mathbb{I}P, \tag{4.52}$$
$$\mathbb{T}_T = \mathbb{I}P + \mathbb{I}\Sigma_T + \tau_T, \tag{4.53}$$

where P is the pressure, Σ_T is the deviatoric and τ_T is the viscous stress of the tumor cells. Deviatoric stress, Σ_T will be responsible for compression of the tumor cells and explained by a heuristic equation [82].

$$\Sigma_T = \begin{cases} k_\Sigma \frac{\varphi_T - \varphi_T^*}{(1-\varphi_T)^2} & \varphi_T > \varphi_{T_MIN}, \\ 0 & \varphi_{T_MIN} \geq \varphi_T, \end{cases} \tag{4.54}$$

where φ_T^* is the maximum volume fraction without compression, and φ_{T_MIN} is the volume fraction after which there is no adhesive interaction.

The viscous stress will be given in terms of μ_T, the viscosity of the tumor,

$$\tau_T = 2\mu_T \, \boldsymbol{\nabla}.\mathbf{u_T}. \tag{4.55}$$

In our model, these stresses are created by friction forces caused by relative motion of the phases. The friction forces are included in the momentum source term. They will be proportional to the volume fractions of two phases that meet to create friction. This will provide no force when the phase is not present in that unit volume. For phases i and j, they will be of the form,

$$\mathbf{F_{ij}} = k_{ij}\varphi_i\varphi_j(\mathbf{u_i} - \mathbf{u_j}). \tag{4.56}$$

Then, the momentum source terms for different phases are given by

$$\mathbf{M_T} = P\boldsymbol{\nabla}\varphi_T - k_{ET}\varphi_E\varphi_T\mathbf{u_T} + k_{LT}\varphi_L\varphi_T(\mathbf{u_L} - \mathbf{u_T}), \tag{4.57}$$

$$\mathbf{M_L} = P\boldsymbol{\nabla}\varphi_L - k_{ET}\varphi_E\varphi_T\mathbf{u_L} + k_{LT}\varphi_L\varphi_T(\mathbf{u_T} - \mathbf{u_L}). \tag{4.58}$$

Since all the momentum sources will add up to zero, $\mathbf{M_E}$ will simply be equal to

$$\mathbf{M_E} = -(\mathbf{M_T} + \mathbf{M_L}). \tag{4.59}$$

4.3.5. Summary: Model Equations

The mass conservation equations are given by

$$\frac{\partial}{\partial t}\varphi_T + \boldsymbol{\nabla}.(\varphi_T \mathbf{u}_T) = \Gamma_T, \tag{4.60}$$

$$\frac{\partial}{\partial t}\varphi_E = \Gamma_E, \tag{4.61}$$

$$\frac{\partial}{\partial t}\varphi_L + \boldsymbol{\nabla}.(\varphi_L \mathbf{u}_L) = \Gamma_L, \tag{4.62}$$

$$\sum_k^N \varphi_k = 1. \tag{4.63}$$

The mass interchange terms are given by

$$\Gamma_T = \begin{cases} k_p(n - n_p) & n \geq n_p \\ 0 & n_p > n > n_h \\ -k_h(n_h - n) & n_h \geq n \end{cases} \tag{4.64}$$

$$\Gamma_E = -k_E m \varphi_E, \tag{4.65}$$

$$\Gamma_L = -(\Gamma_T + \Gamma_E). \tag{4.66}$$

The concentration of biochemicals are given by

$$\frac{\partial n}{\partial t} + \mathbf{u}_L.\boldsymbol{\nabla} n = k_{n1}\boldsymbol{\nabla^2} n - k_{n2}\varphi_T n, \tag{4.67}$$

$$\frac{\partial m}{\partial t} + \mathbf{u}_L.\boldsymbol{\nabla} m = k_{m1}\boldsymbol{\nabla^2} m + k_{m2}\varphi_T\varphi_E - k_{m3}. \tag{4.68}$$

The momentum conservation are given by

$$\boldsymbol{\nabla}(\varphi_T \mathbb{T}_T) + \mathbf{M}_T = 0, \tag{4.69}$$

$$\boldsymbol{\nabla}(\varphi_E \mathbb{T}_E) + \mathbf{M}_E = 0, \tag{4.70}$$

$$\boldsymbol{\nabla}(\varphi_L \mathbb{T}_L) + \mathbf{M}_L = 0. \tag{4.71}$$

Stress tensors are given by

$$\mathbb{T}_L = \mathbb{I}P, \tag{4.72}$$

$$\mathbb{T}_T = \mathbb{I}P + \mathbb{I}\Sigma_T + \tau_T, \tag{4.73}$$

$$\Sigma_T = \begin{cases} k_\Sigma \frac{\varphi_T - \varphi_T^*}{(1-\varphi_T)^2} & \varphi_T > \varphi_{T_{MIN}}, \\ 0 & \varphi_{T_MIN} \geq \varphi_T\,, \end{cases} \tag{4.74}$$

$$\tau_T = 2\mu_T\, \boldsymbol{\nabla}.\mathbf{u}_T. \tag{4.75}$$

Momentum source terms are given by

$$\mathbf{M}_T = P\boldsymbol{\nabla}\varphi_T - k_{ET}\varphi_E\varphi_T\mathbf{u_T} + k_{LT}\varphi_L\varphi_T(\mathbf{u_L} - \mathbf{u_T}), \tag{4.75}$$

$$\mathbf{M}_L = P\boldsymbol{\nabla}\varphi_L - k_{ET}\varphi_E\varphi_T\mathbf{u_L} + k_{LT}\varphi_L\varphi_T(\mathbf{u_T} - \mathbf{u_L}), \tag{4.76}$$

$$\mathbf{M}_E = -(\mathbf{M}_T + \mathbf{M}_L). \tag{4.77}$$

4.4. Extensions to the Model

4.4.1. Blood Vessel Structure and Angiogenesis

Without making major changes, we are able to represent another biological structure within our model. A new variable, a dynamic vessel web density which includes newly produced angiogenic vessels as well as the pre-existing host vessel web can be added to the equation system. From the scale that we look at the tumor, we see a continuous system of blood vessels rather than seeing discrete vessels. This enables us to represent blood vessels with a continuous variable, blood vessel density. Blood vessel density can be approximated as having no volume. As a result, we can include it to our model without taking it as another phase.

The blood vessel density can be initially set as homogeneous. This density is then going to be decrease in the regions where tumor cells are present as pressure builds up as a result of excessive tumor proliferation. The increased pressure will result in collapse of the blood vessels [94]. This mechanical relation can be easily integrated to our model; we can represent vessel density mathematically in a way that decreases with the pressure.

After initiation of angiogenesis, new blood vessels begin to form around and inside the tumor body. This process happens when the tumor angiogenesis factors, TAFs, are present in the IF. Then the production of new vessels will increase the vessel density. The two competitive events, the collapse of the vessels which result in the decrease and the production of new vessels with angiogenesis which results in the increase, govern the evolution of the initially homogeneous vessel web density. In order to simulate this, we need to add another biochemical to our model. The addition of TAFs is very easy at this point because we know how to add chemicals to our model.

The production of TAFs is triggered by the appearance of the hypoxia among the cells. The cells that suffer from lack of nutrients either become hypoxic or evolve by this selective pressure forced by microenvironment which will result in gaining the ability to induce angiogenesis. By claiming that angiogenesis is triggered only by inadequacy of nutrients, the production rate of TAFs is written as

$$\text{production rate of TAF} = \begin{cases} k_{a2}\varphi_T(n_a - n) & n_a > n, \\ 0 & n \geq n_a. \end{cases} \tag{4.78}$$

It is produced when nutrient concentration is dropped below the critical value after which tumor cells initiates the production of TAFs. Thus, TAF concentration, which is denoted by a, can be given by

$$\frac{\partial a}{\partial t} + \mathbf{u}_L.\nabla a = k_{a1}\nabla^2 a + k_{a2}\varphi_T(n_a - n)^+ - k_{a3} \tag{4.79}$$

which includes the diffusion and advection of TAF and a decay term on the rightmost side.

Now, we can build an equation to govern the vessel density which includes angiogenic vessels as well as the pre-existing vessel structure. The vessels will collapse according to the function

$$\text{collapse of vessels} = \begin{cases} k_{v1}v(P - P_c) & P > P_c, \\ 0 & P_c \geq P, \end{cases} \tag{4.80}$$

where P_c is the critical pressure after which a vessel collapses. The vessel density is denoted by v, and given by Equation 4.81,

$$\frac{\partial v}{\partial t} = -k_{v1} v(P - P_c)^+ + k_{v2} a. \tag{4.81}$$

The rightmost term is responsible for the production of new vessels, simply proportional to TAF concentration. Hence, using only two new equations accompanied with two unknowns, vessel density and TAF concentration, we are able to simulate the angiogenesis.

4.4.2. Simulation of MMP Inhibition Treatment

Another chemical can be added to our model with some small alterations in the equations. The synthetic MMP inhibitors, MMPi in short, are clinically experimented for a long time, yet these efforts cannot be turned into a treatment that yields in net benefits. We can use this model to study different aspects of this controversial treatment strategy to cancer. Hence, our model can take part in the in silico experimentation of MMP inhibition treatments about why inhibiting MMP does not yield successful clinical results.

The MMPi treatment is associated with the drug delivery problem, which is the inefficient delivery of drugs to tumor cells by various reasons. The synthetic MMPi is tried to be delivered to the tumor cells by blood circulation. This delivery seems to be failing because of the reasons given below

(i) abnormal circulation within the tumor with a very heterogeneous blood vessel web which composed of damaged pre-existing vessels and irregular new vessels that produced hastily by tumor induced angiogenesis,

(ii) the failure in the passage of chemicals from the blood to the IF which surrounds the tumor cells. This process is managed by the pressure differences of these two flows. Normally blood pressure is high compared to IFP; hence, chemicals are easily be transferred but in tumors IFP is significantly high which prevents this transfer.

Like other chemicals, the concentration of MMP inhibitor, will be governed by advection diffusion equations. The delivery of MMPi will be proportional to the pressure difference between IF and the constant blood pressure that we set for the model. We claim that the existent vessels have an average constant blood pressure, P_b. The density of vessels will also affect the delivery of MMPi. The delivered MMPi in the IF will than react with the MMP which is present in the IF. With this process, both MMP and MMPi will be unusable again, hence; it will lead to a sink term in both equations that govern their concentration. MMPi will be denoted with i and added to the equation set in the form of

$$\frac{\partial i}{\partial t} + \mathbf{u}_L.\boldsymbol{\nabla} i = k_{i1}\boldsymbol{\nabla^2} i + k_{i2} v(P_b - P)^+ - k_{i3} m i. \tag{4.82}$$

Now, we need to modify the concentration equation of MMP, m. The original equation was

$$\frac{\partial m}{\partial t} + \mathbf{u}_L.\boldsymbol{\nabla} m = k_{m1}\boldsymbol{\nabla^2} m + k_{m2}\varphi_T\varphi_E - k_{m3}. \tag{4.83}$$

We need to add another sink term representing the inhibition of MMP with MMPi. We have already done it for the concentration equation of the MMPi. Since the binding between MMP and MMPi occurs one to one, we can use the same form for concentration of MMP,

$$\frac{\partial m}{\partial t} + \mathbf{u}_L.\boldsymbol{\nabla} m = k_{m1}\boldsymbol{\nabla^2} m + k_{m2}\varphi_T\varphi_E - k_{m3} - k_{i3} m i. \tag{4.84}$$

Hence, we are able to add a simulation of MMP inhibition with adding only one new equation with a new variable and a small addition to an existent model equation.

4.4.3. Elaboration on Tumor Cell Motility

In our model, we limited the source of the movements of the cells to the force balances. By this way, we can only see the result of mechanical approach in our simulations. However, tumor cells also gain motility by internal and external stimuli other than physical forces.

Random motility is induced by chemokines in the tumor cells. This random motion can be represented by a diffusion term added to the mass balance equation.

$$\text{diffusion of cancer cells} = k_{rT}\boldsymbol{\nabla^2}\varphi_T. \tag{4.85}$$

Hence, the mass balance equation for tumor turns into

$$\frac{\partial}{\partial t}\varphi_T + \boldsymbol{\nabla}.\left(\varphi_T \mathbf{u}_T\right) = k_{rT}\boldsymbol{\nabla^2}\varphi_T + \Gamma_T. \tag{4.86}$$

Haptotaxis is the movement along ECM by the help of adhesion forces. Thus, the motility term by haptotaxis must include ECM and tumor volume fractions since the binding takes place among them and it must be increased towards the increasing ECM volume fraction. It can contribute to the velocity field of the tumor with term below

$$\text{velocity releated to haptotaxis} = k_{hT}\varphi_T\varphi_E\,\boldsymbol{\nabla}\varphi_E. \tag{4.87}$$

Chemotaxis is the drift of the tumor cells along concentration of chemicals which can be given for a chemical, c

$$\text{velocity releated to chemotaxis} = k_{cT}\varphi_T\varphi_c\,\boldsymbol{\nabla}c\,. \tag{4.88}$$

These terms can be added to the velocity field that we found from mechanical analysis. The new velocity field $\tilde{\mathbf{u}}_T$ will be given by

$$\tilde{\mathbf{u}}_T = \mathbf{u}_L + k_{hT}\varphi_T\varphi_E\,\boldsymbol{\nabla}\varphi_E + k_{cT}\varphi_T\varphi_c\,\boldsymbol{\nabla}c \tag{4.89}$$

where k_{hT} and k_{cT} are motility coefficients related to haptotaxis and chemotaxis.

5. DISCUSSION

While we work on the mixture modeling framework, we realized its flexibility in the modeling of different biological structures and events. Various biological events can be added to this well-structured and mechanically justified model. We added matrix remodeling features as well as a simple but essential blood vessel structure. These two features can be elaborated by addition of more biological phenomena about these two features. However, we need to be careful about not to overcrowd the model because every additional detail costs us computational power. Turning off the unnecessary features of the model, in exploring a special topic, will help to ease this computational burden. The modular structure of the model enables us to make these kinds of alterations easy. Alterations in the equations that govern the behavior of constituents can be made easily. Because of these properties of mixture modeling framework, we think that it is a good platform for in silico experimentation.

During the construction of the model, we ignored the genetic changes that accompanied the tumor progression. Hence, we set the tumor phase genetically static and homogeneous. However, it is known that microenvironmental effects cause change in the genotype and genetic changes cause changes in microenvironment. The genetical effects can never be represented in a continuum picture as good as in a discrete model since mutations are discrete events. However, genetically different tumor populations can be introduced as different phases, each with their own specialized behaviors. They can be confined in a computational domain with varying environmental selective pressures to explore which genetical changes enhance survival in that conditions. They can be confined together to create a competition over nutrients and other substances, like the real, in vivo conditions.

Since we do not take genetical changes into account, we can run our model in time periods in which the genetically static approximation is reasonable. That may correspond to time periods of successive major mutations that create significant alterations in behavior patterns of the tumor cells. Frequencies of major mutations are either known or now studied by scientists, so we can have guidance in biological literature.

Addition of blood vessel density enables us to make in silico experiments about drugs that carried with blood circulation. We included the dynamic density of blood vessel webs of the tumor which will enable us to calculate the spatial and temporal distribution of anti-tumor drugs in the computational domain. Map of blood vessels of the tumor can be supplied with imaging technologies. This map can be converted into initial values of blood vessel densities. Then, the model can be used to simulate the drug delivery through a specific tumor.

In our project, we aimed to find explanations about current issues that cannot be solved in clinical and laboratory experiments. We are especially interested in subjects which are affected by mechanical aspects in order to benefit from our experience in physics. The mechanically relevant issues involve the drug delivery problem and ECM remodeling which we are currently working on.

The ECM remodeling is represented in our model in connection with the activity of the responsible enzyme MMP. MMP activity is a hot topic in cancer research. Scientists are sure that it has a profound effect in tumor progression, but underlying dynamics of its activity is very hard to resolve. That is why treatments that solely based on the idea that "MMP contributes to tumor progression" failed to yield net clinical benefits. Hence, we thought that it deserves special interest and included a simulation of activity of a MMPi in our model. Since we are able to represent abnormal and heterogeneous structure of tumor vessels in our model, we are able to experiment on the drug delivery problem of MMP inhibitors.

We can also turn off the ECM remodeling aspects of the model to use it on other problems, for experimentation with anti-cancer drugs other than MMPis. In order to have a better representation of the reality, blood pressure inside the tumor can be heterogeneous to account for abnormal circulation. Also diffusion constants of drugs can be made anisotropic to represent a more realistic picture.

Determination of parameters for a model is a hard task. We already reflect very little part of reality into models because of the restrictions imposed by computational power. Nevertheless, models can mimic the real life tumors to a surprising degree with this little

amount of detail. However, in order not to get any more far from the reality, we need to find biologically relevant parameters to our models. Especially, the ones that can be found in the biological literature must be chosen to have a more realistic model. In addition to that, biologically relevant parameters will be easier to understand and interpret since they have been used already by biologists. The contribution of biologists to modeling process is very important and this kind of common grounds will provide development of better communication among these interdisciplinary scientists. A model that can only be understood by physicists or mathematicians cannot be successful because in their essence, models aim to improve the experimentation and therapies which will be executed by biologists or oncologists. By taking this into account, we try to choose our parameters from the biologically relevant ones as much as possible.

We hope to take our research one step further by using imaging data from different modalities. Both achieved and newly produced imaging data can be integrated to our model. Parameters can be fixed using imaging data. As a result of this, the models will be more realistic. The imaging data about flow of blood and IF will provide extensive benefits in a good model. Since the delivery of the drugs, distribution of nutrients, chemicals and substrates are all governed by these two flows, exploring the dynamics with models will contribute to cancer research. Imaging data from patients can be used to make predictions. A computational model that runs with the specific data from the patient will have more predictive power than a prediction based on the statistical data about the state of the disease [53].

6. CONCLUSION

We build a mixture model to simulate the tumor growth. In the process, we tried to make a smart design with minimum details possible. The simplest forms of functions that are able to represent the biological event are used and all the complexities are avoided. The model is very flexible; the events that deserve a special interest in a specific research can be elaborated by changing the functions that represent the event. Additional events can be supplemented according to the need. Unnecessary parts can be removed to conduct experiments that aim to see results of the activity of a special part of the structure. This flexibility comes from the modularity of the structure. Each phase and chemicals act as a module. Even when they are removed, tumor model runs properly.

By using coupled equations, we could formulate the complex and interconnected events given below.

(i) The rapid proliferation of the tumor cells, increasing pressure and decreasing nutrients in the environment.
(ii) Energy depletion by excessive proliferation and hypoxia in tumor cells.
(iii) The creation of a velocity field of the tumor phase caused by the stress built up.
(iv) Movements of the tumors to the regions of less pressure.
(v) Drag forces from the relative movement of tumor cells and IF which in turn affect the velocity field.
(vi) Degradation of the ECM with MMP when the tumor and ECM come together.
(vii) Vessel collapse from pressure.
(viii) Angiogenesis in the regions of hypoxia by the help of TAFs.
(ix) Transportation of the drugs by this combined vessel structure.
(x) Passage of the drugs from blood to IF by the help of pressure difference.

By these events, we are able to construct a mechanical framework for the drug delivery problem. We have a vessel structure that evolves with the tumor which opens the door for many different experiment scenarios.

Introduction of MMPi to the system by additional equations is given for simulations of the treatment. The activity of MMP will be visible in the volume change of ECM since it is the only factor that causes alterations on ECM phase. MMPi will prevent remodeling of ECM by inactivation of MMPs. In the absence of MMPs, tumor will be stuck between the fibers of ECM and pressure will build up in the region.

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