ACOUSTIC RADIATION ENHANCED DRUG DELIVERY

by

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ABSTRACT

ACOUSTIC RADIATION ENHANCED DRUG DELIVERY

Drug development is one of the main concerns in cancer research. In recent years, lots of new drugs have been discovered in order to increase the efficiency of cancer treatment and enhance the quality of patient's life. However, anti-cancer drugs, which are effective in laboratory experiments, may not give positive results when they are injected to humans. Because of the abnormal vasculature in tumor microenvironment, drug particles face with several physiological barriers preventing the transport of therapeutic agents homogeneously from the region of administration to the cells in solid tumors. Recent studies have revealed that drug response of a tumor cell is determined by its biological characteristics and its microenvironment regulation. The abnormalities in tumor vasculature result in uneven drug distribution and blood flow within solid tumors. Moreover, the leaky, tortuous and highly permeable tumor vessels and the lack of functional lymphatic vessels lead to the elevated interstitial fluid pressure (IFP). The use of radiation force is for the enhancement of drug delivery is an emerging method. Experimental studies have shown that radiation force can manipulate delivery vehicles in the vasculature by changing their velocities and positions. In the light of these experiments, a mathematical model which associates the effect of acoustic radiation force for the convective transport and the drug distribution in tumors is constructed. Governing equations in the model involve the principles for transvascular and interstitial drug transport as well as conservation laws.

ÖZET

AKUSTİK IŞINIMLA GELİŞTİRİLMİŞ İLAÇ İLETİMİ

Kanser tedavisinde ilaç geliştirilmesi önemli konulardan biridir. Son yıllarda, kanser tedavisinin etkisini ve hastanın yaşam kalitesini artırmak amacıyla çeşitli ilaçlar üretilmiştir. Fakat, laboratuvar ortamında etkili olan ilaçlar insanlar üzerinde olumlu sonuçlar vermemiştir. Tümör mikroçevresindeki düzensiz damarlaşma nedeniyle ilaç parçacıkları homojen iletimi engelleyen çeşitli fiziksel bariyerler ile karşılaşırlar. Son çalışmalar, tümör hücresinin ilaca verdiği tepkinin hem biyolojik özellikleriyle hem de tümör mikroçevresinin düzenlenmesiyle belirlendiğini ortaya koymuştur.

Tümör damar sistemindeki düzensizlikler tümör içinde kararsız ilaç dağılımına ve kan akışına sebep olurlar. Ayrıca, sızıntılı, kıvrımlı ve fazlaca geçirgen tümör damarları ve fonksiyonel lenf damarlarının eksikliği interstisyel sıvı basıncının (İSB) artmasına sebep olur. İlaç iletiminin artırılmasında akustik ışınımın kullanılması yeni geliştirilen bir metottur. Deneysel çalışmalar akustik ışınımın, iletim araçlarının hızını ve konumunu değiştirdiğini göstermiştir. Bu deneylerin ışığı altında, akustik ışınım kuvvetinin etkisini tümörlerde konvektif iletim ve ilaç dağılımıyla birleştiren matematiksel bir model inşa edilmiştir. Modeldeki hakim denklemler, transvasküler ve interstisyel ilaç taşınım ilkeleri ve korunum yasalarını içermektedir.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SYMBOLS

c	speed of sound (m/s)
C_i	Interstitial concentration (g/m)
C_p	Plasma concentration (g/m)
D	Diffusion coefficient (cm^2/s)
F_{Rad}	Acoustic radiation force (pN)
J_f	Fluid leakage from the vessel (m/s)
k_E	Retardation factor, i.e., ratio of solute velocity to interstitial fluid velocity
K	Hydraulic conductivity of the tissue $(cm^2/mmHg.s)$
L_p	Hydraulic conductivity of the blood vessel wall $(cm/mmHg.s)$
P_d	Diffusional permeability of the vessel wall for a particular solute (cm/s)
P	Interstitial fluid pressure $(mmHg)$
P_v	Vascular fluid pressure $(mmHg)$
$\mathbf{q}_F$	Interstitial fluid flow (mm/s)
r	Radius of particle $(\mu$m$)$
S	Vascular surface area (cm^2)
$\mathbf{v}_F$	Interstitial fluid velocity (mm/s)
U_{Rad}	Acoustic radiation potential
Γ_{Fb}	Rate of fluid extravasation from blood vessels to interstitial space $(ml/ml.s)$
Γ_{Fl}	Rate of fluid drainage to the lymphatics $(ml/ml.s)$
λ_d	Vascular diffusion rate of drug molecules
λ_r	The decay rate of drug molecules $(1/s)$
λ_{Fb}	Blood capillary filtration coefficient $(cm/mmHg.s)$
λ_{Fl}	Lymphatic filtration coefficient $(cm/mmHg.s)$
ζ	Drag coefficient
η	Viscosity of the medium $(N.s/m^2)$
ρ_p	Particle density (kg/m^3)

ρ_f	Fluid density (kg/m^3)
κ_p	Compressibility of particle ($1/Pa$)
κ_f	Compressibility of fluid ($1/Pa$)
π_c	Oncotic pressure in the interstitial space
π_p	Oncotic pressure in the blood plasma
ϕ_F	Volume fraction of the interstitial space
σ	Osmotic reflection coefficient

LIST OF ACRONYMS/ABBREVIATIONS

ECM	Extracellular matrix
EPR	Enhanced permeability and retention
FUS	Focused ultrasound
HIFU	High intensity focused ultrasound
IFP	Interstitial fluid pressure
MI	Mechanical index
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
TGF	Tumor growth factor
VEGF	Vascular endothelial growth factor

1. INTRODUCTION

1.1. Overview of the thesis

In the first chapter, we examine the physiological barriers which inhibit the delivery of drug molecules to solid tumors. Specifically, we discuss the abnormal tumor vasculature and transvascular transport mechanism in tumors both of which significantly affect the efficacy of cancer treatment. In addition, we generally discuss the use of ultrasound in the delivery of nanotherapeutics.

In the second chapter, we give a necessary biological background to help us understand the model properly. In order to do this, some biological terms such as extracellular matrix, hypoxia, interstitial fluid and angiogenesis are explained. In the second half of this chapter, we examine the transport mechanism in solid tumors which has a critical role in drug delivery.

In the third chapter, we discuss the applications of ultrasound inthe delivery of drug particles. Firstly, the physical mechanisms of ultrasound which are heat generation, cavitation and acoustic radiation force are examined. Then, the drug delivery vehicles used together with ultrasound are explained.

In the fourth chapter, we present a mathematical model and study the transport of drug molecules by taking into account the acoustic radiation force. Also, the equations used in our model are explained.

In the fifth chapter, we discuss the experimental studies, our model and the future work.

1.2. The barriers to the delivery of nanotherapeutics to solid tumors

Latest improvements in the field of nanomedicine have facilitated the development of nanotherapeutics (e.g., liposomes, polymeric micelles, viruses and quantum dots) for the cancer treatment. Owing to the enhanced permeability and retention effect (EPR), they are passively targeted to tumors which make them appropriate agents for the delivery of cancer drugs.

The EPR effect is a phenomenon that certain size molecules (liposomes, gold nanospheres or macromolecular therapeutic agents) can remain in tumor tissue for longer periods compared to healthy one. In contrast to normal tissues, solid tumors have higher vascular density and irregular blood flow. Due to the abnormal tumor vasculature, tumor vessels have larger pores (100-780 nm) that make macromolecules accumulate in tumor tissues and thus they cannot pass through the normal vessels, causing the lower side effects. In addition, due to the lack of functional lymph vessels, macromolecules cannot be cleared from the tumor tissue. Thus, they stay there longer times.

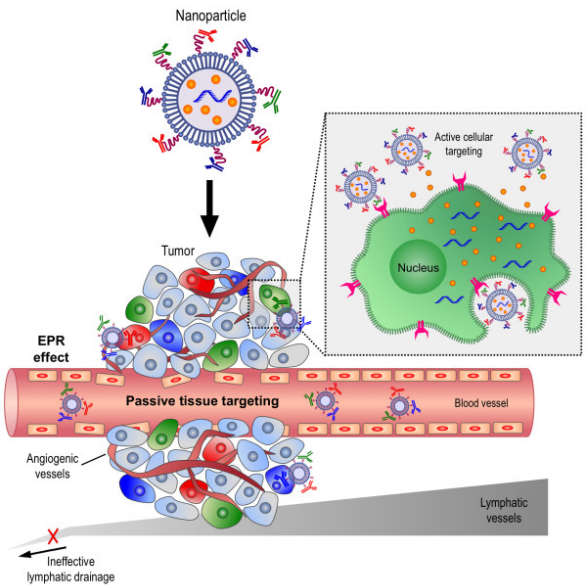

Figure 1.1. The representation of mechanisms for nanoparticle delivery. (Peer, 2014).

Regarding the tumor-targeted drug delivery, passive and active targeting are mostly used targeting strategies. Passive targeting based on the EPR effect can enable macromolecules (liposomes, nanoparticles or polymers) to accumulate in tumor tissue. Active targeting aims to target specific molecules or receptors in tumor cells by using antibodies, glycoprotein, peptides or small molecules.

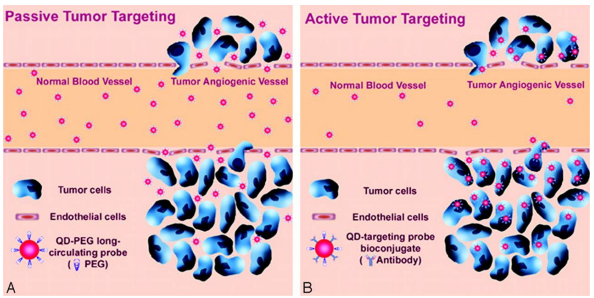

Figure 1.2. a. Passive targeting. b. Active targeting. (Reprinted from Macmillan Publishers Ltd; Nature Biotechnology 2004).

Nanotherapeutics minimize the side effects of chemotherapy by increasing the intratumoral drug concentration. Therefore, it is important to understand the barriers which inhibit the efficiency and homogeneous distribution of nanoparticles to solid tumors. Thus, this can be helpful to develop new methods for the cancer treatment.

Solid tumors form 85% of cancer types. The delivery of nanotherapeutics to solid tumors consists of three parts. Firstly, nanoparticles transport from injection sites by the systemic blood to the different parts of tumors. After extravasation from the tumor vessel wall, they penetrate through tumor interstitium to reach the targeted cells (Jain and Stylianopoulos, 2010, Wang, 2011).

The main transport mechanism of drugs across the vessel wall consists of convection and diffusion. Convection is a mechanism of transport that results from the bulk motion of fluids while diffusion is the random movement of particles from the area of high concentration to the area of low concentration. Diffusion is proportional to the concentration gradient between these areas (Yuan, 1998). For small particles, diffusion is important but when the distance among particles increases, the diffusion time also

increases with the square of the distance so the efficiency of diffusion decreases.

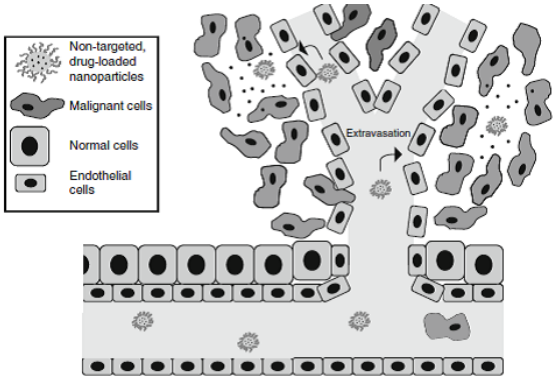

Figure 1.3. Targeting nanoparticles by enhanced permeabilty and retention effect (EPR). (Peer, 2014).

In healthy tissues, convection generally transports macromolecules whereas diffusion mainly transports small molecules. However, in tumor tissues, delivery of nanoparticles is different from the healthy ones because of the differences in their morphologies.

Compared to normal tissues, tumor vasculature is abnormal with tortuous and leaky vessels which have large pore sizes, changing from 100 to 780 nm. These large pores lead to higher hydraulic conductivity and vascular permeability that allow macromolecules to pass into tumors (Jain and Stylianopoulos, 2010). The lymphatic system allows the drainage of particles and interstitial fluid to the blood circulation. It is impaired in solid tumors that results in a decrease in the clearance and an increase in the retention of nanoparticles in tumor interstitium (Wang *et al.,* 2011).

As a result, the lack of functional lymphatics and the leaky, impaired tumor vasculature are the primary reasons for the elevated interstitial fluid pressure (IFP). This elevated IFP causes an increase in the hydraulic conductivity and a decrease in the convective transport of drug molecules from the outer to the inner parts of tumors.

In order to understand the transport mechanism of tumors, Lichtenbeld *et al.* measured the effective microvascular permeability of a human colon adenocarcinoma

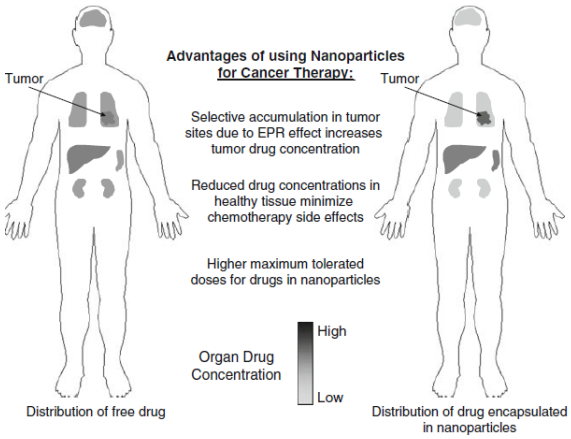

Figure 1.4. Advantages of using nanoparticles for cancer treatment. (Peer, 2014).

(LS174T) which was transplanted in the mouse dorsal skinfold chamber using the single-vessel- perfusion technique (Lichtenbeld *et al.,* 1996). This study has suggested that diffusion is the main transport mechanism in the inner parts of tumors where the microvascular pressure is closely equivalent to the (IFP). On the other hand, convection might have a role in the transport of nanotherapeutics in the periphery of tumors where the interstitial fluid pressure significantly drops.

The pressure difference causes extravasation of drug molecules in the outermost part of solid tumors (Boucher *et al.,* 1990). Therefore, it is observed that there is a higher accumulation of nanotherapeutics at the boundary of normal and tumor tissues (Jain, 1996) leading to a heterogeneous drug distribution in tumors.

1.2.1. Abnormal blood and lymphatic systems

In tumor tissue, blood vessels have a highly irregular structure compared to ones in normal tissue. Tumor vessels are heterogeneous, dilated and tortuous with aberrant branching and leaky walls (Jain and Stylianopoulos, 2010, Jain, 1996, Vakoc *et al.,* 2009) which have abnormal basement membrane, a number of fenestrae and large pores with several hundred nanometers (Hobbs *et al.,* 1998, Hashizume *et al.,* 2000). Due to the solid stress exerting from proliferating tumor cells, blood vessels are compressed

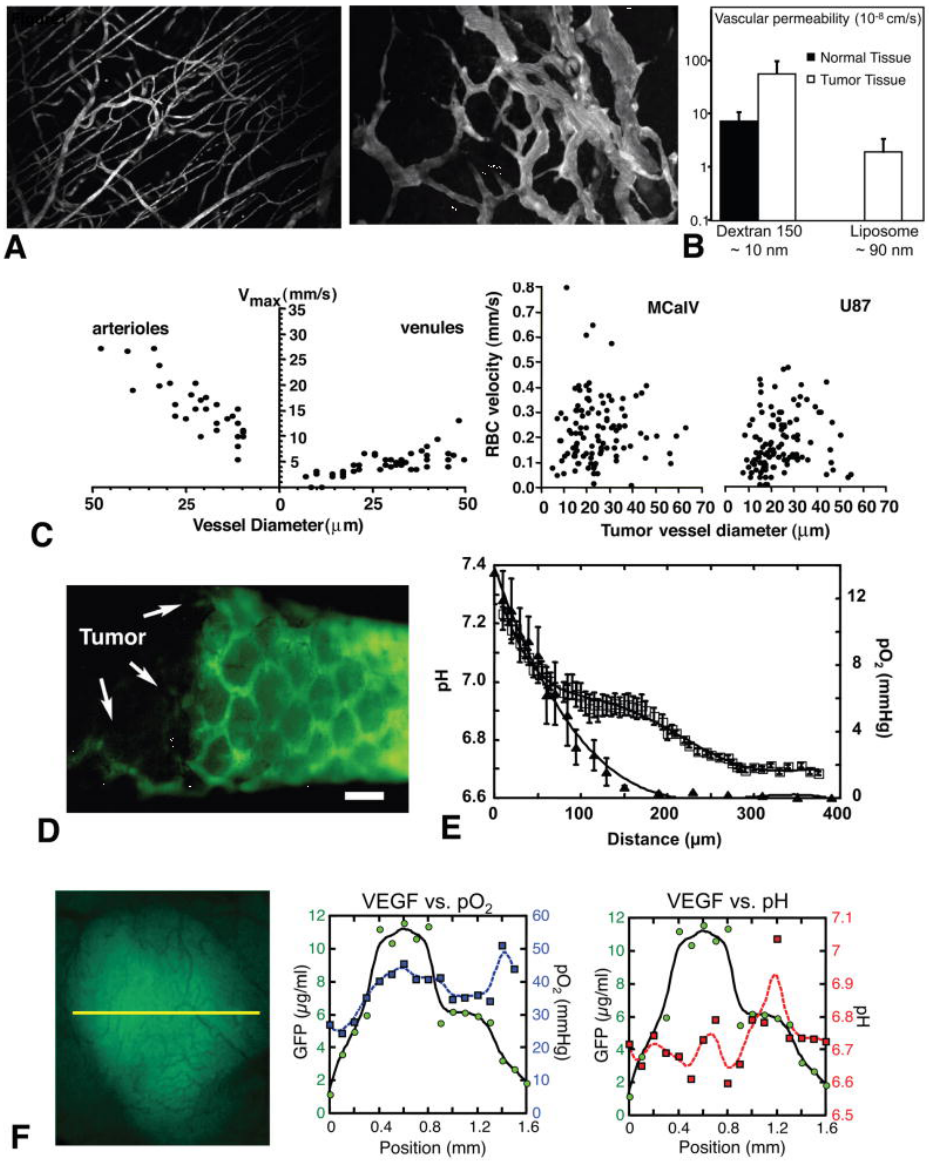

Figure 1.5. Tumor vasculature and microenvironment. (Jain and Fukumura, 2007).

leading to the vessel collapse (Padera *et al.,* 2004, Griffon-Etienne *et al.*, 1999).

In healthy tissues, the function of lymphatic system is to drain excess fluid from tissue to keep interstitial fluid balance. However in tumor tissues, the proliferating cells compress lymph vessels and cause them to collapse at the tumor center. As a result, there are no functional lymphatics at the inner part of tumor, existing only in the tumor periphery (Padera *et al.,* 2002, Leu *et al.,* 2000). The leakiness in tumor vasculature and the inefficient drainage of interstitial fluid both result in the elevated (IFP).

The elevated IFP, irregular blood supply and higher vessel permeability with inefficient lymphatic drainage lead to an abnormal tumor microenvironment. They also have a negative effect on the delivery and efficacy of nanotherapeutics in cancer treatment.

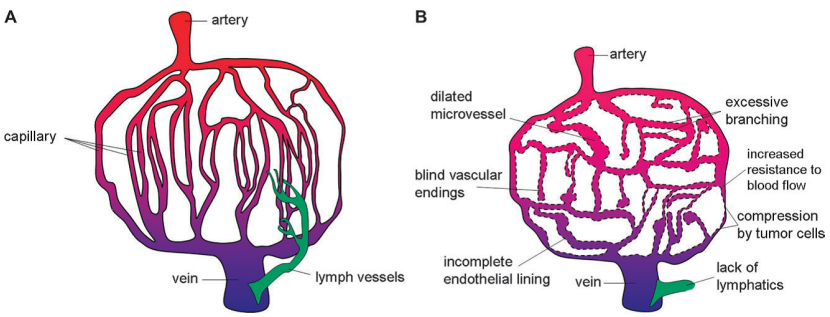

Figure 1.6. Representation of vascular systems. a. Normal tissue. b. Solid tumor. (Tredan *et al.,* 2007).

1.2.2. Abnormal transvascular transport

Tumor tissue has higher hydraulic conductivity and vascular permeability in comparison with normal tissue due to the larger pores in tumor vessel walls. Therefore, nanotherapeutics can easily extravasate from these large pores. Vascular permeability depends on the properties of the transported particle as well as the physiological characteristics of the tumor microenvironment. For instance, as the size of a nanoparticle increases, vascular permeability decreases (Yuan *et al.,* 1994, Yuan *et al.,* 1995).

Cationic nanoparticles have higher vascular permeability compared to neutral and anionic particles (Campbell *et al.,* 2002, Schmitt-Sody *et al.,* 2003, Dellian *et al.,* 2000, Thurston *et al.,* 1998, Krasnici *et al.,* 2003). Furthermore, vascular permeability depends on the region where drug is administered and also changes with time and drug response to treatment (Yuan *et al.,* 1996, Fukumura *et al.,* 1997, Monsky *et al.,* 2002).

IFP which decreases the pressure difference across the vessel wall is another physiological barrier for transvascular transport inhibiting homogeneity of drug delivery. In normal tissues, IFP is almost equal to 0 mmHg but in tumor, there is a substantial increase in IFP (Jain, 1994, Jain *et al.,* 2007) due to the high vessel wall permeability and the lack of functional lymphatic vessels in tumor interstitial space (Jain, 1994, Padera *et al.,* 2002). Correspondingly, the IFP is uniformly elevated in the inner part of a tumor and becomes nearly equal to the microvascular pressure (MVP)(Boucher *et al.,* 1990, Boucher *et al.,* 1991, Baxter and Jain, 1989, Boucher and Jain, 1992, Less *et al.,* 1992, Willett *et al.,* 2004).

As a result, convection is negligible in the interior parts of a tumor. On the other hand, in the tumor periphery, IFP decreases significantly to normal values, resulting in a precipitous pressure gradient (Baxter and Jain, 1989, Jain and Baxter, 1988, Baxter and Jain, 1991). The direction of convection is outward, since IFP gradient is high and inward in the peripheral region. Therefore, drugs delivered into tumors cannot reach in the center of tumor and they can be pushed out, causing drug molecules to accumulate only in the tumor periphery.

1.3. The use of ultrasound in drug delivery system

Ultrasound has been used in the field of medicine such as medical imaging, detection or measurement. Biological effects of ultrasound and their applications have become an emerging concept for over several decades. Especially, therapeutic ultrasound is widely used for the treatment of diseases other than imaging or diagnostics (Fukumura and Jain, 2007). It can be classified as low intensity pulsed and high intensity focused ultrasound.

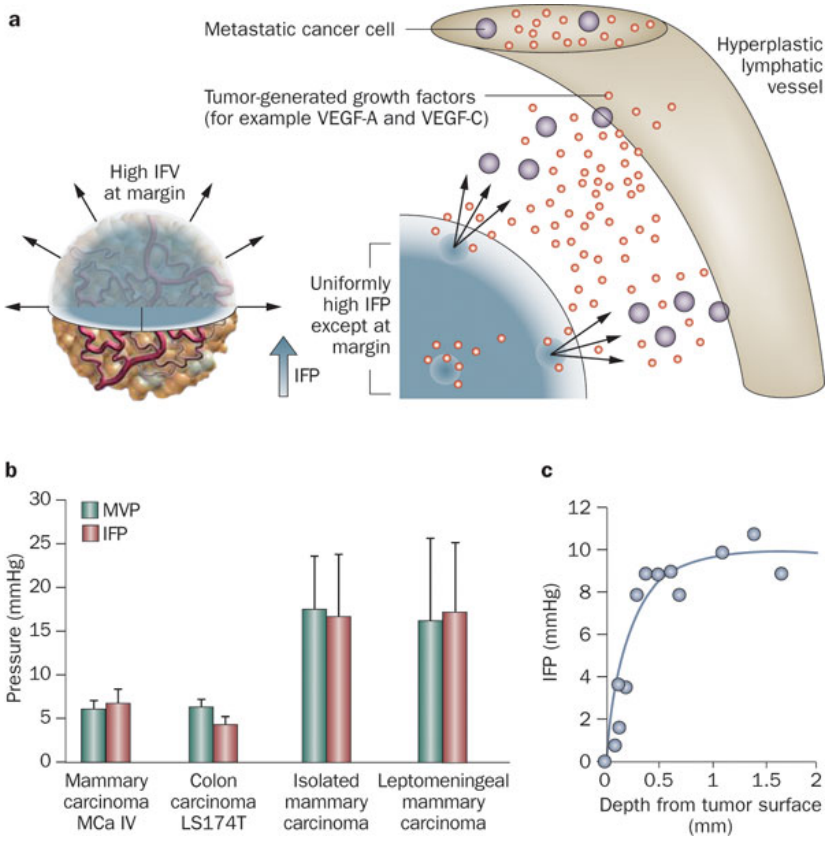

Figure 1.7. High IFP in tumors. a. Uniformly elevated IFP except at the boundaries. (Jain *et al.*) b. Interstitial Fluid and Microvascular Pressures for different cancer types. (Boucher and Jain, Boucher *et al.*) c. The changes in the IFP with respect to the distance from tumor surface. (Boucher *et al.*).

Low intensity pulsed ultrasound is mostly used in diagnostic imaging or in flow studies to get information about the state of matter. Some of its applications contain sonophoresis, sonoporation, gene therapy, and bone healing whereas high intensity focused ultrasound is used to control the state of matter as in cases kidney stone shattering or tumor and fibroid ablation. Its applications contain high intensity focused ultrasound (HIFU) and lithotripsy (Schroeder *et al.,* 2009, Ter Haar, 2007).

Ultrasound is used by itself or with drugs for the treatment of some diseases such as diabetes, cancer, cardiovascular diseases, infections, skin wounds and bone fractures (Mitragotri, 2005). Ultrasound has lots of advantages in drug delivery. Firstly, it is non-invasive so there is no need for surgery or insertion, only acoustic transducers which is in contact with a water-soluble gel are used. Moreover, ultrasound can pass through deep inside the human body and give no harm to the tissue in the beam path. Also it can be regulated and focused on a very small region desired in the body (Ghaleb and Pitt, 2009).

Biophysical effects of ultrasound can be divided into two parts: thermal effect from absorption of sound waves (which is continuous wave exposure) and nonthermal effect from scattering (which is pulsed exposure) (Ter Haar, 2007, Kitchen and Partridge, 1990).

1.3.1. Non-thermal effects

G. Ter Haar has divided nonthermal effects into two parts: cavitation and other mechanical effects such as acoustic radiation forces or acoustic streaming. Here the term cavitation implies acoustic cavitation which is the formation of tiny gas bubbles in the tissue because of the ultrasound vibration (Low *et al.,* 1994). When the sound wave propagates through the medium, its energy transfers to the microscopic gas bubbles causing them to oscillate due to the compression and the rarefaction. Here the rationale is that the steep pressure gradients in and around the cell might have an effect to alter the activity of the cell (Martinez, 2010).

The term acoustic streaming can be defined as the physical forces exerted to molecules or ions by sound waves which have a driving effect on them to displace. In biological system, this results in shear stresses on cell membranes and these stresses form transient pores where drug molecules can be transported (Ter Haar, 2007). Therefore, the temporary increase in permeability of the exposed tissue improves the delivery and thus the efficacy of drugs (Prentice, 1994).

1.3.2. Thermal effects

When the ultrasound waves propagate through the skin, they cause the surrounding tissue to oscillate and this oscillation generates heat in the tissue. Thermal effects can be seen when the temperature inside tissue becomes approximately $40 - 45\,^{\circ}\mathrm{C}$ (Prentice, 1994). High ultrasound intensities which lead to excessive thermal effects might damage tissue (Dyson, 1987).

In order to increase the intensity of ultrasound waves, they can be focused on a small focal zone by using a curved transducer which makes energy pass through inside the body. This type of therapeutic ultrasound is called as High Intensity Focused Ultrasound (HIFU) or Focused Ultrasound (FUS) which have many advantages in cancer treatment: it is non-invasive and can be focused on a focal zone with nearly 1 mm diameter without harming the surrounding tissue. It is safe since it uses ultrasound which is nonradiative.

Furthermore, real time imaging techniques are used which can enable the adjustment of treatment plans. HIFU has thermal (causing hyperthermia by heating tissue locally) and non-thermal effects (acoustic cavitation and radiation force). These effects can increase extravasation and enhance drug delivery that might help to improve treatment response.

According to some studies (O'Neill *et al.,* 2006, O'Neill *et al.,* 2007), it has been shown that the enhancement in drug delivery was seen when there were no acoustic cavitation and hyperthermia. However, it has been claimed that this situation is due to

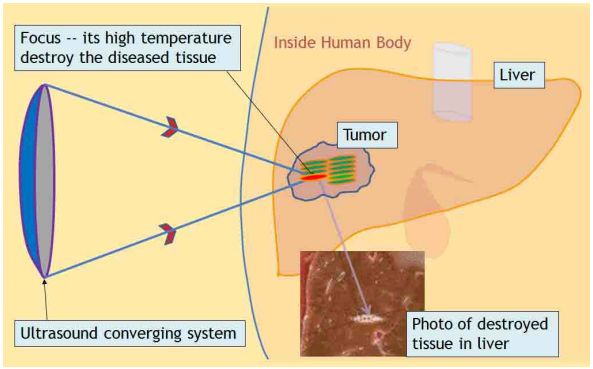

Figure 1.8. The mechanism of HIFU in treatment of cancer. (Reprinted from the www.indiahospitaltour.com).

the displacements produced by radiation forces (Frenkel *et al.,* 2006). The underlying system is like that: the displacement in tissue is not uniform in focal area and the non-uniformity increases in the region between tissue and focal area (Lizzi *et al.,* 2003). Therefore, shear forces generated by the non-uniform displacements of two nearest areas in tissue might lead to changes in the structure of tissue, resulting in the enhancement of tissue permeability.

Moreover, it has been claimed that increased intercellular gaps in solid tumors can have an effect on increasing hydraulic conductivity besides the enhancement of local interstitial transport. Therefore, higher value of hydraulic conductivity can cause a decrease in (IFP) and this means the improvement in the transport of large molecules (Boucher *et al.,* 1998).

2. BIOLOGICAL BACKGROUND

2.1. Tumor Microenvironment

Tumor microenvironment is an important part of a tumor to understand its form and function. It also gives some information about structural features of a tumor. In recent years, lots of studies (Mohla and Witz, 2010, Witz and Levy-Nissenbaum, 2006, Witz, 2009) have been done about tumor microenvironment in order to explain the physical and biochemical characteristics of cancer. The microenvironment of a tumor has attracted a lot of attention since it provides the conditions that tumor cells need to proliferate, grow and metastasize (Mbeunkui and Johann, 2009).

It has been revealed that cells grown in a tumor experience more mutations compared to the ones grown in a normal cell culture. Therefore, we can conclude that tumor microenvironment can lead to some changes in the surrounding cells (Reynolds, 1996).

For anticancer drugs to be effective when administered to the body, they have to distribute homogeneously throughout the tumor vasculature, penetrate into the vessel walls and reach the tumor cells. However, drugs are distributed heterogeneously and a small number of tumor cells receive inadequate amount of therapeutic agents. The tumor microenvironment plays an important role in the heterogeneities in drug distribution, cell proliferation and hypoxic regions and all of them have an effect on the sensitivity of tumor cells to anticancer drugs.

The microenvironment of a solid tumor is a heterogeneous and complex structure which has some properties such as low pH, elevated (IFP) and insufficient oxygen and nutrient supplies. All of these features lead to the abnormal tumor vasculature and heterogeneous blood flow triggering tumor growth and tissue invasion.

Tumor microenvironment generally contains immune cells, blood vessels, fibrob-

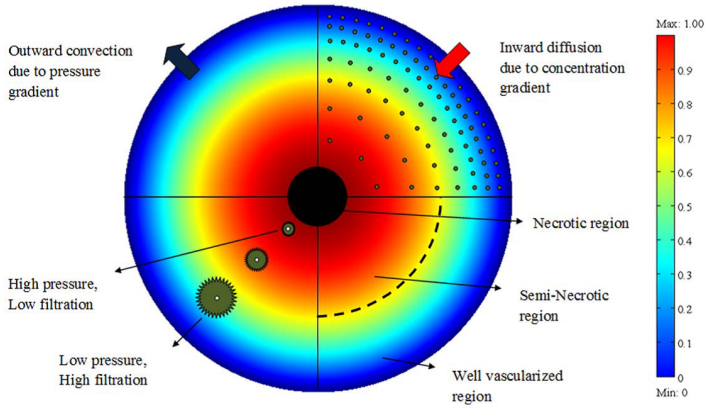

Figure 2.1. Schematic representation of different parts of a tumor. (Soltani and Chen, 2011).

lasts, cytokines, extracellular matrix and host stromal cells. They provide growth factors and help to change connective tissue and get new matrix.

The functions of immune cells are to detect and destroy the particles foreign to the body. Some antigens are expressed only by the tumor cells not by the normal cells (e.g., cytotoxic T-lymphocytes CD8). These antigens make an alert to the immune system and the immune cells start to attack these tumor cells. However, macrophages (a type of white blood cell) and some immune cells can trigger angiogenesis and metastasis by generating growth factors which stimulate tumor growth (Stix, 2007).

Fibroblasts which secrete collagen and the extracellular matrix (ECM), are the main stromal cells in loose connective tissue (epithelial stroma). They promote the formation and growth of tumors (Kim *et al.,* 2011). They can mainly provide connective tissues to keep their structural framework. The number of fibroblasts increases in the stroma of malignant cells whereas they can be rare in healthy tissues.

Host stromal cells play a major role in tumor cell proliferation, angiogenesis and metastasis. However, tumors can alter gene expression in the nearby stroma and this stimulates the growth of tumor cell population. For instance, tumor by triggering some events, causes the host stromal cells to produce matrix metalloproteinase (MMP).

MMP degrades the (ECM) and thereby induces tumor growth.

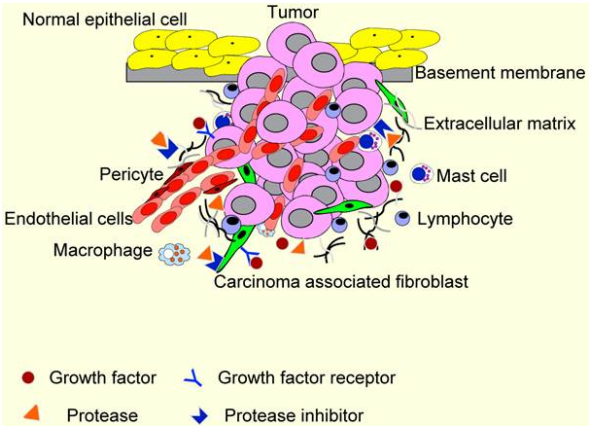

Figure 2.2. The main structure of tumor microenvironment. (Koontongkaew, 2013).

2.1.1. Extracellular Matrix (ECM)

The extracellular matrix is a complex structure composes of collagen, hyaluronan, elastin, proteoglycan and other special proteins. The main component of the ECM is collagens. They are especially formed by fibroblasts also epithelial cells can synthesize these proteins. The extracellular matrix can change from one tumor to another in terms of the composition and the amount of components it contains (Ohtani, 1998). ECM basically fills the spaces among cells and attaches the cells and tissues. It also provides structural support to the cells.

The functions of extracellular matrix change in normal tissues and tumors. In normal tissue, it helps to regulate homeostasis and macromolecule transport, maintain the functional relations among cells whereas in tumors, it behaves like a barrier to drug delivery (Liotta and Rao, 1985). ECM contains vessels and some kind of biochemicals such as growth factors. They also supply nutrients to cells and regulate cell metabolism by signaling respectively.

ECM has a dynamic system that it can be degraded or altered. In the process of ECM remodeling, cell differentiation are organized in some cases such as angiogenesis, wound healing or bone remodeling. Abnormal ECM structure can result in irregular

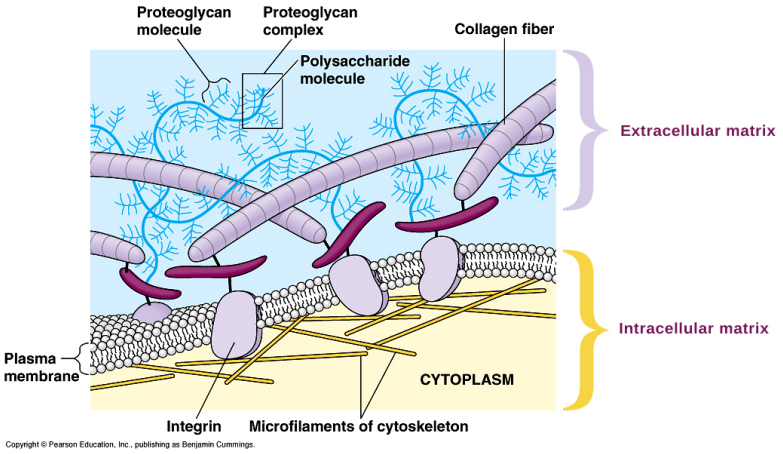

Figure 2.3. The representation of extracellular matrix (ECM).

cell proliferation, invasion or cell death.

Remodeling of ECM induces malignancy and the sensitivity of tumor cells to drug molecules. The physical properties of the extracellular matrix is changed by the (MMPs) that degrade the ECM to provide space for tumor cells to grow. They can affect cell migration by degrading the ECM, alter the cell structures by modulating the ECM microenvironment and change the protease activity.

MMPs also play an important role in invasion and metastasis. With the degradation of the ECM, cancer cells can invade host tissue so that tumor cells can pass through this barrier. Moreover, while degrading the extracellular matrix, MMPs lead to release some growth factors which facilitate the tumor growth and invasion (Weber and Kuo, 2012).

2.1.2. Hypoxia and Low pH

Hypoxia and low extracellular pH are the important consequences of abnormal tumor microenvironment since they play a critical role in tumor growth and in the efficacy of therapies such as chemotherapy, targeted therapy or radiotherapy.

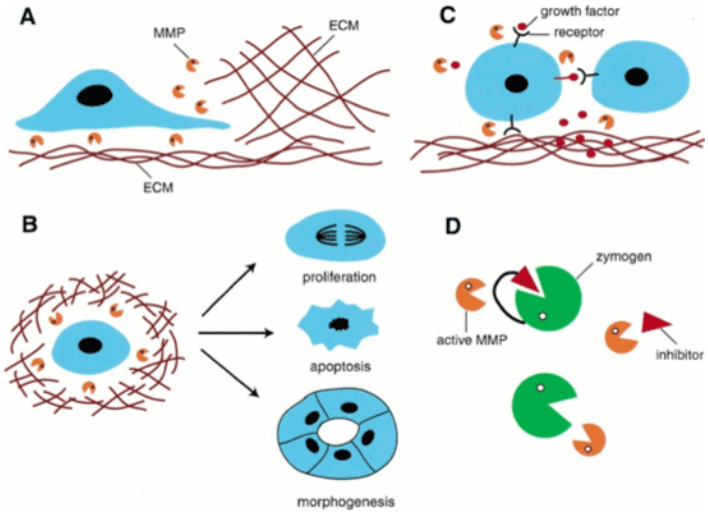

Figure 2.4. The roles of MMPs in ECM remodeling. a. MMPs change cell migration by degrading the ECM. b. MMPs cause proliferation, apoptosis and morphogenesis. c. MMPs alter the activity of growth factors. d. MMPs cause the changes in the activity of proteases. (Ansari *et al.,* 2013).

Hypoxia is a situation where tissue does not have adequate level of oxygen and it adversely affects the functions of cells. Due to the abnormal vasculature in solid tumors, oxygen cannot be delivered sufficiently to the tumor cells. Therefore, there exists an imbalance between the supply and demand of oxygen which causes the formation of large hypoxic regions. The diffusion distance of oxygen is around 100-200 μm and tumor cells far away from this distance cannot get the oxygen and nutrients they need. If cells adjacent to blood vessels are killed in treatment, there will be an increase in the nutrient supply of hypoxic cells, leading to the survival of these cells (Chaplin *et al.,* 1989).

In addition to hypoxia, another important feature of tumor microenvironment is the acidity. In tumors, hypoxic regions do not have adequate nutrients such as glucose. In order to produce ATP to supply the energy for survival and proliferation, tumor cells convert glucose into lactic acid known as glycolysis. Therefore, this causes an increase in CO_2 concentration. The accumulation of these acidic products results in the low interstitial pH (Warburg, 1956).

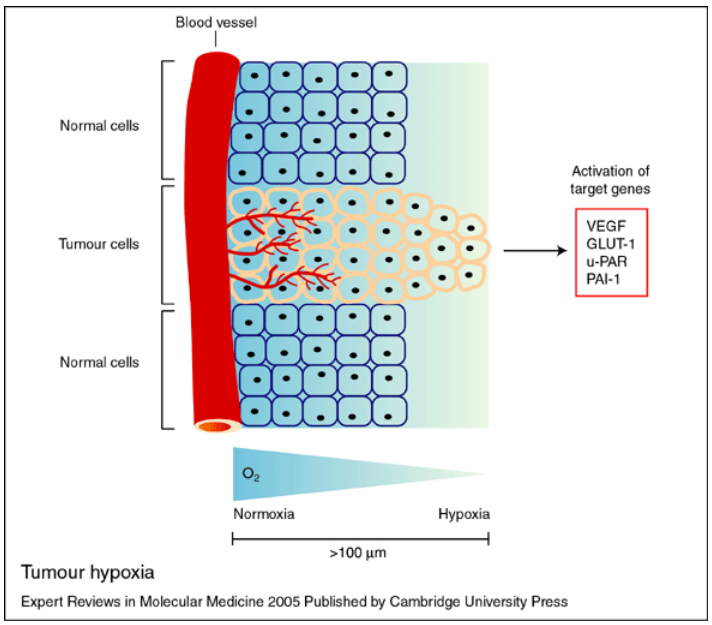

Figure 2.5. Tumor hypoxia.

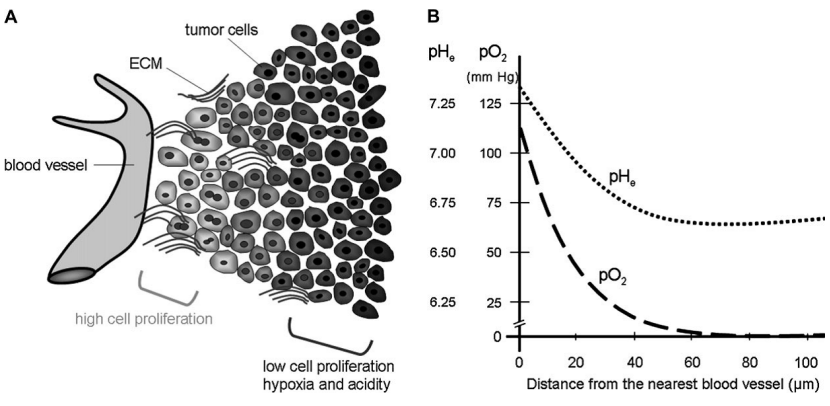

Figure 2.6. Tumor microenvironment. a. Schematic of tumor cells and the extracellular matrix (ECM). b. Gradient of oxygen concentration (pO_2) and pH in terms of distance from blood vessel. (Tredan *et al.*, 2007).

2.1.3. Interstitial Fluid

Interstitial fluid which is the main component of the extracellular matrix, surrounds the cells in tissues. It includes a water solvent containing amino acids, sugars, fatty acids, minerals such as calcium, magnesium and potassium and waste products from the cells. It provides chemicals and nutrients to be taken into the cells and removes waste products resulting from metabolism. The fluid flow between capillaries and interstitial space is controlled by the differences in hydrostatic and osmotic pressures between these two regions and by the permeabilities of each region (Shieh and Swartz, 2011).

Interstitial fluid exerts normal force and shear stress to cell surface. It can cause cells to detach from the ECM by breaking the adhesive bonds between cells and the ECM. Moreover, interstitial fluid flow significantly increases in tumor tissues resulting in the cells to experience more shear stress and create concentration gradients in the direction of flow (Qazi *et al.,* 2011).

Normally, interstitial fluid pressure is less than 10 mmHg and the transcapillary pressure is around 1-3 mmHg which is higher in the vessels to help convection of solutes from the vascular to the interstitial space. However, in tumor tissues IFP is elevated up to 100 mmHg and therefore fluid cannot pass from blood to the stroma easily. The reasons of high IFP are abnormal tumor blood vessels, lack of functional lymphatics and the characteristics of tumor stroma. Since IFP is higher in tumors compared to the normal tissues, fluid flows out from the tumor to the healthy tissue.

Some studies show that tumor induced angiogenesis is also a reason for the elevated IFP in tumor microenvironment. Irregular blood vessels which are formed by angiogenesis, are leaky and have very high permeability. Therefore, fluid and molecules can easily move into the interstitium of the tumor which leads to the highly elevated interstitial fluid pressure. Moreover, remodeled ECM is stiffer which keeps the high IFP inside the tumor.

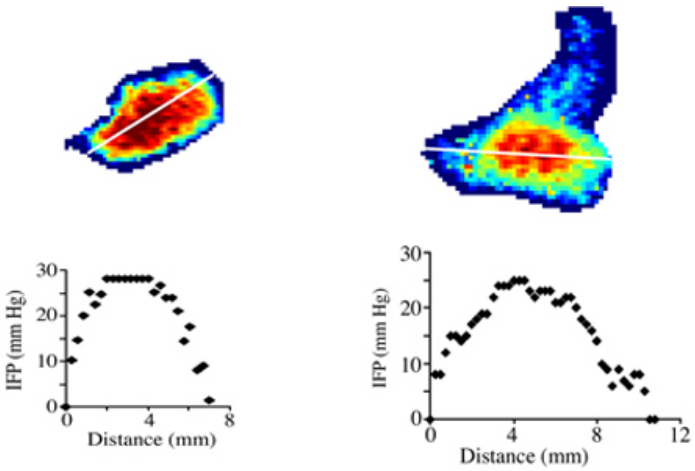

Figure 2.7. The IFP profiles of human non-small cell lung cancer tumors in mice which are calculated by using slow infusion contrast enhanced (MRI). (Hassid *et al.,* 2006).

2.1.4. Angiogenesis

Angiogenesis that is the formation of new blood vessels from pre-existing ones, is an important mechanism in tumor growth, metastasis and wound healing. Formation of new blood vessels is triggered by some anti-angiogenic factors (e.g., vascular endothelial growth factor-VEGF or TGF-β) (Carmeliet and Jain, 2000).

Tumor growth is limited to a certain size, nearly 1-2 mm when tumor cells cannot have adequate oxygen and nutrient supply. Without the formation of new blood vessels, tumor cells can become necrotic or apoptotic (Holmgren *et al.,* 1995). Vascular endothelial growth factor (VEGF) which is secreted by tumor cells, has a significant role in angiogenesis by leading to hyperpermeable blood vessels. It has high concentration around tumor blood vessels and hypoxic regions.

Angiogenesis consists of four steps: Firstly, basement membrane of the existing vessels is destructed, and then angiogenic factors activate endothelial cells. Activated endothelial cells proliferate and migrate. Finally, they lay down a basal membrane. Since tumor needs its own blood supply to grow, a new approach in cancer treatment

has been used which aims to target blood supply instead of tumor cells. By this way, tumor cells can be killed even if they are drug resistant (Gabriel, 2007).

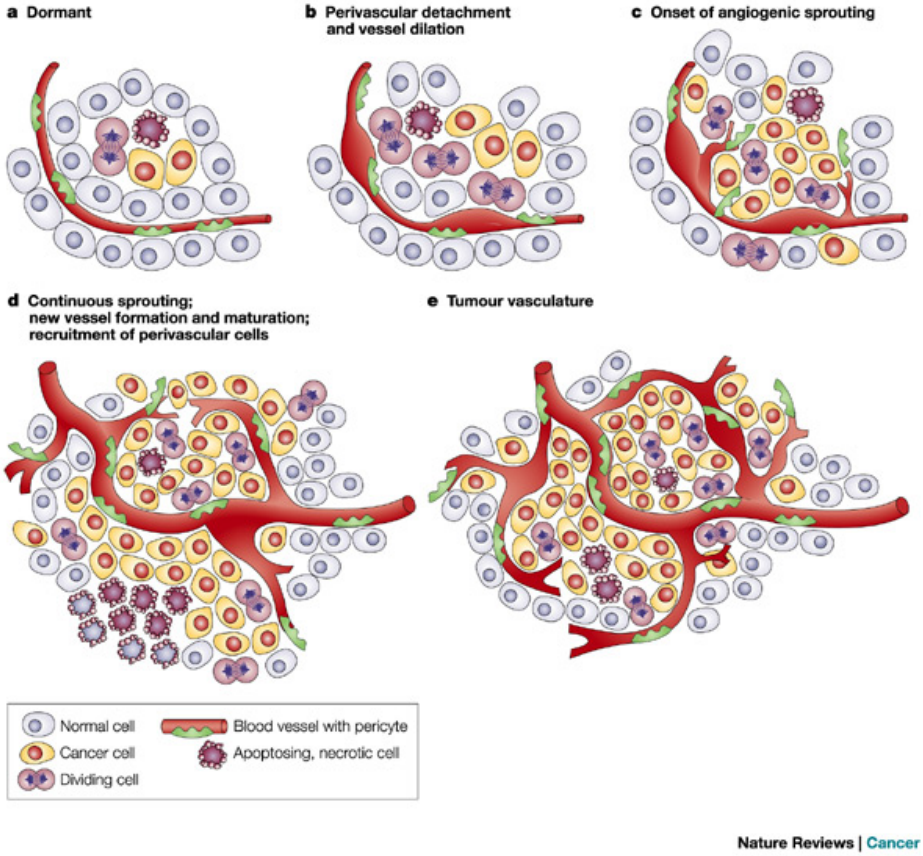

Figure 2.8. The steps of angiogenesis. (Bergers and Benjamin, 2003).

2.1.5. Tumor Vasculature

In healthy tissue, the blood vessels have a regular organized structure containing arterioles, capillaries and venules. Blood enters an organ through arteries which bifurcate arterioles, small arteries and capillaries. Capillaries which is the smallest blood vessels in vascular network, help the exchange of water, nutrient and waste chemical substances between blood and tissues. After capillaries, blood flows to the postcapillary venules, then it enters venules, small veins and large veins, respectively. Finally, large veins send blood from the organs to the systemic circulation. However, in tumor vasculature there is not such an order. It is unorganized with the formation of loops, shunts and the trifurcation of vessels.

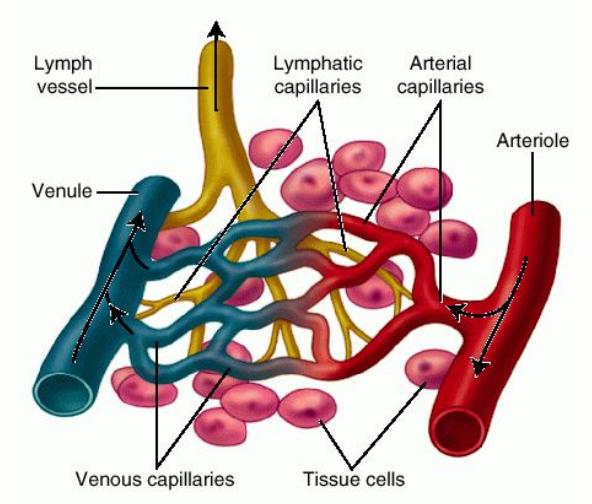

Figure 2.9. Arterioles, capillaries, and venules. (Dorland's Medical Dictionary for Health Consumers, 2007).

In terms of the rate of perfusion, tumor can be divided into four regions which are an avascular, necrotic region, seminecrotic region, microcirculation stabilized region and an advanced tumor region. In tumor vasculature, there are two different vessel populations: the pre-existing host vessels and vessels arising from the angiogenesis. The pre-existing vessels can keep tumor in avascular stage. In this stage, tumor vessels become tortuous, porous and dilated. When tumor grows, some of the pre-existing vessels collapse due to the pressure resulting from excessive proliferation. Neovascularization occurs in the capillaries. These newly-formed vessels can have a lot of structural irregularities which lead to spatially and temporally heterogeneous microcirculation (Endrich *et al.,* 1979). As a result, it can be said that the distribution of drug molecules inside the tumor is uneven and with the increase in tumor growth, the uptake of molecules decreases.

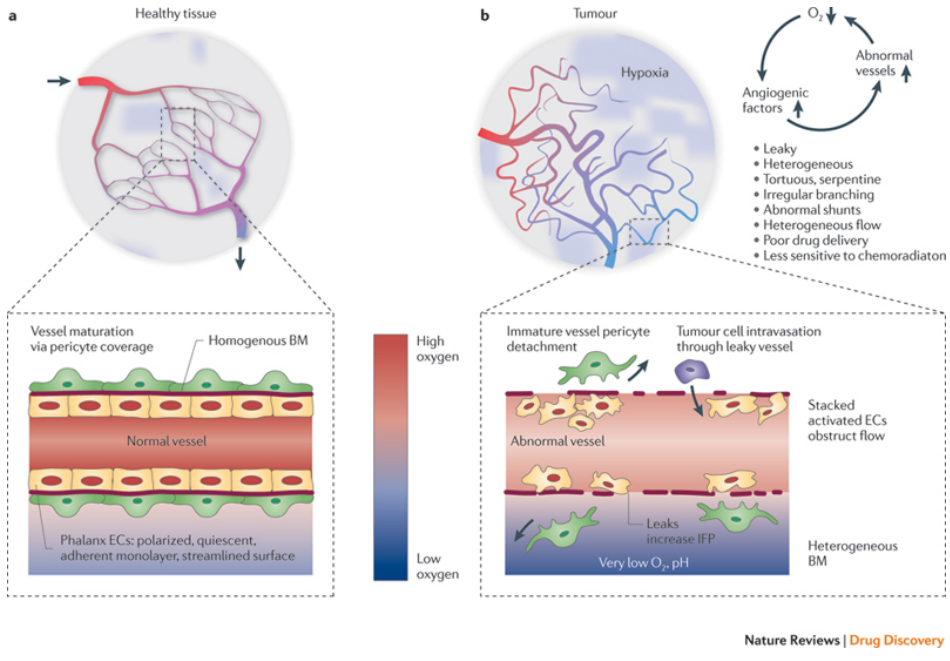

Figure 2.10. The comparison of vascular systems of a. Healthy tissue and b. Solid tumor. (Carmeliet and Jain, 2011).

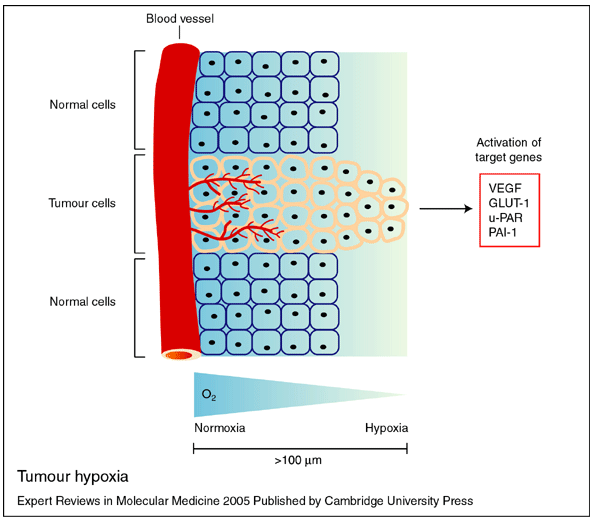

Figure 2.11. a. Distribution of doxorubicin in murine sarcoma, b. Mitoxantrone in a human breast cancer xenograft, c. Cetuximab in a human cervical cancer xenograft, d. Doxorubicin in normal mouse liver. In this figure, blue is drugs, red is vessels and green is hypoxic regions. (Tredan *et al.*, 2007).

2.2. Transport Mechanism in Solid Tumors

Transport of nanotherapeutics is an important part in cancer treatment. Delivery of drug molecules and response of the cells to treatment are changeable in solid tumors. For instance, tumor cells which are sensitive to drug exposure will be killed whereas tumor cells resistant to drug molecules will be alive after treatment.

The delivery of drug molecules to tumor cells has three main obstacles: physiological barriers from the administration side to tumor, the clearance of drug molecules and drug resistance in tumors. Therefore, transport of drug molecules in the body and tumor tissue should be understood clearly to improve cancer treatment.

2.2.1. Transvascular Transport

Transvascular transport is the delivery of therapeutic agents across the vessel walls. It depends on the hydraulic conductivity and the vascular permeability. Drug molecules extravasate from vessel wall by diffusion and convection, respectively. Diffusive flux is determined by the vascular surface area S (cm^2) and the differences between the plasma and interstitial concentrations, $C_p - C_i$ (g/m). Convective flux depends on the fluid leakage J_f (m/s), from the vessel. This term is proportional to vascular surface area S, the difference between vascular and interstitial hydrostatic pressures, $p_v - p_i$ $(mmHg)$, osmotic reflection coefficient and the vascular and interstitial pressure gradients. Vascular permeability and hydraulic conductivity depend on porosity, elasticity of vessel wall and basement membrane as well as physicochemical properties of drug molecules (Chauhan *et al.,* 2011, Jain, 2001).

In tumors, vascular permeability is heterogeneous and has larger values compared to normal tissues. In addition, there are larger pores in the vessel wall which facilitate the transport of macromolecules and therapeutic agents. The cutoff pore size is around 2 microns in diameter. However, in normal vessels, this value is less than 20 nm.

Tumor vascular permeability can be affected by cytokines such as vascular en-

dothelial growth factor (VEGF), also known as vascular permeability factor (VPF). VEGF is generally overexpressed in tumors by hypoxia and growth factors (Dvorak *et al.,* 1995, Brown *et al.,* 1997). Some studies have shown that eliminating VEGF in tumors leads to a decrease in tumor vascular permeability and there is a relation between the VEGF expression and tumor vascular permeability (Yuan *et al.,* 1996). Thus, it is considered that the overexpression of VEGF is one of the main reasons for hyperpermeability in solid tumors (Yuan *et al.,* 1996, Jain, 2001, Dvorak *et al.,* 1995).

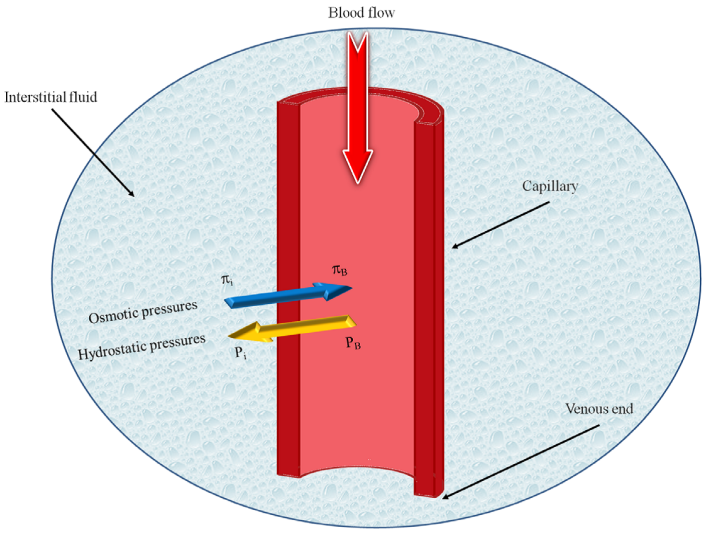

Figure 2.12. Capillary microcirculation. (Soltani and Chen, 2011).

2.2.2. Interstitial Transport

Interstitial transport is the distribution of drug molecules through the interstitial space toward the targeted cells by diffusion and convection. Interstitial space is filled with interstitial fluid and extracellular matrix. As mentioned previously, the matrix consists of elastins, collagens, proteoglycans and some structural glycoproteins (Robert, 2002). Diffusive transport is proportional to the interstitial concentration gradient with diffusion coefficient D (cm^2/s) which relates the diffusive flux to the concentration gradient. Similarly, convective transport depends on the interstitial fluid velocity v_i (cm/s) and the concentration gradient in the interstitium. Additionally, v_i is proportional to the interstitial hydraulic conductivity K ($cm^2/mmHg.s$) and hydrostatic pressure gradient ∇p_i.K is determined by the concentration of interstitium and

properties of drug molecules such as size or configuration (Chauhan *et al.,* 2011, Jain, 2001).

The main transport mechanisms in the interstitium are diffusion and convection. Diffusion between two near blood vessels is dominant for small molecules due to the fact that diffusion coefficient depends on the size of molecules. Yet, convection is important for the interstitial transport of large molecules. Due to the high levels of IFP in the center of tumors and low levels in the periphery and surrounding tissue, it is reasonable to say that interstitial fluid flows from the tumor's center to the periphery.

According to a laboratory experiment, nearly 6-14% plasma which crosses into the tumor has left from the periphery of tumor (Jain, 1987, Jain, 1989) and therefore the leakage of this fluid causes radially outward interstitial fluid velocity around 0.1-0.2 μm/s at the tumor boundary (Jain, 1989). Because of the sharp decrease in the interstitial fluid pressure at the tumor periphery, convective transport oozes drug molecules from the inner to the outside of tumor, thus preventing, rather increasing, the transport of therapeutic agents to tumor cells.

To sum up, we can say that interstitial transport is one of the problematic steps in systemic and local drug delivery (Jain, 1999) due to the molecular and cellular characteristics of solid tumors: i) the high IFP and the lack of functional lymphatics, ii) low levels of convective transport, iii) radially outward IFP which leads to the convection of drug molecules from the inner part to the boundary of tumors, iv) the diffusion distances among blood vessels and some proteins expressed by the tumor such as fibroblasts or macrophages.

2.2.3. Vascular Transport

Vascular transport can be described as the delivery of drug molecules through blood into the tumor tissue. It consists of the movement of the drug molecules into a tumor and the distribution of these molecules inside the tumor by means of tumor vasculature. Tumor blood flow is unevenly distributed, it changes with time and also

the flow of direction is reversed, therefore poor perfusion is commonly seen.

Vascular transport is influenced by the perfusion rate of blood to the parts of tumor. The blood perfusion rate is defined as q=Q/V, here Q is volumetric flow rate and V is the tissue volume. The volumetric flow rate Q is represented by this formula Q=Δp/R, where R is resistance and Δp is the pressure drop. The amount of the transported drug molecules to tumor tissue is determined by the drug flux J_v in tumor tissue by blood vessels and the heterogeneous distribution in tumor vasculature (Chauhan *et al.,* 2011). The parameters Q and J can be calculated by using some techniques such as multiphoton microscopy (Kamoun *et al.,* 2010, Brown *et al.,* 2001) and optical frequency domain imaging (Vakoc *et al.,* 2009).

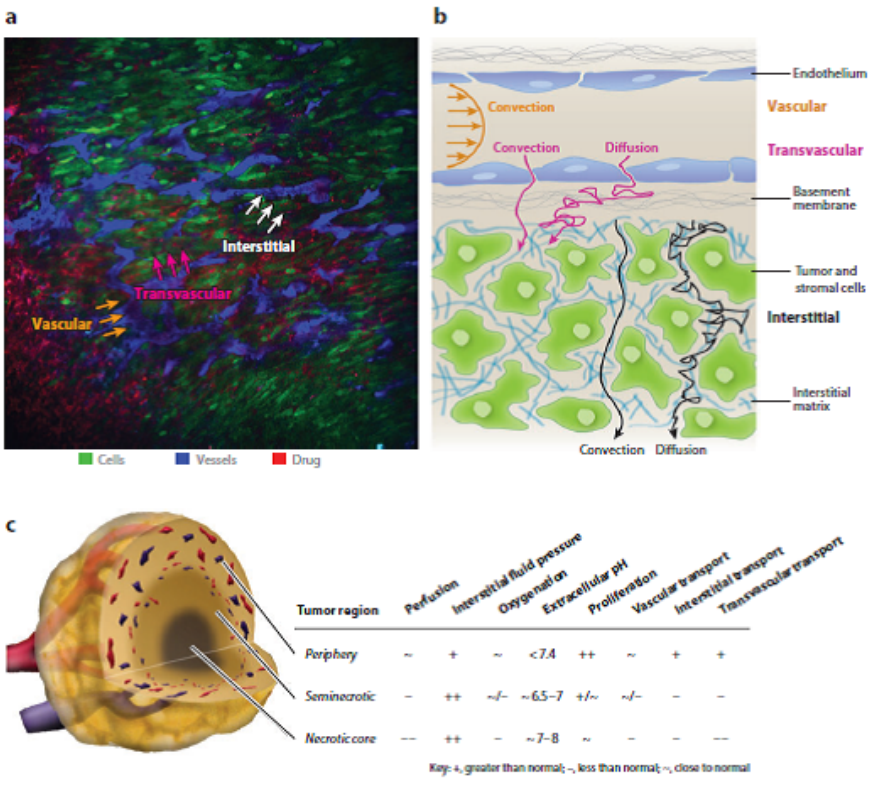

Figure 2.13. A representation of transport in tumors. a. Drug transport of an orthotopic mammary tumor in a mouse. b. Transport processes of a tumor. c. The properties of a tumor's regions. (Chauhan *et al.,* 2011).

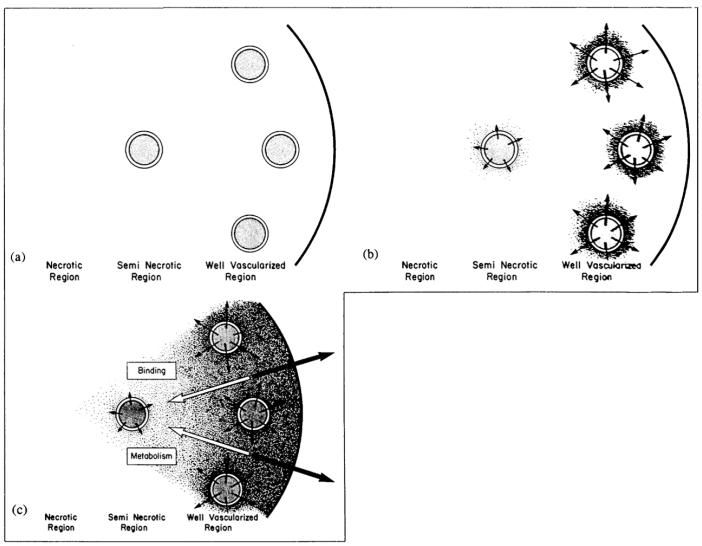

Figure 2.14. Physiological barriers for the transport of drug molecules (Jain, 1994).

3. THE APPLICATIONS OF ULTRASOUND IN DRUG DELIVERY

One of the primary goals of the drug delivery systems is to reach the sufficient amount of drug to the desired site without affecting the healthy tissues. It has been shown that local drug delivery is an effective way to achieve this goal. Ultrasound can be applied in many different techniques to enable local drug delivery. It provides to control the drug release location, improves the release of therapeutic agents, enhances transvascular transport and the transport from extracellular matrix. It also facilitates the uptake of drug molecules by increasing the cell membrane permeability due to mechanical or thermal effects (Deckers *et al.,* 2008). To understand the ultrasound mediated drug delivery mechanism, it is important to examine the physics of ultrasound and the drug delivery vehicles which are used together with ultrasound.

3.1. The physics of ultrasound

Ultrasound is the transmission of pressure waves similar to normal sound but at frequencies upper than human hearing or above 20 kHz. These pressure waves are reflected, refracted, focused and absorbed. Ultrasound can induce physical changes in the structure of the biomolecules or cells. As the medium gets expanded at low pressure or compressed at high pressure, ultrasound can lead to the movement of molecules.

For local drug delivery, it is important to apply the trigger locally and noninvasively to the region where the drug is needed. The radiation beam should reach the deep inside the human body and interact with the target tissue. According to figure 3.1, it can be said that only X-ray, radiofrequency and acoustic radiation can be absorbed and penetrate deep inside the body. Since it is a type of ionizing radiation which is harmful to living tissues, X-ray is not used in drug delivery systems. Radiofrequency waves are not focused into very small regions due to having the wavelength that is on the order of 1 meter or above. However, ultrasound having a wavelength on the order

of a millimeter, can be applied locally to a small region of interest without harming the healthy tissues (Deckers *et al.,* 2008). Thus, ultrasound is the only source of radiation which allows noninvasive local drug delivery. The physical mechanisms of ultrasound mainly consist of three parts: heat generation, cavitation and acoustic radiation force.

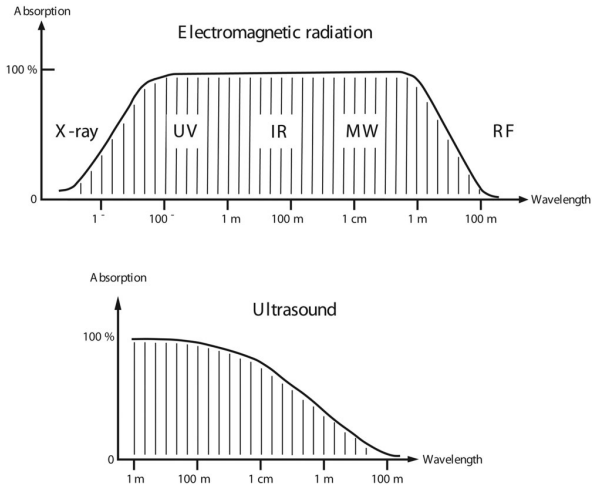

Figure 3.1. Attenuation of radiation by human tissue. (Ernst *et al.,* 1987).

3.1.1. Heat Generation

The intensity of an ultrasonic beam can be described as the power transferred per cross section area and it may be attenuated due to absorption. When an ultrasonic beam is focused into a small region, the power per area becomes so large that a considerable amount of thermal energy is absorbed by the target tissue leading to the heating. The amount of heat mainly depends on the intensity of the ultrasound, the exposure time and the absorption characteristics of the tissue.

It has been revealed that around 2-15 MHz which is the frequency range used in medical applications of ultrasound, the attenuation coefficient of the tissues becomes directly proportional to the frequency (Hueter, 1948). For a plane wave in tissue, at a point x the intensity I_x with respect to the initial one can be written as

$$I_x = I_0.10^{-\frac{\alpha x}{10}}. \tag{3.1}$$

In this equation, α is the attenuation coefficient of amplitude of the wave and generally around 50-350 dB $m^{-1}MHz^{-1}$ in soft tissues (Deckers *et al.,* 2008). For instance, the use of 2 MHz ultrasound will result in 50% decrease in the intensity at 50 mm penetration for muscle. The rate of absorption increases with the higher frequencies. At low intensities, thermal effects can result in the changes in the drug carriers with high intensities.

Furthermore, hyperthermia has been used in drug delivery systems with the heating of the drugs, drug carrying vehicles and the tissues which drug molecules are expected to reach. When the temperature of tissue increases, the blood flow in tumors will also start to increase (Karino *et al.,* 1988).

Moreover, some studies have revealed that the rise in temperature can result in larger vessel pore size which leads to the increase in the extravasation of drug molecules/carriers from tumor vasculature (Gaber *et al.,* 1996, Kong *et al.,* 2001). A study by (Kong *et al.,* 2000) has revealed that hyperthermia does not affect the extravasation of liposomes from the normal tissues. As a result, the increased liposomal extravasation due to the thermal effects can be a useful mechanism in drug delivery systems.

3.1.2. Cavitation

Cavitation can be described as the formation, oscillation and collapse of the gas filled bubbles in a medium which is subjected to rapid changes of pressure. These gas bubbles can pre-exist in the fluid or they can be formed when the pressure is relatively small. Acoustic cavitation can result in shear stresses, an increase in the fluid velocity and temperature so its biological effects especially in the field of drug delivery make it

an important ultrasound mechanism.

Basically, there are two types of cavitation: non-inertial and inertial cavitation. Non-inertial cavitation is the stable oscillations of bubbles over a large number of cycles. The size of the oscillating bubble can reach an equilibrium value. The oscillation around the surface of the bubble can result in the local fluid convection which is called micro-streaming and it leads to the shear stresses in the fluid (Schroeder *et al.,* 2009).

Inertial cavitation is the rapid growth and collapse of the bubbles in a half-cycle. The bubble can expand 2-3 times higher than its resonant size. This cavitation can be harmful for the tissues since the bubble collapse can induce high shear stresses and produce shock waves. If the collapse of the bubble occurs near a solid boundary (e.g. cell membrane), it will be an asymmetrical collapse that leads to a high speed micro jet fluid where the jet velocity is around 100 m/s.

This kind of cavitation can also be used to change the permeability of cell membrane for the improved drug and gene delivery which is called sonoporation. In this process, sound energy is used to enhance the membrane permeability by creating large pores. Fechheimer et al. used this technique for the first time by using the ultrasound for the cells of slime mold. The fluorescein-labeled dextrans were used since they were not permeable to the cells. After the ultrasound exposure, it was observed that nearly 40% of the dextrans were taken by the cells and after this study, the mechanism was also used for the delivery of DNA to mammalian cells (Frenkel, 2008).

High pressure (p on the order of MPa) and low frequency (f on the order of MHz) are the main determinants for the intensity of inertial cavitation. The relation between these two factors can be called the mechanical index (MI) described as :

$$MI = \frac{p}{\sqrt{f}}. \tag{3.2}$$

Mechanical index gives information about the risks of nonthermal effects especially cavitation. It is mostly applied in the use of ultrasound contrast agents. Inertial cavitation can cause the irreversible damage in the cells and an increase in the vessel wall permeability. Therefore, it is assumed that the main mechanism for changing the cell structure is the inertial cavitation although it has been asserted that the tissue damage can stem from the non-inertial cavitation (Frenkel, 2008).

Collapse cavitation might be detrimental for the tissues so some studies have been done in order to determine the factors which are necessary for the cavitation process. Generally, physical properties and the size of the bubbles affect the cavitation. An in vitro study has been done in order to investigate the effect of physical parameters (pulse duration, pulse repetition frequency and pulse length) on ultrasound mediated gene delivery. The results have shown that the efficient gene delivery is dependent on the acoustic pressure which is around 0.1-0.5 MPa. Moreover, the sufficient space for the bubble formation and the bubble growth is also required in cavitation. The vasculature has this necessary space for the formation of cavitation when a high pressure field exists.

3.1.3. Acoustic Radiation Force

Acoustic radiation force is other widely used mechanism for the enhanced drug delivery in addition to heat generation and cavitation. It is also one of the main topics of this thesis. There are some studies about that the acoustic radiation force can enable the targeted drug and gene delivery by using HIFU (Dittmar *et al.,* 2005, Yuh *et al.,* 2005). The some of applications of acoustic radiation force in biological systems are the targeted gene and drug delivery, investigating the viscoelastic properties of tissues and fluids, examining lesions during ultrasound therapy.

Momentum transfer can occur from the propagating wave to the medium due to the effect of ultrasound exposure with high amplitudes and this process produces acoustic radiation forces. There are two types of radiation force : The primary radiation force created by the incident pressure field, is in the direction of the propagating

wave and the secondary radiation force which can be an attractive or a repulsive force between bubbles, is produced by the scattered pressure field. The primary radiation force can be exploited to manipulate delivery vehicles near the vessel wall which have a smaller velocity compared to the ones in the center of the vessel (Dayton *et al.,* 1999). Moreover, the secondary radiation force is an attractive force between the particles that leads to the large microbubble concentration near the vessel wall (Dayton *et al.,* 1997) and this can be useful for the targeted drug delivery.

Radiation force can induce the translation of vehicles resulting in the shear stresses between displaced and nondisplaced regions. The resulting strain may bring about the gaps among the endothelial cells which enhance the extravasation of drug molecules from the vessels. Also, the strain can increase the intracellular spaces in epithelial tissue that improves the diffusion of drug molecules inside the cell (Frenkel *et al.,* 2000).

The amount of the applied energy and the absorption coefficient of the medium have a positive effect on the radiation force while the velocity of sound wave in the medium affects it negatively. For the large radiation force, the displacements of the tissue near to the focal zone of the ultrasound beam can occur and this displacement can be regulated by the tissue elasticity.

Radiation forces can also produce steady flows in a fluid medium which is called acoustic streaming. The velocity of this streaming can depend on the attenuation coefficient of the fluid medium, the intensity of the ultrasound wave and the speed of sound in the medium (Frenkel, 2008). Acoustic streaming effects can be used together with cavitation or heat generation to improve the delivery of therapeutic agents.

There are lots of biological applications of acoustic radiation forces especially related to the enhancement in drug delivery. It has been shown that radiation force can manipulate the particles in the vasculature by changing their velocities and positions (Dayton *et al.,* 2002). Studies carried out by using murine have revealed that these forces can increase the extravasation of nanoparticles and interstitial transport (O'Neill

et al., 2006; O'Neill *et al.,* 2007). Moreover, it has been observed that in tumors, there is an increase in the extravasation of nanoparticles, monoclonal antibodies and fluorescent dextrans (Frenkel *et al.,* 2006, Yuh *et al.,* 2005).

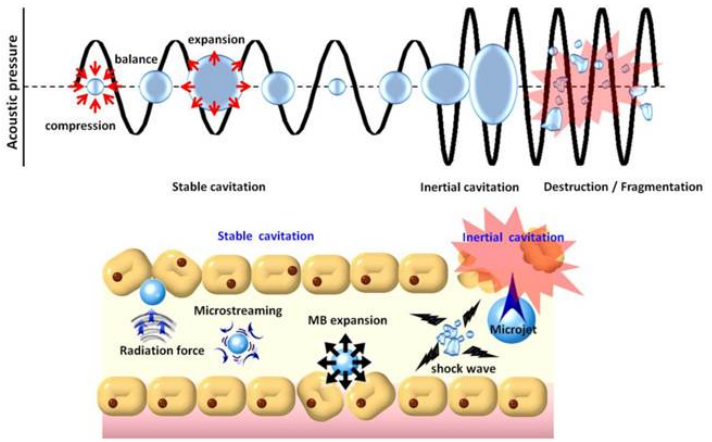

Figure 3.2. Microbubble destruction under the acoustic radiation force. (Liu *et al.,* 2014).

3.2. Ultrasound-Activated Drug Delivery Vehicles

Some kinds of vehicles can be used for thermal or mechanical applications of ultrasound in drug delivery systems. The mostly-used ones activated by ultrasound are micelles, liposomes and microbubbles (See Figure 3.2). These vehicles have their own characteristics with respect to the size and the chemical structure. Therefore, the interaction of each of them with ultrasound can differ in relation to their distributions and clearance rates in the targeted regions. It has been observed that these vehicles can increase the localized drug delivery to solid tumors up to 5-10 fold (Rapoport, 2007).

3.2.1. Micelles

Micelles are formed by amphiphilic co-polymers that consist of both hydrophilic and hydrophobic regions. They are used as drug carriers and play an important role in targeting. Since they have the proper sizes (Diameters are in the range of 10-100

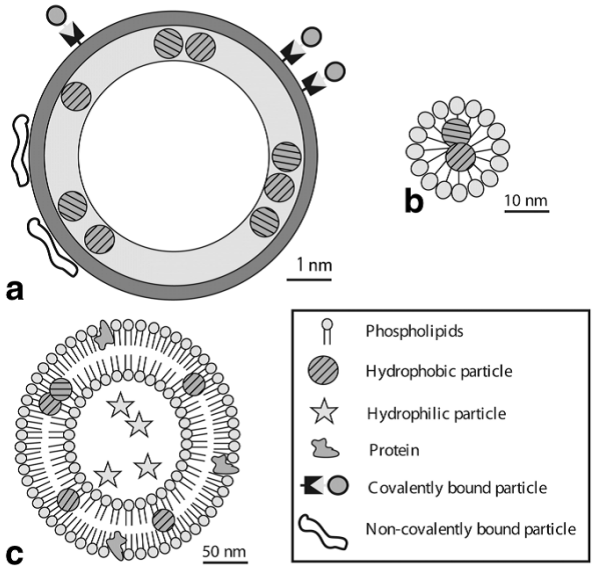

Figure 3.3. The main delivery vehicles activated by ultrasound. In this figure, a. microbubble, b. micelles and c. represent liposomes. (Deckers *et al.,* 2008).

nm.) for the EPR effect, a passive targeted mechanism in solid tumors, micelles have an ability to target solid tumors.

Moreover, micelles can stay longer in bloodstream since they are not captured by the cells in the reticuloendothelial system (Deckers *et al.,* 2008). They also have two phases that each of them is essential for drug delivery systems. The inner core determines the pharmacological activities in drug release while the outer shell is responsible for the pharmacokinetic activities and the distribution of drug molecules.

3.2.2. Liposomes

Liposomes are generally composed of phospholipids with lipid chains. They are biocompatible and non-toxic and also have the ability to deliver hydrophilic and hydrophobic drugs in their aqueous and lipophilic environment respectively. They can be used as drug carriers for cancer and other diseases by crossing the cell membrane and transporting drug molecules to the targeted regions.

Liposomes are spherical vesicles, ranging from 15-1000 nm in diameter. Although micelles and liposomes are spherical and lipid based structures, micelles consist of fatty acids but liposomes are formed by phospholipids. Moreover, a micelle has a single lipid layer whereas a liposome has bilayer of lipids allowing the charged particles inside.

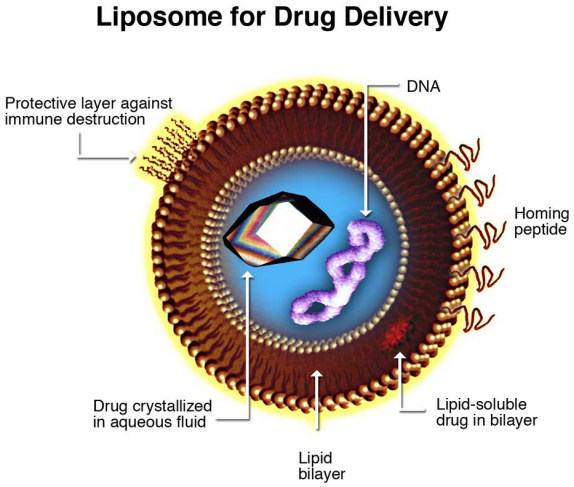

Figure 3.4. The liposome in ultrasound-mediated drug delivery. (Torchilin, 2012).

In ultrasound-mediated drug delivery systems, mechanical forces or thermal energy are used for the activation of liposomes. Since they are lack of a gas-filled nucleus, microbubbles should be attached to them in order to exploit the cavitation effects for the enhanced drug delivery. In recent studies, acoustically active liposomes have been started to use for drug encapsulation and ultrasound-triggered drug delivery (Huang and MacDonald, 2004, Tartis *et al.,* 2006). Due to their acoustic activity, they can release the drugs by the use of ultrasound (Deckers *et al.,* 2008).

3.2.3. Microbubbles

Microbubbles ranging from 1-10 μm in diameter, are widely used contrast agents for the ultrasound imaging and drug carriers for targeted drug delivery. Microbubbles are mainly filled with gas or perfluorocarbon and stabilized by a lipid shell. The main structure of a microbubble is shown in figure 3.6. The gas core plays an important role in ultrasonic drug delivery and the shell can consist of proteins, lipids or polymers.

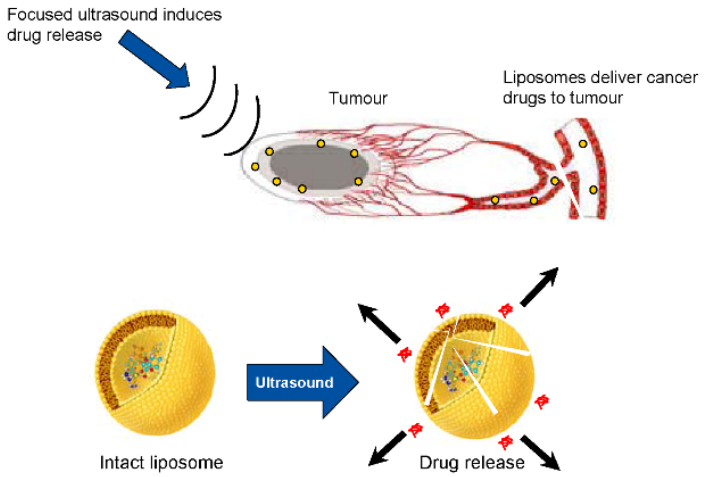

Figure 3.5. The use of liposome in ultrasonic drug delivery. (From the website: www.ntnu.edu/physics/biophysmedtech/drugdel).

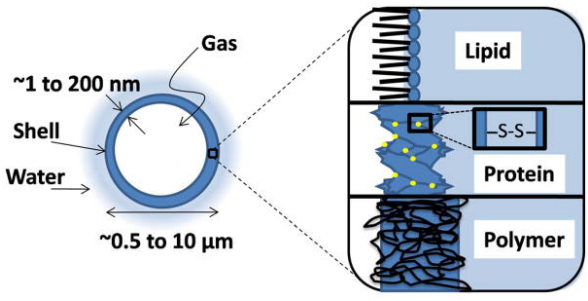

Figure 3.6. The structure of a microbubble. (Sirsi and Borden, 2009).

There are some methods for the use of microbubbles in drug delivery. Drug molecules can be attached to the cell membrane which surrounds the microbubble (Bekeredjian *et al.,* 2005), Mukherjee *et al.,* 2000), they can be combined inside the membrane (Unger *et al.,* 1998) or they can be loaded into the microbubble (Unger *et al.,* 1998). In addition to drug delivery, microbubbles are widely used in gene delivery. In this application, DNA and microbubble are combined and the resulting vehicle is administered within a tumor or vessels.

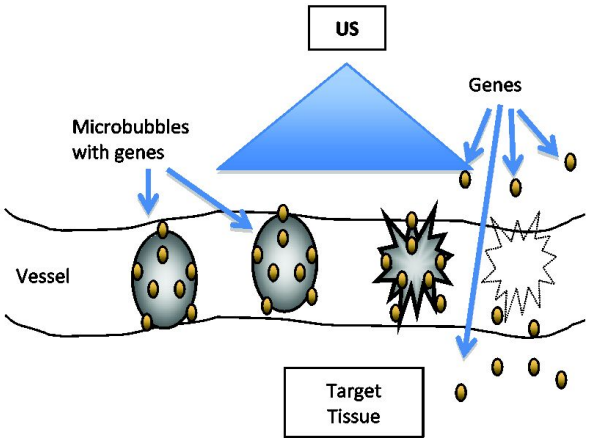

Figure 3.7. The ultrasound-mediated gene delivery. (Matsumoto *et al.,* 2011).

In ultrasonic drug delivery, microbubbles are used in two different ways. In the first approach, by using ultrasound, the microbubbles are destructed that causes the cavitation thereby an increase in the vessel wall permeability and the extravasation of the released drug molecules. This method can be effective in microvasculature. However for the large vessels, a considerable amount of drug can reach the center of the vessel but not to be delivered to the extravascular spaces. For this reason, it is useful to locate the delivery vehicles near to the vessel wall then ultrasound pulses should be sent for the microbubble destruction.

In the second way, microbubbles can be loaded with drug molecules and then ultrasound pulses are applied causing the microbubble destruction. This results in an increase in the vessel pores causing the release and the extravasation of molecules (Deckers *et al.,* 2008).

The use of microbubbles in drug and gene delivery has some advantages such that microbubbles as carriers can improve the drug/gene transport across vessel walls and using with ultrasound, microbubbles can enable the targeted drug and gene delivery to solid tumors (Sirsi and Borden, 2009).

4. MODELING OF ACOUSTIC RADIATION FORCE IN DRUG DELIVERY

This chapter consists of two parts. In the first part, interstitial fluid flow and the transport of drug molecules in the tissue following an intravenous bolus injection are investigated. In the second part, acoustic radiation force is applied after the distribution of drug particles within the tumor.

We try to construct a mathematical model to investigate the effect of acoustic radiation force in drug delivery. In order to do this, firstly the transport of interstitial fluid are examined by using mass balance equations and some important laws in transport phenomena which are *Starling's Law* and *Darcy's Law* describing the fluid flow in porous media. Then, the convection-diffusion-absorption equation is introduced to describe the delivery of drug molecules. After the transport of drug molecules, the acoustic radiation force term will be added to the convection-diffusion-absorption equation to investigate its impact to the delivery system.

4.1. Transport of interstitial fluid and drug molecules

We consider that blood and lymph vessels are the sources of sinks of fluid which are distributed uniformly. The mass balance equation for interstitial fluid that has a velocity ($\mathbf{v}_F$) is:

$$\boldsymbol{\nabla} \cdot (\phi_F \mathbf{v}_F) = \Gamma_{Fb} - \Gamma_{Fl}, \tag{4.1}$$

where ϕ_F is the volume fraction of interstitial fluid, Γ_{Fb} (1/s) is the supply of fluid from blood vessels to the interstitial space and Γ_{Fl} (1/s) is the drainage of fluid from interstitial space to the lymph vessels.

In order to describe transvascular fluid flow, Starling's Law is used:

$$\Gamma_{Fb} = \lambda_{Fb} \left[P_0 - P - \sigma(\pi_c - \pi_i) \right], \tag{4.2}$$

and

$$\Gamma_{Fl} = \lambda_{Fl} P, \tag{4.3}$$

where λ_{Fb} and λ_{Fl} are hydraulic conductivities of blood and lymph vessel networks (1/mmHg·s), respectively. P_0 is vascular fluid pressure (mmHg), P is the interstitial fluid pressure (IFP, mmHg), σ is the osmotic reflection coefficient, π_c and π_i are the capillary and interstitial oncotic pressures (mmHg), respectively. The hydraulic conductivities of blood and lymph vessel networks depend on the vessel wall hydraulic conductivities (L_p) and vessel surface densities ($\frac{S}{V}$) by the following expression, $\lambda_{Fb,Fl} = L_p \frac{S}{V}$. Since lymph vessels have high permeability (i.e., $\sigma \approx 0$), osmotic pressure contribution is neglected in equation (4.3) and it is also assumed that lymph vessel pressure is 0 mmHg (Goh *et al.,* 2001).

On the other hand, interstitial fluid flow in the tissue is given by Darcy's Law:

$$\mathbf{q}_F = -K \boldsymbol{\nabla} P, \tag{4.4}$$

where ($\mathbf{q}_F$) is the interstitial fluid flux (IFF, mm/s) and K is the hydraulic conductivity of the tissue (cm^2/s·mmHg).

IFF is related to the velocity of the interstitial fluid ($\mathbf{v}_F$) by

$$\mathbf{q}_F = \phi_F \mathbf{v}_F. \tag{4.5}$$

Combination of equations (4.2), (4.3) and (4.4) with equation (4.1), gives us:

$$\boldsymbol{\nabla} \cdot (-K \boldsymbol{\nabla} P) = \lambda_{Fb} \left[P_0 - P - \sigma(\pi_c - \pi_i) \right] - \lambda_{Fl} P. \tag{4.6}$$

Drug molecules reach tumor tissue through blood and extravasate from blood vessels by diffusion and convection within interstitial fluid. In order to reach tumor cells, drug molecules travel within interstitial space and they are removed from there by biological decay or being drained to lymph vessels with the interstitial fluid.

Transport of drug molecules is satisfied by using the following convection-diffusion equation:

$$\frac{\partial c}{\partial t} + \boldsymbol{\nabla} \cdot (-D \boldsymbol{\nabla} c) + \boldsymbol{\nabla} \cdot (\mathbf{q}_F c k_E) = \Gamma_{Fb}(1 - \sigma) c_p + \lambda_d \left(c_p - c \right) - \Gamma_{Fl} c - \lambda_r c \,. \tag{4.7}$$

In this equation, c and c_p are the tissue and plasma drug concentrations ($1/\mathrm{mm}^3$), respectively. Also, D is the diffusion coefficient in the tissue (cm^2/s), k_E is the retardation coefficient for interstitial convection, σ is the solvent drag reflection coefficient for transvascular convection, λ_d is the transvascular diffusion coefficient ($1/\mathrm{s}$), and λ_r is the decay rate of drug molecules ($1/\mathrm{s}$). The retardation coefficient for interstitial convection (k_E) comes from the physical obstructions which are present in the tissue structure against flow. (k_E) is equal to solute convective velocity/solvent convective velocity (Jain, 1987).

The right hand side of equation (4.7) can be described as the extravasation of drug molecules by convection, by diffusion, clearance by the lymphatic vessels and biological decay of the drug molecules, respectively.

It is assumed that the capillary network is distributed continuously and inter-capillary drug concentration is not influenced by the extravasation of drug molecules. Plasma drug concentration c_p decays exponentially such that $c_p(t) = c_0 e^{-kt}$ where k

is a nonnegative number and c_0 is the peak plasma concentration of the drug after injection, respectively. If we take natural logarithm of both sides of this equation, we get $\log(c_p(t)) = \log(c_0) - kt$. The half life of drug concentration $t_{1/2}$ can be calculated as:

$$t_{1/2} = \frac{\log(c_p) - \log(\frac{c_p}{2})}{k} = \frac{\log 2}{k}. \tag{4.8}$$

Therefore, the plasma drug concentration after injection can be written as:

$$c_p(t) = c_0 e^{-\ln(2)t/t_{1/2}} \qquad t \geq 0\,. \tag{4.9}$$

For tumor domain and boundary conditions, we consider a spherically symmetric tumor embedded in normal tissue. Problem parameters are given by assuming uniform and distinct values within the tumor domain (Ω_T) and normal tissue domain (Ω_N). At the boundary between the tumor and normal tissue, we assume the mass continuity relations for the interstitial fluid:

$$\begin{aligned}
[P]_{r=R_0} &= 0\,, & (4.10)\\
\left[-K\frac{\partial P}{\partial r}\right]_{r=R_0} &= 0\,, & (4.11)\\
\left[-D\frac{\partial c}{\partial r} - K\frac{\partial P}{\partial r}(1-\sigma)\,c\right]_{r=R_0} &= 0, & (4.12)
\end{aligned}$$

where R_0 is the tumor radius and $[\cdot]$ denotes jump at the boundary. At the tumor center and in the far field, symmetric boundary conditions are applied:

$$\begin{aligned}
\left.\frac{\partial P}{\partial r}\right|_{r=0,R^\infty} &= 0, & (4.13)\\
\left.\frac{\partial c}{\partial r}\right|_{r=0,R^\infty} &= 0. & (4.14)
\end{aligned}$$

4.2. The application of acoustic radiation force

Following to the previous part in which the drug molecules are transported and distributed within the tumor tissue, ultrasound is applied in order to investigate the effect of the resulting radiation force on the distribution of drug molecules inside the tumor.

In most advanced models, Helmholtz wave equation has been solved to determine the pressure amplitude of the sound wave and potential flow for the velocity amplitude of the acoustic oscillation (Laurell, 2007; Neild, 2007). When the pressure and velocity amplitudes of the acoustic wave have been found, the acoustic radiation force acting on the drug particles can be determined by solving the Gor'kov's equation since it explains the acoustic force potential exerted on the particles by the acoustic field.

The acoustic radiation force can be obtained from the acoustic radiation potential by using the following equation:

$$F_{Rad} = -\nabla U_{Rad}. \tag{4.15}$$

In this equation, the acoustic radiation potential has been calculated by Gor'kov as (Gor'kov, 1962):

$$U_{Rad} = \frac{4\pi}{3} r^3 [f_1 \frac{1}{2\rho_f c_f^2} \langle p^2 \rangle - f_2 \frac{3}{4} \rho_f \langle v^2 \rangle], \tag{4.16}$$

$$f_1 = 1 - \frac{\kappa_p}{\kappa_f}, \tag{4.17}$$

$$f_2 = \frac{2(\rho_p - \rho_f)}{2\rho_p + \rho_f}, \tag{4.18}$$

where f_1 and f_2 are the monopole and dipole contributions. The terms $\langle p^2 \rangle$ and $\langle v^2 \rangle$

in the equation (4.16) represent the mean square pressure amplitude fluctuation of the sound field and of the particle velocity, respectively. κ_p and κ_f are the compressibility of particle and fluid, respectively. Moreover, ρ_p is the particle density and ρ_f is the fluid density.

Helmholtz wave equation can be used for the calculation of the pressure amplitude and the potential flow for the velocity amplitude:

$$\nabla^2 P - \frac{w^2}{c^2} P = 0, \tag{4.19}$$

$$v = -\frac{i}{w\rho} \nabla P. \tag{4.20}$$

In equations (4.19) and (4.20), the terms c and ρ are the speed of sound and the density of the fluid, respectively. Also, w represents the angular frequency and P represents the acoustic pressure amplitude. Solving the Helmholtz equation allows us to determine $\langle p^2 \rangle$ and $\langle v^2 \rangle$, which are needed to calculate the primary acoustic radiation force by using the equations (4.16), (4.17) and (4.18).

After the modeling of the acoustic field and the calculation of the Gor'kov's potential, the convection-diffusion-absorption equation is modified by being added the force term to understand its effect on the concentration of drug particles. In this model, we assume that the size of the drug molecules (which are chosen liposomes) is on the order of the micrometer. Typical administration interval of liposome-based anti-cancer drugs is around 2-4 weeks. Therefore, the drug accumulation in the tumor cells after 2 weeks is considered.

Therefore, the resulting convection-diffusion-absorption equation with the force term can be described as:

$$\frac{\partial c}{\partial t} + \boldsymbol{\nabla} \cdot (-D \boldsymbol{\nabla} c) + \boldsymbol{\nabla} \cdot (\mathbf{q}_F c k_E) + \boldsymbol{\nabla} \cdot \left(-\frac{\boldsymbol{\nabla}\, U_{Rad}}{\xi} c \right) = 0\,. \tag{4.21}$$

The right hand side of the equation (4.21) is zero which means that there is no source and sink in the tissue. Drug molecules are delivered in tumor tissue with the intravenous bolus injection which is explained in the previous section. Therefore, there is a specific initial drug concentration inside the tumor. For this situation of the tissue, ultrasound is applied. Thus, we set the right hand side of the equation equal to zero.

For a spherical particle with radius r, moving through a viscous fluid which means at the low Reynolds number from the Stokes' law, drag coefficient ζ can be expressed as:

$$\zeta = 6\pi\eta r, \tag{4.22}$$

where η is the viscosity of the medium. Moreover, for low Reynolds number, mobility μ or the ratio of the particle's drift velocity to the applied force, $v = \mu$F, is the inverse of drag coefficient. Therefore, it can be written as:

$$v = \mu F = \frac{F}{\zeta} = \frac{F}{6\pi\eta r}. \tag{4.23}$$

If the order of magnitude calculation is done, the radiation force is on the order of pN and the viscosity of medium is around 10^{-3} (Ns/m^2), the velocity of the micron-sized particles inside the medium can be found around $\sim 10^{-6}$ (m/s) when the acoustic radiation force is applied.

For diffusion of spherical particles through a medium with low Reynolds number, Stokes-Einstein equation is used:

$$D = \frac{k_B T}{6\pi\eta r}, \tag{4.24}$$

where D is diffusion constant, k_B is Boltzmann's constant and T is the absolute temperature. For this time, if the order of magnitude calculation is done for the diffusion of drug particles, it can be found that diffusion constant is around $\sim 10^{-13}$ (m^2/s).

In equation (4.21), the second and the third terms in the left hand side are neglected since their effects are too small during the application time of the radiation force which is on the order of seconds. Therefore, the diffusion equation can be expressed as:

$$\frac{\partial c}{\partial t} + \boldsymbol{\nabla} \cdot \left(-\frac{\boldsymbol{\nabla}\, U_{Rad}}{\xi} c \right) = 0 \,. \tag{4.25}$$

5. CONCLUSIONS

In this study, we aim to examine the applications of acoustic radiation force in drug delivery to solid tumors. Since the heterogeneous and ineffective drug distribution is a problematic issue in cancer treatment, it is important to develop new methods to overcome this problem. Because of the abnormal vasculature in tumor microenvironment, drug particles are faced with several physiological barriers which prevent transport of therapeutic agents homogeneously from the region of administration to the cells in solid tumors. Experimental studies have shown that radiation force can manipulate the delivery of vehicles in the vasculature by changing their velocities and positions.

In a recent study, by using mouse cells as a model, the uptake of calcein has been examined (Pitt *et al.,* 2004). The results have revealed that the cellular uptake has increased at changing acoustic energies. Also, in another study (Treat *et al.,* 2007), the transport of doxorubicin has enhanced with the ultrasound exposure using a center frequency 1.6 MHz and the systemically injected drug. It has been observed that the non-cavitational exposures result in an increase in the diffusion rate of silver chloride (AgCl) in a fish skin (Frenkel *et al.,* 2000).

In the light of these experiments, we develop a mathematical model to investigate the concentration changes of drug particles by solving the convection-diffusion-absorption equation which takes into account the radiation forces. In the first step, a bolus drug injection is introduced, the transport and the distribution of drug particles within the tissue are examined. In the second step, by using the Helmholtz equation, pressure and velocity amplitudes are determined. This helps us to calculate the acoustic force potential and thereby the acoustic radiation force. In the final step, the convection-diffusion-absorption equation is introduced with the radiation force term to determine the changes in the concentration of drug particles.

We can also extend our study to investigate the effect of acoustic radiation force by injecting the drug intravenously and sending the pressure wave at the same time. In order to provide a certain amount of drug inside the tissue before decaying, the drug injection and the force are applied simultaneously. It is also an interesting problem in terms of understanding the viscoelastic properties of tumor tissue, fluids and their behaviors during the application of the force. In this case, there will be also source and sink terms on right hand side of the equation (4.21).

We hope to take our research one step further by simulating this mathematical model. This allows us to make predictions about the problem and use the simulation results in the cancer treatment. A computational model which runs with the specific data taken from a patient will give us more realistic results compared to the statistical data about the state of the disease.

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