

A multi-physics model for thermosensitive liposome drug delivery to solid tumour

By

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Abstract

Thermosensitive liposome (TSL)-mediated drug delivery in combination with focused ultrasound (FUS) induced hyperthermia has been considered as a promising alternative to conventional chemotherapy. Tumour cells residing in tumour interstitial space can only be killed when they are exposed to therapeutic levels of drug concentration. Interstitial transport, as an essential step to carry drug molecules to tumour cells, must be examined in detail to gain mechanistic understandings of TSL delivery in combination with hyperthermia exposure.

The process starting from the infusion of drug-loaded TSL to the uptake of chemotherapy drug by tumour cells involves multiple physical and biochemical steps; these include acoustic wave propagation in tissue upon the application of FUS, heat transfer in response to FUS heating, diffusive and convective transport of TSL and released drug from the blood stream and across the vessel wall into the interstitial space, as well as the transfer of bioavailable drug across the cell membrane and its eventual uptake by tumour cells. To understand the transport mechanism and the interactions among the different steps, mathematical models have been developed to simulate the TSL drug delivery system.

First, a multi-compartmental model is developed and applied to idealised tumour cord geometry with an aim to identify the key transport parameters that affect the predicted therapeutic outcome. Results suggest that hyperthermia duration and drug release rates are the most influential parameters. Based on this finding, multivariable optimisation is carried out in searching for optimal conditions that would maximise tumour cell killing while minimising the risk of side effect. This is followed by the development of a multiphysics continuum-based model for detailed examination of spatial and temporal distributions of key variables (e.g. temperature and drug concentration) in realistic tumour geometry reconstructed from magnetic resonance images. The results provide more insights into the spatial profiles of temperature upon the application of FUS and hyperthermia-induced drug release. Comparisons are made between two TSL delivery systems, a conventional TSL loaded with doxorubicin (DOX) and a novel imageable TSL (iTSL) loaded with topotecan (TOP). Results show that the iTSL-TOP system achieves much higher cancer cell killing fraction than the conventional TSL-DOX system.

Since non-uniform temperature profile and heterogeneous drug distribution at the tumour site are identified as limiting factors, initial attempts are made to determine how the number and location of ultrasound focal points may influence temperature distribution in a targeted tumour. Although the computational models employed in the present study have several limitations, they are able to provide detailed spatiotemporal information that cannot be obtained *in vivo*. The modelling framework can serve as a useful tool for evaluation and optimisation of therapeutic efficacy of FUS-TSL drug delivery systems.

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Declaration

I hereby declare that the work represented in this thesis was originated entirely from me. Information derived from the published and unpublished work of others has been acknowledged in the text and references are given in the bibliography.

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Chapter 1: Introduction

With the development of new drugs and advancement in technology, a variety of diseases can now be cured. However, cancer is still one of the most common causes of death. All traditional treatment methods including surgery, radiation therapy and chemotherapy have some limitations. Surgery is aimed to remove the tumour tissue as much as possible, but some tumours are not amenable to surgery due to their size and location of the tumour. While radiation therapy and chemotherapy are effective in some patients, they could also cause serious side effects. Therefore, considerable research efforts have been made towards developing novel therapies for more effective treatment of different types of cancer. One of the emerging technologies is focused ultrasound (FUS) which has shown great potential in cancer treatment (Lyon et al., 2018).

FUS has been employed as the heat source for thermal ablation and mild hyperthermia since it can heat the tissue non-invasively. When used under the guidance of magnetic resonance imaging (MRI), the heating process with FUS is highly controllable. With the advancement in nanotechnology, a variety of nanoparticles have been developed to serve as drug carriers to deliver anticancer drugs to the tumour site. Nanoparticle-based drug delivery systems are designed to improve the therapeutic efficacy, while reducing undesired damage to normal tissue. The release of drug from nanoparticles can be triggered by different mechanisms, e.g. through elevation in temperature or changes in shear rate or PH value. Hyperthermia induced by FUS is a promising way to trigger drug release from thermosensitive liposomes (TSL) at the tumour site (Sercombe et al., 2015).

1.1 Cancer

Cancer is a group of cells that grow uncontrollably. They may invade other parts of the body (King and Mike W., 2006). The cause of cancer is still not fully understood. Many factors such as smoking, radiation and pollution may increase the probability of cancer. The developed tumour forms a microenvironment, which is unique and resists to both chemotherapy and radiotherapy (Mader, 2007). Cancer cells grow much faster than normal cells, causing immature and irregular vascular structure in the tumour. The irregular blood vessel network makes the transport of oxygen and nutrition difficult, resulting in the formation of a necrotic core in some tumours. As cancer cells grow, they migrate to other parts of the body, and that is the primary cause of death. Cancer cells can also damage healthy cells and lead to organ failure.

Over 200 types of cancer have been discovered, which increases the difficulty of anticancer treatment. Statistic results show that from 2014 to 2016, 363484 new cases of cancer have been diagnosed leading to 163444 death in 2016 in the UK(Cancer Research UK,2017). The prediction based on statistical results shows that the number of people living with cancer increases year by year, as shown in Table 1.1. The situation is even worse for the older people (aged 65 and above), with the number of older people living with cancer increased by 23% in the past five years. Despite major progress in the development of new therapies, cancer is still the second leading cause of death. The quality of life of cancer patients is significantly affected not only by the disease itself but also the anticancer treatment. Therefore, novel treatment method is urgently required to improve the survival rate and the quality of life for these patients.

Table 1. 1: Estimated number of people living with cancer

	2010	2015	2020	2030
England	1,700,000	2,000,000	2,400,000	3,400,000
Wales	110,000	130,000	160,000	220,000
Scotland	190,000	220,000	260,000	360,000
Northern Ireland	53,000	63,000	74,000	100,000
UK	2,100,000	2,500,000	2,900,000	4,000,000

(Cancer Research UK, 2017)

1.2 Anticancer therapies

There are three traditional methods for cancer treatment, namely surgery, chemotherapy and radiotherapy. Approximately 45% of cancer patients in the UK take surgery as the primary way of treating cancer, chemotherapy and radiotherapy sharing a similar proportion. The treatment selection is highly dependent on the stage of the tumour. For patients with stage 1 and 2 disease, surgery is the most effective treatment since the tumour can be removed directly. However, for the late stage cancers, chemotherapy is preferred due to the size of the tumour and the physical conditions of the patient. Radiotherapy can be applied to all stage to kill the tumour cells locally and non-invasively. Treatments are usually combined to achieve the best therapeutic efficacy.

Table 1. 2: The proportion of patient having different anticancer treatment

Treatment method	Percentage of selection
Surgery	45%
Chemotherapy	28%
Radiotherapy	27%

(Cancer Research UK, 2017)

Surgery is the most common and direct way of treating cancer, whereby tumour tissue is removed from a patient locally. It is a very efficient way of curing local tumour before metastases. The operation not only removes the tumour but also cuts off some of the nearest healthy tissue and lymph nodes to prevent metastases. In some cases, surgery cures cancer completely. Surgery could also be helpful even if cancer cells have spread. It may prolong a patient's life and reduce the pain they suffer. Surgery may also be applied to prevent cancer. For example, patients who have a high risk of breast cancer could choose to remove the gland to prevent cancer. Surgery treatment has its drawbacks. At some locations, the tumour cannot be easily cut off. Surgery may stimulate the remaining cancer cells causing them to regrow and spread. Some patients may be too weak to undergo an operation. Since surgery is invasive, it is also associated with pain and prolonged recovery time.

Chemotherapy uses several drugs to kill cancer cells and prevent them from proliferating. Its effectiveness depends on the pharmacodynamics of the drugs and their transport properties. Some drugs kill cancer cells efficiently, but they are not able to penetrate the tumour. In this case, the overall efficacy of the drug would be

reduced. Different drugs exert their effects on cancer cells through different mechanisms. They may damage the gene of cancer cells or interrupt tumour cell division.

On the other hand, anticancer drugs can also kill healthy cells, which may lead to serious side effects. Many anticancer drugs are not designed to target a specific region. Drugs are circulated in the circulatory system, causing neurotoxicity, myelosuppression and cardiomyopathy (King and Mike W., 2006).

Anticancer drugs usually suppress the generation of blood cells. With low levels of red blood cells, patients may suffer from lack of nutrition. Anticancer drugs are often designed to target cells with a high proliferation rate, which may cause hair loss. Doxorubicin is a widely used drug which can damage cancer cells RNA and is suitable for almost all types of cancer. The most common side effects associated with doxorubicin is cardiomyopathy. As a result, the dose is limited to 550mg per unit body surface area to minimise the side effects (Laurence et al., 2017). Reducing drug toxicity is challenging. Therefore, targeted drug delivery is required to improve treatment efficacy and to reduce side effects.

Radiation is an efficient way to damage cancer cells DNA (Baskar et al., 2012) and can be applied to kill malignant tumour cells. Radiation therapy would not work if cells are anoxic, which commonly happens at the tumour centre. The unique microenvironment of tumour protects tumour cells from radiation since it is difficult for oxygen and nutrient to reach the tumour core.

Radiotherapy can be applied externally and internally. When radiation is used externally, the treatment area is well identified before treating. By doing so, the damage to healthy tissues is minimised. For internal application, radioactive implants are infused close to the tumour before the treatment. The radioactive agents would accumulate in tumour and be active when radiation is applied. Radiotherapy is regarded as a local treatment. However, it still causes some side effects. Patients often feel tired after treatment due to the irreversible damage to normal cells.

Thermosensitive liposomes (TSL) involve the encapsulation of anticancer drugs in temperature-sensitive liposomes, which will only release the encapsulated agents at temperatures above the phase transition temperature of the liposome (Kennedy, 2005). The average phase transition temperature for drug-loaded liposomes is in the range of 37 to 42°C, depending on the liposome formulation (Kennedy, 2005). Ligand and antibodies can be attached to the surface of liposomes to achieve targeted delivery. External heating is usually provided by high-intensity focus ultrasound (HIFU), which is non-invasive. Under MRI guidance, the heating area and temperature can be controlled accurately, without causing severe damage to the surrounding (Kennedy,

2005). TSL-mediated drug delivery has several potential advantages over traditional chemotherapy: drugs would only be released locally in the tumour region; as such, it may reduce side effects and improve therapeutic efficacy. TSL encapsulated doxorubicin (ThermoDox) has been applied clinically and shown an improvement in drug uptake by 3.7 times compared to the treatment without FUS (Lyon et al., 2018). However, our understanding of detailed transport mechanisms of the complex process is still limited, and the use of mathematical models offers a great potential to design and optimise treatment protocols for enhanced therapeutic efficacy.

1.3 Structure of the thesis

The thesis has seven chapters including introduction, literature review, and methodology, results obtained with the multi-compartmental model for microscopic drug distributions, results obtained with the integrated multiphysics model, the influence of acoustic focal point on temperature distribution and the conclusion.

Chapter 1 gives a brief introduction to background knowledge of cancer and cancer treatment. The advantage of TSL drug delivery system is identified. Chapter 2 reviews a wide range of literature on the tumour microenvironment, existing microscopic and macroscopic drug delivery models, TSL drug delivery systems, the bio-heat transfer equation in living tissue and the simulation of acoustic pressure field produced by HIFU. This chapter finishes with a statement of the aim and objectives of the study. In chapter 3, the mathematical models employed in this study are described. The model geometry and critical parameters of the model are described. Chapters 4 and 5 show the results and analysis of two different models, namely the multicompartmental model applied to a tumour cord model, and the coupled bio heat transfer and drug transport model applied to a realistic tumour geometry. In Chapter 6, the influence of HIFU focal point location on temperature distribution is investigated. Finally, conclusions and future work are summarised in Chapter 7.

Chapter 2: Literature review

2.1 Tumour microenvironment

To simulate the drug delivery process in a solid tumour, we must understand the internal structure and microenvironment of it. The inner structure of tumour is very different from that of healthy tissue. The complicated microenvironment in tumour creates additional barriers to drug transport, and poor drug penetration not only leads to ineffective treatment but also increases the risk of side effects. Therefore, it is essential to understand the microenvironment of tumour and design new drugs to overcome this difficulty.

Compared to normal tissue, tumour tissues have a poorly organized vascular structure, resulting in irregular blood flow (Minchinton and Tannock, 2006). This is largely attributed to the fast growth of tumour cells. Tumour cells have much higher proliferation rate than normal cells, and the rapidly growing cells force vessels to be apart, resulting in low vascular density and poorly organized vessel architecture (Padera et al., 2004). The transport properties are significantly altered due to the irregular vascular structure — the closer to the tumour centre, the lower the vascular density. Less oxygen and nutrition are transported to the centre of the tumour. As a result, oxygen and nutrition levels at the core of the tumour are very low, and cell proliferation rate decreases towards the tumour centre. The hypoxia condition naturally resists radiotherapy and some drugs that require sufficient oxygen levels to exert their effects (Chaplin et al., 1989). Table 2.1 compares the morphological feature of tumour and normal microvasculature.

Table 2. 1: morphological features of tumour and normal microvasculature (Less et al., 1997, 1991; Skinner et al., 1990; Yamaura and Sato, 1974)

	Diameter of capillary (μm)	Diameter of venules (μm)	Average Length (cm/mm^3)	Average Surface area (mm^2/mm^3)	Average Vascular volume fraction (%)
In normal tissue	5-20	15-70	36	70	50
In tumour	5-8	12-50	160	20	20

In addition to the poorly organized vascular structure, the lymphatic system in tumours is usually non-functional due to the compression of fast-growing tumour cells (Dewhirst et al., 1998; Jain et al., 2002; Leu et al., 2000).

Therefore, interstitial fluids cannot be effectively drained, leading to excessive accumulate in tumour tissue and high interstitial fluid pressure (IFP). Since the force that drives drug penetration through the blood vessel wall to interstitial space is the pressure difference across the vessel wall, the higher the IFP, the more difficult for the drugs to pass through.

After crossing the vessel wall, the movement of drugs would be slowed down by the extracellular matrix (ECM). ECM is a complex group of molecules that exist in the tissue outside cells. It is composed of collagen and hyaluronic acid (Brown et al., 2003; Davies et al., 2002). Water molecules and water-soluble drugs are trapped in ECM and cannot transport into cancer cells. Furthermore, metabolic products including lactic and carbonic acids quickly build up in tumour causing an acidic microenvironment in the extracellular space (Gatenby and Gillies, 2004; Raghunand et al., 2003; Tannock and Rotin, 1989). Some anticancer drugs are inactive in an acidic environment. Figure 2.1 shows a schematic diagram of tumour microenvironment. To summarise, the challenges for drug transport in solid tumour are poorly organized vascular structure, non-functional lymphatic system, high IFP, and ECM drug trapping.

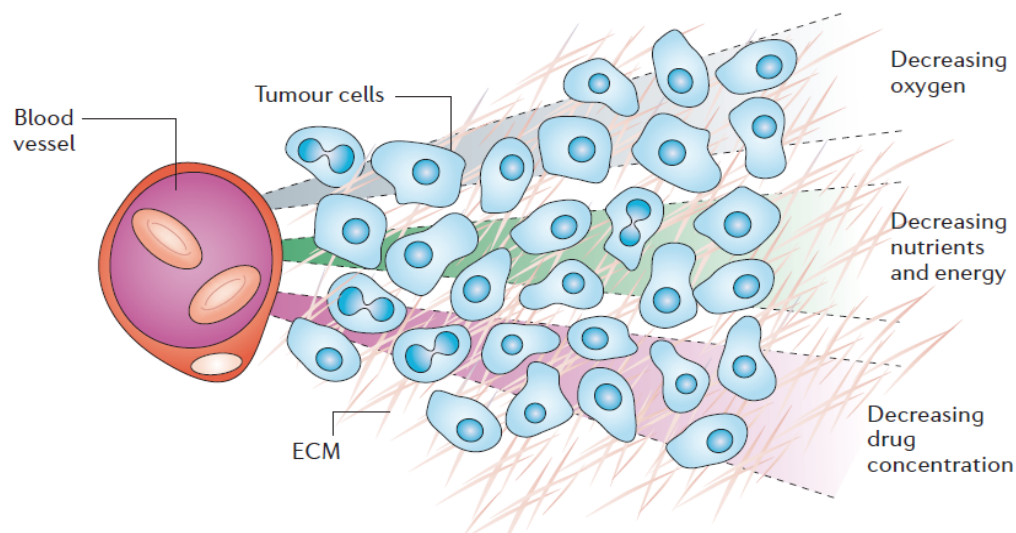


Figure 2. 1: Schematic of tumour microenvironment. Extracted from Minchinton and Tannock (2006)

Apart from drug transport related properties, it would also be interesting to know the role of mechanical forces in tumour progression. It has been shown that externally applied and growth-induced stresses affect tumour progression (Jain and Martin, 2014). Experimental results obtained with spheroid cancer cells at different concentrations of agarose gel show that the higher the concentration, the greater forces are applied to the spheroid externally (Jain et al., 2014). Experimental results demonstrate that the compressive force inhibits cell growth. However, the effect is temporary which means that the growth rate resumes after the force is removed.

External stress inhibits cell growth by triggering cell death via the mitochondrial pathway (Helmlinger et al., 1997; Raghunand et al., 2003; Stylianopoulos et al., 2012). Therefore, it also plays a role in determining the shaping of the spheroid. The part of the tumour with lower stress grows faster and vice versa. However, compression would enhance the invasive phenotype and remodel the ECM.

In addition to externally applied forces, there is growth-induced solid stress which is stored during cell growth (Stylianopoulos et al., 2012). If a tumour is cut along the longest axis without external stress, the stored force would be released, causing the centre of the tumour to swell and the boundary to retract (Stylianopoulos et al., 2013), indicating tension at the tumour periphery and compression at the tumour centre. Figure 2.2 demonstrates this phenomenon. Growth-induced stress in a human tumour ranges from 35 to 142 mmHg (4.7 to 1839 kPa) which accounts for 30% of the total stress acting on the tumour (Roose et al., 2003; Sarntinoranont et al., 2003). Similar to external force, higher growth-induced stress corresponds to a lower cell proliferation rate. Growth-induced forces are stored in ECM in two different ways. The collagen fibres are stiffer in tension and provide tensile strength. They resist stretching when neighbour cells are pulled apart. On the other hand, hyaluronic acid traps water molecules. Since water is incompressible, the hyaluronic acid resists compression force. Therefore, collagen and hyaluronic acid have different roles in storing stress (Chauhan et al., 2013). Both externally applied stress and growth-induced stress compress the tumour vascular structure, reducing the transportation of oxygen and nutrition to tumours. This may shield tumour from the immune system. One of the hypotheses is that the immune system requires a well- developed vascular network to patrol the human body and detect abnormal tissues. However, a compressed vascular structure would prevent these molecules from reaching the tumour and indirectly protect tumour from the immune system (Jain, 2013).

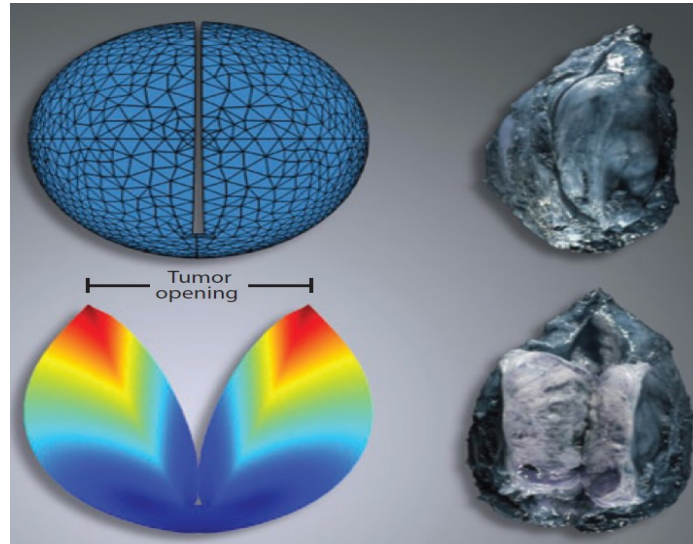


Figure 2. 2: Cutting an excised tumour along its longest axis causes surface retraction and interior swelling (Jain et al., 2014)

Fluid stress also has an impact on tumour microenvironment. Because of the abnormal shape and leakage of tumour blood vessels, the blood flow velocity is slower than that in the normal vessel. Lower flow rate induces lower shear stress on the vessel wall. Shear stress is essential for angiogenesis, and it could be sensed by the glycocalyx layer which is connected to endothelial lining. Endothelial layer is connected to the cytoskeleton of endothelial cells. Therefore, angiogenesis is regulated by stresses acting on the vessel wall. When the shear stress is low, the vessel would mistakenly consider itself to be static and consequently signal endothelial cells to sprout seeking new sources of blood flow (Song and Munn, 2011).

On the other hand, high shear stress could cause vessel branching which reduces the shear stress acting on individual endothelial cells. Shear stress also causes blood shunting. It happens typically near the arteriovenous connection where blood flow can bypass the downstream vessel. Shear stress at the shunt is usually high which leads to shunt dilatation (Pries et al., 2010). Thus, more blood passing through the shunt and less blood flow to the downstream vascular network causing the diameter of the downstream vessel to reduce further. In a solid tumour, the abnormal shear stress gives rise to low perfusion rate which resists drug delivery.

Interstitial fluid pressure (IFP) in a tumour is critical in drug delivery since drugs have to pass through the interstitial space to be taken up by cells (Kalluri and Zeisberg, 2006; Nagy et al., 2012). Hydrostatic and osmotic pressure gradient between vascular and interstitial space are the driving force for interstitial flow. External forces compress the ECM into a dense and tortuous network that blocks fluid flow (Carmeliet and Jain, 2000; Jain, 2005). The interstitial flow velocity is about 0.2 to 0.8 $\mu\text{m/s}$ which corresponds to a very low Reynolds number (Chary

and Jain, 1989). It is necessary to elevate the hydraulic conductivity to increase the interstitial fluid velocity. Hydraulic conductivity is defined as the resistance to fluid flow in porous media. In a tumour, the hydraulic conductivity depends on the volume fraction, surface charge and architecture of the fibres. EMC contains a significant amount of collagen that may cause low hydrostatic conductivity (Ng et al., 2005). Low hydrostatic conductivity and non-functional lymphatic system further increase IFP, which leads to positive feedback in building up IFP. Based on the analysis above, two possible treatment strategies have been proposed namely stress-alleviation and vascular normalization (Goel et al., 2011) Both aim to reduce IFP and enhance drug delivery. Figure 2.3 demonstrates the effects of these two treatment methods.

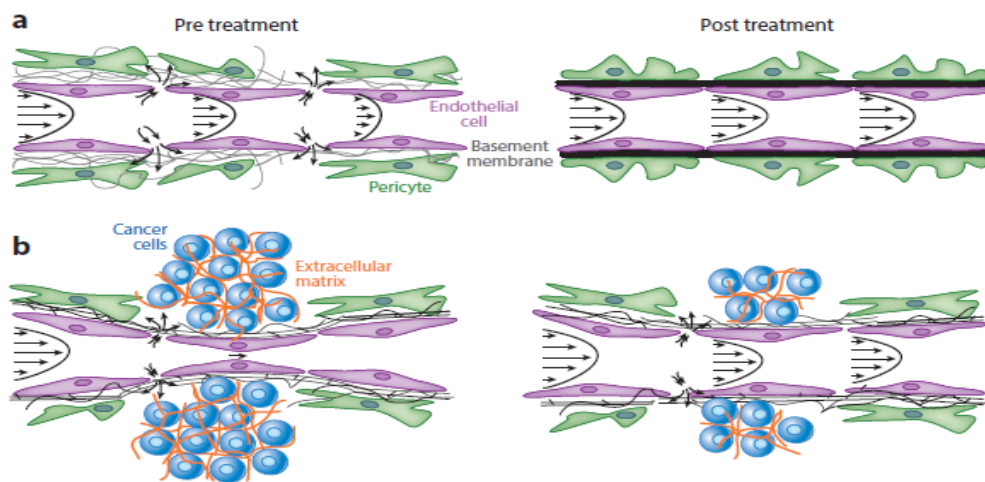


Figure 2. 3: (a) Angiogenesis reduces the leakage of artery wall which decreases the permeability and improves perfusion (b) Stress alleviation reduces compression and improves perfusion (Jain, 2005)

2.2 Liposome drug delivery

Liposomes are lipid-based spherical vesicles with an aqueous core and at least one lipid bilayer, as shown in Figure 2.4. Liposomes are suitable as drug carriers, and without external stimulations, the encapsulated drug would not usually be released. Various liposome formulations have been developed and they can be designed to be heat sensitive, pH sensitive or shear sensitive for targeted drug delivery. This technique has a great potential for application in cancer chemotherapy. Anticancer drugs are usually toxic to normal cells. By encapsulating drugs in liposomes and releasing them at the tumour site, it would be possible to reduce drug dose, thereby effectively reducing side effects.

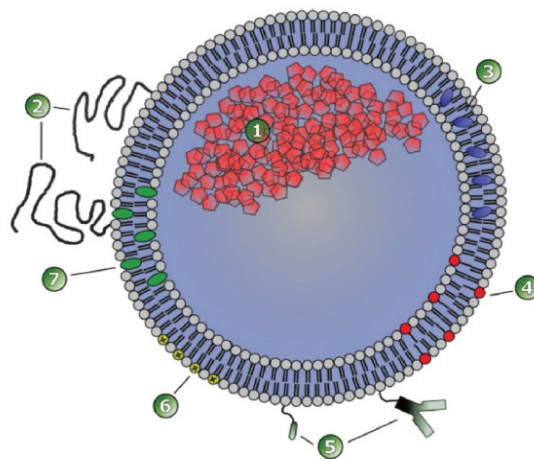


Figure 2. 4: Schematic drawing of liposome vesicle 1. Encapsulated drug; 2. polymers attached at liposome surface; 3. Liposome bilayer; 4. Antibodies and ligands; 5. Cationic lipids; 6. Cholesterol; 7. Amphiphilic molecules forming lamellar structure (Kneidl et al., 2014)

2.2.1 Types of liposomes

Figure 2.5 demonstrates four types of liposomes namely conventional liposomes, sterically- stabilised liposomes, ligand-targeted liposomes and a combination of the above. The bilayer of conventional liposomes is composed of cationic, anionic or neutral lipids and cholesterol. The aqueous volume is enclosed in the centre of lipids. Clinical studies have shown that conventional liposomes can reduce drug toxicity, but they are not stable enough and a large amount can be taken up by macrophages of the reticuloendothelial system (RES) causing drug losses (Gabizon et al., 1982; Hua and Wu, 2013; Koning and Storm, 2003; Metselaar and Storm, 2005).

More stable liposomes need to be developed to overcome the blood clearance problem, sterically-stabilized liposomes are one of the options. By adding hydrophilic polymer, polyethene glycol (PEG) to conventional liposomes, their circulation time can be increased significantly. The half-lives of the liposome in a human body can be as long as 45 hours depending on the particle size and coating polymers (Ishida et al., 2001; Northfelt et al., 1996; Torchilin et al., 1992).

In order to achieve targeted drug delivery, ligand-targeted liposomes have been designed. Ligands, usually monoclonal antibodies, are implanted on the surface of liposomes. Liposomes can be delivered to a specific site of the body using the binding effects of the antibody. However, ligand-targeted liposomes have a similar problem to conventional liposomes where blood clearance is quick. Thus, developing a new liposome which combines properties of both sterically-stabilised and ligand-targeted liposomes is essential.

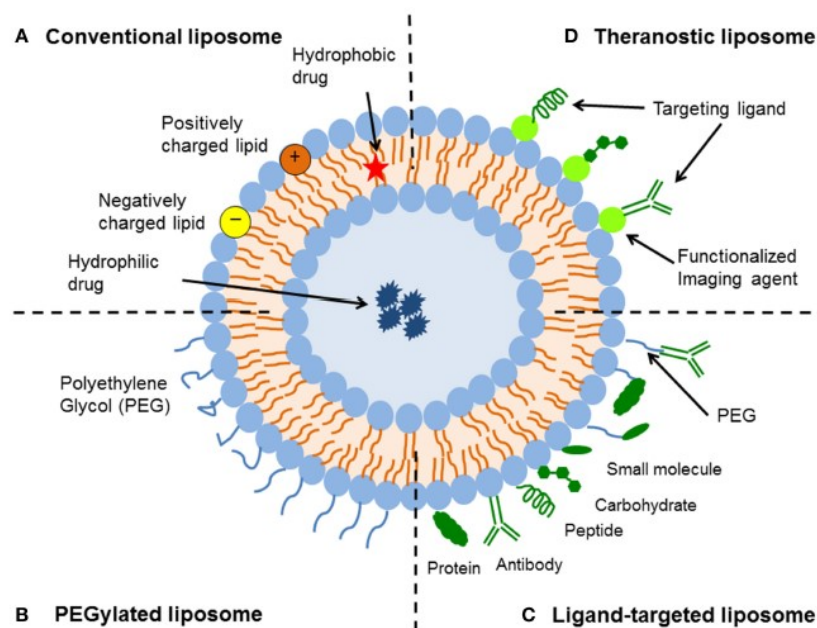


Figure 2. 5: (a) Conventional liposome contains bilayers, positive and negative charged lipid; (b) PEGylated liposome, more stable in vivo, increases circulation time; (c) ligand-targeted liposome, can be used as specific targeting drug carrier; (d) theranostic liposome contains targeting ligand and imaging agent (Sercombe et al., 2015)

2.2.2 Formulations of thermosensitive liposomes

Although liposome-mediated drug delivery has many advantages, it has a limitation that affects its therapeutic efficacy which is the enhanced protective effect. The drug loaded in liposomes is well protected by the drug carrier with only a small amount being released at the tumour site. Various solutions have been proposed to solve this problem; these include liposome formulations that respond to different external stimuli, namely temperature, electromagnetic fields and light. Among these, thermosensitive liposomes have drawn special attention due to their stability in the circulation and quick drug release when heating is applied. A comprehensive review of thermosensitive liposomes can be found in Bi et al., (2019)

Several different formulations of thermosensitive liposomes have been developed to improve chemotherapy efficiency. A proper formulation should have a long circulation time which means the carrier is stable at normal body temperature (37°C) and have quick release at phase transition temperature (42°C). David Needham and his group developed a low-temperature sensitive liposome (LTSL) which is composed of three different lipids. Each lipid affects the material properties of the LTSL. Overall, the LTSL has sharp thermal transition and rapid drug release. Experimental results illustrated that 4 mol% DSPR-PEG2000 raised the transition temperature by

1 °C (Needham et al., 2013), while inclusion of monopalmitoylphosphatidylcholine (MSPC) slightly lowered the phase transition temperature. The primary function of MSPC is to increase drug release rate. By adding 9.3 mol% MSPC, the liposome achieved 80% drug release in 3 min (Needham et al., 2013). The third lipid, PEG-lipid, also enhances drug release by increasing membrane permeability. Ta et al. (2014) tried another formulation including a polymer named p(NIPAAm-co-PAA) which gives sensitivity to both temperature and pH. The new formulation requires less thermal dose than that of the old formulation. It could be combined with MRI-guided focus ultrasound to trigger local drug release.

Kim et al. (2014) proposed a liposome with elastin-like-polypeptide (ELP) and arginine-glycine-aspartic-acid. The ELP has thermal sensitivity, while the acid can bind with $\alpha_v\beta_3$ integrin which is present in tumour where new blood vessels are formed. Liposomes containing arginine-glycine-aspartic-acid would passively accumulate in tumour. This formulation allowed drug release at 42 to 45°C. Flow cytometry results showed that the cell uptake rate increased 8 to 10 folds compared to that of non-targeting liposomes. The authors concluded that the stable, target specific liposome offers a promising tool to enhance therapeutic efficacy (Kim et al., 2014).

In addition, a new formulation of thermosensitive liposome, namely imageable thermosensitive liposome (iTSL) was developed to allow drug distribution to be observed in vivo named. By adding near infrared fluorescence (NIRF), the drug distribution can be tracked using MRI. The brighter the image the higher the concentration. Figure 2.6 shows an in vivo tracking performed by Centelles et al (2018a).

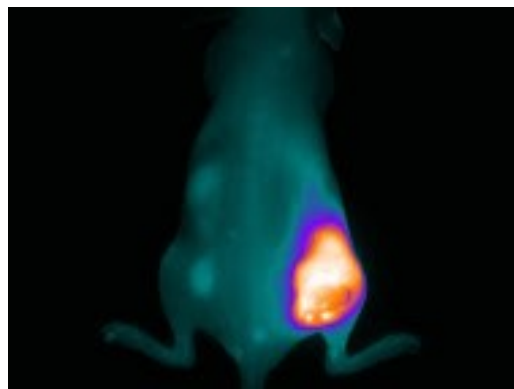


Figure 2. 6: In vivo tracking of drug distribution using iTSL (Centelles et al., 2018a)

2.3 Focused ultrasound and ultrasound simulation

2.3.1 Transducer

Ultrasound transducer is a single device or an array of devices that vibrate to produce sound waves above the hearing range. The evolution of transducer can be divided into four generations. (1) The first generation of

transducer is a single piston connected with a single thermocouple. (2) The second generation is a group of separately controllable stationary transducers arrays. The transducer arrays are coupled with multiple thermometers for temperature measurement. (3) The third generation has mechanical scanning of the ultrasound beams. (4) the most recent devices integrate with non-invasive, real-time MR thermometry.

For treatment using the first-generation devices, the ultrasound frequency is within the range of 1 to 3 MHz (Marmor and Hahn, 1978). A piston covered by a latex membrane is placed directly on the skin over the tumour. Cooling water is circulated through the transducer housing. The temperature is measured invasively by inserting a thermocouple in the centre of the tumour. Energy output from the transducer surface is relatively uniform, and the manufacture of the device is simple. The main limitation of the first-generation transducer is that the spatial and temporal distribution of heating is untraceable.

The second-generation devices have better temperature control than that of the first-generation. An example of a commercialised system is Sonothrem 1000 which contains 4 or 16 elements ultrasound array (Benkeser et al., 1989). The penetration depth is 6 cm with 1 MHz and a higher frequency, 3.4 MHz, is employed when treating a superficial site (2-3 cm). The energy output of each transducer is well controlled by a feedback loop where 2-16 needle thermocouples are implanted invasively near the treatment area. The drawbacks of the second-generation devices are the heavy applicators, complex operating system and invasive temperature measurement. Besides, only 19% of cases achieve an average temperature higher than 42°C at the tumour site. Therefore, a more powerful way to deliver the energy is required for thermal ablation proposes.

For the first and second-generations, the ultrasound transducer is static. The third-generation device incorporates a mechanical scanning strategy which makes the transducer array mobile. The scanning is achieved by two ways, namely mechanically scanning an acoustic reflector and mechanical scanning the transducer itself (Moros et al., 1995). The movement of the transducer array is controlled by software which distributes the power input based on variations in tumour thickness to ensure a more evenly distributed temperature field. However, the temperature measurement is still invasive via small thermocouples.

In order to achieve non-invasive, real-time temperature measurement, the ultrasound generating devices are integrated with MR imaging which is commonly known as MRgHIFU. With MRI temperature mapping, the tissue temperature can be accurately controlled (Hijnen et al., 2012). Pre-clinical animal studies show that the MRgHIFU is safe (Chu et al., 2016). MRgHIFU is the method of choice for hyperthermia-triggered TSL drug delivery systems. Clinical studies demonstrate that MRgHIFU is capable of triggering the release of drug safely and successfully (Chu et al., 2016; de Smet et al., 2011).

In conclusion, the development of HIFU technology from a single transducer to multi transducer array, from single invasive thermocouple to MRI real-time temperature mapping, leads to a more controllable hyperthermia process making HIFU become a reliable way of non-invasive heating at tumour site. However, some challenges remain unsolved where cold spots exist even in the most favourable cases(Overgaard, 1989). Improving the temperature measurement technique is necessary as current method does not provide temperature measurement volumetrically. The controller reduces the power input when one or more spots reach the desired temperature while other regions are not heated sufficiently (Diederich and Hynynen, 1999). Further development on the measurement and control technology is essential for achieving a homogeneous temperature distribution.

2.3.2 Applications

HIFU is able to heat up the targeted tissue effectively, accurately and non-invasively when applied under MRI guidance, making it an ideal choice for applications in clinical hyperthermia, ablation and thermosensitive liposome drug delivery.

2.3.2.1 Hyperthermia

Mild hyperthermia has been shown to enhance the efficacy of chemotherapy (Lyon et al., 2018) . It is essential to be able to control the thermal dose and heating area accurately. For accurate temperature control and prevention of tissue damage due to overheating, it is necessary to develop *in vivo* temperature monitoring technique and an effective control system. MRI offers a non-invasive approach for *in vivo* temperature measurement, which can be used to guide the ultrasound transducer. MRI temperature mapping is based on the principle of proton resonance frequency (PRF). The resonant frequency of proton in a molecule is related to the local magnetic field and can be calculated according to the Larmor equation. The Larmor equation relates the resonance frequency to the local magnetic field, the gyromagnetic of hydrogen and the shielding constant. The shielding constant accounts for the shielding effect of the electron in water molecules which reduces the effect of the magnetic field. The shielding constant has a linear relationship with temperature independent of tissue type. Changing the shielding constant will change the PRF. By using a normal gradient-recalled echo (GRE) imaging pulse sequence, the change in PRF can be detected via phase change in the heated area (Jing et al., 2012). The temperature change can then be calculated according to changes in PRF.

It has been demonstrated that ultrasound transducer could produce relatively uniform heating under the MR guidance (Tillander et al., 2016). In their experiment 11 porcine legs were heated from room temperature to 42.5°C in 10 to 60 min. Experimental results showed that the temperature of the target area was 42.0±0.6°C after heating with the application of HIFU. The average depth that ultrasound reaches from the skin was 86.9mm

(Tillander et al., 2016). It was demonstrated that the heat produced by HIFU was able to penetrate deeply and distribute fairly uniformly (Tillander et al., 2016). Similar experiments were performed on tumours in rabbits (Lam et al., 2016) where four rabbits were treated with focus ultrasound and the temperature at the focal point was successfully raised to 42°C. Both experiments showed that focused ultrasound is capable of heating tissues locally and non-invasively (Lam et al., 2016).

2.3.2.2 TSL drug delivery

Focused ultrasound can also be employed to control drug release of TSLs which are designed to release majority of the encapsulated drugs upon mild hyperthermia. It is hoped that the drugs would not release at body temperature and after reaching and accumulating in a target area, high-intensity ultrasound would be applied to raise the local temperature to the temperature required for drug release. Consequently, most of the TSL encapsulated drugs would release locally in the targeted area. Drug release induced by HIFU was tested by Lai et al (2013) who added fluorescent dye in low-temperature sensitive liposomes (LTSLs). The liposomes were mixed with agarose powder, regular evaporated milk, Dulbecco's phosphate-buffered saline (DPBS), n-propanol, and silicon carbide powder. A dual-mode linear array ultrasound transducer produced ultrasound with frequency 1.54MHz. The total power input was 2W, and the acoustic pressure was 1.5MPa. The heating was stopped when the core of the phantom reached 32°C. After that the single focal heating was employed to further heat the core to 35, 37 and 39°C, respectively. Immediately after reaching the desired temperatures, the phantom was cooled in 0°C water and sliced every 2.0mm along the depth direction. The slices were further analysed by using an optical system and their results illustrated that the transition temperature of the LTSLs was 35°C, and 90% of drug release was achieved at 37°C (Lai et al., 2013). The phase transition temperature can be different depending on the formulation of liposomes and the environment.

2.3.3 Simulation methods

In order to predict the temperature distribution in a tumour, it is essential to include simulation of ultrasound hyperthermia. Simulating the ultrasound effect on living tissue requires mathematical descriptions of both the acoustic energy propagation and bio-heat transfer in living tissues. Ultrasound produces a pressure field which can be converted into acoustic energy and absorbed by living tissues. The acoustic energy converts to thermal energy and raises the local tissue temperature where blood perfusion helps remove the heat and cools the tissue down. The final temperature profile is a result of these combined effects. In this section, four methods for the simulation of acoustic pressure field are briefly described. Three methods are based on the linearised wave equation, and the last one considers the non-linear effect.

2.3.3.1 Point source method

Point source method was developed by O'Neil in 1948. In this method the transducer is treated as a collection of points. By simulating the effect of each point and summing them up, the performance of the whole transducer can be estimated (O'Neil, 1949). It was found that under certain conditions, the normal velocity of the transducer is represented by u which is proportional to $e^{i\omega t}$, the velocity potential can be approximately presented by equation 2.1:

$$\psi = \iint \frac{u}{2\pi s} e^{-iks} dS \quad (2.1)$$

where ω is excitation frequency, t is time, $k = \frac{\omega}{c} = \frac{2\pi}{\lambda}$, λ is wave length, c is the velocity of propagation, s is the distance from a source point Q_1 to the field point Q . This expression is derived from the linearised wave equation. Equation 2.1 is theoretically correct if the transducer is a plane with an infinite plane baffle around it. If the surface is curved, the integral neglects the fact that some of the waves are diffracted by other part of the surface. However, if the curvature is small, the diffraction can be ignored. The acoustic pressure is directly related to the velocity potential, as described by Equation 2.2.

$$p = \frac{\rho \partial \psi}{\partial t} = ikc\rho\psi \quad (2.2)$$

where acoustic pressure is given by the real part, based on which the local acoustic intensity can be calculated using equation 2.3.

$$I = \frac{pp^*}{2\rho c} \quad (2.3)$$

The acoustic intensity is related to acoustic energy (Q_{FU}) via equation 2.4,

$$Q_{FU} = 2\alpha_{ABS}I = \alpha_{ABS} \frac{pp^*}{\rho c} \quad (2.4)$$

where p^* is the complex conjugate of p , α_{ABS} is an absorption coefficient depending on tissue properties and ρ is tissue density. Point source method is suitable for simulating a single transducer with a single focal point. However, it involves solving a double integral to compute the velocity potential, which is computationally intensive. Therefore, alternative methods have been sought in order to reduce the computational demand.

2.3.3.2 Impulse response method

The impulse response for a circular piston is defined as $h(r, z; t)$. By performing a Fourier transform, $h(r, z; t)$ can be converted to $H(r, z; k)$ and the expression for pressure is

$$p(r, z; k) = -i\omega\rho c e^{i\omega t} H(r, z; k) \quad (2.5)$$

Therefore, if $H(r, z; k)$ can be expressed as a single integral, it would greatly simplify the solution for equation 2.5. Using formulas in tables of integral transforms combined with Bessel function, Oberhettinger (Oberhettinger, 1961) successfully reduced the double integral problem to a single integral equation, with $H(r, z; k)$ written as

$$H_1(r, z; k) = \begin{cases} \frac{e^{-jkz} - e^{-jk\sqrt{z^2+a^2}}}{jk}, & r = 0 \\ I_1 + \frac{e^{-jkz} - e^{-jk\beta_2}}{jk}, & 0 < r < a \\ I_1, & r \geq a \end{cases} \quad (2.6)$$

where

$$I_1 = \frac{1}{\pi} \int_{\beta_2}^{\beta_3} \cos^{-1} \left\{ \frac{\beta^2 - z^2 + r^2 - a^2}{2r\sqrt{\beta^2 - z^2}} \right\} e^{-jk\beta} d\beta \quad (2.7)$$

$$\beta_1 = z \quad (2.8)$$

$$\beta_2 = \sqrt{z^2 + (r - a)^2} \quad (2.9)$$

$$\beta_3 = \sqrt{z^2 + (r + a)^2} \quad (2.10)$$

It is worth noting that the transfer function $H(r, z; k)$ is different in different regions. The three expressions in equation 2.6 correspond to the centre of the piston, the surface of the piston and outside the piston. This transfer function can be written in a simpler form as suggested by Stepanishen and derived by Archer-Hall et al (Archer-Hall et al., 1979; Stepanishen, 1970).

$$H_2(r, z; k) = \frac{a}{jk\pi} I_2 + \frac{1}{jk} e^{-jkz} \times \begin{cases} 1, & r < a \\ \frac{1}{2}, & r = a \\ 0, & r > a \end{cases} \quad (2.11)$$

$$I_2 = \int_0^\pi \frac{(r \cos \psi - a)}{r^2 + a^2 - 2ar \cos \psi} e^{-jk\sqrt{r^2 + a^2 - 2ar \cos \psi + z^2}} d\psi \quad (2.12)$$

Although the impulse response method has reduced the simulation time significantly compared to the point source method, there is still room for further improvement. The transfer function has a singularity at the point where $r \approx a$ and $\psi \approx 0$. The singularity causes not only long simulation time but also increases numerical errors near the centre of the piston. To further improve the simulation results, Hutchins proposed the fast near field method which successfully removes the singularity (Hutchins et al, 1986).

2.3.3.3 Fast near field method

The expression given below was derived from Green's function approach and the double integral is simplified using Hankel transform tables.

$$H_3(r, z; k) = \frac{a}{j2\pi k} \int_{-\pi}^{\pi} \frac{a - re^{-j\psi}}{a^2 + r^2 - 2ar\cos\psi} \times \left(e^{-jkz} - e^{-jk\sqrt{r^2 + a^2 - 2ar\cos\psi + z^2}} \right) d\psi \quad (2.13)$$

Analytically, H_3 and H_2 are equivalent. However, the numerical properties are different. H_3 eliminates the singularity by combining the two terms in H_2 . H_3 can be further simplified by changing the interval from $[-\pi, \pi]$ to $[0, \pi]$. The final expression is shown in equation 2.14 (McGough et al., 2004a).

$$H_4(r, z; k) = \frac{a}{j2\pi k} \int_0^{\pi} \frac{rcos\psi - a}{a^2 + r^2 - 2ar\cos\psi} \times \left(-e^{-jkz} + e^{-jk\sqrt{r^2 + a^2 - 2ar\cos\psi + z^2}} \right) d\psi \quad (2.14)$$

Simulation results show that fast near field method is three times (McGough et al., 2004b) faster than the impulse response method with less numerical errors.

2.3.3.4 Non-linear wave equation

When a sound wave propagates through inhomogeneous biological tissue, the linearised wave equation is no longer accurate. The inhomogeneous wave equation can be employed to simulate the pressure field.

$$\left(\nabla^2 - \frac{1}{c^2} \frac{\partial^2}{\partial t^2} \right) p - \frac{1}{\rho} \nabla p \cdot \nabla \rho + \frac{\delta}{c^4} \frac{\partial^3 p}{\partial t^3} + \frac{\beta}{\rho c^4} \frac{\partial^2 p^2}{\partial t^2} = 0 \quad (2.15)$$

where c is the local sound speed, ρ is the local density, δ is the local acoustic diffusivity, β is the local coefficient of nonlinearity which depends on the properties of the medium. The first term in equation 2.15 describes the propagation of the wave in space and time. The second term is the ambient inhomogeneity in medium density, which would be zero if the medium is homogeneous. The third term is the pressure loss due to the viscosity of a fluid. The last term accounts for the non-linear wave propagation effect (Beyer, 1974).

2.3.4 Limitations

Although with the development of temperature mapping using MRI, HIFU shows great potential in cancer treatment, it still has certain limitations. As bones and lungs oppose the penetration of ultrasound, it is difficult for ultrasound beams to reach a certain area of the liver parenchyma where it is adjacent to a rib. Also, ultrasound cannot penetrate gases in the bowel. The high energy wave is reflected towards the transducer, which could lead to burns in the intervening tissue (Yufeng et al., 2011).

HIFU penetration to the skull is also very limited due to the aberrations and absorption induced by the skull. Although adaptive correction of the distortions induced by the skull has been performed using 3D computational

tomography (CT) scan of the structure, the fast algorithm of aberration correction is still in development (Shen et al., 2014).

HIFU treatment may disturb the tumour microenvironment and increase the risk of metastases. Fry et al. (1978) reported metastases rate increased by 17% to 44% after HIFU treatment. However, the feature was not reported in other studies. The mechanism and significance of HIFU induced changes in the tumour microenvironment need to be investigated further.

2.4 Bio-heat transfer equation

Heat transfer in living tissue is a complex process involving conduction, convection, blood perfusion and metabolic reaction. Several different mathematical models have been developed to describe these process, and three models are outlined below.

2.4.1 Theoretical models

2.4.1.1 Pennes model

Pennes model was developed in 1948 to describe heat transfer in living tissue. It was initially validated against temperature measurements in human forearms(Pennes, 1948). The expression is shown in equation 2.16.

$$\rho c \frac{\partial T}{\partial t} = \nabla k \cdot \nabla T + (\rho c)_b \omega_b (T_a - T) + q_m \quad (2.16)$$

where T is tissue temperature, k is thermal conductivity, q_m is metabolic heat production, ρ is blood density, c is blood heat capacity, ω_b is blood perfusion rate, and T_a is temperature of blood. Pennes equation assumes that heat exchange between blood and tissue mainly occurs at the wall of capillaries. Another important assumption is that blood entering the capillary bed at temperature T_a and reaches equilibrium with the surrounding tissue instantaneously. Blood leaves the capillary bed at tissue temperature T . Therefore, the total energy exchange can be modelled as a non-directional term proportional to blood perfusion rate ω_b . Since blood perfusion rate is difficult to measure in living tissues, in the original work (Pennes, 1948) the forearm temperature profile was measured and predicted temperature profiles were fitted to the measurements to find the corresponding blood perfusion rate. Pennes equation has been widely used and is commonly referred to as the bio-heat transfer equation (BHT).

2.4.1.2 Wulff's model

Wulff (1974) pointed out that Pennes model was inaccurate by arguing that the assumptions made by Pennes were invalid. Equation 2.16 combined the local energy storage with global convection, which is not permissible. Also, Pennes assumed that tissue, blood in and blood out occupy the same point in space which is physically

impossible. Wulff stated that the blood could also remove heat from tissue depending on the local temperature gradient, and BHT failed to describe that feature. Based on the above arguments, Wulff proposed a new version of BHT (Wulff, 1974).

$$\rho c \frac{\partial T}{\partial t} = \nabla k \cdot \nabla T + (\rho c)_b U_h \cdot \nabla T + q_m \quad (2.17)$$

where the blood perfusion term is different from that in Equation 2.16. ∇T in equation 2.17 allows heat to transfer along the local temperature gradient. The blood perfusion rate ω_b is replaced by blood mass flux $\rho_b U_h$. More information associated with flow condition is required to estimate the mass flux. He solved both equations by assuming steady state and no metabolic heat production to testify the effect of blood flow on the tissue temperature profile. Results showed that blood flow had more impacts when Wulff's model was applied (Wulff, 1974)

2.4.1.3 Incomplete Counter current heat exchange model (WJ model)

Studies show that vessels with a large diameter exchange little heat with the surrounding tissue due to their small specific contact area. Small vessels are in thermal equilibrium with the surrounding tissue; therefore, no heat is transferred. Only vessels with a diameter of 50 to 500 μm are thermally important (Wang, 2008). These vessels are usually present in countercurrent pairs as shown in Figure 2.7 (Weinbaum et al., 1984).

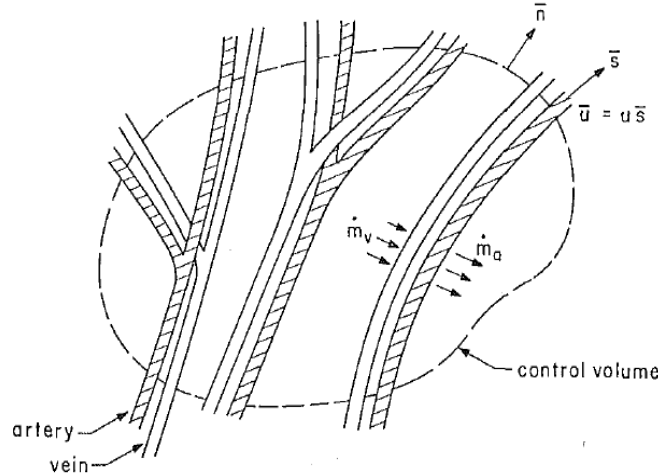


Figure 2. 7: Schematic illustration of counter current heat exchanging network (Weinbaum and Jiji, 1985)

By applying mass and energy balance including a heat source term, Weinbaum developed a new expression for bio-heat transfer equation.

$$\overline{\rho C} \frac{\partial \theta}{\partial t} - \frac{\partial}{\partial x_i} \left[(k_{ij})_{eff} \frac{\partial \theta}{\partial x_j} \right] = - \frac{\pi^2 n a^2 k_b^2}{4 \sigma k} P e^2 l_j \frac{\partial l_i}{\partial x_i} \frac{\partial \theta}{\partial x_j} + q_m \quad (2.18)$$

Where $\overline{\rho C}$ is average blood tissue heat capacity times average density, θ is tissue temperature. $(k_{ij})_{eff}$ is effective thermal conductivity as defined in equation 2.19 (Perl and Hirsch, 1966a). k_b is thermal conductivity of blood, σ is shape factor, Pe is Peclet number and l_i is the length along i direction.

$$(k_{ij})_{eff} = k \left(\delta_{ij} + \frac{\pi^2}{4\sigma k^2} n a^2 k_b^2 Pe^2 l_i l_j \right) \quad (2.19)$$

Where δ_{ij} is Kronecker delta. The second term on the left-hand side is the enhancement due to counter current convection. Equation 22 can be simplified if counter current vessels are straight. Straight vessels mean that $\frac{\partial l_i}{\partial x_i}$ would be zero and the first term on the right-hand side vanishes. There is no blood perfusion term in the counter current heat exchanging model. The blood perfusion effects are included in the enhancement factor $(k_{ij})_{eff}$ which makes the equation easy to solve. This model assumes that the tissue temperature is the average temperature of the arteriole and venule. This assumption is essential for eliminating blood temperature in the WJ model, but it was challenged by Wissler (Baish et al., 1986a) who opposed the idea that blood and tissue temperatures are closely related (Baish et al., 1986b). Wissler calculated the temperature profile for a small volume of tissue containing one pair of counter current vessels. He demonstrated that the WJ model failed to predict the temperature profile in many conditions (Baish et al., 1986b, 1986a). Weinbaum modified their assumptions and proposed new ways to derive the WJ model. By assuming that the temperature gradient of tissue is equal to that of the blood vessel and the second derivative of them are equal, the modified WJ model is applicable to vessels smaller than 0.3 mm (Weinbaum and Jiji, 1989).

2.4.2 Model comparisons

All three models described above have their pros and cons when applied to different situations. Pennes equation is convenient and can be readily coupled with drug transport equations. In order to ensure the accuracy of the simulation results, an appropriate estimation of the blood perfusion rate would be required. BHT model is suitable for vessels with a diameter smaller than 0.3 mm and thermal equilibrium ratio less than 0.6 ($\epsilon < 0.6$). ϵ is defined as the ratio between thermal equilibrium length and actual vessel length (Arkin and Ross, 1994). Since Pennes model describes the capillary diffusion by a source like perfusion term, it is not suitable to simulate tissue that is adjacent to vessels because these vessels may introduce local convection which is not stated in Pennes model. Pennes model is widely applied to different tissues such as kidney and deep muscle. The modified version of the Pennes equation proposed by Wulff considers the local convection effect by adjusting the blood perfusion term. It allows heat transfer to all directions. However, the blood velocity term in his equation is difficult to solve since the vascular structure is complicated. It can be applied to all situations where Pennes model is valid (Baish

et al., 1986b; Chary and Jain, 1989). The Incomplete Countercurrent heat exchange model provides a new understanding of the heat transfer mechanism in living tissue. However, it has a narrower application range. It is applicable when vessel diameter is less than 0.3mm and thermal equilibrium ratio is smaller than 0.3 (Baish et al., 1986b). Although all three methods have their advantages, none of them can be applied to all tissues. In most simulations, the Pennes model is still used to predict temperature profile since it has acceptable accuracy and relatively low complexity which makes it easy to implement and is computationally less demanding.

2.5 Computational models for drug delivery

Drug transport in solid tumours has been studied theoretically and by using computational models based on transport equations. It is essential for us to understand the transport mechanisms and their dynamic interactions in order to improve drug penetration and therapeutic efficacy. Several insightful models have been developed focusing on different aspects of drug transport, namely PBPK/PD models, microscopic transport models and macroscopic transport models.

2.5.1 PBPK/PD model

Pharmacokinetic (PK) models use mass balance equations to describe the absorption, distribution, metabolism, and excretion of drugs within the human body. To investigate the drug effects on a particular organ or tissue, a physiologically based pharmacokinetic (PBPK) model is employed. PBPK models provide a more detailed analysis of the inter-compartment connections through the circulatory system. When combining with the pharmacodynamics (PD) model, the time-course of drug effects can be described quantitatively (Danhof et al., 2008). PBPK/PD models can not only simulate conventional chemotherapy but can also be modified to simulate nanoparticle-mediated drug delivery. A schematic diagram of nanoparticle-mediated drug delivery PBPK/PD model is shown in figure 2.8 (Zhan et al., 2018). Figure 2.8 shows that the model is composed of multiple compartments including normal tissue (NT), blood in the circulatory system (BP), tumour intravascular space (IVS), tumour extracellular space (ECS) and intracellular space (ICS). Nanoparticles, free drugs, and protein bounded drugs are shown as NP, FD, and BD respectively. Only the free drug can access the ICS. All drugs are removed continuously by the blood clearance effects in the BP compartment.

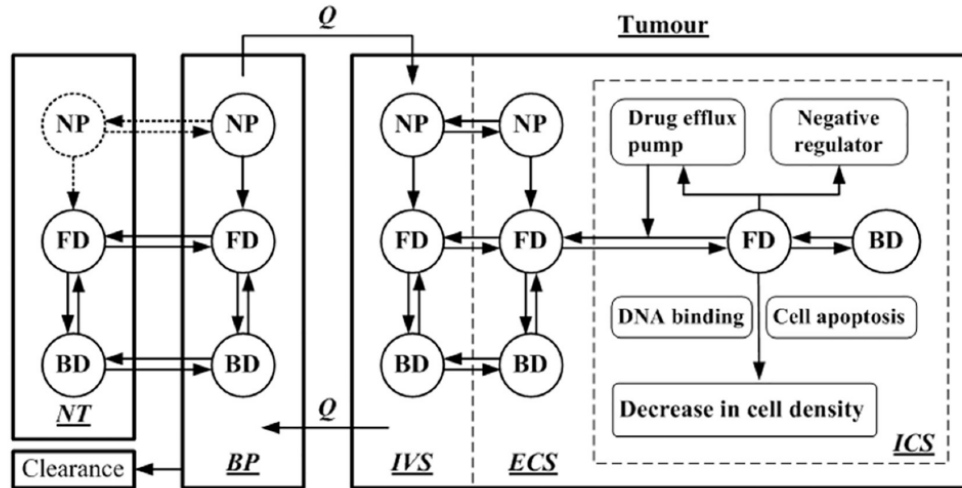


Figure 2. 8: Schematic diagram of a multi-compartmental PBPK/PD model(Zhan et al., 2018)

PK/PD models can be used to provide different outputs. For example, by incorporating an empirical correlation for cell killing into a conventional PK/PD, it would be possible to estimate the cell survival rate. The latter is usually related to the peak intracellular concentration of free drug (El-Kareh and Secomb, 2002) or the area under the concentration-time curve (AUC) (Skipper, 1965). However, for different anticancer drugs and tumour types, the empirical correlations for cell survival rate would be different. For anticancer drugs that affect the cell-cycle, more detailed killing functions have been developed based on the type of drug and tumour (Barnum and O'Connell, 2014).

PBPK/PD model serves a good starting point for us to understand the drug distribution in different compartments. It gives the prediction of cell survival rate as a function of time. It is also very flexible and can be modified to investigate more phenomena such as drug resistance and drug infusion mode (O'Connor et al., 2007).

2.5.2 Microscopic transport model

Although PBPK/PD models can shed light on the temporal distribution of drugs among different compartments, they provide no information about the spatial distribution. It is critical for us to understand the spatial distribution of bioavailable drugs since it has a direct influence on treatment efficacy. Microscopic transport model involves the use of partial differential equations (PDEs) to describe the fluid flow and drug transport mechanism. Based on various assumptions different microscopic models have been developed focusing on different transport aspects.

2.5.2.1 Tumour cord model

Tumour cord model is an idealised model that assumes a uniform distribution of blood vessels and a solid tumour consists of many tumour cords. The geometry is simplified to be a straight cylindrical blood vessel surrounded by tumour tissue. Drug, oxygen and nutrients are supplied from the central vessel to the surrounding tumour tissue — all concentrations decay toward the periphery of the cord. Figure 2.9 shows a schematic drawing of a single tumour cord.

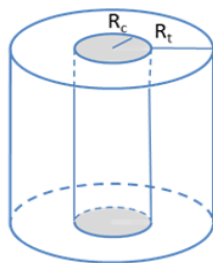


Figure 2. 9: Schematic drawing of a single tumour cord

A tumour cord model can be combined with a PBPK/PD model to provide spatial drug concentration distributions in different compartments. Convection and diffusive transport can be implemented in a tumour cord model, however, due to the high interstitial pressure in the tumour tissue, drug transport is usually dominated by diffusion. Eikenberry [31] evaluated the relationship between therapeutic efficacy and drug dose, and their results revealed that for low drug doses, bolus injection performed better. In contrast, continuous infusion would be preferred for high doses (Eikenberry, 2009a). Recent study shows that the higher the diffusivity of the drug the more uniform the spatial concentration distribution (Hubbard et al., 2017).

The assumption of uniformly distributed vasculature may lead to inaccuracy in drug transport simulations. Because of the fast growth of tumour cells, tumours usually have a highly irregular vascular structure which affects the drug distribution significantly. Angiogenesis models can be employed to simulate irregularly formed blood vessels (Anderson and Chaplain, 1998; Stéphanou et al., 2005). Results show that the drug concentration distribution is more heterogeneous if the simulation accounts for realistic tumour vascular structure. An example of the tumour vascular structure produced by high-resolution ex-vivo technique is shown in figure 2.10.

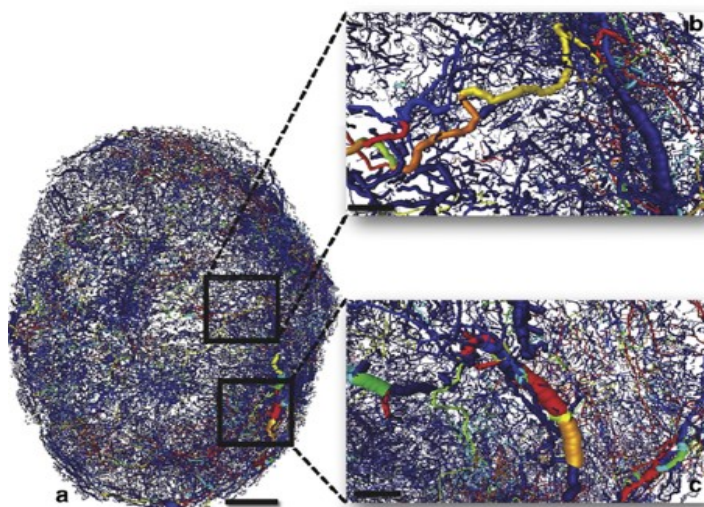


Figure 2. 10: An example of tumour vascular structure (Stamatelos et al., 2014)

Microscopic models can be built to account for transvascular transport and heterogeneity of the tumour extracellular space which reflects the tumour microenvironment explicitly. However, extending the model to a realistic sized-tumour to obtain macroscopic drug distribution would be computationally very challenging.

2.5.2.2 Macroscopic transport model

At the macroscopic level, a solid tumour can be treated as a porous medium for interstitial fluid flow and drug transport. The tumour vasculature is not usually modelled explicitly, but rather treated as a uniformly distributed source term in the transport equations. In addition, homogeneity in the tumour interstitial space is usually assumed. This assumption can be relaxed by dividing a tumour into multiple regions with each having a different vascular density to accommodate heterogeneous vascular distribution, as shown by Zhan et al (Zhan et al., 2014). Macroscopic drug transport models can provide drug concentration distributions in space and time. When coupled with a PBPK/PD model, cell survival fraction can also be evaluated which gives insight into the therapeutic efficacy (Zhan et al., 2014).

One of the advantages of macroscopic models is that they can be used to simulate drug distribution in real tumour geometry reconstructed from patient images. High resolution computed tomography (CT) and magnetic resonance images are useful resources to build the tumour geometry as shown in Fig 2.13 (Zhan et al., 2014).

2.5.3 Modelling thermosensitive liposome-mediated drug delivery

As mentioned previously, conventional chemotherapy has two main issues namely systemic toxicity and insufficient drug accumulation at the tumour site. Thermosensitive liposome-mediated drug delivery provides a

promising alternative way that reduces the side effect and elevates the therapeutic efficacy. PBPK-based compartmental models are usually employed to simulate and predict the efficacy of thermosensitive liposome-mediated drug delivery. When coupled with the transport equations describing diffusion and convection, these models can provide spatial and temporal distributions of drug concentration at both microscopic and macroscopic levels.

A previous computational study showed that the drug release rate from thermosensitive liposomes (TSLs) is a key parameter that determines the peak intracellular drug concentration (Gasselhuber et al., 2012b). A parametric sensitive analysis demonstrated that the drug release rate and heating duration could be optimised to achieve maximum drug concentration in tumour intracellular space and minimum drug concentration in systemic plasma (Liu and Xu, 2015a). Comparisons of predicted intracellular drug concentration and systemic drug concentration with those of the conventional chemotherapy showed that TSL drug delivery had lower drug accumulation in normal tissue while achieving a higher drug concentration in tumour intracellular space.

Since drug release from TSL is triggered by the application of high intensity focused ultrasound (HIFU), the heating effect can be integrated into computational models. Heating is usually treated as a source term in bio-heat transfer equations. In some models an instantaneous heating assumption is made to decouple the bio-heat transfer equation from the system to save the computational cost (Liu and Xu, 2015a). This assumption states that when HIFU is applied the temperature in the tumour compartment reaches 42°C instantaneously. The assumption is valid when the simulation scale is within micrometres (Liu and Xu, 2015a). However, for a macroscopic simulation, the BHT equation has to be integrated since the temperature cannot reach the liposome phase transition temperature instantaneously. Also, the spatially distribution of temperature is no longer uniform. The spatial distribution of is a critical factor that controls the drug release and distribution. Wenbo has developed a macroscopic liposome-mediated drug delivery. His model coupled the ultrasound heating effect with a single focal point at the centre of the tumour. Although the side effect is proven to be reduced, the therapeutic efficacy improved limitedly comparing with the free drug infusion due to the insufficient heating. The study shows that a single focal cannot heat the whole tumour to the phase transition temperature of the liposome (Zhan et al., 2019). His study sheds light on improving the therapeutic efficacy with the help of ultrasound and thermosensitive liposome. Therefore the multifocal heating, which improves the temperature distribution, is considered to achieve a better treatment efficacy (Liu and Xu, 2015b).

2.6 Objective of the study

This project aims to develop mathematical models to simulate TSL-mediated drug delivery to a solid tumour, by incorporating the heating effects induced by high intensity focus ultrasound (HIFU). These models are then used to identify the key parameters that affect the therapeutic efficacy and the risk of side effect associated with this delivery system. The ultimate goal is to optimise the treatment outcome and provide guidance on the clinical treatment strategy.

TSL-mediated drug delivery is a multiphysics process and modelling such a complex system is a significant challenge. It involves simulating the acoustic pressure field generated by the FUS transducer array, incorporating the acoustic pressure field into the bio-heat transfer equation, predicting temperature distributions in the tumour and blood compartments, and coupling the heating effects with the drug transport model. The latter includes drug transport within blood vessels, extravasation and interstitial penetration via convection and diffusion, drug uptake by tumour cells and evaluating the survival fraction of cancer cells based on pharmacodynamics. The mathematical models contain a large number of parameters which can be divided into two groups, namely physiochemical properties of the drugs and the biological properties of the tumour. Critical parameters of drugs include the diffusivity, permeability, equilibrium constant of binding to proteins. Tumour properties such as cell density, tumour size, and interstitial fluid pressure can affect therapeutic efficacy. In addition, changes in temperature induced by HIFU also influence the transport properties and predicted outcomes.

In order to achieve the overall project aim, the following objectives have been specified,

- (1) To develop a multicompartmental model for TSL drug delivery with a focus on microscopic drug distribution in a tumour cord model.
- (2) Using the above model to identify the key transport parameters that affect the predicted outcomes.
- (3) To make an initial attempt towards optimising the treatment outcome by varying the parameters identified in objective 2.
- (4) To develop a multiphysics model by incorporating the effects of focus ultrasound (FUS) heating into a drug transport model, and apply this integrated model to realistic tumour geometry.
- (5) Using the model developed in objective 4 to examine how the location of focal point may affect the temperature distribution in tumour tissue.

Chapter 3: Methodology

This chapter describes the mathematical models for two types of simulations covered in this thesis: (1) a multi-compartmental model applied to the tumour cord geometry, and (2) a multi-physics continuum-based model applicable to realistic tumour geometries. The mathematical equations are described, which include those for interstitial fluid flow, diffusion and convection of anticancer agent in tumour interstitial space, as well as drug uptake by tumour cells. Acoustic effects upon the application of high intensity focus ultrasound (HIFU) and bio-heat transfer equations are incorporated into the continuum-based model for simulations of HIFU-induced heating in tumour and its influence on drug release from thermosensitive liposomes (TSL). Details of model parameters are explained and the choice of their values discussed. The numerical methods employed to solve the equations are also described.

3.1 Multi-compartmental model

A schematic diagram of the computational model describing the interactions of multiple transport steps is shown in Figure 3.1. A multi-compartment modelling approach is employed to simulate the PK of TSLs and anticancer drugs at the systemic level as well as drug transport in the tumour compartment that is composed of tumour plasma, tumour extravascular extracellular space (EES) and tumour cells. The computational model has been improved upon a previous model developed in our group (Liu and Xu, 2015b) by accounting for the spatial distribution of free drug (bioavailable) in tumour interstitial space, while drug-loaded TSLs are injected intravenously and triggered to release their encapsulated contents in response to hyperthermia exposure at the targeted tumour site. There are eight variables representing drug concentrations in either liposomal or free form in different compartments and the mass conservation equation for each variable incorporates kinetic transfer and reaction processes. An idealised tumour cord geometry is employed for simplification and computational efficiency, which consists of a cylindrical tube representing the capillary vessel (with a radius $R_c = 10 \mu m$) and its surrounding tumour interstitium (with a radius $R_t = 120 \mu m$). Given the assumption of axis-symmetry and homogeneous distribution along tumour microvessels, one-dimensional drug transport in the radial direction is formulated.

Hyperthermia exposure is the required external stimulus to trigger anticancer drug release from TSLs. Although the spatio-temporal distribution of temperature in tumour tissue can be predicted by coupling bio-heat transfer models (e.g. Pennes' model) with drug transport models (Gasselhuber et al., 2010, 2012a; Zhan et al., 2019), the

focus of the multi-compartmental model is on resolving drug distributions in the tumour microenvironment (i.e., capillary and its surrounding interstitial space). Therefore, a uniform temperature is assumed. This simplification allows for efficient computation, which is advantageous especially for model-based optimisation that involves numerous iterative steps.

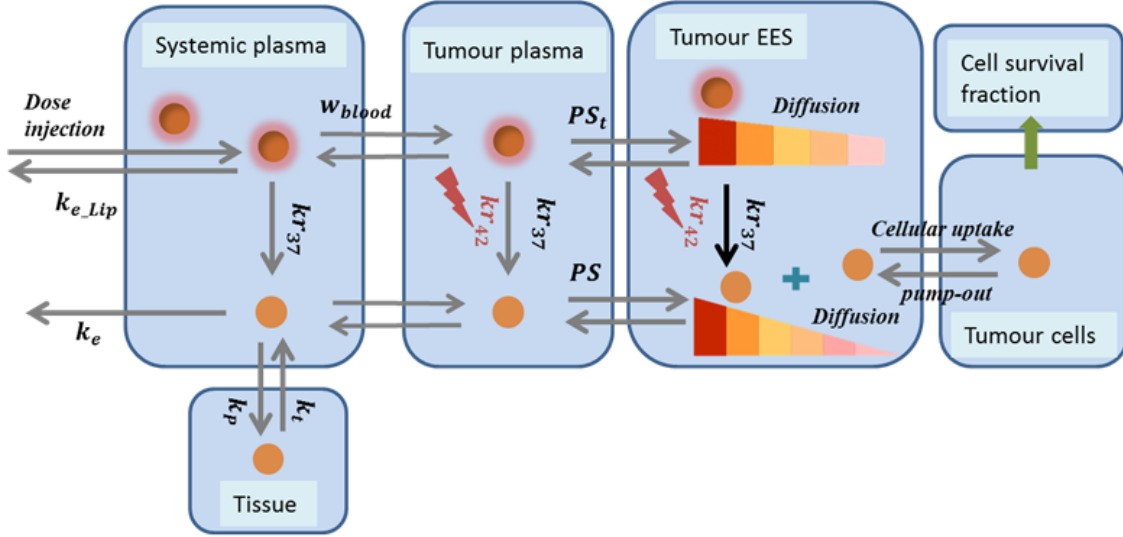


Figure 3. 1: Overview of the multi-compartmental model used in this study: two compartments of “systemic plasma” and “tissue” for systemic effects of drug and the tumour compartment comprising of “tumour plasma”, “tumour extravascular extracellular space (EES)” and “tumour cells”. (Liu and Xu, 2015b)

3.1.1 Systemic drug transport: Pharmacokinetics of TSLs and anticancer drug

Pharmacokinetics of TSLs and an anticancer drug can be described by a two-compartment model, with the assumption that only anticancer drug can move between the central (systemic plasma) and peripheral compartments (lumped tissue). Temporal variations in the systemic concentrations of TSL and anticancer drug and the tissue-level concentration of anticancer drug for bolus injection of TSL can be described by:

$$V_P^B \frac{dc_{P,Lip}^B}{dt} = -k_{e,Lip} c_{P,Lip}^B V_P^B - kr_{37} c_{P,Lip}^B V_P^B + F_{PV}^T V_P^T c_{P,Lip}^T - F_{PV}^T V_P^T c_{P,Lip}^B \quad (3.1.1)$$

$$V_P^B \frac{dc_P^B}{dt} = kr_{37} c_{P,Lip}^B V_P^B - k_e c_P^B V_P^B - k_p c_P^B V_P^B + k_t c_t^B V_t^B + F_{PV}^T V_P^T c_P^T - F_{PV}^T V_P^T c_P^B \quad (3.1.2)$$

$$V_t^B \frac{dc_t^B}{dt} = k_p c_P^B V_P^B - k_t c_t^B V_t^B \quad (3.1.3)$$

where V is the volume of each compartment, c the concentration of TSL or drug, kr_{37} the drug release rate at the body temperature, k_e the elimination rate from the central compartment, and k_p and k_t are the rate

constants for drug transfer between the central plasma and tissue compartments. The subscripts P, t, Lip denote the plasma, tissue and liposome, respectively. The superscripts B and T represent the systemic level and the tumour compartment, respectively. F_{PV}^T is the plasma flow per tumour plasma volume, defined as: $F_{PV}^T = w_{blood}(1 - H_{ctt})/v_P^T$ in which w_{blood} , H_{ctt} and v_P^T are the perfusion rate in [mL/s/mL], haematocrit and volume fraction of tumour plasma, respectively.

3.1.2 Drug transport in the tumour compartment

3.1.2.1 Transport in tumour plasma

The release of anticancer drug from TSLs in tumour plasma depends on temperature and concentration of TSLs, which can be described by the first-order kinetics with the release rate constants kr_{37} and kr_{42} at the body temperature and mild hyperthermia upon heating, respectively. The dynamic concentrations of TSLs and anticancer drug are described by:

$$V_P^T \frac{dc_{P,Lip}^T}{dt} = F_{PV}^T V_P^T c_{P,Lip}^B - F_{PV}^T V_P^T c_{P,Lip}^T - R \cdot c_{P,Lip}^T V_P^T \quad (3.1.4)$$

$$V_P^T \frac{dc_P^T}{dt} = F_{PV}^T V_P^T c_P^B - F_{PV}^T V_P^T c_P^T + R \cdot c_{P,Lip}^T V_P^T \quad (3.1.5)$$

$$R = \begin{cases} kr_{42}, & \text{for } T_c \leq t \leq T_c + T_h \\ kr_{37}, & \text{otherwise} \end{cases} \quad (3.1.6)$$

where R is the release rate constant determined by temperature and T_c and T_h are the initial time and duration of hyperthermia, respectively.

3.1.2.2 Transport in the tumour interstitial space

Both diffusive and convective transport mechanisms are important in determining the accumulation of drug in tumour interstitium. Anticancer drugs are also taken up and pumped out by tumour cells, which can be modelled using the first-order kinetics and Michaelis-Menten kinetics for both passive diffusion and active transport. Therefore, the concentration of anticancer drug in tumour interstitium can be described by the convection-diffusion-reaction equation, whereas the transport of TSLs is assumed to be diffusion-dominated.

$$\frac{\partial c_e}{\partial t} = D \nabla^2 c_e - c_t \left(k_{1ci} c_e + \frac{V_{max} c_e}{c_e + K_e v_e^T} - \frac{V_{max} c_i}{c_i + K_i} \right) - u_i \cdot \nabla c_e \quad (3.1.7)$$

$$\frac{\partial c_i}{\partial t} = k_{1ci} c_e + \frac{V_{max} c_e}{c_e + K_e v_e^T} - \frac{V_{max} c_i}{c_i + K_i} \quad (3.1.8)$$

$$\frac{\partial c_{e,Lip}}{\partial t} = D_{Lip} \nabla^2 c_{e,Lip} - R \cdot c_{e,Lip} \quad (3.1.9)$$

where c_e is the extracellular concentration, c_i the intracellular anticancer drug, D the diffusivity, v_e^T the volume fraction of tumour EES, k_{1ci} the rate constant for passive transport, V_{max} the maximum rate for transmembrane transport, K_e and K_i the Michaelis constants, and R is the release rate constant defined in Eq. (3.1.6). The blood

flow velocity u_i in the interstitial space is determined by the permeability of vessel walls (K) and pressure difference between the blood vessel and tumour interstitial space (P_i) via Darcy's law.

$$u_i - \nabla \cdot (KP_i) = 0 \quad (3.1.10)$$

$$\nabla u_i = 0 \quad (3.1.11)$$

Solving Eqs. (3.1.7), (3.1.10) and (3.1.11) requires boundary conditions for c_e and u_i . Starling's law and Kedem-Katchalsky equations are used to prescribe the transmural blood flow velocity and drug flux at the capillary walls, i.e., at $r = R_c$. It is assumed that osmotic pressure is negligible compared to the pressure gradient in determining the transmural velocity in solid tumour (Li et al., 2011). These boundary conditions are expressed as:

$$u_i = J_F = L_p(P_v - P_i), \text{ at } r = R_c \text{ and for } t > 0 \quad (3.1.12)$$

$$-D \frac{\partial c_e}{\partial r} = P(c_p^T - c_e) + J_F(1 - \sigma_F) \frac{c_v - c_e}{\ln(\frac{c_v}{c_e})}, \text{ at } r = R_c \text{ and for } t > 0 \quad (3.1.13)$$

where J_F is the transmural velocity, P_v the vessel pressure, c_v is the drug concentration in the vessel side, L_p the hydraulic permeability and σ_F the reflection coefficient. At the outer boundary of the tumour cord ($r = R_t$), no flux is prescribed for c_e as expressed in Eq. (3.1.14).

$$\frac{\partial c_e}{\partial r} = 0, \text{ at } r = R_t \text{ and for } t > 0 \quad (3.1.14)$$

3.1.3 Parameterisation and initialisation

Values of parameters in the model equations are adopted from the literature or estimated from relevant *in vitro* experimental data (see Table 3.1). Parameters characterising TSLs, namely release rates at body temperature and during hyperthermia (kr_{37} and kr_{42}), are strongly dependent on the TSL formulation. Release rates used in this study are estimated as approximation of linear kinetics by fitting to release curves of TOP-iTSL (Centelles et al., 2018b), and the same values are used for DOX-TSL. Also, the elimination rate of TOP and DOX from the systemic plasma is assumed the same, as their average half-life is similar, 2-3 hours for TOP (Heron, 1998) and 1-3 hours for DOX (Gabizon et al., 2003). Other parameters for iTSLs are assumed to be identical to those for TSLs, found in the literature listed in Table 3.1. Finally, initial values for all variables are set to zero, except for TSLs concentration in systemic plasma for bolus injection, which is determined by $Dose/V_p^B$.

Table 3. 1: Parameters and baseline values used in the computational model

Symbol	Description	Value	Reference
$k_{e,Lip}$	Rate constant of TSLsclearance	9.417×10^{-6} [1/s]	(Gasselhuber et al., 2012a)
k_e	Rate constant of drug clearance	1.1×10^{-3} [1/s]	(Gasselhuber et al., 2010)
k_p	Transfer constant from systemic plasma to tissue	1.6×10^{-3} [1/s]	(Gasselhuber et al., 2010)
k_t	Transfer constant from tissue to systemic plasma	4.68×10^{-5} [1/s]	(Gasselhuber et al., 2010)
kr_{37}	Release rate constant from iTSLs at body temperature	3×10^{-4} [1/s]	(Centelles et al., 2018b)
kr_{42}	Release rate constant from iTSLs during HT (at 42°C)	0.114 [1/s]	(Centelles et al., 2018b)
K_e	Michaelis constant for transmembrane transport	2.19×10^{-4} [$\mu\text{g}/\text{mm}^3$]	(El-Kareh and Secomb, 2002)
K_i	Michaelis constant for transmembrane transport	1.37 [$\text{ng}/10^5$ cells]	(El-Kareh and Secomb, 2002)
$V_{\max}$	Maximum rate for transmembrane transport	0.28 [$\text{ng}/(10^5 \text{ cells} \cdot \text{min})$]	(El-Kareh and Secomb, 2002)
k_{1ci}	Rate for passive intracellular uptake	6.33×10^{-4} [1/s]	Fit to (Gasselhuber et al., 2010)
V_p^B	Volume of systemic plasma	3.04 [L]	(Gasselhuber et al., 2012a)
V_t^B	Volume of body tissue	64.47 [L]	(Gasselhuber et al., 2012a)
V^T	Volume of tumour tissue	8.82×10^{-2} [L]	Estimated based on a spherical tumour with a radius of 2.7cm
v_p^T	Volume fraction of tumour plasma	0.07452	(Gasselhuber et al., 2012a)
v_e^T	Volume fraction of tumour EES	0.454	(Gasselhuber et al., 2012a)
v_i^T	Volume fraction of intracellular space	0.454	(Gasselhuber et al., 2012a)

w_{blood}	Blood perfusion rate	0.018 [1/s]	(Gasselhuber et al., 2012a)
H_{ctt}	Haematocrit for tumour microvasculature	0.19	(Gasselhuber et al., 2012a)
PS_t	Permeability surface area product for drugs	2.53×10 ⁻³ [1/s] (TOP) 7×10 ⁻³ [1/s] (DOX)	Estimated based on molecular size (Gasselhuber et al., 2012a)
PS_L	Permeability surface area product for TSL	4.76×10 ⁻⁶ [1/s] (TOP) 2.38×10 ⁻⁵ [1/s] (DOX)	Estimated using the PS_t , P and P_{lip} of DOX and TOP respectively
P	Diffusive permeability for drugs	3.61×10 ⁻⁷ [m/s] (TOP) 1×10 ⁻⁶ [m/s] (DOX)	Estimated based on molecular size (El-Kareh and Secomb, 2002)
P_{lip}	Diffusive permeability for TSLs and iTSLs	3.4×10 ⁻⁹ [m/s]	(El-Kareh and Secomb, 2002)
D	Diffusion coefficient	4.123×10 ⁻¹⁰ [m ² /s] (TOP) 1.578×10 ⁻¹⁰ [m ² /s] (DOX)	Estimated based on molecular size (Eikenberry, 2009a)
D_{lip}	Diffusion coefficient for TSLs and iTSLs	9×10 ⁻¹² [m ² /s]	(Zhan and Xu, 2013)
R_c	Tumour capillary radius	10 [μm]	(Eikenberry, 2009a)
R_t	Tumour cord radius	120 [μm]	(Eikenberry, 2009a)
V_c	Volume of single tumour cell	1×10 ⁻⁶ [mm ³ /cell]	(El-Kareh and Secomb, 2002)
$Dose$	Total dose	49 [mg]	Calculated at a dose of 0.7 mg/kg in a 70 kg human

3.1.4 Numerical methods

Spatial derivatives in the model equations are discretised via the finite difference methods. As a result, the partial differential equations become a set of ordinary differential equations to resolve temporal concentrations at each discretised segment. The system of ordinary differential equations for TSL and drug concentrations in each compartment is solved numerically by means of an in-built solver in MATLAB (ode15s). Mesh sensitivity tests are carried out and the final grid size is $\Delta r = 0.4 \mu m$ and time step $\Delta t = 0.1s$.

3.1.5 Optimisation

Using the developed mathematical model, an optimisation framework is established in order to identify the best drug release properties of TSL and heating schedule that can achieve reduced side effects and enhanced treatment outcome. Specifically, we aim to optimise the drug release rates (kr_{37} and kr_{42}) and hyperthermia duration (T_h) for maximised intracellular drug concentration (c_i) and minimised systemic drug concentration (c_{BP}) over time, which are indicative of therapeutic efficacy and the risk of side effects, respectively. For this purpose, a multi-objective optimisation problem is formulated, which can be solved by the weighted sum method and described as follows:

$$\begin{aligned}
 & \underset{kr_{37}, kr_{42}, T_h}{\text{Minimise}} \begin{cases} J_1 = \frac{\bar{c}_B^P}{\bar{c}_{B,base}^P} \\ J_2 = -\frac{\bar{c}_i}{\bar{c}_{i,base}} \end{cases} = \underset{kr_{37}, kr_{42}, T_h}{\text{Minimise}} (w_1 J_1 + w_2 J_2) \\
 & \text{subject to } w_1 + w_2 = 1 \\
 & \text{Eqs. (1)-(15)} \\
 & kr_{37,lb} \leq kr_{37} \leq kr_{37,up} \\
 & kr_{42,lb} \leq kr_{42} \leq kr_{42,up} \\
 & T_{h,lb} \leq T_h \leq T_{h,up}
 \end{aligned} \tag{3.1.15}$$

where J_1 and J_2 are the objective functions to be minimised that represent time-averaged systemic plasma drug concentration and intracellular drug concentrations normalized by their respective baseline values (as defined in Table 3.1), and w_i the weighting factor for the i -th objective function value. The model equations presented in Eqs. (3.1.1) to (3.1.14) serve as equality constraints and each optimisation variable is bounded by its lower and upper limits defined as: $0.1kr_{37.ref} \leq kr_{37.ref} \leq 10kr_{37.ref}$, $0.1kr_{42.ref} \leq kr_{42.ref} \leq 10kr_{42.ref}$ and $0 \leq T_h \leq 3600$ sec. The weighting factor w_1 is varied between 0 and 1 and optimisation results for different weighting factors are compared. Also, multiple sets of initial guesses of the optimisation variables are chosen to ensure global optimality. The above optimisation problem is solved via an in-built function in MATLAB (fmincon), designed for constrained nonlinear multivariable problems.

3.2 Continuum-based model for drug transport

A multiphysics continuum-based model has been developed to simulate the drug delivery process mediated by thermosensitive liposomes (TSL). This model integrates fluid flow, drug transport between systemic blood and tumour tissue, diffusion and convection of drug within the tumour interstitial space, the acoustic field upon application of focused ultrasound, bio-heat transfer, and drug uptake by tumour cells via a pharmacodynamics

model. Due to the complexity of this system, some reasonable assumptions are made. Firstly, the tumour is assumed to be homogeneous with uniform transport properties. Secondly, tumour blood vessels are treated as a uniformly distributed source term (no necrotic core) in the transport equation rather than being modelled explicitly. Thirdly, all tumour cells are alive initially and their movements are negligible. Finally, all tumour properties are constant within the simulation window, as the latter is much shorter compared to the tumour growth time. Detailed mathematic equations are described below.

3.2.1 Mass conservation of interstitial fluid

The conservation of mass in a living tissue needs to account for fluid supply from blood vessels (source term) and drainage through the lymphatic system (sink term). This can be written as

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho v) = (F_v - F_{ly})\rho \quad (3.2.1)$$

Where ρ and v are the density and velocity of interstitial fluid. F_v is fluid that transfers from blood vessel per unit volume of tumour tissue. F_{ly} is fluid drained by the lymphatic system per unit volume of tumour tissue. Both of them are described by Starling's law.

$$F_v = K_v \frac{S}{V} [p_v - p_i - \sigma_T(\pi_v - \pi_i)] \quad (3.2.2)$$

$$F_{ly} = K_{ly} \frac{S_{ly}}{V} (p_i - p_{ly}) \quad (3.2.3)$$

Where K_v and K_{ly} are hydraulic conductivity of microvascular wall and lymphatic wall respectively. $\frac{S}{V}$ and $\frac{S_{ly}}{V}$ are the surface area of blood vessels per unit volume of tumour tissue and the surface area of lymphatic vessels per unit volume of tumour tissue respectively. σ_T is the average osmotic reflection coefficient for the plasma protein. p_v , p_i and p_{ly} are pressures correspond to vascular, interstitial and lymphatic respectively. π_v and π_i are osmotic pressures of plasma and interstitial fluid respectively.

3.2.2 Momentum conservation of interstitial fluid

In the drug transport process, the distance that the drug travels is typically 2 to 3 orders of magnitude larger than the intercapillary distance. Therefore, the tumour and its surrounding tissue can be treated as porous media (Less et al., 1991; Yuan et al., 1994). Applying Navier-stokes equations ignoring the gravitational term, the conservation of momentum is shown in equation 3.2.4.

$$\frac{\partial(\rho v)}{\partial t} + \nabla \cdot (\rho v v) = -\nabla p_i + \nabla \cdot \tau + F \quad (3.2.4)$$

Where τ is the stress tensor and $\mathbf{F}$ is the Darcian resistance to flow through porous media. These are expressed by equations 3.2.5 and 3.2.6.

$$\boldsymbol{\tau} = \mu[\nabla \mathbf{v} + (\nabla \mathbf{v})^T] - \frac{2}{d}\mu(\nabla \cdot \mathbf{v})\mathbf{I} \quad (3.2.5)$$

$$\mathbf{F} = W\mu\mathbf{v} + \frac{1}{2}C\rho|\mathbf{v}|\mathbf{v} \quad (3.2.6)$$

Where ρ and μ are density and dynamic viscosity of the interstitial fluid. $\mathbf{I}$ represents the unit tensor, and W is the diagonal matrix composed by the inverse of the permeability of the interstitial space. It is calculated in equation 3.2.7.

$$W = \kappa^{-1} \quad (3.2.7)$$

Where κ is the permeability of the interstitium. $\mathbf{C}$ in equation 3.2.6 is the matrix representing the inertial loss. The interstitial flow velocity is very slow means that $|\mathbf{v}|$ is much less than 1. Therefore, the inertial loss term is negligible compared to the viscous loss term. Equation 3.2.6 is reduced to equation 3.2.8.

$$\mathbf{F} = W\mu\mathbf{v} \quad (3.2.8)$$

3.2.3 Drug transport

When modelling TSL-mediated drug delivery, there are several species that need to be described: drug-loaded TSL, released drug in its free form and drug in bound form. Moreover, these species can be present in the circulating blood or tumour interstitial space, with inter-exchanges between different compartments. In what follows, an overview of drug transport in different compartments is given first. This is followed by mathematical equations describing the transport of TSL and different forms of drugs.

The system is divided into three different compartments, namely systemic blood compartment, tumour extracellular space and intracellular space. Figure 3.2 shows a schematic drawing of the blood and tumour extracellular space. In all the simulations presented in this thesis, drug is administered through bolus injection which provides an initial liposome concentration in the systemic blood compartment. Liposomes can be removed by blood clearance effects, disrupted to release the encapsulated drug or move to the extracellular space compartment. Some of the free drugs in the blood compartment will bind with proteins to form bound drugs. The binding is reversible and depends on the equilibrium constant varying from drug to drug. Some of the free drugs will pass through the capillary wall and enter the extracellular space while the rest of the free drugs would

either remains in the systemic circulation or be cleared out of the system. Similarly, bound drugs can move to the extracellular space or be removed by blood clearance effects.

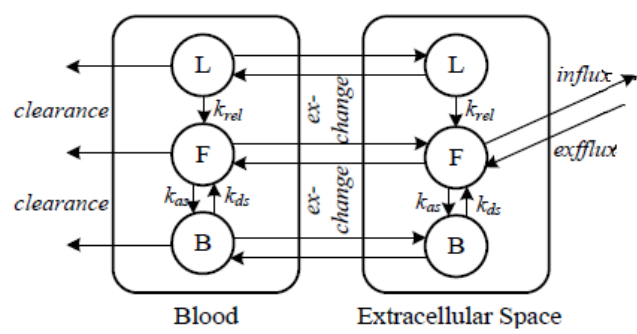


Figure 3. 2: Drug transport between blood and tumour extracellular space

In the extracellular space compartment, only free drugs can pass through the cell membrane owing to their small molecular size. Other forms of drug either accumulate in this compartment or reversely transfer back to the blood compartment and being removed. In the intracellular compartment, free drugs can be consumed by tumour cells to induce cell killing according to the pharmacodynamics model (shown in Figure 3.3). Intracellular drugs can also be pumped out of cells by membrane transporter proteins.

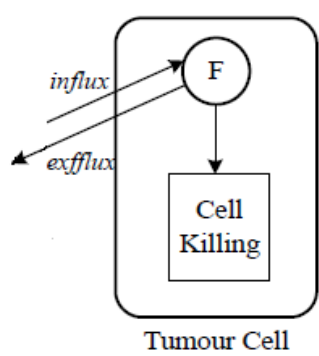


Figure 3. 3: Tumour intracellular compartment

Within systemic blood and the extracellular space, the drug transport is described by the diffusion and convection equations. The release of drug from TSL is triggered by hyperthermia when focused ultrasound heating is applied. The spatial-temporal temperature distribution is described by bio-heat transfer equations where acoustic power acts as a source term. Mathematical equations describing the transport of TSL and different forms of drugs are given below.

In the circulating blood, the liposome concentration (C_{lp}) is given by

$$\frac{dC_{lp}}{dt} = -CL_{lp}C_{lp} - \frac{V_T}{V_B}F_{lp} - S_r \quad (3.2.9)$$

Where CL_{lp} is the blood clearance rate of liposome. S_r represents the rate at which the encapsulated drug is released from TSL as free drug. The drug release rate depends on the drug release constant and liposome concentration as shown in equation 3.2.10.

$$S_r = k_{rel}C_{lp} \quad (3.2.10)$$

k_{rel} in equation 3.2.10 is the drug release constant which is temperature and formulation dependent. The release constant will be discussed further in model parameters.

F_{lp} in equation 3.2.9 represents the amount of liposome extravasating from blood plasma to the extracellular space by crossing the capillary wall. V_T and V_B are volumes of the tumour and the blood plasma respectively. F_{lp} is described by the pore model for trans-capillary exchange [49,50] .

$$F_{lp} = F_v(1 - \sigma_l)C_{lp} + P_{le}\frac{S}{V}(C_{lp} - C_{le})\frac{Pe_l}{e^{Pe_l} - 1} \quad (3.2.11)$$

The exchange rate depends on the osmotic reflection coefficient of liposome (σ_l), the permeability of vasculature wall to liposome (P_{le}) and the trans-capillary Peclet number Pe_l which is expressed as

$$Pe_l = \frac{F_v(1 - \sigma_l)}{P_{fe}\frac{S}{V}} \quad (3.2.12)$$

An initial liposome concentration in the blood compartment is defined based on the total drug dose. The release of the encapsulated drug is treated as the source of free drug. Therefore, the free drug concentration in blood plasma is expressed as,

$$\frac{\partial C_{fp}}{\partial t} = S_r - \frac{V_T}{V_B}F_{fp} - CL_{fp}C_{fp} - (k_a C_{fp} - k_d C_{bp}) \quad (3.2.13)$$

Equation 3.2.13 shows that the accumulation of free drug in blood is related to drug release in blood plasma, the trans-capillary exchange, the blood clearance effect, the drug association and dissociation with protein in the blood. The trans-capillary exchange is described similarly to equation 3.2.11 with the corresponding transport parameters. k_a and k_d are the association and disassociation rates with proteins.

Similar to the free drug concentration, the bound drug concentration is given by

$$\frac{\partial C_{bp}}{\partial t} = (k_a C_{fp} - k_d C_{bp}) - \frac{V_T}{V_B} F_{be} - CL_{bp} C_{bp} \quad (3.2.14)$$

The free drug association term works as the source of the bound drug. F_{be} represents the trans-capillary exchange of the bound drug which is described using pore model. The expression is similar to equation 3.2.11 with the relevant transport parameters.

At this point, the concentration of three different forms of the drug in blood plasma compartment is fully described. The diffusion and convection terms are introduced in governing equations in tumour extracellular space compartment to give the spatial distribution. The liposome concentration in extracellular space is described in equation 3.2.15.

$$\frac{\partial C_{le}}{\partial t} + \nabla \cdot (C_{le} \mathbf{v}) = D_l \nabla^2 C_{le} + S_l \quad (3.2.15)$$

The transport equation contains four terms namely the accumulation, convection, diffusion and a source term. D_l is the diffusion coefficient of the liposome. The source term S_l is composed by two parts,

$$S_l = S_v - S_r \quad (3.2.16)$$

Where S_r is the drug release and S_v is the net gain of liposome from blood plasma and lymphatic vessel.

$$S_v = F_{lp} - F_{ll} \quad (3.2.17)$$

Where F_{lp} is described in equation 3.2.18. F_{ll} is related to the fluid drained by lymphatic system and liposome concentration in tumour extracellular space.

$$F_{ll} = F_{ly} C_{le} \quad (3.2.18)$$

Free doxorubicin concentration in interstitial fluid (C_{fe}) is described in equation 3.2.19 which includes the accumulation, diffusion, convection and a source term.

$$\frac{\partial C_{fe}}{\partial t} + \nabla \cdot (C_{fe} \mathbf{v}) = D_{fe} \nabla^2 C_{fe} + S_i \quad (3.2.19)$$

Where D_{fe} is the diffusion coefficient of free drug.

S_i is the net rate of doxorubicin gained from the other compartments. There are three different sources of gaining free DOX namely blood/lymphatic vessels (S_v), association/dissociation with bound doxorubicin-protein (S_b) and influx/efflux from tumour cells (S_u). Equation 3.2.20 illustrates the source term explicitly.

$$S_i = S_v + S_b + S_u \quad (3.2.20)$$

Where S_v is further expressed as the differences between the DOX gained from the blood plasma compartment (F_{fp}) and the DOX loss to the lymphatic system (F_{fl}).

$$S_v = F_{fp} - F_{fl} \quad (3.2.21)$$

To calculate F_{fp} and F_{fl} , the pore model for trans-capillary exchange is employed (Baxter and Jain, 1990, 1989). The expressions of F_{fp} and F_{fl} are similar to F_{lp} and F_{ll} with corresponding transport parameters.

The binding and unbinding processes are first-order reactions respecting to the bounded drug concentration (C_{be}) and the free drug concentration (C_{fe}) respectively.

$$S_b = k_d C_{be} - k_a C_{fe} \quad (3.2.22)$$

Where k_d and k_a are the DOX-protein binding and dissociation rate constants.

S_u is the free drug exchanging between interstitial and intracellular space is described in equation 3.2.23.

$$S_u = D_c \varepsilon - D_c \zeta \quad (3.2.23)$$

Where D_c is the cell density of tumour, ε and ζ are the cell efflux and influx functions respectively. The transmembrane process is described in equation 3.2.24 and 3.2.25.

$$\varepsilon = V_{max} \frac{C_i}{C_i + k_i} \quad (3.2.24)$$

$$\zeta = V_{max} \frac{C_{fe}}{C_{fe} + k_e \varphi} \quad (3.2.25)$$

where V_{max} is the rate of transmembrane transport, and C_i is the free drug intracellular concentration. k_i and k_e are measured from experiments and φ is the extracellular space volume fraction.

Not all free drugs are available for cellular uptake since the free drug would bind to the protein in blood to form bound drug which cannot pass through the cell membrane. The bound drug is unavailable for tumour cell killing. Bound-doxorubicin concentration in extracellular space is described in equation 3.2.26.

$$\frac{\partial C_{be}}{\partial t} + \nabla \cdot (C_{be} \mathbf{v}) = D_{be} \nabla^2 C_{be} + F_{be} - S_b \quad (3.2.26)$$

Where D_{be} is the diffusion coefficient for bound doxorubicin-protein and S_b is the binding and unbinding term which is described in equation 3.2.22. F_{be} is the bound doxorubicin crossing the capillary wall into the interstitial fluid. It is expressed in a similar way to the free drug crossing the capillary wall (F_{fe}).

The intracellular drug concentration is given by

$$\frac{\partial C_i}{\partial t} = \zeta - \varepsilon \quad (3.2.27)$$

Where ζ and ε are the cell uptake and efflux respectively, as described in equations 3.2.24 and 3.2.25.

3.2.4 Pharmacodynamics model

By solving all the transport equations, the intracellular drug concentration can be obtained from equation 3.2.27. The relationship between DOX intracellular drug concentration and cell killing rate is described using a pharmacodynamics model in equation 3.2.28.

$$\frac{dD_c}{dt} = -\frac{f_{max}C_i}{EC_{50} + C_i}D_c + k_pD_c - k_gD_c^2 \quad (3.2.28)$$

Where f_{max} is cell kill rate constant and EC_{50} is the drug concentration corresponding to 50% f_{max} . k_p and k_g are cell proliferation rate and physiologic degradation rate. The cell death achieved by the treatment is estimated using equation 3.2.28.

3.2.5 Acoustic energy

To trigger drug release, focused ultrasound is applied at the tumour site. The acoustic effect is simulated using the fast near field method which is reviewed in the previous chapter. Since the tumour is treated as a homogeneous tissue and high frequency ultrasound is applied (1 MHz), the non-linear effect of ultrasound is negligible. The governing equations are shown in equations 3.2.29 and 3.2.30.

$$p(r, z; k) = -i\omega\rho c e^{i\omega t} H(r, z; k) \quad (3.2.29)$$

The acoustic pressure p is related to the excitation frequency ω , propagation velocity c , tissue density ρ and the Fourier transform of impulse response function H . The impulse response function can be further expressed as

$$H(r, z; k) = \frac{a}{j2\pi k} \int_0^\pi \frac{r \cos\psi - a}{a^2 + r^2 - 2ar \cos\psi} \times \left(-e^{-jkz} + e^{-jk\sqrt{r^2 + a^2 - 2ar \cos\psi + z^2}} \right) d\psi \quad (3.2.30)$$

where ψ is the velocity potential and a is the radius of the circular boundary.

By solving equations 3.2.29 and 3.2.30, the acoustic pressure p can be obtained. The acoustic pressure field acting on a living tissue will convert to acoustic energy absorbed by the tissue. The acoustic energy is given by

$$Q_{FU} = 2\alpha_{ABS}I \quad (3.2.31)$$

where α_{ABS} is an absorption coefficient depending on tissue properties and I is the acoustic intensity which is related to the acoustic pressure.

$$I = \frac{pp^*}{2\rho c} \quad (3.2.32)$$

where p^* is the complex conjugate of p .

The acoustic energy is coupled with the bio-heat transfer equation as a source term.

3.2.6 Bio-heat transfer equation

In order to obtain the temperature distribution, the bio-heat transfer equation (Pennes, 1948) is employed. By performing energy balance on blood plasma and tumour extracellular space, the follow expressions can be obtained.

$$\rho_t c_t \frac{\partial T_t}{\partial t} = k_t \nabla^2 T_t - \rho_b c_b w_b (T_t - T_b) + q_t \quad (3.2.33)$$

$$\rho_b c_b \frac{\partial T_b}{\partial t} = k_b \nabla^2 T_b + \rho_b c_b w_b (T_t - T_b) - \rho_b c_b w_b (T_b - T_n) + q_b \quad (3.2.34)$$

where ρ , c , k are the density, heat capacity and thermal conductivity respectively. q is the acoustic energy absorbed by blood and tumour tissue. The subscripts b , t , and n represent blood, tumour and normal tissue, respectively.

3.2.7 Temperature control

Upon the application of focused ultrasound, tissue temperature in the vicinity of the focal point can rise rapidly. The desirable temperature to trigger drug release from TSL is 42°C. To ensure temperature at the tumour site is within a desired range and to avoid overheating, a temperature controller is essential which is usually equipped with a feedback loop to achieve controllable heating. The temperature controller monitors the maximum temperature in both blood and tumour compartments. If the temperature at any point exceeds 43°C, the power input from the ultrasound will be ceased until the temperature is lower. The controller equation is given by (Lin et al., 1990)

$$P(t) = k_{cp}[T_{target} - T_{max}(t)] + k_{ci} \sum [T_{target} - T_{max}(t)] \quad (3.2.35)$$

Where k_{cp} and k_{ci} are the control parameters for the Proportional-Integral controller.

3.3 Parameters for the continuum-based model

A large number of model parameters are involved in the multiphysics continuum model. These can be divided into three groups: drug related properties, tissue related properties, as well as focus ultrasound and heat transfer properties. The definition and choice of these parameters are detailed below.

3.3.1 Transport parameters related to drug properties

Vascular permeability (P)

Vascular permeability reflects the ability of the blood vessel wall to allow a substance to pass through the wall. It is determined by the vessel wall structure and size of the molecule (Yuan et al., 1995). TSL, free and bound drugs are included in this study.

DOX has a molecular weight of 580 Da whereas Sulforhodamine has a similar molecular mass with a permeability of $3.4 \times 10^{-7} m/s$ in normal tissue (Wu et al., 1993b). Another drug named Patent weighted 566 Da has a permeability of $3.95 \times 10^{-7} m/s$ in normal tissue of frog (Curry et al., 1983). Therefore, the permeability of DOX is estimated to be $3.75 \times 10^{-7} m/s$. Gerlowski and Jain (Gerlowski and Jain, 1986) showed that the permeability of the vessel wall in tumour is around 8 times higher than that of normal tissue. Hence, the permeability of DOX in tumour is estimated to be $3 \times 10^{-6} m/s$. Permeability of bound drug in tumour and normal tissue are $7.8 \pm 1.2 \times 10^{-9} m/s$ and $2.5 \pm 0.8 \times 10^{-9} m/s$ respectively, according to the measurements by Wu et al (Wu et al., 1993b). Permeability of the liposome are $3.42 \pm 0.78 \times 10^{-9}$ in tumour and $9 \times 10^{-10} m/s$ in normal tissue based on measurements on polyethylene glycol coated liposome at 37°C (Wu et al., 1993a).

Diffusion coefficient (D)

Diffusion coefficient is a measure of the rate of diffusion, with a high diffusion coefficient corresponding to faster diffusion. The diffusion coefficient of a substance in water is related to its molecular weight (Granath and Kvist, 1967; Jain, 1987) as expressed in equation 3.2.36, and the diffusion coefficient in tumour is directly proportional to the diffusion coefficient in water as given in equation 3.2.37.

$$D_w = a_{d1}(MW)^{-a_{d2}} \quad (3.2.36)$$

$$\frac{D}{D_w} = a_{d3}MW + a_{d4} \quad (3.2.37)$$

where a_{d1} , a_{d2} , a_{d3} and a_{d4} are determined by experiment. Similar to equation 3.2.36, the diffusion coefficient in normal tissue is given by

$$D = 1.778 \times 10^{-8}(MW)^{-0.75} \quad (3.2.38)$$

The diffusion coefficients for DOX in water, tumour and normal tissue are $3.83 \times 10^{-10}m^2/s$, $3.40 \times 10^{-10}m^2/s$ and $1.58 \times 10^{-10}m^2/s$.

Free drugs can combine with proteins in the blood, especially albumin, which has a significantly higher molecular mass than the free drug. Therefore, the diffusion coefficient of a bound drug approximately equals to the diffusion coefficient of the protein. In tumour, the diffusion coefficient of albumin is measured to be $8.89 \times 10^{-12} m^2/s$ (Eikenberry, 2009b). By applying equation 3.2.38, diffusion coefficient in normal tissue is estimated to be $4.17 \times 10^{-12}m^2/s$.

The diffusion coefficient of liposome in water is estimated using Einstein-Stokes equation (Jain, 1987).

$$D_w = \frac{k_b T^*}{6\pi\mu r} \quad (3.2.39)$$

where T^* is the temperature in Kelvin, k_b is the Boltzmann's constant, μ is viscosity and r is the radius of the particle. Experimental work shows that the diffusion coefficient for 90 nm liposome is $1.0 \pm 0.8 \times 10^{-11}m^2/s$ (Yuan et al., 1995). Since the diffusion coefficient is inversely proportional to the particle radius, the diffusion coefficient of liposome with an average radius of 100 nm is estimated to be $9.0 \pm 7.2 \times 10^{-12}m^2/s$.

Drug dose (D_d)

Anticancer drugs are usually associated with side effects. The most severe side effects induced by DOX is cardiomyopathy (Saltiel and McGuire, 1983; Singal and Iliskovic, 1998) which can cause heart failure. Therefore, a dose limit must be set which is approximately 550 mg/body surface area (Laurence et al., 2017). In all simulations presented in this thesis, the total dose is $50 \text{ mg}/m^2$. The body area is calculated using equation 3.2.40 (51).

$$\text{Body surface area} = \left(\frac{\text{Weight in kg}}{70} \right)^{0.73} (1.73m^2) \quad (3.2.40)$$

Reflection coefficient (σ)

The pores on the vascular wall and the sizes of the drug determine the reflection coefficient which is the efficiency of the oncotic pressure gradient in driving transport across the vascular wall. The reflection coefficient of albumin is measured to be 0.82 ± 0.08 (Wolf et al., 1987). The molecular size of albumin is 3.5 nm. In this study, the reflection coefficient of liposome is estimated to be 0.95 (Aukland and Reed, 1993). The coefficient is affected by the type of tissue. Our model assumes the tumour as a homogenous tissue. Therefore, the reflection coefficient remains constant.

3.3.2 Transport parameters related to tissue properties

Blood vessel surface area to tissue volume ratio (S/V)

The amount of drug transport from blood plasma to the extracellular space depends strongly on the blood vessel surface area to the tissue volume ratio. A large surface to volume ratio leads to a higher drug concentration in interstitial fluid. Since the growth of tumour leads to a highly irregular vasculature in the tumour, the S/V varies with the type of tumour and the stage of tumour. In the absence of tumour-specific measurements for S/V, mean values of 70 cm^{-1} for normal tissue and 200 cm^{-1} for tumour tissue have been used (Baxter and Jain, 1989).

Tumour cell density

Tumour cell density is related to the volume fraction of extracellular space and the size of tumour cells. The volume fraction of extracellular space in tumour tissue is approximately 0.2 to 0.6 (Nugent and Jain, 1984). Eikenberry [90] estimated the cell density based on the extracellular volume fraction which is ranged from 9.55×10^{15} to $1.53 \times 10^{16} \text{ cell m}^{-3}$. This value is used to define the initial tumour cell density. During a computational simulation, tumour cell density varies with time as it is affected by cell death triggered by the anticancer drug, cell proliferation and physiological degradation.

3.3.3 Focus ultrasound and heat transfer properties

In the multiphysics model, the bio-heat transfer equations are coupled with the mass transfer equations for TSL and the release drug. The parameters used for the acoustic field and bio-heat transfer simulations are summarised in Table 3.2.

Table 3. 2: Parameters for bio-heat transfer and acoustic field simulations

Symbol	Description [Unit]	Value			Source
		Blood	Tumour	Normal tissue	
v_a	Ultrasound speed m/s	1540	1550	1550	(Sheu et al., 2011)
f	Ultrasound frequency Hz		1.1×10^6		(Sheu et al., 2011)
ρ	Density Kg/m ³	1060	1000	1055	(Sheu et al., 2011)
c	Specific heat J/kg/k	3770	3800	3600	(Sheu et al., 2011)
k	Thermal conductivity W/m/k	0.53	0.552	0.512	(Sheu et al., 2011)
α	Absorption constant	$1.5 f$	$9 f$	$9 f$	(Sheu et al., 2011)
w_{b0}	s^{-1}	-	0.002	0.018	(Vaupel et al., 1989)
R	J/mol/K	-	8.314	8.314	(Schutt and Haemmerich, 2008)
ΔE	J/mol	-	6.67×10^5	6.67×10^5	(Schutt and Haemmerich, 2008)
A_f	s^{-1}	-	1.98×10^{106}	1.98×10^{106}	(Schutt and Haemmerich, 2008)

 f represents ultrasound frequency

3.4 Implementation of the multiphysics model

An overview of the implementation of the multiphysics model is shown in Figure 3.4. First, the mass and momentum equations are solved to obtain fluid velocity and pressure in the interstitial space. Under the assumption that the infusion of drug will not disturb the flow field, the steady-state solutions are used as input to the drug transport equations. In addition to this, the acoustic energy absorbed by tumour and blood is required. The acoustic pressure is solved in MATLAB using an open source code, FOCUS, developed by Michigan state university (McGough et al., 2004b). An interface is built between MATLAB and ANSYS FLUENT to import the resolved acoustic energy to ANSYS FLUENT. Following this, an initial value of drug concentration is given to the system based on the drug infusion mode and dose. Further details of the numerical methods and simulation setup are described below.

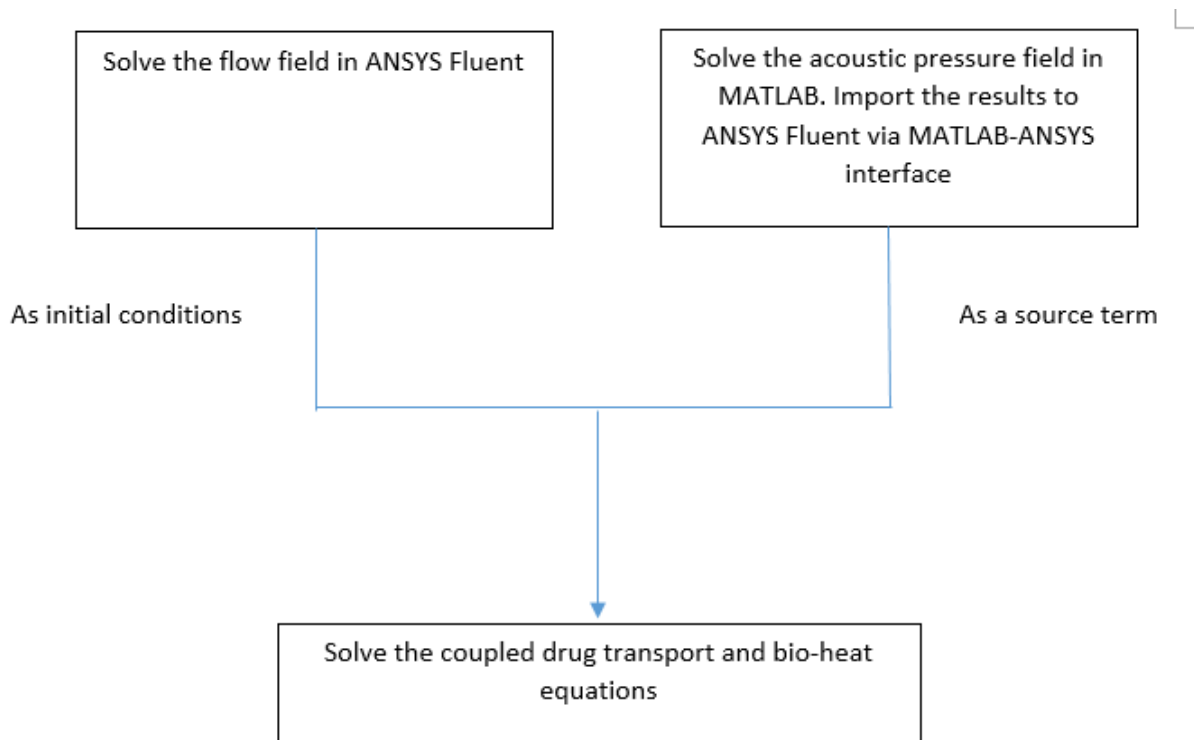


Figure 3. 4: Schematic representation of the adopted workflow

3.4.1 ANSYS FLUENT and user-defined functions

ANSYS FLUENT is employed to solve the partial differential equations employed in the complex Multiphysics model. It is a general purpose CFD code based on the finite volume method (FVM) where the computational domain is divided into a large number of small grids. All variables of interest are solved in the centroid within the

control volume based on the governing equations. The residual between two consecutive iterations is calculated and compared with a given tolerance. When the residual is smaller than the defined tolerance, the iteration is completed. All conservation laws are obeyed in each control volume.

All transport equations are spatially discretised using the second order UPWIND scheme, while a fully implicit second order backward Euler scheme is used to discretise time-dependent terms. The SIMPLEC algorithm is employed for pressure-velocity coupling. The nodal point value is updated by the Gauss-Seidel method.

The default transport equation in ANSYS FLUENT is not sufficient to handle the complex Multiphysics model. Therefore, a customised equation sets are employed. These equations are implemented via User Defined Function (UDF) coded in C programming language.

UDF for drug transport

The customised mass transport equations are implemented using UDF and loaded with FLUENT. The pressure field obtained by solving the mass and momentum equations is used as input to the mass transport equations. The second order UPWIND scheme is employed to discretise the mass transfer equation. Numerical convergence is reached when residual of the mass transfer equations is below 1×10^{-8} .

UDF for heat transfer

The bioheat transfer model is coded and integrated with FLUENT via UDF. The temperature field is obtained by solving the heat transfer equations, and when the local temperature reaches the desired hyperthermia drug release in the system is triggered. The second order UPWIND scheme is also applied to discretise the PDEs. The residual tolerance is set to be 1×10^{-10} .

Ultrasound heating

The effect of applying focus ultrasound is described through the acoustic energy absorbed by living tissue, which is represented as a source term in the bioheat transfer equations. The acoustic pressure is obtained by employing the fast near field method in FOCUS (Chen and McGough, 2008) coded in MATLAB. An interface between MATLAB and FLUENT is established to load all the acoustic pressure information to ANSYS FLUENT.

3.4.2 Boundary conditions

Boundary conditions are essential in setting up numerical simulations. They need to not only satisfy the mathematical requirement to ensure a unique numerical solution, but also be physiologically relevant. Since boundary conditions can be case dependent, more detailed description of problem-specific boundary conditions

will be given in each case study. In this thesis, the most common boundaries are the outer boundary of the normal tissue, the interface between tumour and normal tissue interface, and tumour-tumour interface.

3.4.3 Heating point selection

A uniformly distributed temperature profile is essential for ensuring therapeutic efficacy. However, the focal points generated by the ultrasound devices are usually small compared to the volume of the tumour. Therefore, multifocal heating is required to achieve uniform temperature distribution. To achieve optimal heating, the percentage area above a specific temperature (e.g. 41°C) is evaluated and the influence of the number and location of focal points is examined.

Chapter 4: Compartmental model

4.1 Introduction

This chapter aims to investigate drug transport from a capillary vessel to its surrounding tumour tissue in the TSL drug delivery system and to assess the treatment efficacy for different TSL properties and hyperthermia duration by means of computational modelling. The multi-compartmental model described in Chapter 3 was used to predict spatio-temporal distributions of drug concentration in a single tumour cord. Numerical predictions were compared with experimental data in the literature, where imageable thermosensitive liposomes (iTSLs) loaded with fluorescence labels were used to observe the spatial and temporal evolution of drug concentration in a murine cancer model (Jain, 1987). Computational studies of two chemotherapy drugs were performed: doxorubicin (DOX) and topotecan (TOP). Simulation results for different agents and therapeutic conditions (by varying drug release rates and heating duration) were compared. Finally, model-based optimisation was performed in an attempt to maximise the intracellular drug concentration while minimising the systemic drug concentration, which are indicative of therapeutic effectiveness and cytotoxicity, respectively. Three parameters were chosen to be optimised: drug release rate at the body temperature (37°C), drug release rate at the targeted mild hyperthermia temperature (42°C) and heating duration.

4.2 Model geometry

In this simulation study, the tumour cord model is adopted, which assumes a uniform distribution of blood vessels in the tumour. Figure 4.1 shows the geometry of a single tumour cord. A straight capillary vessel is surrounded by tumour tissue where drug is supplied by the central capillary. The cylindrical annulus represents the capillary (with a radius $R_c = 10\ \mu\text{m}$) and its surrounding tumour interstitium (with a radius $R_t = 120\ \mu\text{m}$). Given the assumption of axis-symmetry and homogeneous distribution along tumour micro vessels, one-dimensional drug transport in the radial direction is formulated. A no flux boundary condition is applied at the edge of tumour cord, which means no mass is exchanged between tumour cords.

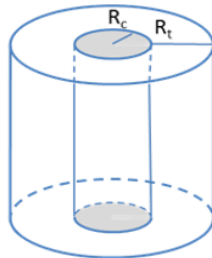


Figure 4. 1: Tumour cord model

4.3 Model parameters

Tumour-related and drug-related transport parameters are provided in Chapter 3 Table 3.1. More details about the drug infusion mode, drug release rate and transport parameters related to TOP and the liposome carriers are discussed here. Considering the toxicity of anticancer drugs, the drug dosage of TOP and DOX for each treatment cycle is 31.5 and 50mg/m² respectively (Armstrong, 2004; Laurence et al., 2017). Body surface area is estimated based on equation 4.1 (Goh et al., 2001).

$$\text{Body surface area} = \left(\frac{\text{Body Weight kg}}{70 \text{ kg}} \right)^{0.73} (1.73)^2 \quad (4.1)$$

The dosage of TOP and DOX for a 70 kg patient is 54.5 and 86.5 mg respectively. A low dose corresponding to 50 mg/m² is adopted in this study, for which the bolus injection mode is employed (Laurence et al., 2017).

TSL-related properties are taken from the iTSL developed by Centelles et al. (Centelles et al., 2018a) as much as possible. Based on their measured light intensity over time at different temperatures and the assumption of a linear relationship between the light intensity and percentage of drug release, the drug release rate at different temperatures can be obtained. Figure 4.2 shows the variation of drug release rate with temperature. The release rate of TOP from iTSL is 0.003s⁻¹ at 37°C, and increases to 0.114s⁻¹ at 42°C which corresponds to 98% of the encapsulated drug (Centelles et al., 2018b).

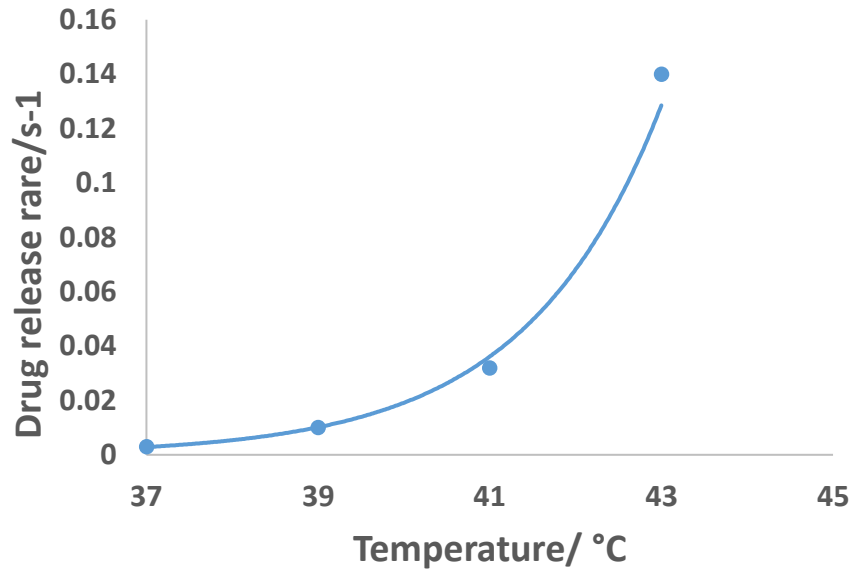


Figure 4. 2: TOP release rate from iTSL at different temperatures (data extracted from (Centelles et al., 2018b))

4.4 Numerical method and optimisation

As described in Chapter 3, drug transport in blood plasma, tumour plasma and intracellular space is described by a set of ordinary differential equations (ODEs), while partial differential equations (PDEs) are used to resolve drug concentration in tumour extracellular space. The PDEs are discretised using the finite difference method and converted to ODEs. A forward difference scheme is used at the capillary wall, while central difference and backward difference schemes are applied in the middle and at the edge of the tumour cord, respectively. The system of ODEs for TSL and drug concentrations in each compartment is solved numerically by means of an in-built function in MATLAB (ode15s). To ensure computational accuracy, grid sensitivity tests have been performed. The final grid size is $\Delta r = 0.4\mu\text{m}$ and time step $\Delta t = 0.1\text{s}$.

The multivariable optimisation problem is solved by using an in-built function in MATLAB (fmincon). The optimisation aims to find the global optimal point with the best therapeutic efficacy and least side effect, which means the highest average intracellular drug concentration and the lowest drug concentration in systemic plasma. The objective functions and constraints are illustrated in chapter 3.1.6. Three essential parameters are carefully selected, which are drug release rate at body temperature, drug release rate at liposome phase transition temperature and hyperthermia duration. By assigning different weighting factors in the objective function, we investigate the operating conditions that fulfil different requirements. The higher the weighting factor assigned to a variable means, the more we value the effect. The optimised results would give insight into the clinical operation. Multiple sets of initial guesses of the optimisation variables are chosen to ensure global optimality.

4.5 Results and discussion

Simulation results are presented for the baseline case first. This is followed by a comparison of two different chemotherapy drugs, and a parametric study of release rate constants and heating duration. Finally, optimisation of the key parameters is discussed.

4.5.1 Simulation results for the baseline scenario

The baseline case is designed to provide detailed information on spatio-temporal drug distributions in different compartments. We chose topotecan (TOP) loaded TSLs for the baseline case, as the release rate constants were derived from the experiments for TOP-iTSL (Centelles et al., 2018b). In the baseline scenario, a bolus of $16\mu\text{g/mL}$ TOP-iTSLs is intravenously injected into the systemic and tumour plasma, and heating is applied at 30 min for 3 mins and at 90 min for 5 mins after the treatment begins, following the work described in (Centelles et al., 2018b). Figure 4.3 (a) shows TSL concentrations in both systemic and tumour plasma gradually decline from its initial concentration over time mainly due to systemic clearance and transfer to other compartments. As TSLs move

between the systemic plasma and tumour plasma compartments rapidly, an equilibrium of TSL concentration in both compartments is quickly established. However, there are sharp falls in TSL concentration in the tumour plasma compartment at 30 min and 90 min by approximately 4 $\mu\text{g/mL}$ and 1 $\mu\text{g/mL}$, respectively, due to elevated temperature by heating and rapid breakdown of TSL at 42°C. In Figure 4.3 (b), TOP concentrations in different compartments are displayed. During hyperthermia, a large amount of TOP is released, resulting in peaks of TOP level in the tumour plasma. This also affects the tumour extracellular and intracellular space, where concentration peaks also appear at the same time instants, as in Figures 4.3 (b) and (d), respectively. Furthermore, temporal distributions of TOP at different radial positions are presented in Figure 4.3 (d). It shows that TOP concentration near the tumour core is more sensitive to hyperthermia than at locations further away from the core, with the edge of the tumour being hardly influenced by hyperthermia, i.e., no concentration peaks at the time of heating. Figure 4.3 (c) shows the spatial drug distributions at different time points, $t = 0.55$ hrs, $t = 1.63$ hrs and $t = 7$ hrs, the times immediately after the first hyperthermia, second hyperthermia, and the end of the treatment, respectively. As can be seen from both Figures 2 (c) and (d), TOP is not uniformly distributed within the tumour cord especially in the first hour of the treatment, with very high TOP concentration near the core close to the blood vessel (at $r = 0$). As time proceeds, TOP starts to distribute more uniformly.

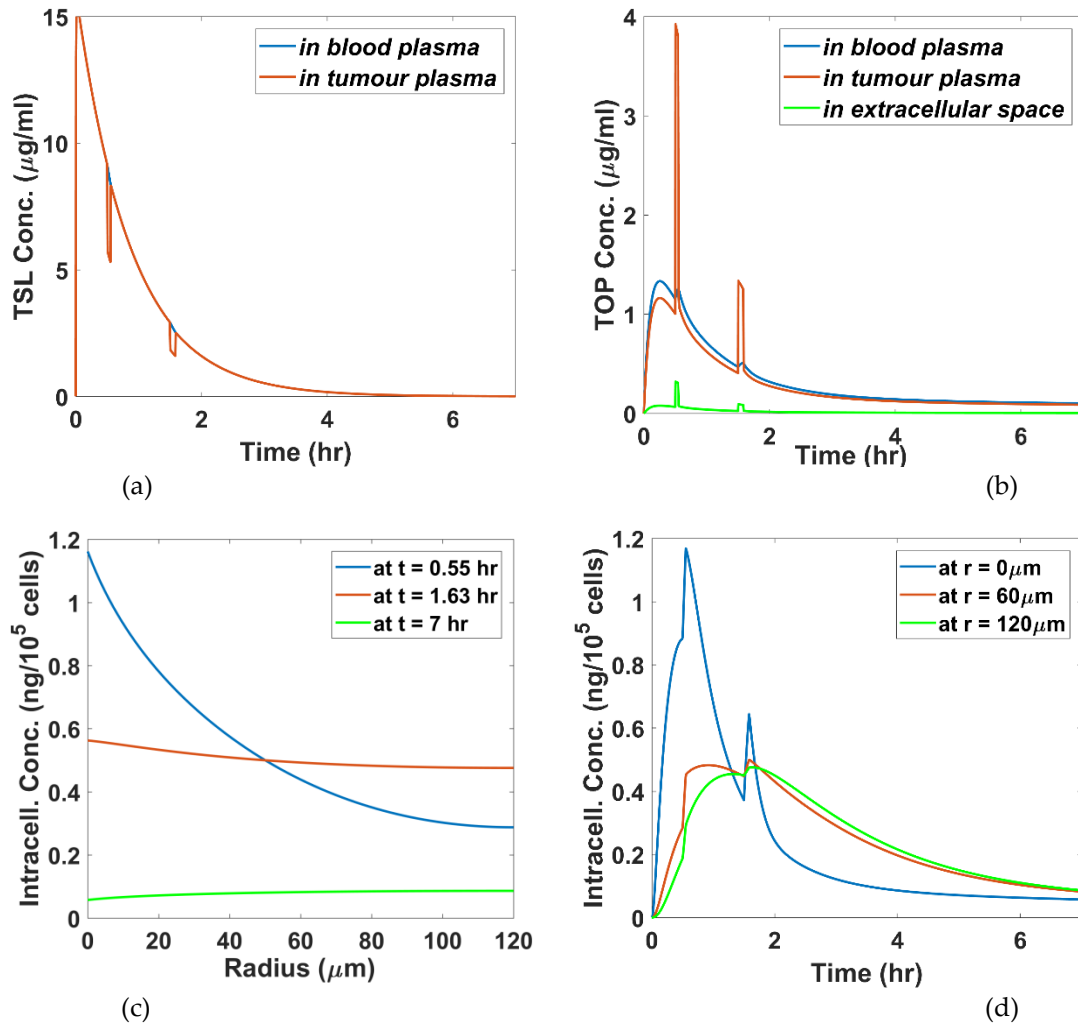


Figure 4. 3: Spatio-temporal distributions of TSL and TOP concentrations in different compartments. (a) TSL concentrations in the systemic and tumour plasma compartments, (b) released TOP concentration in the systemic plasma, tumour plasma and extracellular space, (c) spatial profiles of TOP intracellular concentrations at different time points and (d) temporal profiles of TOP intracellular concentrations at different radial positions.

This simulation represents an initial effort of using mathematical models to understand the micro-distribution of bioavailable drug following TSLs-mediated drug delivery. For validation purposes, the model predictions are compared with animal experiments performed by Centelles et al. (Centelles et al., 2018b) who tested TOP-loaded iTSLs on a murine cancer model. A good qualitative agreement can be found between the experimental results and the simulation results in capturing the sharp increase in TOP concentration after each hyperthermia (Figures 4.3 (b) and (d) in this work and Figure 5 in (Centelles et al., 2018b)); the murine cancer model also exhibited higher TOP-related fluorescence intensity immediately after hyperthermia was applied, indicating a burst release of TOP from iTSL. Quantitative comparisons are not made, because it was not possible to measure TOP concentrations in the experiment (Centelles et al., 2018b).

4.5.2 Comparison of DOX and TOP

The same model has been used to simulate DOX-TSL, assuming the same liposomal properties and heating duration. Hence, any difference in drug concentration would be mainly attributed to the different transport properties (i.e. diffusivity and permeability) that are estimated from their molecular sizes.

Distributions of DOX and TOP concentrations in the systemic plasma, tumour plasma and intracellular space are shown in Figure 4.4. Due to the same release rate for TOP-TSL and DOX-TSL, there is hardly any difference in systemic drug concentration. In the tumour and intracellular compartments, DOX concentrations are lower than TOP concentrations, meaning that more TOP can reach tumour cells. This is because TOP has a smaller molecular size than DOX, which results in higher diffusivity and permeability. These results reiterate that small molecule drugs would transport more easily through the tumour without increasing their exposure at the systemic level (Ozcelikkale et al., 2013; Park, 2015a; Rippley and Stokes, 1995).

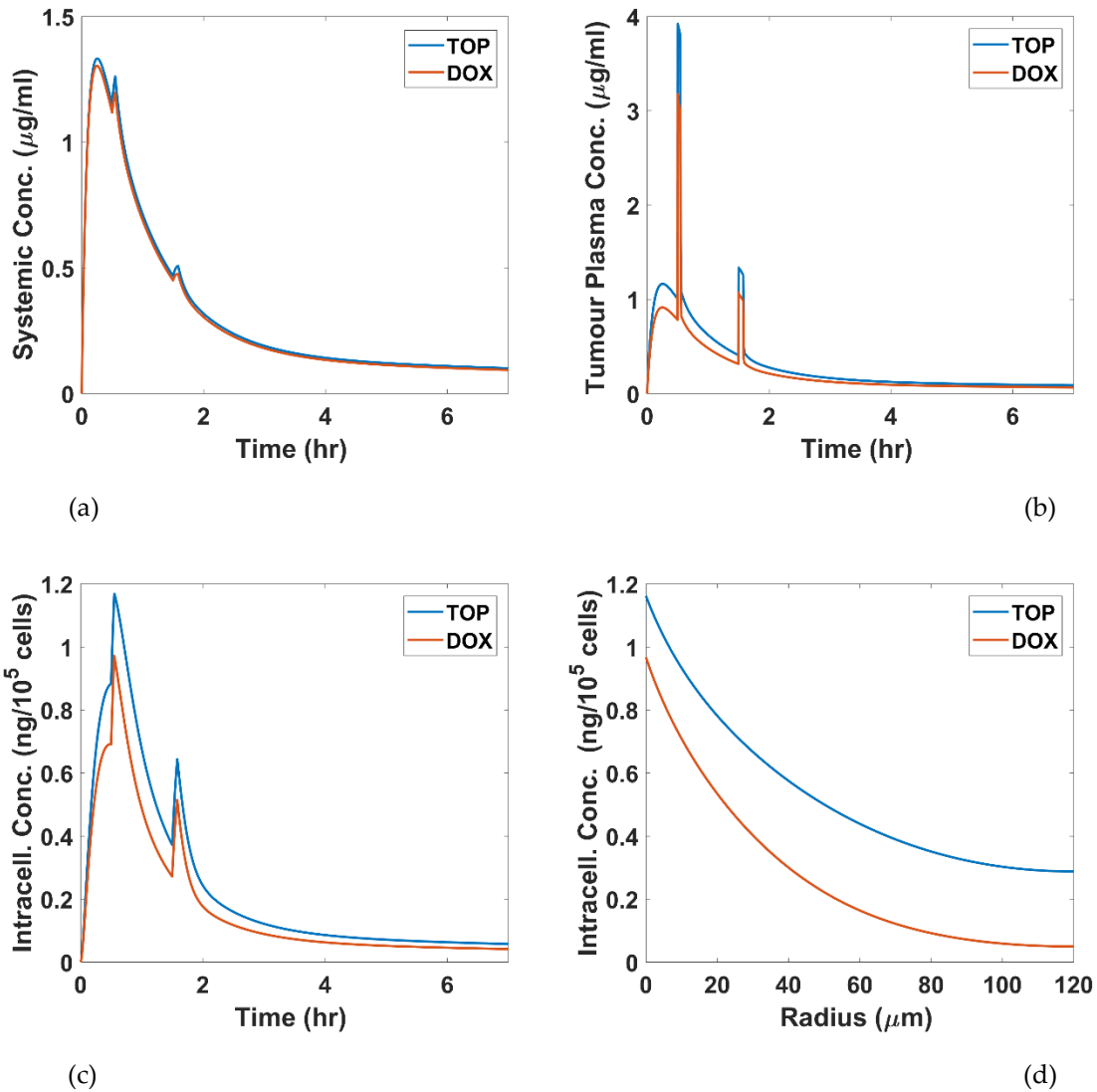


Figure 4. 4. Simulation results for two different drugs, DOX and TOP. (a) Free drug concentrations in systemic plasma, (b) free drug concentrations in tumour plasma, (c) temporal intracellular drug concentrations in the intracellular space at $r = 0 \mu\text{m}$ over the course of treatment and (d) spatial intracellular drug concentrations at $t = 0.55$ hrs.

4.5.3 Effects of drug release rates

The rate of drug release from TSL is temperature dependent and varies with the liposome formulation. In our current model, two release constants are required: one at physiological temperature (37°C) and another at mild hyperthermia (42°C). We chose TOP as the anticancer agent for simulations presented hereafter. In Figure 4.5, drug concentrations in the systemic plasma and tumour intracellular compartments are shown with different combinations of release rate constants. The systemic drug concentration displayed in Figure 4.5 (a) has a direct impact on the risk of side effects of the treatment, whereas the intracellular drug concentration shown in Figure 4.5 (b) is considered representative of therapeutic efficacy. The contour maps show that a low value of kr_{37} would

be needed in order to keep the systemic concentration low. On the other hand, a high kr_{42} would be desirable for enhanced cancer cell killing. Results in Figure 4.5 (b) show that intracellular drug concentration is highly sensitive to kr_{42} , but its effect on systemic concentration is rather trivial, especially at moderate and high kr_{37} .

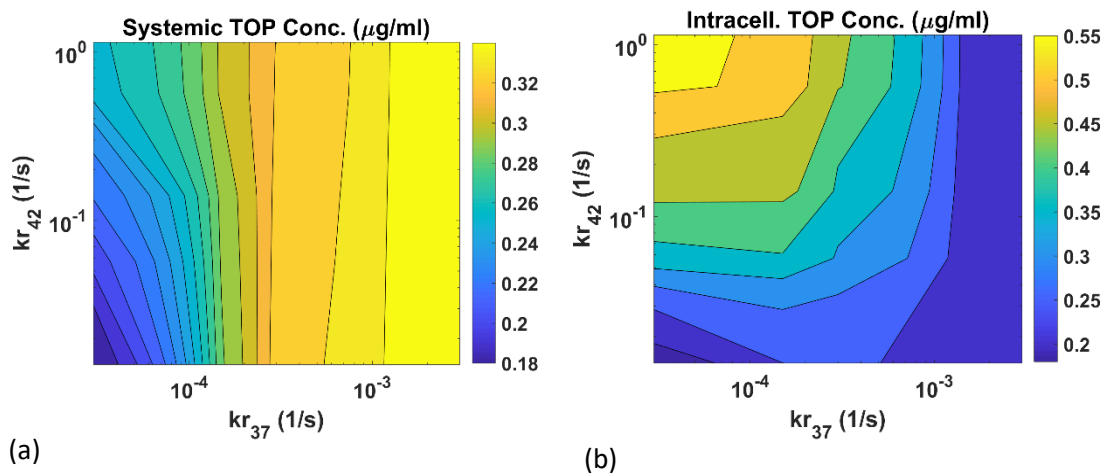


Figure 4. 5: Drug concentration averaged over the course of the treatment with different combinations of release rate constants kr_{37} and kr_{42} (a) in the systemic plasma and (b) in intracellular space.

The extent of drug leakage at body temperature is controlled by kr_{37} (Kong et al., 2000). Ideally, this should be kept as low as possible in order to achieve controlled drug release triggered by hyperthermia. As kr_{37} increases, more encapsulated drugs would be released before hyperthermia is applied, rendering the process less temperature sensitive. The rate of drug release at hyperthermia is controlled by kr_{42} . A very high kr_{42} value corresponds to instantaneous drug release, which triggers a sharp rise in free drug concentration in the tumour plasma compartment upon hyperthermia, as shown in Figures 4.3 (b) and 3 (b). This would lead to a significant difference in drug concentration between the tumour plasma and extracellular space, providing substantial driving force for diffusive drug transport into the intracellular space.

4.5.4 Effects of hyperthermia (HT) duration

Simulations are undertaken for different heating schedules while keeping the release rate constants unchanged. Three additional heating schedules are simulated: a two-step heating of 12 min and 20 min, a two-step heating of 24 min and 40 min and a single stage heating for 64 min. The two-step heating schedules consist of a short heating duration followed by a longer heating duration, following the heating protocol adopted in the experimental work (Centelles et al., 2018b). The time points when hyperthermia is applied are also kept the same as in the baseline case, i.e., at 30 and 90 min from the start of the treatment.

Figure 4.6 displays drug concentrations in the systemic plasma and intracellular space. It is interesting all heat schedules achieve the same peak systemic drug concentration of $1.4 \mu\text{g/mL}$ at the first hyperthermia, as depicted in Figure 4.6 (a). However, upon application of the second hyperthermia, different peak values are achieved, at between 0.75 and $1 \mu\text{g/mL}$. The longer the first heating, the lower the drug concentration reached during the second heating. This is due to the remaining amount of encapsulated drug being different after the first hyperthermia. Figure 4.6 (b) displays the temporal variation of intracellular drug concentration. The longer the heating duration, the higher the intracellular drug concentration, e.g, when the heating time is increased by 8 times relative to the baseline case, there is almost a 4-fold increase in the intracellular drug concentration. This means that an extended heating duration might be beneficial in enhancing the treatment efficacy due to high intracellular drug concentration.

Figures 4.6 (c) and (d) give the average and peak concentrations in the systemic plasma and intracellular space. It shows that the single heating protocol achieves the highest peak and average concentrations in both the systemic plasma and intracellular compartments. When the single heating protocol is compared with the two-step heating protocol of the same total heating duration, i.e, $24 \text{ min} + 40 \text{ min}$, there is no difference in the systemic drug concentration, but the level of drug in the intracellular space is higher with the single heating than the two-step heating protocol. This indicates that the risk of cytotoxicity might not be affected by the choice of heating protocols as long as the total heating duration is the same, whereas the therapeutic efficacy could be enhanced by continuous heating.

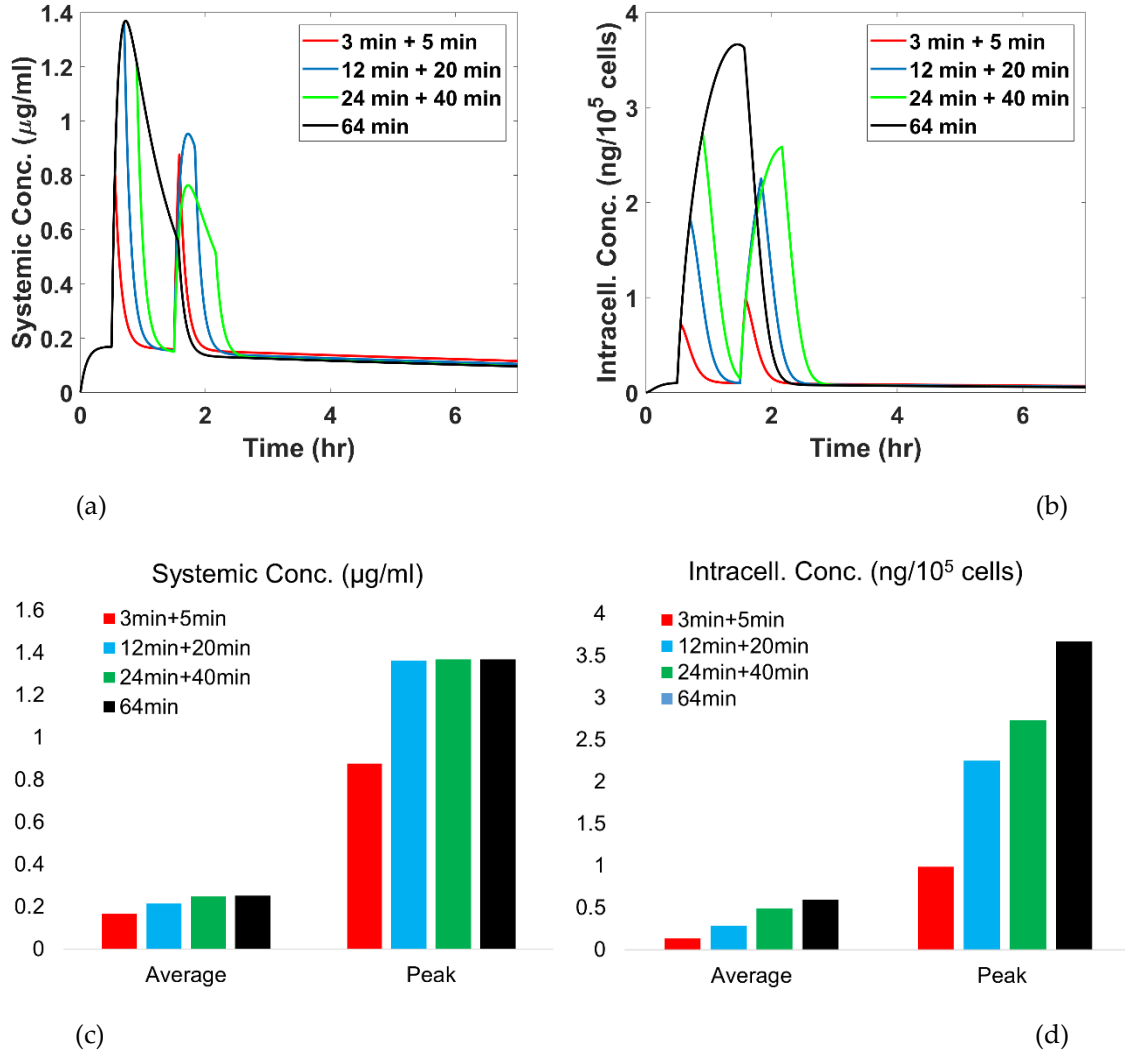


Figure 4. 6: Temporal concentrations of drug with different hyperthermia schedules. (a) Free drug concentration in systemic plasma, (b) drug concentration in intracellular space at $r = 0 \mu\text{m}$, (c) average and peak drug concentration in systemic plasma and (d) average and peak topotecan concentration in intracellular space at $r = 0\mu\text{m}$

4.5.5 Optimisation results

An initial optimisation exercise is performed for TOP-loaded TSL delivery with a single heating protocol. Three optimisation variables, kr_{37} , kr_{42} and Th , are simultaneously manipulated during the computer-based optimisation until the objective function value, the weighted sum of systemic plasma and intracellular concentrations as described in Eq. (3.1.15), reaches its minimum. The weighting factors are also varied for global optimisation and optimisation results are compared in Table 4.2. The higher the weighting factor w_1 , the more

we value the minimisation of systemic drug concentration, and vice versa. As the weighting factor, w_1 , changes from 0 to 1, the optimisation problem turns from the maximisation of intracellular concentration to the minimisation of systemic plasma concentration. Table 4.1 indicates that regardless of the release constants and weighting factor, the optimal heating duration is found to be at its upper limit, 3,600 s. Also, a similar behavior for kr_{37} can be seen; the optimal kr_{37} value is at its lower bound regardless of the weight factor and k_{42} , which means that a low release rate at 37 °C is always beneficial. On the other hand, the optimal value for k_{42} depends strongly on the weight factor. As w_2 increases (i.e., only maximising the intracellular drug concentration), kr_{42} approaches its upper bound. When the weight factors are equal (i.e., $w_1 = w_2 = 0.5$), a moderate kr_{42} would be optimal. However, the impact of kr_{42} on systemic drug concentration is weak, and the rate of increase in intracellular concentration slows down significantly after kr_{42} reaches 0.7 s⁻¹, suggesting that further increase in kr_{42} would not enhance intracellular concentration. It is worth noting that our optimisation results have limitations especially arising from the current formulation of objective function; the average systemic and intracellular drug concentrations are used to indicate the extent of cytotoxicity and cancer cell killing efficacy, respectively. The concentration terms in the objective function can be replaced by more direct and/or accurate predictors of cytotoxicity and cancer cell killing efficacy. In addition, the optimization is based on the assumption of uniform temperature in tumour interstitium.

Table 4. 1: Optimisation results with different weighting factors in the objective function

Weighting factor, w_1 , for the systemic plasma conc.	Weighting factor, w_2 , for the intracellular conc.	kr_{37} (10^{-4} s ⁻¹)	kr_{42} (s ⁻¹)	Hyperthermia duration (s)	Systemic plasma concentration (µg/ml)	Intracellular concentration (ng/10 ⁵ cells)
0	1	0.3	1.14	3600	0.26	0.56
0.25	0.75	0.3	1.1	3600	0.25	0.56
0.5	0.5	0.3	0.7	3600	0.24	0.55
0.75	0.25	0.3	0.13	3600	0.21	0.48
1	0	0.3	0.0114	3600	0.18	0.19

4.6 Comparison with experimental data

The simulation results for the baseline case are compared qualitatively with those reported by Centelles et al (Centelles et al., 2018a), who designed iTSL, a thermosensitive liposome with fluorescence properties by adding lipids, as the drug carrier. The anticancer drug used in this study is topotecan (TOP) because the fluorescence properties of the lipid labelled drug are significantly different between the encapsulated state and the released

state. Therefore, the release of the drug is traceable using optical equipment. Figure 4.7 shows the experimental procedure adopted by Centelles et al, where an initial hyperthermia was applied for 3 minutes at 30 minutes after the injection of the drug. After allowing the drug to circulate for another 1 hour, the second hyperthermia was applied with a heating duration of 5 minutes.

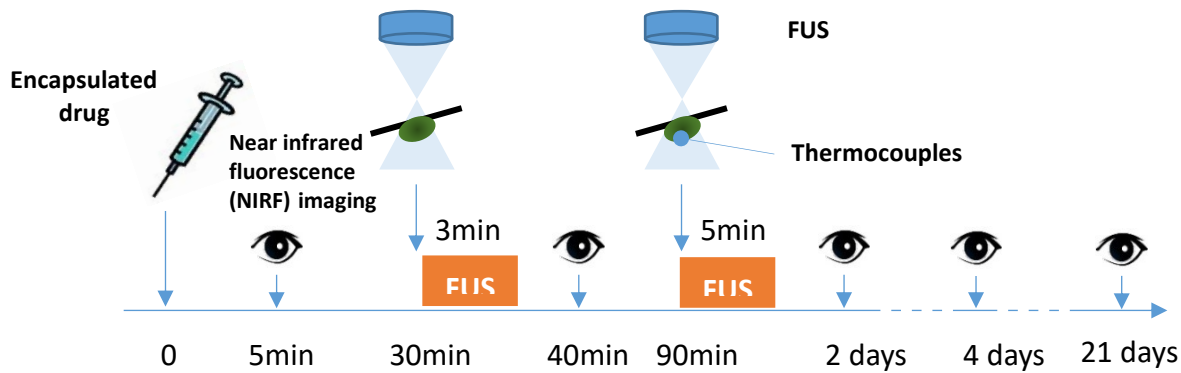


Figure 4. 7: Schematic of a double focused ultrasound application to the right-hand tumour in mice. It consists of an initial heating (3 min; $\leq 42^{\circ}\text{C}$) at 30 min and a slightly longer second heating period (5 min; $\leq 42^{\circ}\text{C}$) one hour after. The light intensity which corresponds to the drug concentration distribution is measured at 5 min, 40 min, day 2, day 4 and day 21. (extracted from (Centelles et al., 2018a))

The concentration distribution of TOP is plotted by measuring the light intensity of the fluorescence. As shown in Figure 4.8, very little TOP is detected at 5 min but there is a dramatic increase in light intensity after the first hyperthermia (35 min), indicating that the iTSL has a low release rate at body temperature but a high release rate upon heating. Since drug concentration was not measured directly in the animal study, only qualitative comparisons can be made with the model prediction. As already shown in Figure 4.3, TOP concentration before hyperthermia is low and it peaks at 35 min. After the first hyperthermia, the light intensity decreases steadily due to blood clearance and decomposition by cell metabolism (between 35 min and 1h10min); this is well captured by the computational model. At 1h35, when the second hyperthermia is applied, light intensity in the treatment area increases again, indicating further release of the encapsulated drug. The second peak is again well predicted by the computational model, although its value is much lower than the first peak at 35min. However, Figure 4.8 shows the value of the second peak is very close to that of the first peak. The differences between the experimental results and the model prediction could be caused by the overestimation of the drug release rate at the liposome phase transition temperature. The model predicts that most of the drug is released at the first hyperthermia with a massive release rate. However, experimental results illustrate that a significant level of the drug remains capsulated after the first hyperthermia and is released during the second heating. After the second hyperthermia, light intensity drops gradually due to blood clearance effects and this is well predicted

by the model. Another reason for the differences observed between the model prediction and experimental results could be due to the factor that the model employed in this chapter does not provide information on the spatial distribution at the tumour scale. Therefore, the model cannot capture any differences in spatial distribution of the drug between the first and second heating.

In order to make a quantitative comparison, several improvements are required. First of all, we need an accurate calibration between light intensity and drug concentration as the relationship between light intensity and drug concentration may not be linear. Converting the light intensity to drug concentration would create a map that can be directly compared with the simulation results. Secondly, more measurements could be taken at multiple time points to provide more detailed information on the variation of drug concentration with time. Finally, a macro-scale model of the tumour mimicking the mouse tumour model employed in the experimental work should be built to allow direct comparisons of spatio-temporal distributions of bioavailable drug.

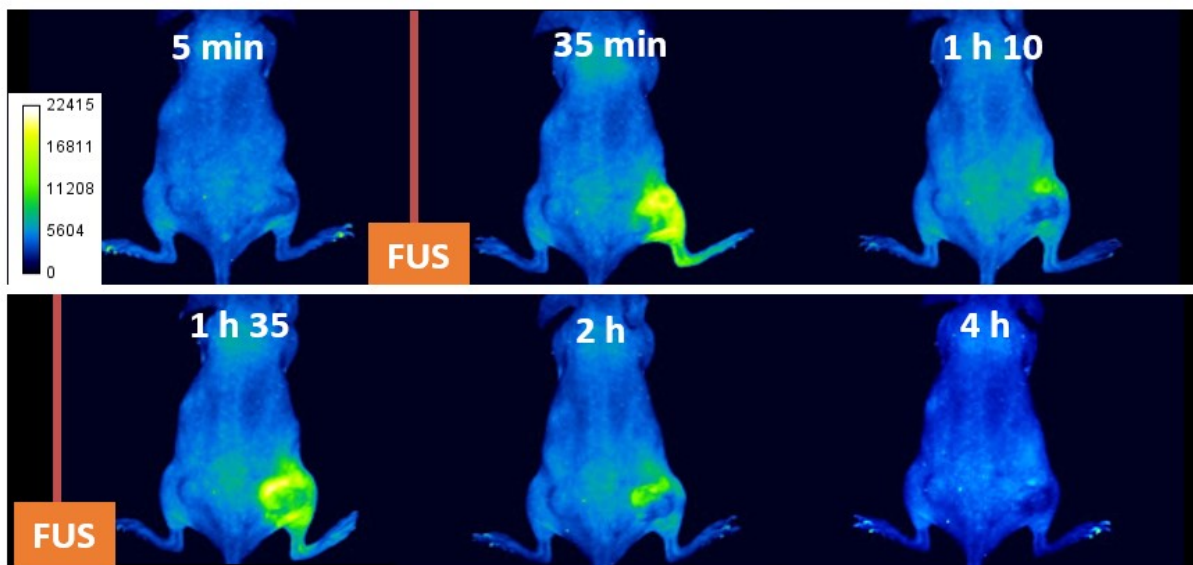


Figure 4. 8: Distribution of TOP concentration represented by light intensity in the treatment area (extracted from (Centelles et al., 2018b))

4.7 Summary and conclusion

TSLs delivery in combination with hyperthermia exposure has been considered as a promising alternative to conventional chemotherapeutics. Tumour cells residing in tumour interstitial space can only be killed when they are exposed to therapeutic levels of drug concentration. Interstitial transport, as an essential step to carry drug

molecules to tumour cells, must be examined in detail to gain mechanistic understandings of TSL delivery in combination with hyperthermia exposure (Park, 2015b).

In this chapter, a multi-compartmental model is used to simulate the multiple transport processes involved in TSLs-DOX/TOP systems by integrating PKs of TSLs and anticancer drugs at the systemic level and multiple transport steps in tumour tissues, namely extravasation and interstitial penetration. The model has been applied to a single tumour cord geometry as an attempt to reveal micro-scale distributions of DOX and TOP around capillary vessels following a bolus injection of TSLs and upon application of hyperthermia. The impacts of different parameters, namely, TSL release rate at 37°C (kr_{37}), TSL release rate at 42°C (kr_{42}), heating duration (T_h) and type of drugs (DOX vs. TOP) are also investigated. Finally, an initial attempt is made with model-based optimisation aimed at achieving a high drug concentration in tumour intracellular space, while keeping the systemic plasma drug concentration minimal, by controlling the release rate constants (dependent on liposome formulations) and heating duration.

Our simulation and optimisation results have shed light on the performance of TSL-mediated drug delivery systems as well as their dependence on a variety of factors, including TSLs formulation related parameters (e.g. drug release rate), properties of encapsulated drugs and hyperthermia exposure duration. The modelling and optimisation framework are expected to serve as a useful tool in the design of TSLs and treatment schedules for various TSLs therapies.

The model can be further improved by incorporating the bioheat transfer equations, so that the spatial distribution of temperature in response to the application of focused ultrasound could be predicted. This would allow the effect of heterogeneous temperature distribution to be included in the model prediction. The current computational model for microvascular transport can be extended relatively easily to incorporate fluid flow and intravascular drug transport, as demonstrated by Liu et al (Liu et al., 2011). In addition, cell killing models can be employed to directly predict the therapeutic efficacy of anticancer drugs. Since cell killing models are different for different cancer cells and anticancer drugs, tumour-specific and drug-specific models would be required in order to make the prediction more patient-specific (Liu et al., 2014).

Chapter 5: continuum-based drug transport model

5.1 Introduction

The continuum-based drug transport model has been coupled with the bio-heat transfer equations in order to simulate thermosensitive liposomal (TSL) drug delivery systems. In this multiphysics model, fluid flow in the interstitial space is resolved first, and the obtained interstitial velocity and pressure are employed as the initial conditions for the simulation of drug transport. Focused ultrasound (FUS) heating is simulated using a separate code, FOCUS, implemented in MATLAB. A MATLAB-ANSYS interface is developed to import the acoustic power field obtained from FOCUS in MATLAB to ANSYS Fluent. The model can be used with and without FUS heating depending on the treatment protocol. When FUS heating is switched on, the acoustic field will be applied via a source term in the bio-heat transfer equations. The source term will not be needed when heating is switched off, or when the temperature in the computational domain exceeds the maximum temperature set by the temperature controller. The latter is designed to simulate the function of a simple temperature controller to avoid over-heating in clinical applications. The adopted workflow is summarised in Figure 5.1.

The model has been applied to a realistic tumour geometry reconstructed from magnetic resonance (MR) images of a cancer patient. A treatment period of 12 hours is simulated with drug-loaded TSL being administered via bolus injection. Spatial and temporal distributions of TSL, temperature, released drug and cancer cell survival fraction are recorded and analysed in detail. Simulation of a baseline case, mimicking the delivery of TSL-DOX to a prostate tumour, is performed first. After establishing a qualitative understanding of the multiphysics drug delivery process, the model is used to investigate the influence of key parameters on the predicted treatment outcome, including heating duration and drug release rate. Furthermore, the transport of imageable TSL loaded with TOP (Centelles et al., 2018a) is investigated, and comparisons of drug concentration profile and cancer cell survival fraction are made between these two systems.

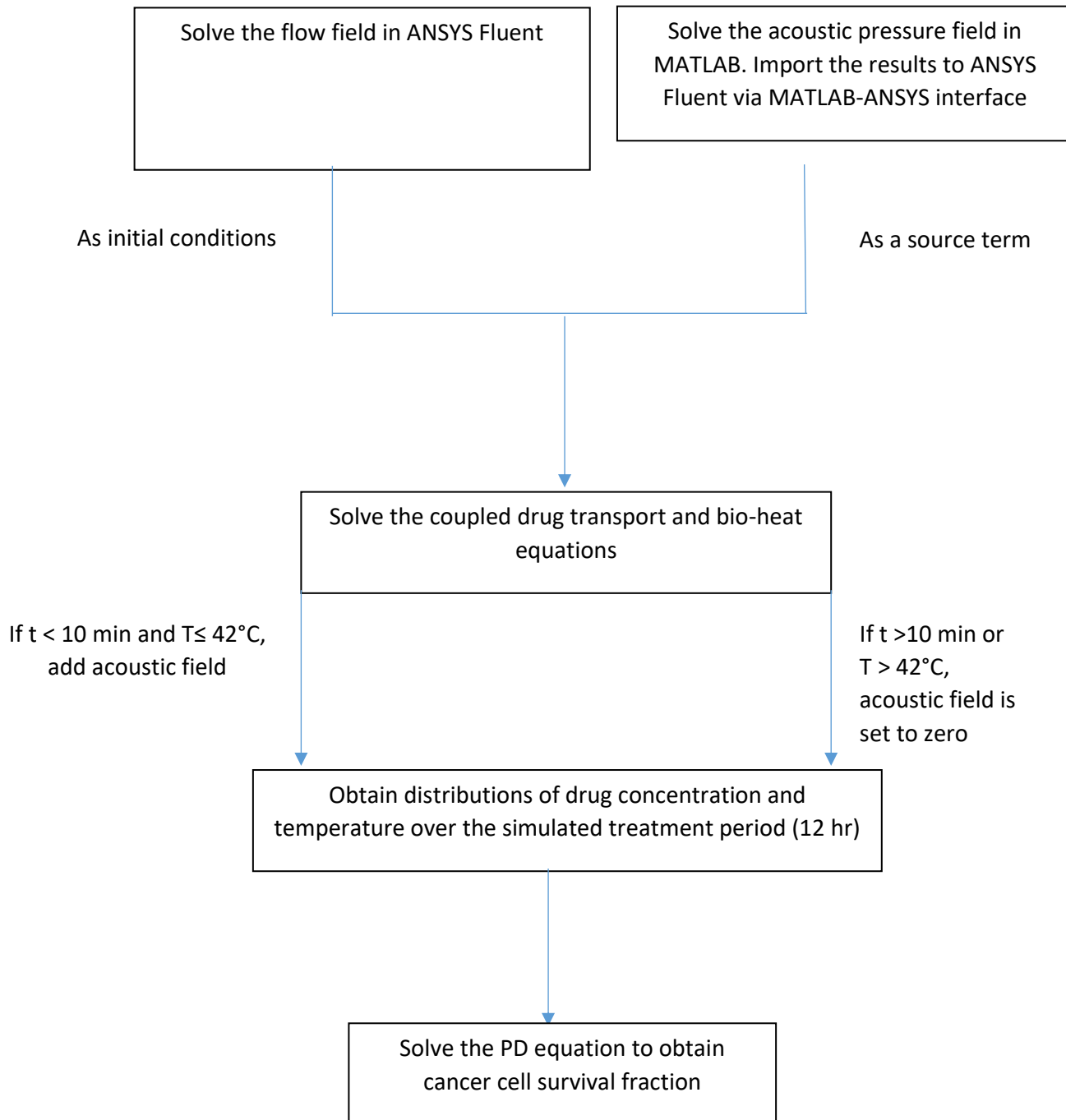


Figure 5. 1: Schematic representation of the adopted workflow

5.2 Model geometry

MR images of a patient with prostate cancer were acquired using a 3.0-Tesla MR scanner. The tumour is segmented from the surrounding normal tissue according to the signal intensity value. The section which shows the maximum width and maximum depth is selected to reconstruct a 2D model of the tumour. The reconstructed geometry shown in Figure 5.2 is imported into GAMBIT for mesh generation. The tumour region (shown in red) has a dimension of 47 mm (maximum width) by 38 mm (maximum depth). The tumour region is subdivided into two blocks: a central rectangle block and the rest of the tumour. The final mesh consists of 125751 quadrilateral elements in the central block and 93770 triangular elements in the rest of the tumour. The normal tissue (shown in blue) consists of 17150 triangular elements. A mesh sensitivity study was performed to ensure that the simulation results are independent of the grid.

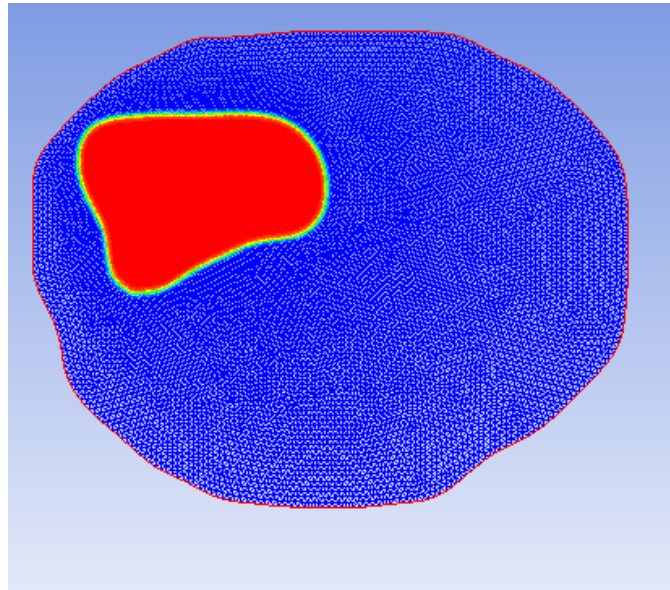


Figure 5. 2: The 2D tumour model and the computational mesh

5.3 Model parameters

5.3.1 Drug dosage

As discussed in section 4.3, the recommended dose level of $50 \text{ mg}/\text{m}^2$ is employed. For an average adult of 75 kg in body weight and 3.15 m^2 in body surface area, the estimated drug dosage per treatment is 86.5 mg.

5.3.2 Acoustic and thermal parameters

In the multiphysics model, the bio-heat transfer equations are coupled with the mass transfer equations for TSL and the release drug. The parameters used for the acoustic field and bio-heat transfer simulations are summarised in Table 5.1.

Table 5. 1: Parameters for bio-heat transfer and acoustic field simulations

Symbol	Description [Unit]	Value			Source
		Blood	Tumour	Normal tissue	
v_a	Ultrasound speed m/s	1540	1550	1550	[74]
f	Frequency Hz		1×10^6		
ρ	Density [Kg/m ³]	1060	1000	1055	[74]
c	Specific heat J/kg/k	3770	3800	3600	[74]
k	Thermal conductivity W/m/k	0.53	0.552	0.512	[74]
α	Absorption constant	$1.5 f$	$9 f$	$9 f$	[74]
w_{b0}	Perfusion rate of blood at 37°C s^{-1}	-	0.002	0.018	[45]
R	Universal gas constant J/mol/K	-	8.314	8.314	[141]
ΔE	Activation energy J/mol	-	6.67×10^5	6.67×10^5	[141]
A_f	Frequency factor s^{-1}	-	1.98×10^{106}	1.98×10^{106}	[141]

5.3.3 Ultrasound transducer

The ultrasound transducer employed in this study is a linear phased array consisting of identical elements. A phased array can change the focal point without moving the antenna, and is often used clinically in ultrasound hyperthermia (Zhu et al., 2019). The transducer parameters are summarised in Table 5.2. The transducer array can produce multifocal heating with 2 or more focal points.

Table 5. 2: Parameters of transducer array

Symbol	nx	ny	w	h
Description	Number of elements in the x direction	Number of elements in the y direction	Width of a single element	Height of a single element
Value	128	1	3×10^{-3}	5×10^{-2}
Unit	-	-	m	m

5.3.4 Temperature controller

Since the phase transition temperature of the simulated TSL is 42 °C (Tagami et al., 2012, 2011), local tissue temperature must reach 42 °C to ensure sufficient drug release at the targeted tumour site. However, temperatures exceeding 43 °C may cause vascular shutdown (Grüll and Langereis, 2012). Therefore, local hyperthermia needs to be applied within a narrow temperature range. To ensure safe operation and avoid overheating the tissue, a feedback temperature controller is employed in practice. A typical controller design equation is given in equation 5.1.

$$P(t) = k_{cp}[T_{target} - T_{max}(t)] + k_{ci} \sum [T_{target} - T_{max}(t)] \quad (5.1)$$

where control parameters k_{cp} and k_{ci} are set as 5.0 and 0.006, respectively (Staruch et al., 2011). In the computational model, the mechanism of the temperature controller is described using a simple switch function, whereby FUS heating is switched off when the maximum temperature in the heating area exceeds 42 °C. This allows the system to cool down via blood circulation.

5.3.5 Permeability

Permeability of free and bound drug varies with temperature as described by Equation 5.2 .

$$\log P = z_1 T + z_2 \quad (5.2)$$

where z_1 and z_2 are 0.07 and 2.43 respectively [144]. DOX, bound DOX, TOP and bound TOP are assumed to follow the same relation.

Permeability of TSL follows a different pattern. Based on experimental data [145], a curve fitting method is applied to describe the relationship between TSL permeability and temperature.

$$m_p = m_{p0} + m_{p11}e^{(T-T_0)/m_{p12}} + m_{p21}e^{(T-T_0)/m_{p22}} \quad (5.3)$$

where m_p is the fold increase in permeability of TSL as a function of temperature. m_{p0} , m_{p11} , m_{p21} and m_{p22} are curve fitting parameters which are -10.54, 5.76, 5.78 and 5.46, respectively. T_0 is the baseline temperature, which is 34°C.

5.3.6 Diffusivity

Drug diffusivity is another parameter that varies with temperature. To estimate the fold increase in diffusivity as a function of temperature, Stoke-Einstein equation is employed.

$$\frac{D_1}{D_2} = \frac{T_1^* \mu_2}{T_2^* \mu_1} \quad (5.4)$$

where D is diffusivity and μ is the viscosity of solvent. In this case viscosity of water is used, which is also temperature-dependent as described by Equation 5.5.

$$m_d = e^{(m_{d1} + m_{d2}T + m_{d3}T^2)} \quad (5.5)$$

where m_d is the fold increase in viscosity as a function of temperature. m_{d1} , m_{d2} and m_{d3} are parameters obtained by curving fitting, which are 5.10, -0.03 and 1.04×10^{-4} respectively.

5.3.7 Transmembrane parameter

El-Kareh and Secomb show that hyperthermia increases drug uptake by cells [55]. Experimental results on DOX indicate a 2.2-fold increase in transmembrane parameter at 42°C compared with that at 37°C [147]. A relationship between fold increase in transmembrane parameter and temperature is obtained by linear interpolation.

$$k_{tv} = 0.24T - 7.88 \quad (5.6)$$

5.3.8 Perfusion rate

The relationship between blood perfusion rate and temperature can be described as:

$$w = w_0 DS \quad (5.7)$$

where w_0 is the blood perfusion rate at 37°C, and DS is the degree of vascular stasis which depends on temperature.

$$DS = e^{(-\int_0^t A_f e^{\frac{-\Delta E}{RT^*}} d\tau)} \quad (5.8)$$

where A_f is the frequency factor, T^* is the absolute temperature, R is the universal gas constant and ΔE is the activation energy.

5.3.9 Drug release rate from TSL

Drug release rate from TSL varies significantly with temperature since the liposome is designed to release the encapsulated drug rapidly at phase transition temperature. The percentage release can be expressed as a function of time via Equation 5.9 (Afadzi et al., 2010).

$$\%R(t) = R_c(1 - e^{-k_{rel}t}) \quad (5.9)$$

where R_c is the total percentage of release at a certain temperature. By fitting to the experimental data reported Centelles and Tagami (Centelles et al., 2018a; Tagami et al., 2012), the release constant k_{rel} can be calculated at different temperatures in the range of 37 - 42°C. The results are summarised in Table 5.3.

Table 5. 3: Release constants for different TSL formulations (Centelles et al., 2018a; Tagami et al., 2012)

Temperature (°C)	37	38	39	40	41	42
k_{rel} of encapsulated DOX (s^{-1})	0.00417	0.00545	0.01492	0.02815	0.04250	0.05409
k_{rel} of encapsulated TOP (s^{-1})	0.00325	0.00668	0.01372	0.02817	0.05783	0.1140

Curve fitting method is employed to obtain a relationship between release rate and temperature.

$$k_{rel} = Ae^{BT} \quad (5.10)$$

where A and B are curve fitting parameters depending on the liposome formulation. Values for A and B are 4×10^{-12} , 0.5603 and 9×10^{-15} , 0.7193 for TSL-DOX (Tagami et al., 2012) and TSL-TOP (Centelles et al., 2018a), respectively.

5.4 Numerical methods

ANSYS FLUENT, a finite-volume based computational fluid dynamics code, is employed to solve the mathematical equations. User Defined Scalar functions are used to customise the governing equations of heat and mass transfer. Extra source terms are introduced into the original transport equations as detailed in Chapter 3. The 2nd order UPWIND scheme is selected to discretise the momentum, drug transport and heat transfer equations. The SIMPLEC algorithm is adopted for pressure-velocity coupling. Residual tolerances are set to control the convergence of the simulation, and these are 1×10^{-5} , 1×10^{-8} and 1×10^{-10} for the momentum equation, mass transport equation and heat transfer equation respectively.

Since the simulated treatment time (12 hr) is not long enough to cause tumour growth, it is reasonable to assume that the geometry of normal and tumour tissue is fixed. The interface between tumour and normal tissue is treated as an internal boundary where all variables are continuous. No flux condition is applied at the outer boundary of normal tissue, where the relative pressure and temperature are set to be 0 Pa and 37°C, respectively.

5.5 Results and discussion

5.5.1 Baseline case analysis

A baseline case is analysed first to establish qualitative understanding of the temporal and spatial distributions of temperature, drug concentration and cancer cell survival fraction. The infusion mode applied in the baseline case is bolus injection and the drug dosage is $50 \text{ mg}/\text{m}^2$. The duration of FUS heating is 10 min, starting at the same time as the drug infusion. A transducer array which produces two focal points is employed.

5.5.1.1 Temperature profile

Temperature is an important factor in the transport process of TSL drug delivery systems. This is because key transport parameters, namely diffusivity, permeability and transmembrane parameters, are strongly dependent

on temperature. More importantly, drug release from TSL is triggered and controlled by hyperthermia. Therefore, understanding the spatial distribution of temperature and its variation over time is essential.

Figure 5.3 shows the location of the focal points and the acoustic pressure map in the tumour model. It shows that two focus points are located near the centre of the tumour. The acoustic pressure is the highest at the focus points, and elevated in very narrow regions in the vicinity of the two focus points.

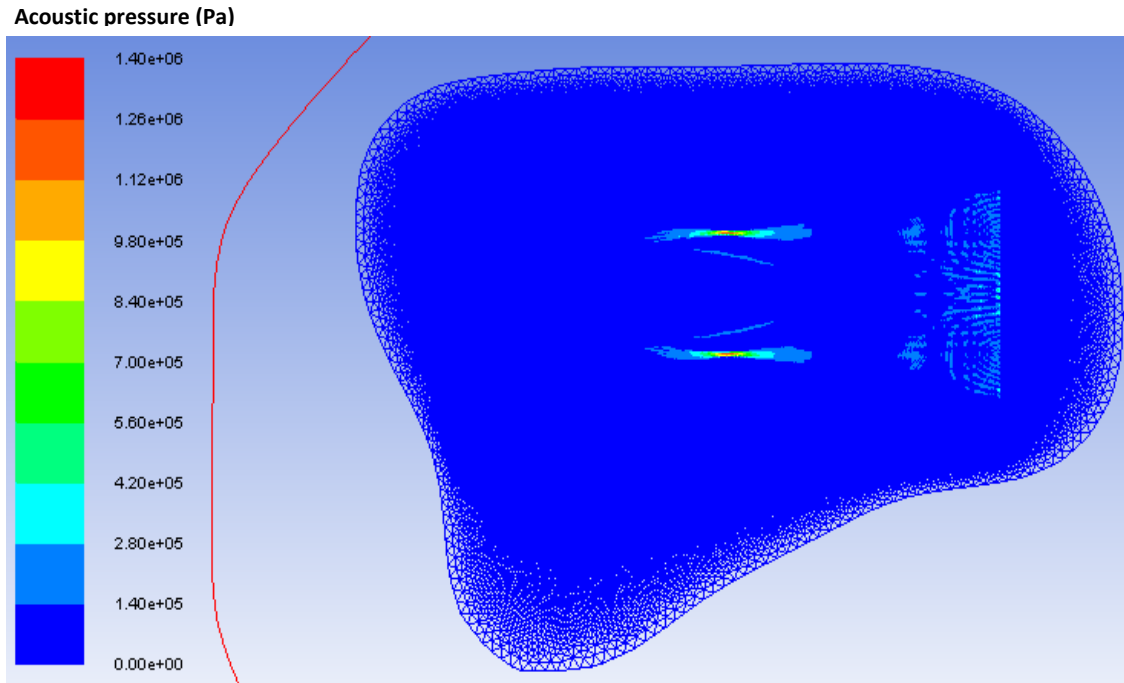


Figure 5. 3: Predicted acoustic pressure field for a transducer with 2 focal points located within the tumour

After the application of FUS for 10 minutes, the predicted distribution of temperature in tumour interstitial space and blood is shown in Figure 5.4. It shows clearly that there is a core region in the tumour where temperature reaches 315K (42°C). Over 80% area of the tumour area is heated to above 312K (39°C). However, the top left and bottom left corners remain unheated due to the irregular shape of the tumour. The temperature profile in the blood compartment follows a similar trend, but the magnitude of temperature rise is much smaller, with the maximum temperature elevation being up to 1°C. This is because the circulating blood works as a cooling fluid (i.e. heat sink) which removes the heat generated by FUS from the system. Larger temperature rise in the tumour interstitial space than in blood is desirable, as it would lead to a rapid drug release within the tumour and a slow release in blood. This would enhance drug accumulation in tumour cells, while reducing the level of drug (and toxicity) in the systemic circulation.

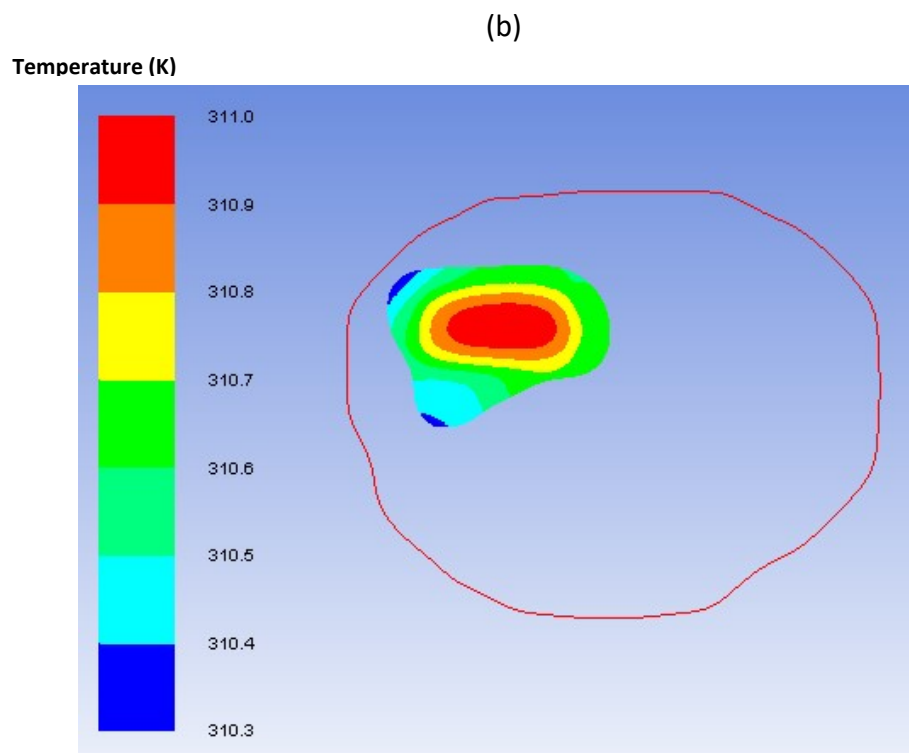
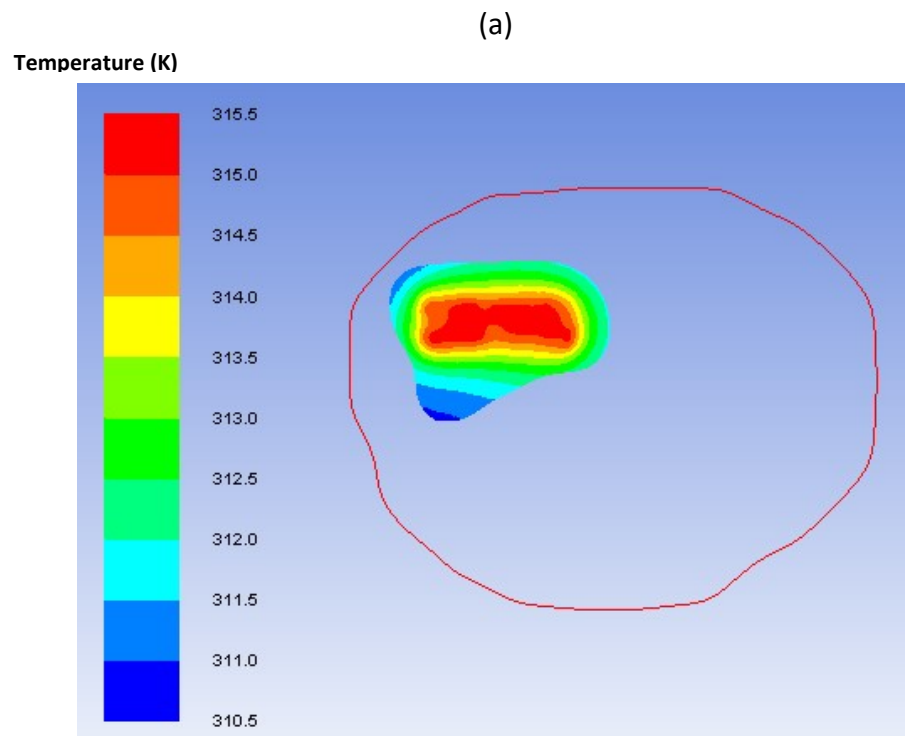


Figure 5. 4: Temperature distribution at 10 min in (a) tumour interstitial space, and (b) blood

Owing to the function of the temperature controller, predicted temperatures in the entire computational domain are below 42.5°C . However, it is noted that the temperature distribution is not uniform, which would lead to heterogeneous drug distribution in the targeted tumour. Particularly, there would be little drug release in the unheated corners. To optimise the temperature profile, the position of the focus points and the design of the temperature controller are further explored and these will be discussed in Chapter 6. The effect of hyperthermia duration is investigated in section 5.5.2.

In addition to its spatial distribution, temporal variation of temperature is also important for the control of drug release. Figure 5.5 shows the variation of spatially averaged temperatures in tumour interstitial space and blood. Upon the application of FUS, there is a sharp increase in average temperature which peaks at 600 s (10 min) when FUS heating is terminated. This is followed by a gradual reduction until the initial body temperature is reached at 15000 s (4.2 hours) due to heat removal by the blood. The average temperature in tumour plasma rises slightly to 37.5°C during FUS heating.

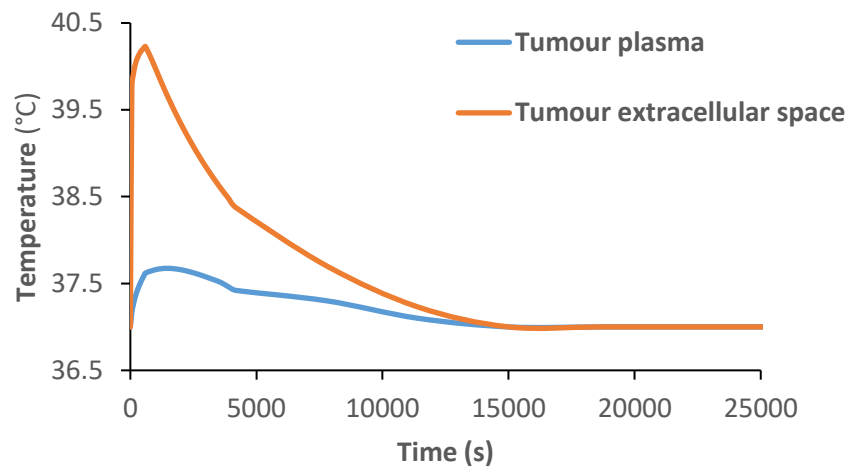


Figure 5. 5: Variation of spatially averaged temperatures in tumour interstitial space and blood

5.5.1.2 Drug concentration distribution

Drug-loaded TSL is administered by bolus injection and carried by the bloodstream into the tumour site and normal tissue. The drug release rate is very low at body temperature (37°C). The application of FUS causes temperature elevation, which triggers more rapid release of drug in the tumour extracellular space. Figure 5.6 shows the variation of spatially averaged concentrations of TSL in tumour plasma and tumour extracellular space. Liposome concentration in tumour plasma falls gradually due to the blood clearance effect whereby drugs are removed continuously by blood circulation. There is no TSL left in the system at around 1000 s (16.7 min after

drug administration). Liposome concentration in tumour extracellular space increases rapidly at the beginning of the treatment from 0 to 60 s. During this period, temperature in the tumour extracellular space is below the phase transition temperature of TSL, therefore, the encapsulated drug is released slowly. TSL transport from tumour plasma to tumour extracellular space is driven by the concentration difference. Since there is no TSL in the tumour extracellular space at the beginning of the treatment, the rate of transport is at its maximum. Therefore, liposome concentration peaks at around $t = 60$ s. After 60 s, the temperature in the tumour extracellular space rises to the phase transition temperature, leading to disruption of TSL and rapid release of the encapsulated drug. As a result, TSL concentration in tumour extracellular space falls dramatically from 60 s to 600 s (1 - 10 min). Figure 5.6 suggests that by the end of FUS heating at 10 min, 95% of the encapsulated drug has been released.

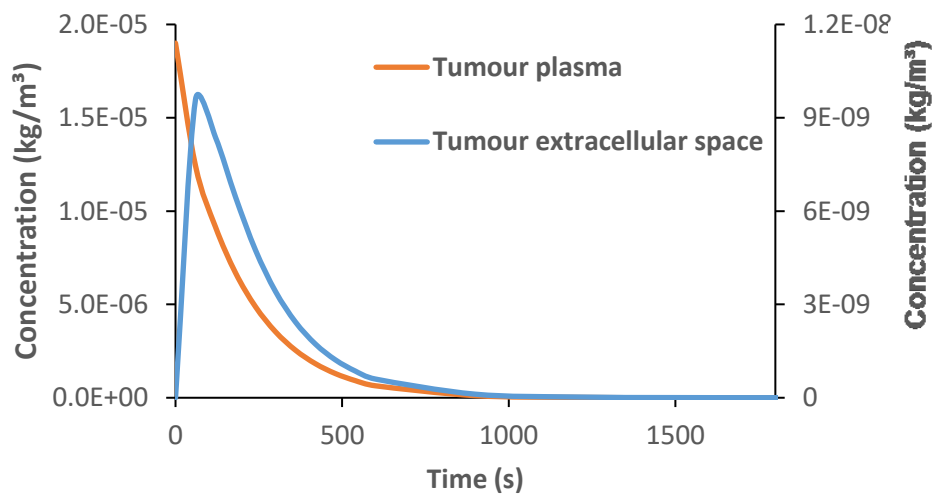


Figure 5. 6: Spatially averaged liposome concentrations in tumour plasma (scale on the left) and tumour extracellular space

The released drug is present in the system in two forms: free drug which is bioavailable and can enter tumour cells to exert its effect, and bound drug which is formed when free drug binds with protein. Due to its large molecular size, bound drug cannot pass through the cell membrane to cause cancer cell death. Figure 5.7 (a) shows the variation of spatially averaged free and bound drugs. Because of the equilibrium constant of the binding reaction is high, 60% of the free drug becomes a bound drug. The binding effect reduces the therapeutic efficacy significantly since less drug is available for cancer cell killing. Figure 5.7 (b) shows the average free drug concentration in tumour plasma and tumour extracellular space to illustrate the drug transport mechanism. During the hyperthermia period, free drug concentration in both compartments increases with time due to the

high temperature triggered drug release. Although the drug release rate in tumour extracellular space is much higher than that in tumour plasma due to temperature difference, concentrations of free drug in both compartments are similar. This is because there are far more TSLs in tumour plasma than in tumour extracellular space, as shown in Figure 5.6. As a result of the slow and inefficient transportation of liposome from tumour plasma to tumour extracellular space, a large amount of drug is removed by blood clearance, thereby limiting the therapeutic efficacy. It has been suggested that the transport of liposome could be improved by angiogenesis (Carmeliet and Jain, 2000; Goel et al., 2011; Jain, 2013) and stress alleviation (Jain, 2013, 2005; Padera et al., 2004).

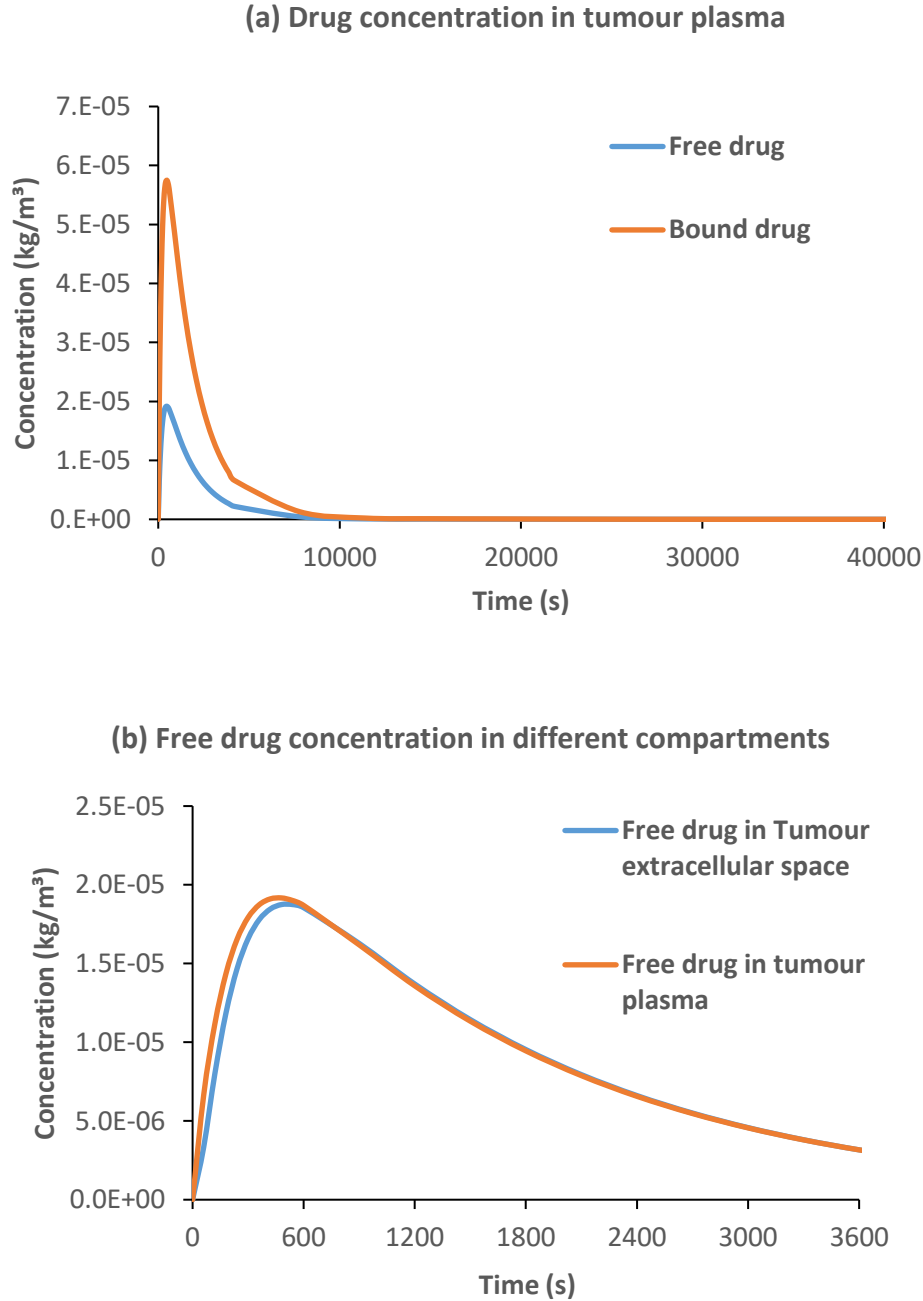


Figure 5. 7: (a) Spatially averaged free and bound drug concentrations in tumour plasma, and (b) spatially averaged free drug concentrations in tumour plasma and tumour extracellular space

Figure 5.8 (a) shows the spatially averaged drug concentration in the intracellular space. As expected, intracellular drug concentration increases with time during hyperthermia. It continues to rise after hyperthermia and peaks at 7800 s (2.2 hours). This is in contrast to the extracellular drug concentration and indicates that transmembrane transport is much slower than other transport steps. To enhance cell killing, a high area under the curve (AUC) value of intracellular drug concentration is essential according to the PD model employed. The

AUC value can be augmented by either increasing the peak concentration or widening the curve through a more gradual reduction in intracellular concentration. To achieve that, it is essential to maintain a high level of extracellular drug concentration. Methods to accomplish the aim, such as extending the hyperthermia duration and increasing the release rate, will be discussed later in this chapter.

In addition to intracellular drug concentration, which has a direct impact on therapeutic efficacy, free drug concentration in normal tissue is also of interest as it corresponds to the extent of potential side effects. Figure 5.8 (b) shows the drug concentration in normal tissue, which is about 10% of the drug concentration in tumour extracellular space (Figure 5.7 (b)), suggesting that the risk of side effect is likely to be low with TSL-mediated drug delivery.

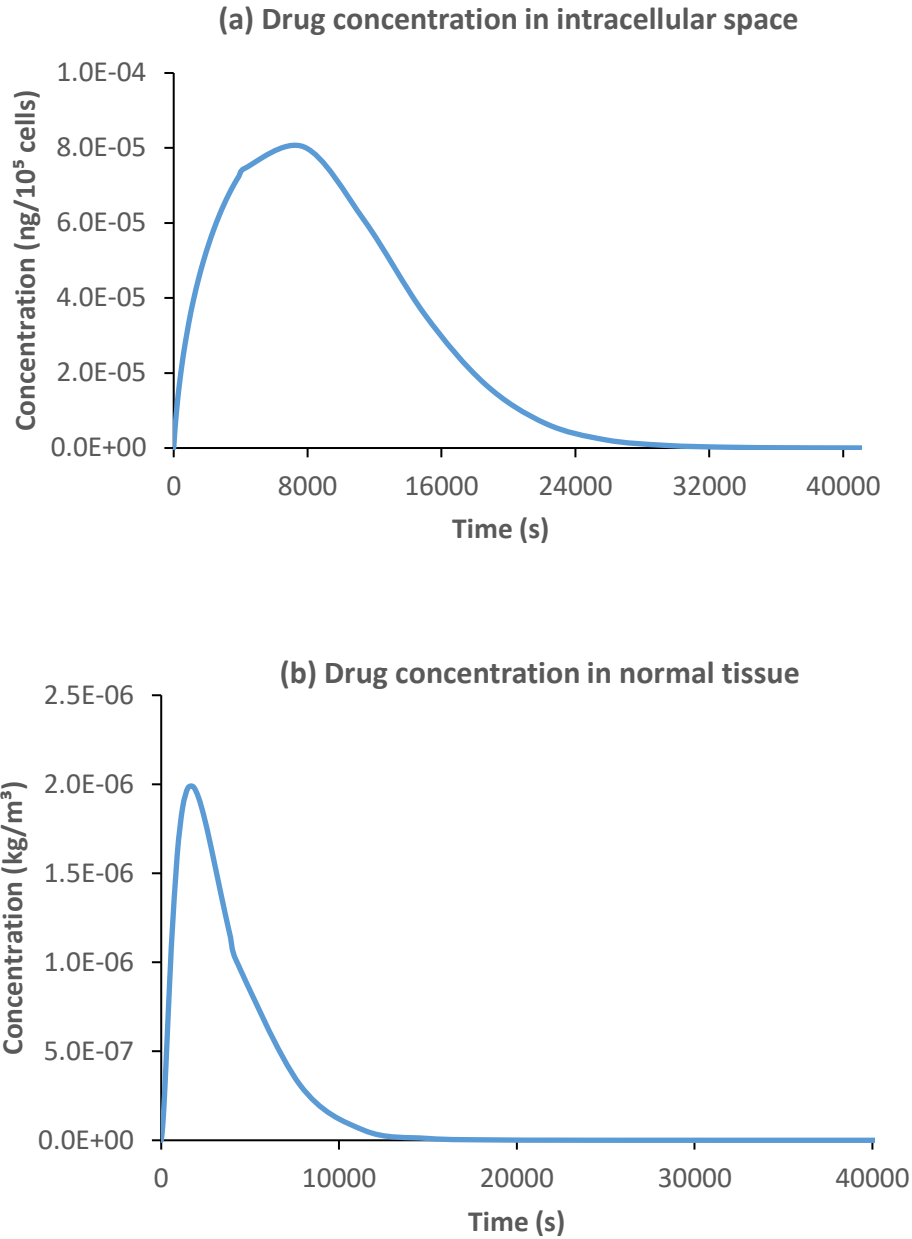
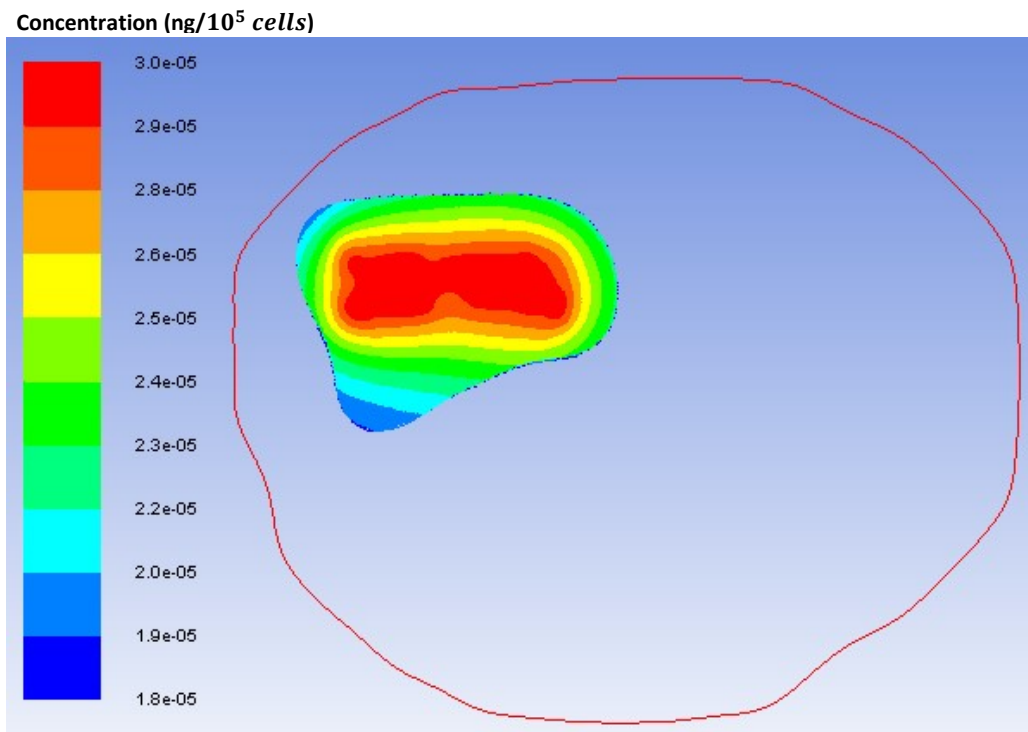


Figure 5.8: Spatially averaged drug concentration in (a) intracellular space, and (b) normal tissue

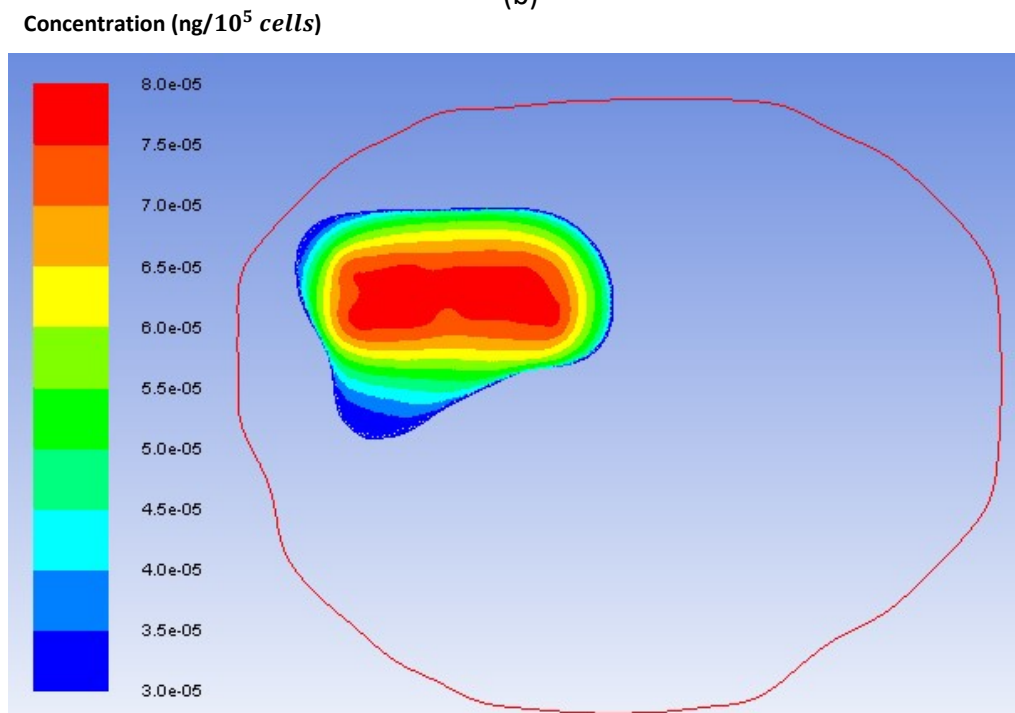
Having examined the temporal profiles of spatially averaged drug concentrations, let's turn to spatial distributions of the drug. A homogeneous drug concentration in tumour intracellular space is desirable as it would lead to uniformly distributed tumour cell death. Figure 5.9 presents spatial distributions of the intracellular drug concentration at 600 s (10 min), 7800 s (2.2 hours) and 43200 s (12 hours), which correspond to immediately after hyperthermia, at peak intracellular concentration and the end of the treatment, respectively. The results show that drug accumulates at the centre of the tumour, but less drug reaches the edges the tumour, especially at the bottom left corner. Comparing with the spatial temperature profile in Figure 5.4 (a), it is clear that more

drugs accumulate in regions with higher temperature. Therefore, we conclude the temperature distribution has a significant impact on drug distribution. The more region reaches the phase transition temperature, the better the results of treatment.

(a)



(b)



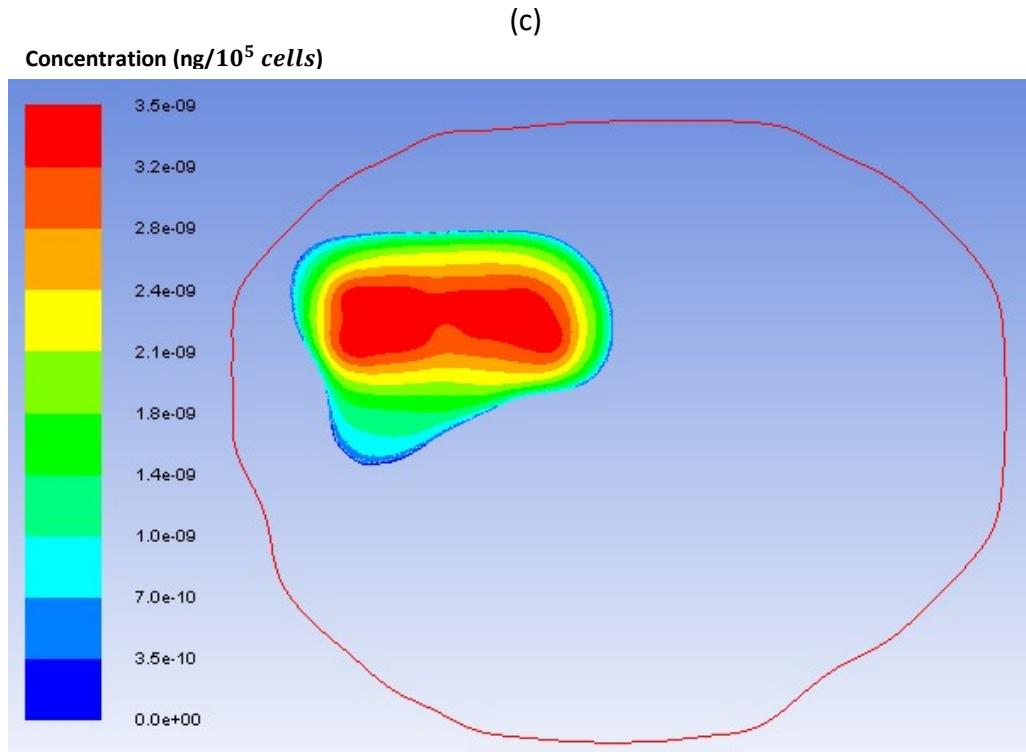


Figure 5. 9: Spatial distributions of drug concentration in tumour intracellular space: (a) immediately after hyperthermia, at $t = 600$ s (10 min), (b) peak intracellular concentration, at $t = 7800$ s (2.2 hours), and (c) end of the treatment, at $t = 43200$ s (12 hours).

5.5.1.3 Cancer cell survival fraction

Drug accumulation in the intracellular compartment triggers cell killing. The rate of killing can be estimated using an appropriate PD model as discussed in chapter 3. Figure 5.10 shows the predicted cell survival fraction using a general PD model for DOX. The survival fraction of tumour cells decreases gradually to 70% until 33000 s (9.2 hours). After 9.2 hours, the level of intracellular drug concentration is insufficient to cause cell death. Therefore, the survival fraction slowly increases as cancer cells regrow.

Figure 5.11 shows the spatial distribution of cancer cell density at 600 s (10 min), 7800 s (2.2 hours) and 43200 s (12 hours). It is consistent with the intracellular drug concentration profile in that more cancer cells survive at the top-left and bottom-left corners, where intracellular drug concentration is the lowest. At the end of the treatment, the percentage difference of cancer cell density between the corner and the centre of the tumour is approximately 13%. Comparing with the temperature distribution, it can be found that the region with a temperature above 40°C achieves more uniform and effective cancer cell killing. To further improve the cancer

cell killing fraction, a high level of intracellular drug concentration should be maintained. However, this is limited by insufficient drug release and drug binding with proteins.

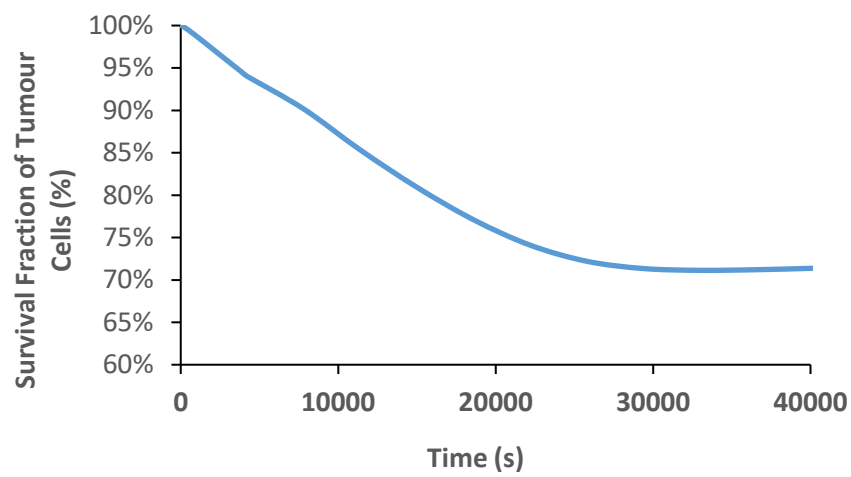
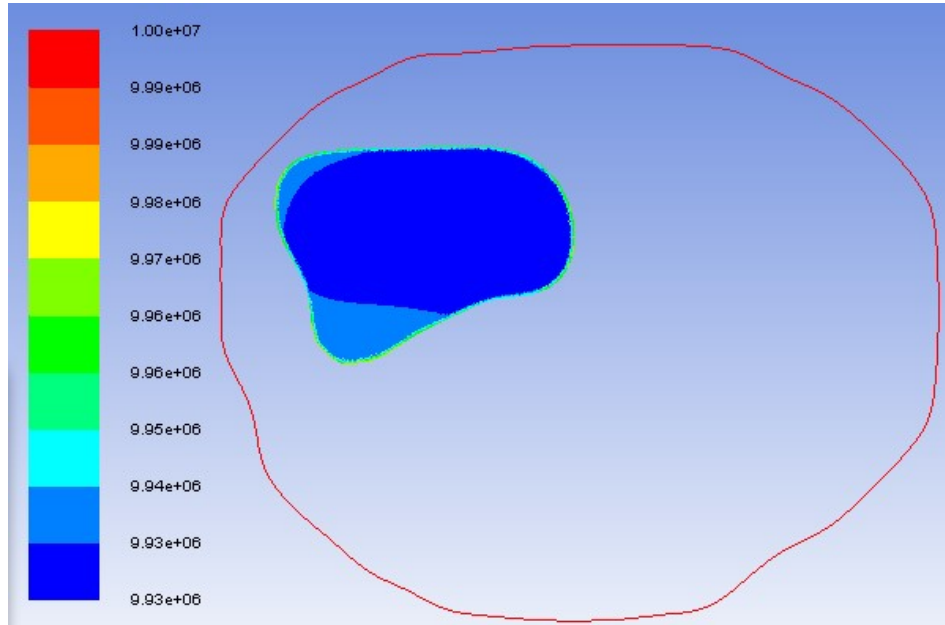


Figure 5. 10: Predicted cancer cell survival fraction using a general PD model for DOX for baseline case

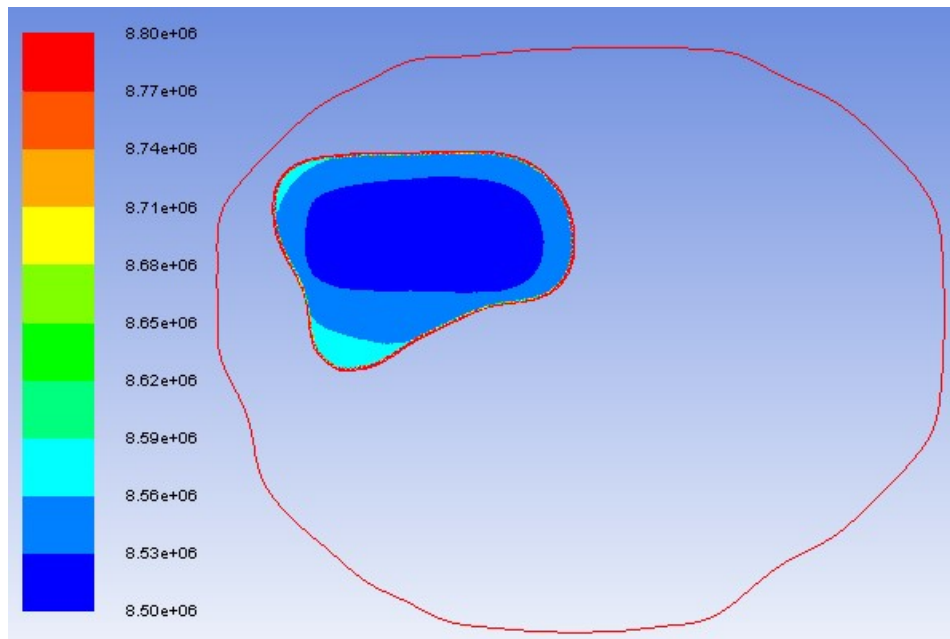
(a)

Cell density (cells/ m^3)



(b)

Cell density (cells/ m^3)



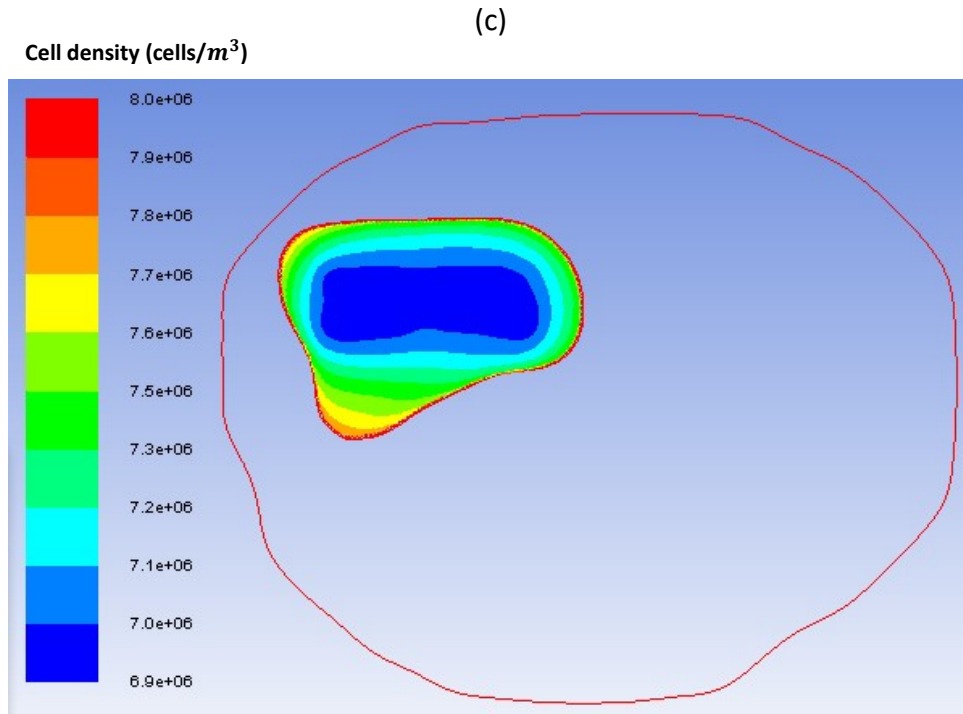


Figure 5.11: Spatial distributions of cancer cell density at different time points: (a) immediately after hyperthermia, $t = 600$ s (10 min), (b) peak intracellular concentration, $t = 7800$ s (2.2 hours), and (c) end of the treatment, $t = 43200$ s (12 hours).

5.5.2 Effects of hyperthermia duration and drug release rate

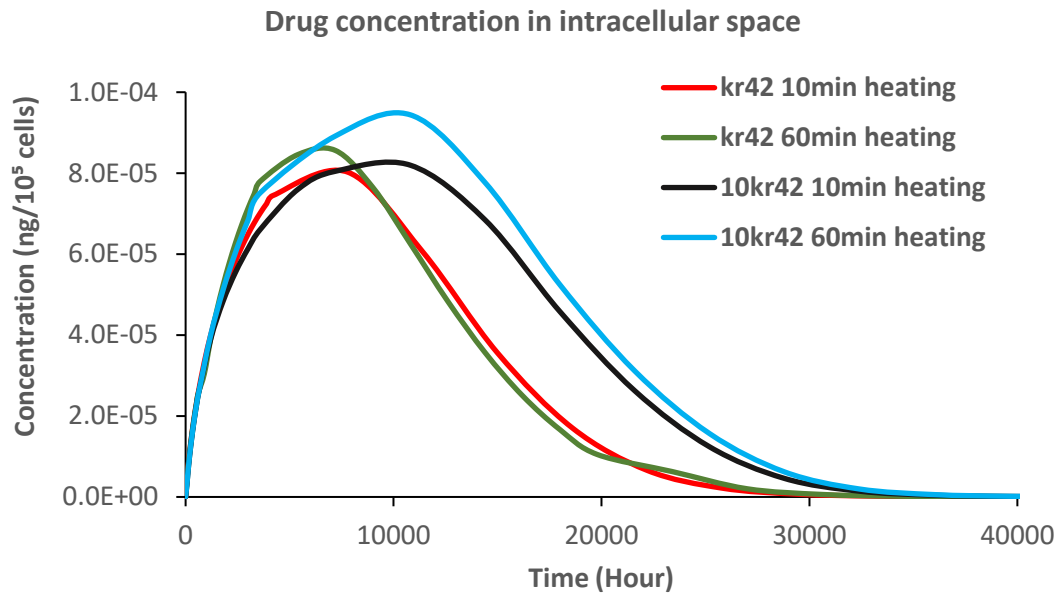
The baseline case analysis identifies two parameters, namely hyperthermia duration and drug release rate, that are important in determining the therapeutic efficacy and side effects. The maximum clinical hyperthermia duration (60 min) is simulated to compare with results obtained with 10 min heating in the baseline case. It has been reported in the literature that the drug release rate from TSL can be manipulated by changing the liposome formulation. Therefore, a 10-fold increase in release rate at 42°C is chosen to determine its effects on temporal variations of drug concentration in intracellular space and normal tissue.

Figure 5.12 (a) shows that extending the heating duration from 10 min to 60 min increases the peak intracellular concentration by 6.3%. This is due to a combination of additional amount of drug release and changes in transport parameters. However, the difference in intracellular concentration is fairly modest. Prolonging heating duration increases the total amount of drug release in tumour extracellular space, but has little influence on the instantaneous value which determines the driving force for transport between the extracellular space and

intracellular space. Therefore, longer hyperthermia duration has limited impacts on intracellular drug concentration. Figure 5.12(b) shows that drug concentration in normal tissue is hardly affected by the hyperthermia duration.

Increasing the release constant at 42°C ($10k_{r42}$) has a more profound effect on intracellular drug concentration as shown in Figure 5.12. A faster release leads to a spontaneous increase in drug concentration in tumour extracellular space, which increases the driving force for transmembrane drug transport. Compared to the results obtained with the baseline release rate (k_{r42}), the intracellular drug concentration curves with $10k_{r42}$ are wider with delayed peak values; all these help to augment the AUC value. On the other hand, the free drug concentration in normal tissue increases slightly with the higher drug release rate. The effect of increasing the AUC of intracellular drug concentration gives a higher fraction of cancer cell killing, which is shown in figure 5.13. Increasing the drug release rate at 42°C by 10 fold improves the cancer cell killing fraction by 5%.

(a)



(b)

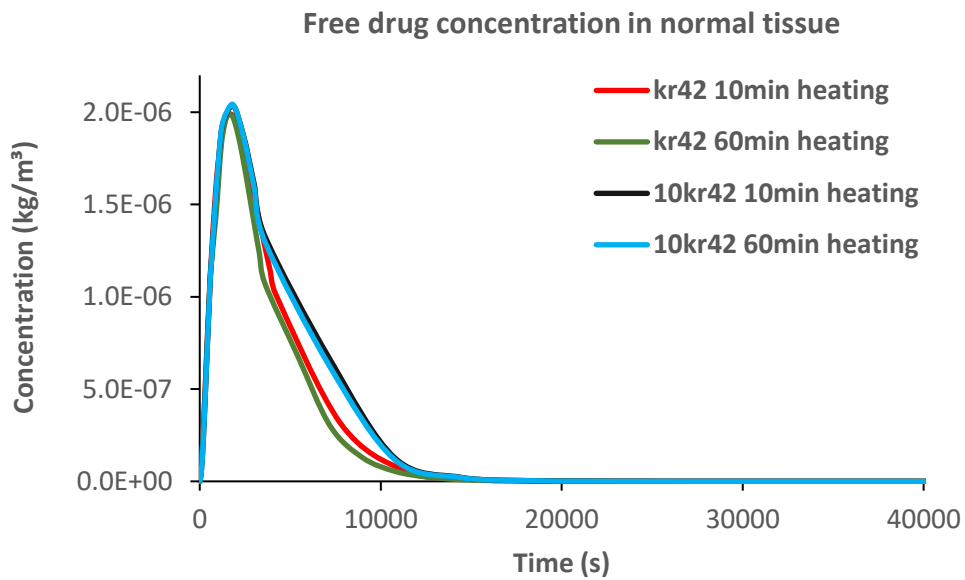


Figure 5.12: Spatially averaged drug concentrations at different conditions in (a) intracellular space, and (b) normal tissue.

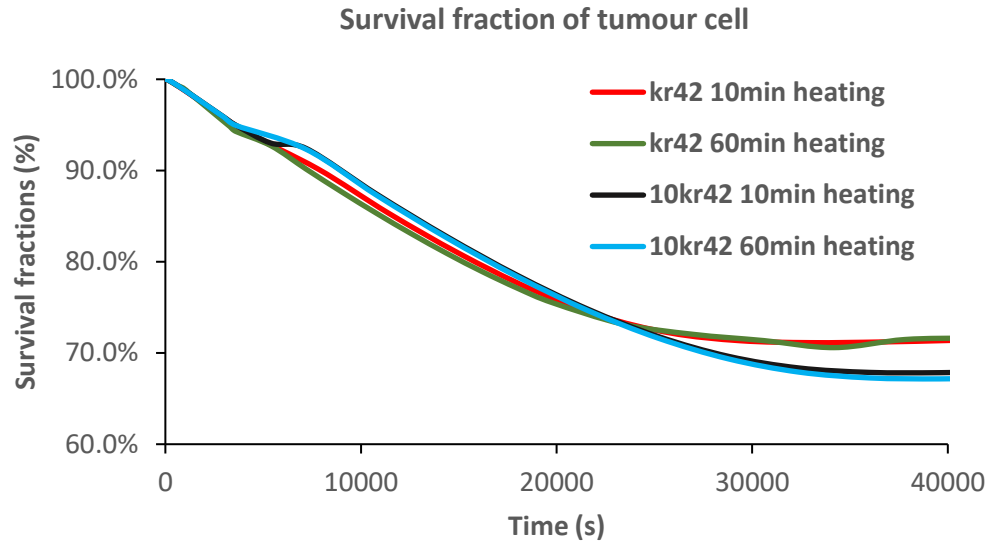


Figure 5.13: Predicted survival fraction of tumour cells with different liposome formulations and heating durations

Although Figure 5.13 suggests that 10kr42 with 10 min and 60 min heating lead to a similar cancer cell survival fraction, the spatial distribution of the survived tumour cell is different as shown in Figure 5.14. Comparing the spatial distribution of tumour cell density at the end of the treatment between 10 min and 60 min hyperthermia reveals that more tumour cells are killed in the core region with a longer heating duration. This is likely attributed to the effect of temperature on transport properties. Longer heating duration allows more efficient drug transport in the high temperature region for an extended period, further enhancing the local therapeutic efficacy. These results differ from the original expectation that extending the heating duration might improve the uniformity in temperature and allow more drugs to reach the corners of the tumour. To help interpret the results, the temperature profiles of the two cases are compared. Figure 5.14 shows that the temperature profile with 60 min heating is the same as that with 10 min heating shown in Figure 5.14 (baseline case heating). Hence, hyperthermia duration does not improve the spatial distribution of temperature.

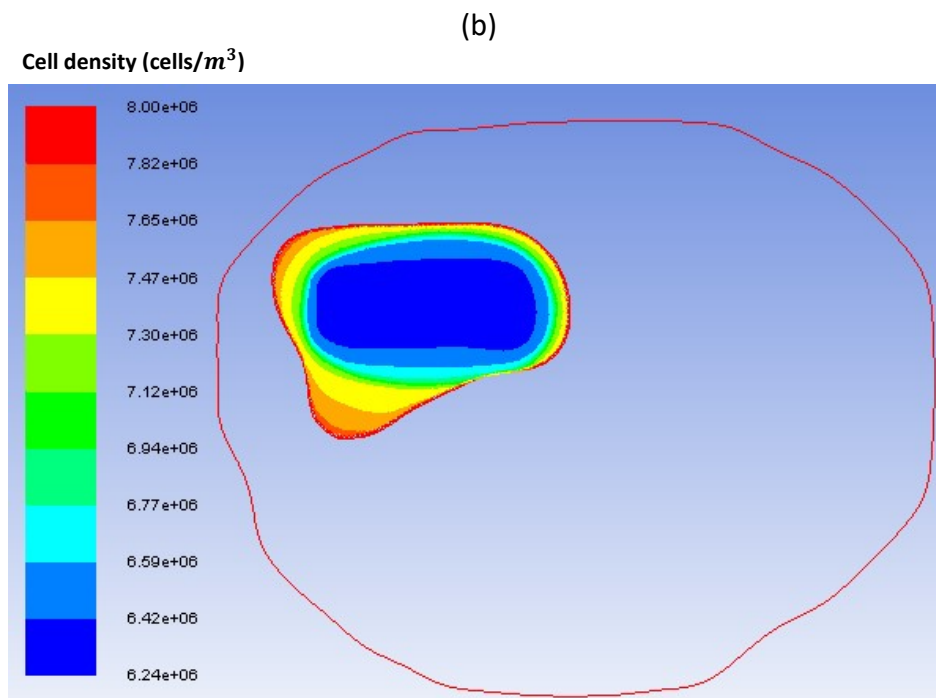
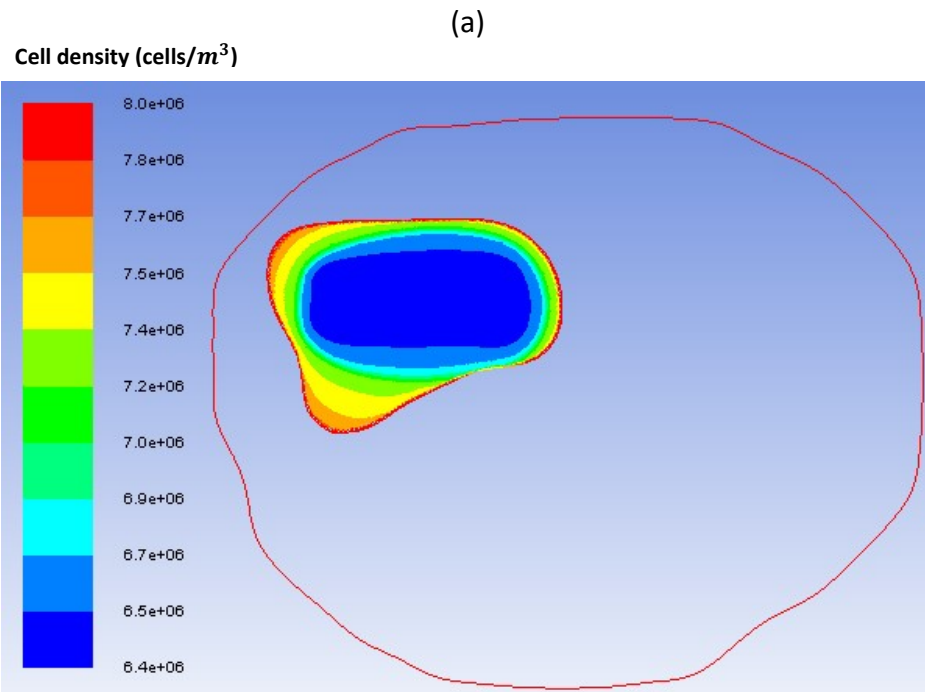


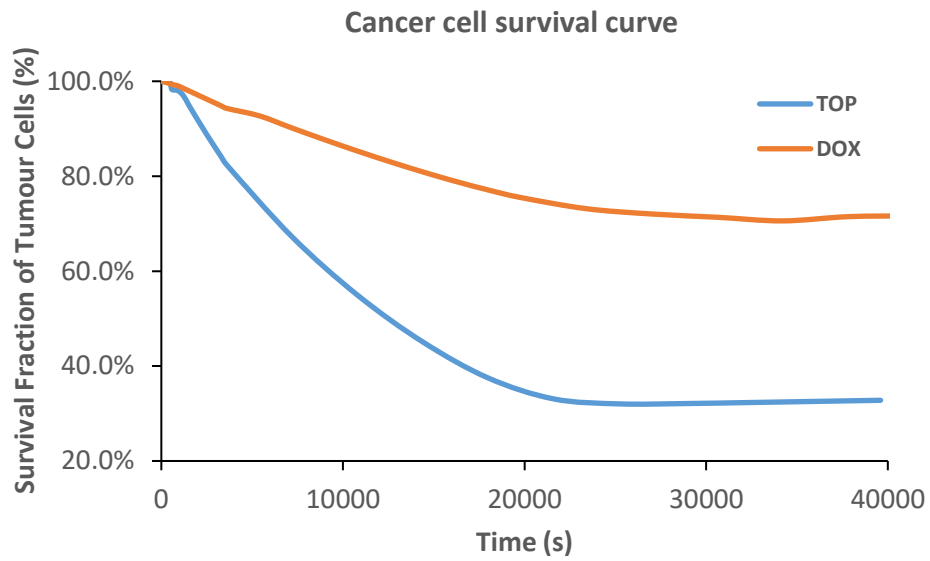
Figure 5. 14: Spatial distribution of cancer cell density with 10kr42 at 12 hours for (a) 10 min heating, and (b) 60 min heating.

5.5.3 Effects of liposome formulation and anticancer drug

Different liposomes and drugs can also be simulated using the macroscopic drug delivery model. The imageable thermosensitive liposome (iTSL) loaded with TOP (Centelles et al., 2018a) is simulated to compare with the results for TSL-DOX discussed above. The therapeutic efficacy and side effects are evaluated based on the fraction of tumour cell survival and drug concentration in normal tissue, respectively. A hyperthermia duration of 60 min is applied to show the maximum potential of cancer cell killing. Figure 5.15 (a) compares the cancer cell survival fraction of TSL-DOX and that of iTSL-TOP. At the end of the treatment, iTSL-TOP achieves 70% cancer cell killing, which is 40% higher than that of TSL-DOX. The improvement in the predicted therapeutic efficacy can be attributed to four factors. Firstly, the drug release rate of iTSL is slightly higher than that of the conventional TSL, which enhances the driving force for drug transmembrane transport. Secondly, TOP has a smaller molecular size than DOX. Therefore, its diffusivity and permeability are higher than those of DOX, so that TOP can extravasate more easily from the blood to the tumour extracellular space, and the diffusion for TOP in extracellular space is faster than that of DOX. Thirdly, the equilibrium constant of the free and bound drug for TOP is approximate 2:1 whereas that of DOX is 1:3 meaning that more free TOP is available in the system. Since only free drug can pass through the cell membrane, a lower binding rate would enhance cancer cell killing potential. Finally, TOP has a much lower EC_{50} (the concentration of a drug that gives half maximal response) than DOX, meaning that less TOP is required for the same fraction of tumour death. Therefore, iTSL-TOP is predicted to have better therapeutic efficacy than TSL-DOX.

On the other hand, Figure 5.15 (b) reveals that the free TOP concentration in normal tissue is twice as much as the concentration of DOX, suggesting that iTSL-TOP may be associated with more severe side effect compared to TSL-DOX. The high concentration in normal tissue is caused by more free drug availability in both blood and tumour extracellular space. The PD model for normal cell killing is highly depended on tissue properties, which is not included in our model. The drug concentration in normal tissue is used to assess the risk of potential side effects.

(a)



(b)

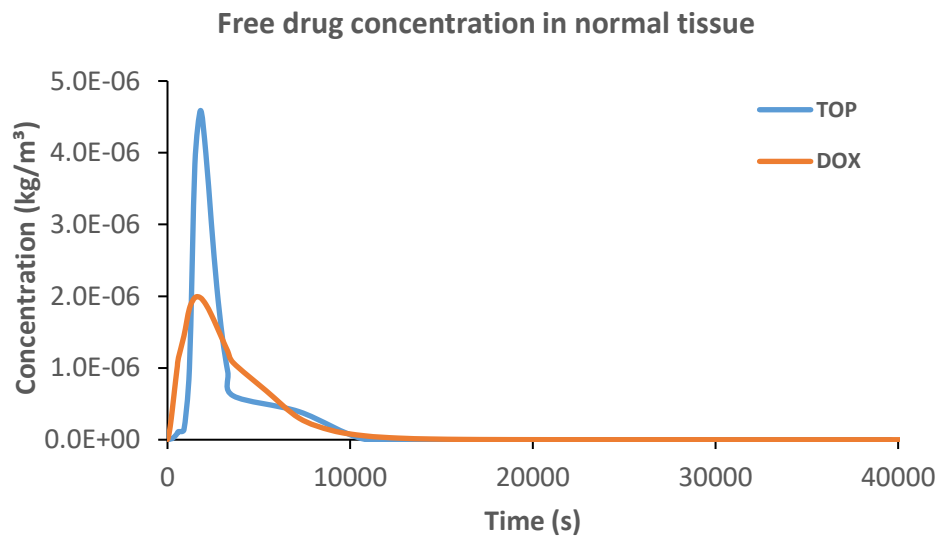


Figure 5. 15: (a) Predicted cancer cell survival fraction, and (b) free drug concentration for different drugs.

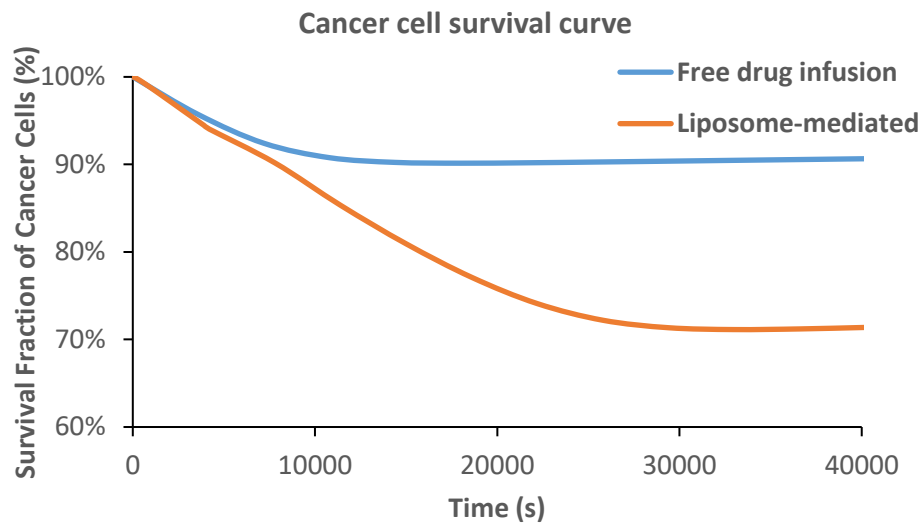
5.5.4 Comparison with conventional delivery of DOX without using TSL

Results for TSL-DOX and iTSL-TOP have been analysed and compared so far. It would be of interest to evaluate the effectiveness of TSL as a chemotherapy drug carrier by comparing results for TSL-DOX with those without TSL-mediated delivery. For this purpose, the delivery of free DOX without the use of FUS and bio-heat transfer equation is simulated. The same drug infusion mode (bolus infusion) and drug dosage (50mg/kg) are applied.

Figure 5.16 shows the fraction of cancer cell survival and free drug concentration in normal tissue. The predicted cell survival fraction with the delivery of free DOX is much higher than that with TSL-DOX. The blood clearance rate of free drug is much higher than that of the liposome. Therefore, a large amount of free drug is removed by the circulation system in the blood plasma compartment, meaning that less drug is available in tumour extracellular space and intracellular space. Less free drug leads to lower cancer cell killing. Another reason for the reduced therapeutic efficacy is related to temperature. Without hyperthermia, temperature in the tumour region is constant at body temperature (37°C) where the diffusivity and permeability of drug are lower than those at a higher temperature. Therefore, the amount of drug reaching tumour intracellular space is less than that achieved at hyperthermia.

On the other hand, the free drug concentration with free DOX delivery is higher than that with TSL-mediated drug delivery (Figure 5.16 (b)). At the beginning of treatment, the free drug concentration in blood plasma is high. Therefore, the driving force of drug transport between blood and normal tissue is strong. The free drug is transferred quickly from blood to the normal tissue. However, as the time proceeds, the free drug concentration drops fast in blood plasma due to the blood clearance effect, causing the rapid decline of drug concentration in normal tissue. These results suggest that the main issues that limit the therapeutic efficacy of free drug delivery are high clearance rate, high initial drug concentration in blood compartment and low transport parameters.

(a)



(b)

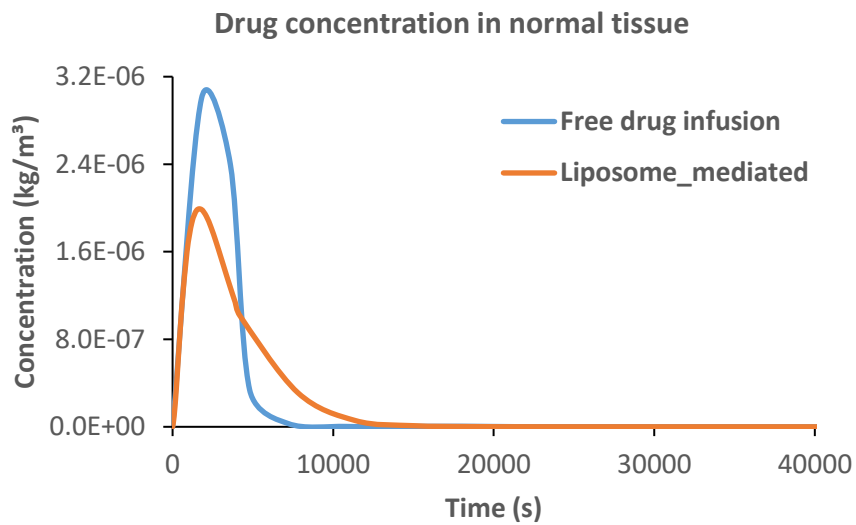


Figure 5. 16: Comparison between free drug infusion and liposome-mediated drug delivery: (a) cancer cell survival fraction, and (b) free drug concentration in normal tissue.

5.6 Summary

In this chapter, the continuum-based macroscopic drug delivery model has been applied to real tumour geometry to evaluate the therapeutic efficacy of TSL-mediated drug delivery. The model is coupled with FUS heating and bio-heat transfer equations to provide information on spatial and temporal distributions of temperature and drug concentration in each compartment. The baseline case simulation, which employs the conventional TSL loaded with DOX as the anticancer agent, reveals the influence of temperature profile on drug concentration distribution. The more area of tumour achieves the required hyperthermia, the more effective the cancer cell killing.

The baseline case gives a 70% cancer cell survival fraction at the end of the treatment, which can be improved by modifying some parameters of the system. The hyperthermia duration, drug release rate at phase transition temperature and type of anticancer drug are varied in an attempt to search for optimal treatment efficacy. The simulation results suggest that a high drug release rate, a low binding equilibrium constant and a drug with high cytotoxicity would give the most desired treatment outcome based on the predicted fraction of cancer cell survival. However, there is a trade-off between cancer cell killing and damage to the normal tissue; the latter is assessed based on drug concentration in normal tissue.

The macroscopic model can be easily adapted to simulate different drug delivery systems. Patient images with specific transport parameters can be employed to give a more realistic estimation of the therapeutic efficacy on a patient-specific basis. The assumption of homogeneous transport properties in tumour regime and uniform distribution of blood vessels can be replaced by heterogeneous transport properties and realistic blood vessel distribution. Furthermore, the number and location of focal points can be optimised to achieve more uniform temperature distribution, which will be discussed in the next chapter.

Chapter 6: Focal points selection

6.1 Introduction

The release of drug encapsulated in TSLs is triggered by hyperthermia, hence the temperature profile in tumour will have a direct influence on the level of drug concentration and its distribution. The latter determines the therapeutic efficacy and risk of side effects. Therefore, it is essential to gain detailed information on temperature profiles within the tumour and its surrounding tissue, which would help to develop methods to control this important parameter.

FUS is employed to heat the tumour to the desired temperature (42°C) to trigger local drug release. However, the size of the focal point produced by the ultrasound transducer array is much smaller than that of a real tumour, making it difficult to achieve uniform heating and a homogenous temperature profile at the tumour site. An unevenly distributed temperature field would result in non-uniform release and a heterogeneous distribution of anticancer drug, which would impair the therapeutic efficacy of TSL drug delivery systems.

Ultrasound transducer arrays can be employed to produce multiple focal points which could be spread around the tumour to improve the uniformity of temperature distribution. The number and location of focal points have a strong influence on the temperature profile; therefore, these should be selected with careful consideration.

A mathematic model is developed to serve the purpose of predicting the temperature distribution in tumour upon the application of FUS hyperthermia. The model is capable of simulating the heating process with multiple focal points and predicting the temperature distribution in tumour and blood compartments by coupling the acoustic wave equation with the bio-heat transfer equations. The simulation of acoustic wave propagation and bio-heat transfer is accomplished in MATLAB and ANSYS Fluent, respectively. In order to ensure the safety of FUS heating, a feedback control loop is included in the model. When the temperature of any point in the tumour exceeds 42°C, FUS heating would be stopped until the system cools down. The model has been applied to idealised tumour geometries with circular and elliptic shapes. By changing the number and location of focal points, we are aiming to achieve the following: (i) more tumour area reaches the desired hyperthermia for drug release from TSL, and (ii) more uniform temperature distribution in tumour.

6.2 Model geometry and parameters

Figure 6.1 shows the geometry of the idealised models where the tumour region is shown in red and the normal tissue in blue. Figure 6.1 (b) and (c) are elliptical with aspect ratios of 1.5 and 2, respectively. Although the shape of the tumour is different, the area of the tumour is kept the same to eliminate the influence of this factor. Table 6.1 summaries the relevant geometric parameters, while model parameters used in this chapter are the same as those in Table5.1 and 5.2 in Chapter 5.

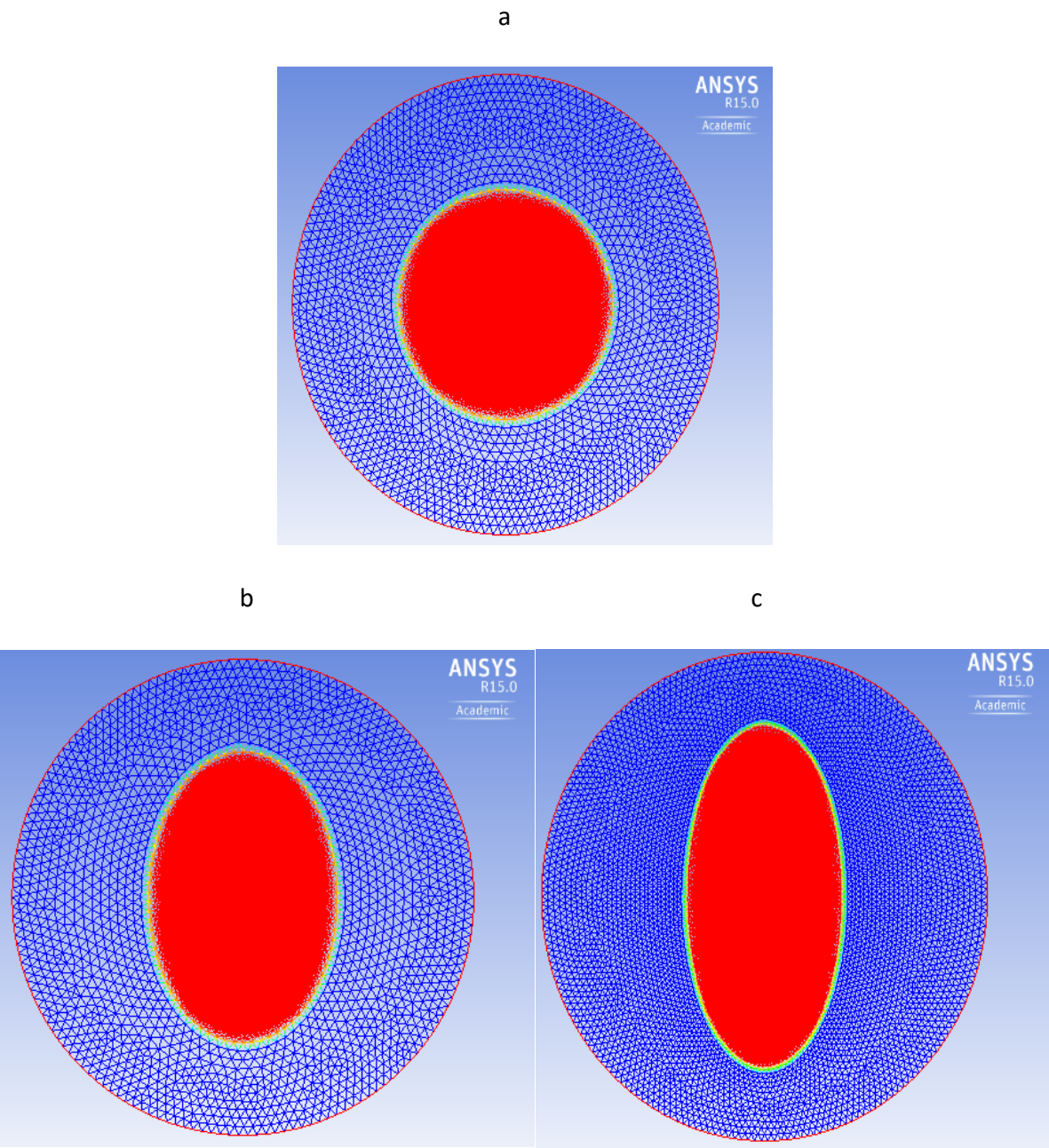


Figure 6. 1: Model geometry (a) Circular tumour, (b) ellipse A with aspect ratio 1.5, (c) ellipse B with aspect ratio 2

Table 6. 1: Geometric parameters of the models for focal point selection simulations

	Circle	Ellipse A	Ellipse B
Major axis (cm)	2.50	3.06	3.54
Minor axis (cm)	2.50	2.04	1.78
Aspect ratio	1	1.5	2

6.3 Methods

The standard approach for the simulation of acoustic wave propagation is by using the point source method. However, this method involves a double integral over the transducer geometry, which is inaccurate and computationally expensive (Zemanek, 1971). The impulse response method was developed to overcome these issues by replacing the double integral with a single integral. Although it reduces the computational demand, the simulation results are still inaccurate due to a singularity in the near field (Lockwood and Willette, 1973; McGough et al., 2004b). In this study, the fast near field method is employed, which has been shown to be more accurate, while also being computationally more efficient than the other two methods (McGough, 2004; McGough et al., 2004b).

An open source code named FOCUS, developed by Michigan State University, was employed to solve the acoustics equation. FOCUS is a MATLAB based code and uses the fast near field method to simulate the FUS effects. Furthermore, it is capable of simulating multifocal heating generated by an ultrasound transducer array (McGough et al., 2004b).

The obtained acoustic pressure field is converted to an acoustic power field, which is then applied as a source term in the bio-heat transfer equation in ANSYS FLUENT. It is essential to import the results from MATLAB to ANSYS FLUENT accurately. In order to achieve this, an interface was built between MATLAB and ANSYS FLUENT. A rectangle area in the tumour is selected and named as heating zone. The same shape, mesh and size of the geometry are generated in MATLAB. The acoustic field is simulated and converted to acoustic power on the geometry built in MATLAB. The acoustic power is then imported as a source term in ANSYS FLUENT by a mapping function. Figure 6.2 shows the acoustic pressure field obtained in MATLAB and the one applied in ANSYS FLUENT, demonstrating that the mapping is done correctly.

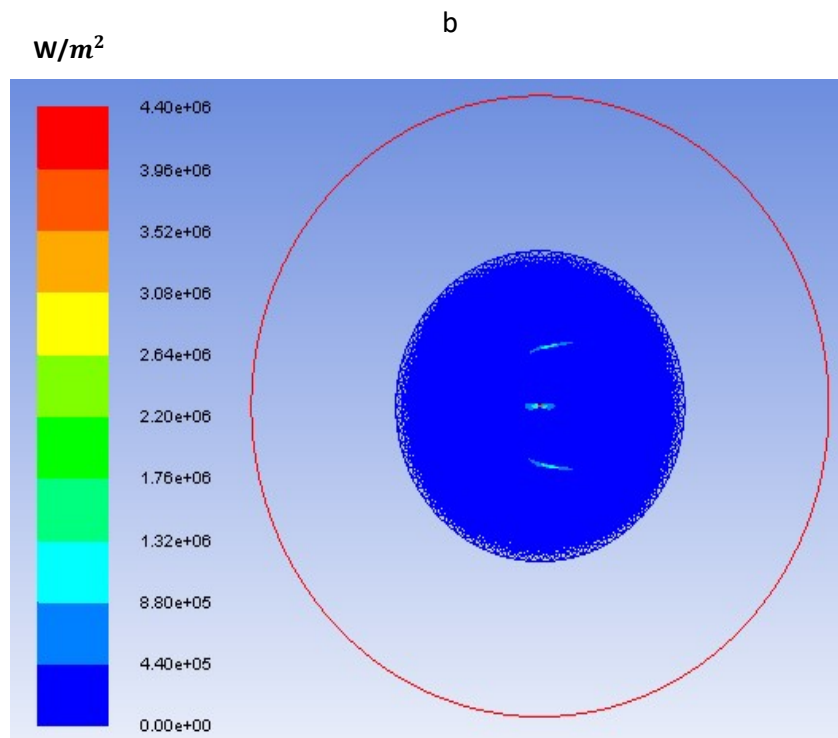
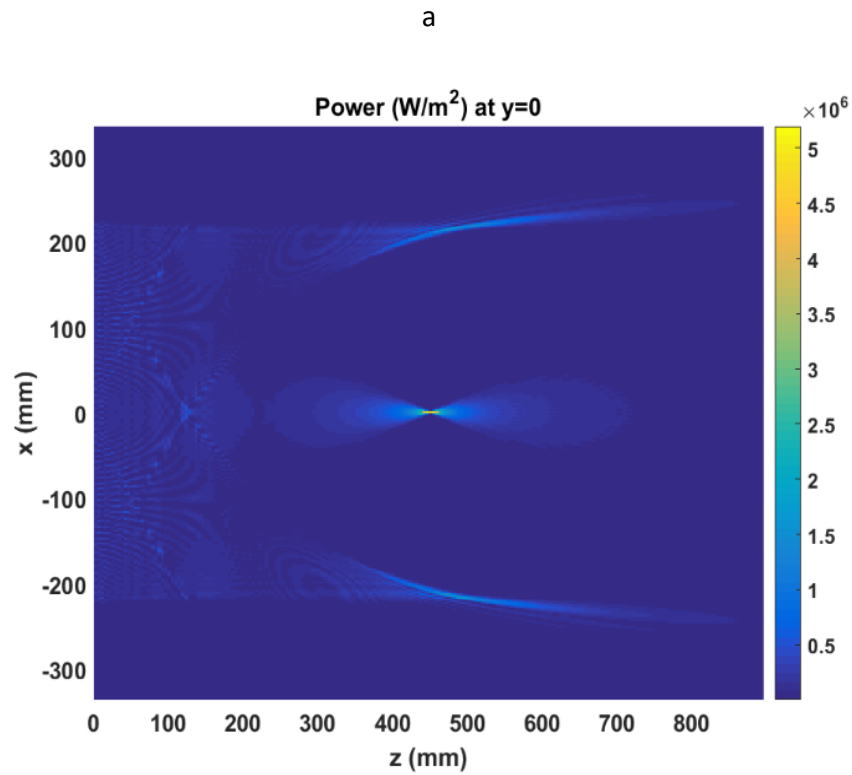


Figure 6. 2: Acoustic power field with a single focal point. (a) MATLAB simulation results, (b) ANSYS FLUENT mapping results

By solving the bio-heat transfer equations in ANSYS FLUENT, temperature distributions in the tumour, blood and normal tissue can be obtained. Computational mesh is generated using a software named Gambit (Ansys Inc. Canonsburg, Pennsylvania). The tumour region consists of quadrilateral elements in order to match with the rectangular grid in MATLAB. Triangle mesh is adopted in the normal tissue. The number of elements for the circular model, ellipse A and B are 121597, 114891 and 112965, respectively. Mesh sensitivity tests have been performed. The second order UPWIND scheme is selected for spatial discretisation of the heat transfer equations. Residual tolerance of the heat transfer equation is set to be 1×10^{-10} . The initial temperature of the tumour, the normal tissue and the blood is 37°C . At the interface between the normal and tumour internal boundary, all variables are continuous, while temperature at the outer surface of the normal tissue is fixed at 37°C .

6.4 Results and discussion

6.4.1 Baseline case

The circular model with a single focal point located at the centre is chosen as the baseline case. Figure 6.3 shows the spatial temperature distribution in the tumour region after 1-hour FUS heating. The maximum temperature is 315K (42°C) because of the temperature controller. Regions close to the transducer array and the focal point experience higher temperature, while the rest of the tumour, especially the periphery of the tumour, remains unheated.

Based on the relationship between release rate and temperature for the TSL described in Chapter 5, drug release rate at 40°C is fivefold of the release rate at body temperature (37°C). Therefore, 40°C is chosen as a threshold to indicate sufficient heating. Areas with temperature at 40°C or above are defined as sufficient heating zones (SHZ), as shown in Figure 6.3 (b). The percentage of sufficient heating is calculated as the ratio of the area of all the SHZ to the total area of the tumour. The percentage of sufficient heating for the baseline case is 33%, indicating that 67% of the tumour area is under-heated. Insufficient heating will cause low drug release, leading to poor therapeutic efficacy and unevenly distributed cancer cell survival.

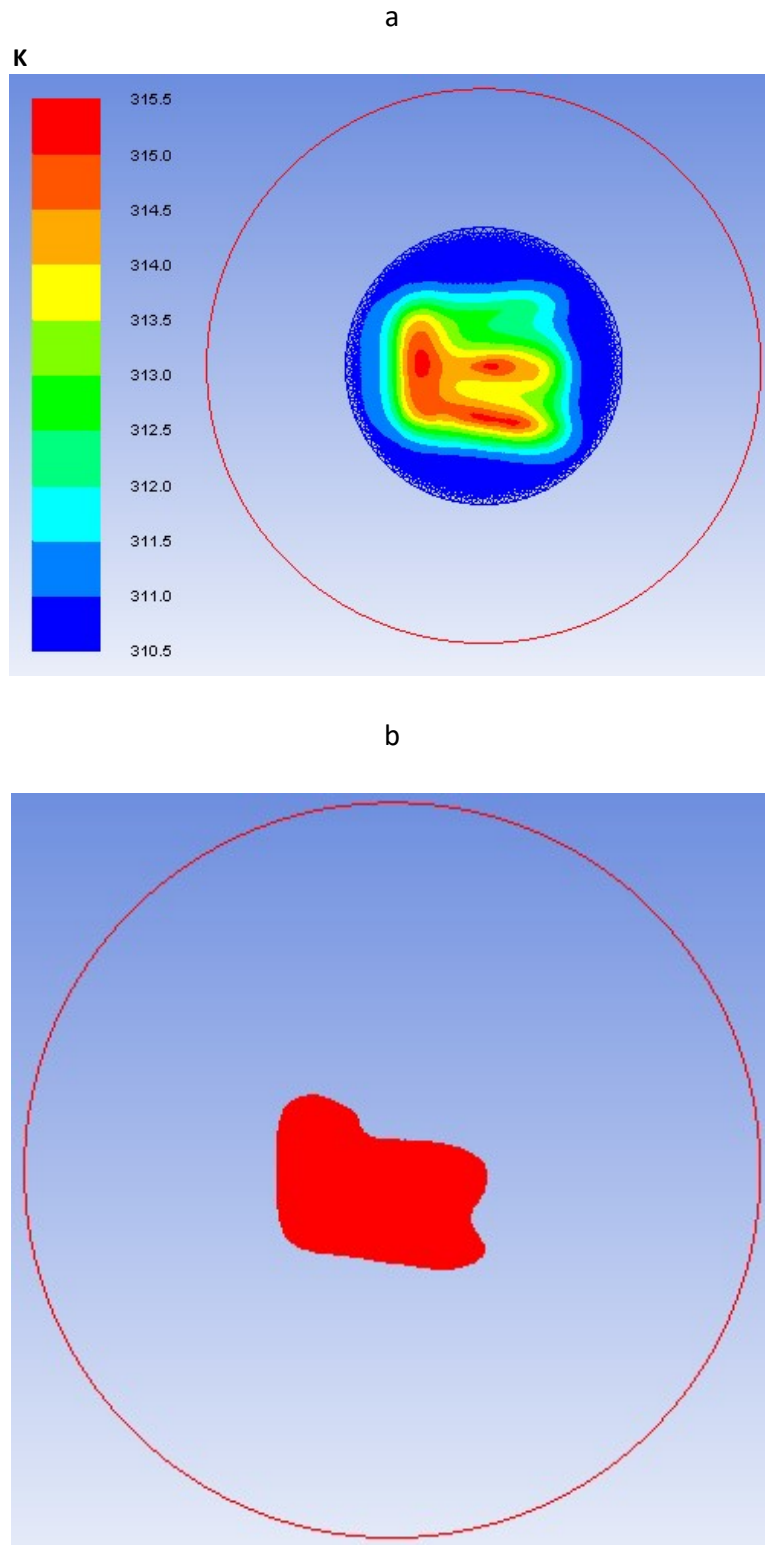
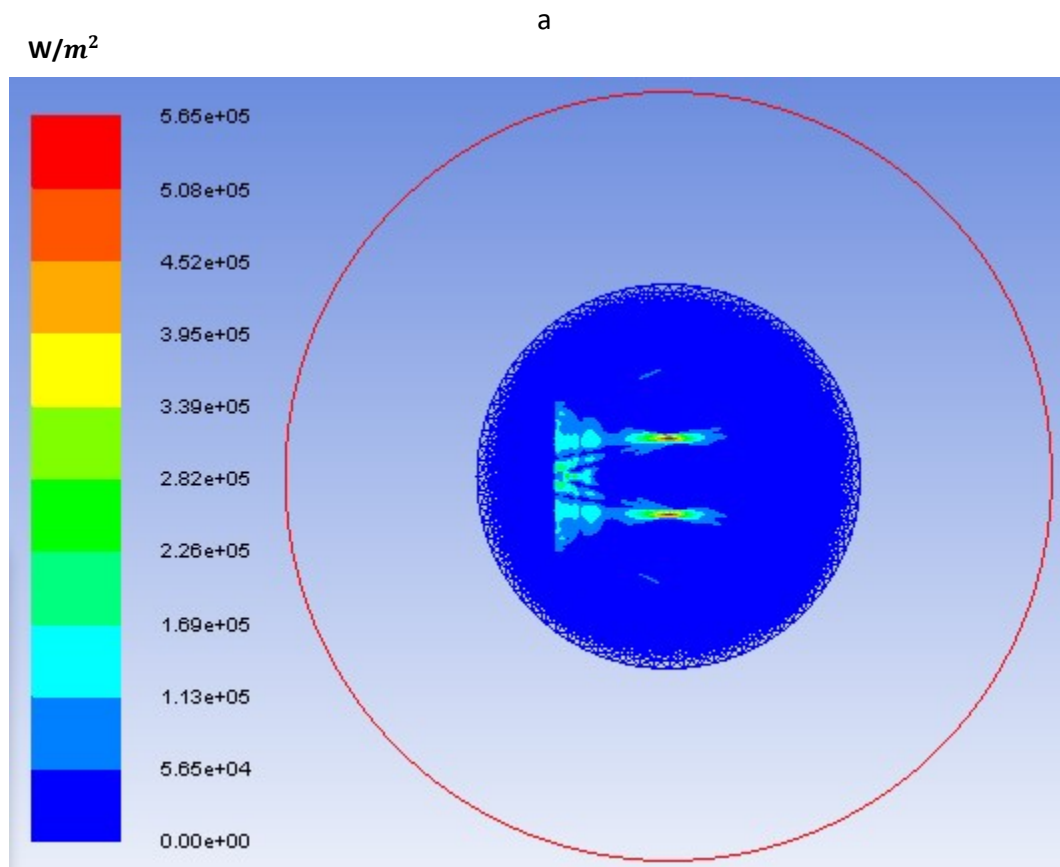


Figure 6. 3: (a) Spatial temperature distribution for the baseline case. (b) The sufficient heating zone where temperature is above 313K.

6.4.2 Effect of multifocal heating

FUS heating with a single focal point yields 33% sufficient heating, indicating that a single focal point is not adequate for the size of the tumour. Multifocal heating with two and three focal points is tested in an attempt to improve the fraction of sufficient heating. Figure 6.4 (a) and (b) show the acoustic power field generated by the ultrasound transducer array at the tumour site for two and three focal points, respectively. As the number of focal points increases, the interference of the acoustic waves becomes more obvious. The acoustic pressure field converges along a thin belt through each focal point.



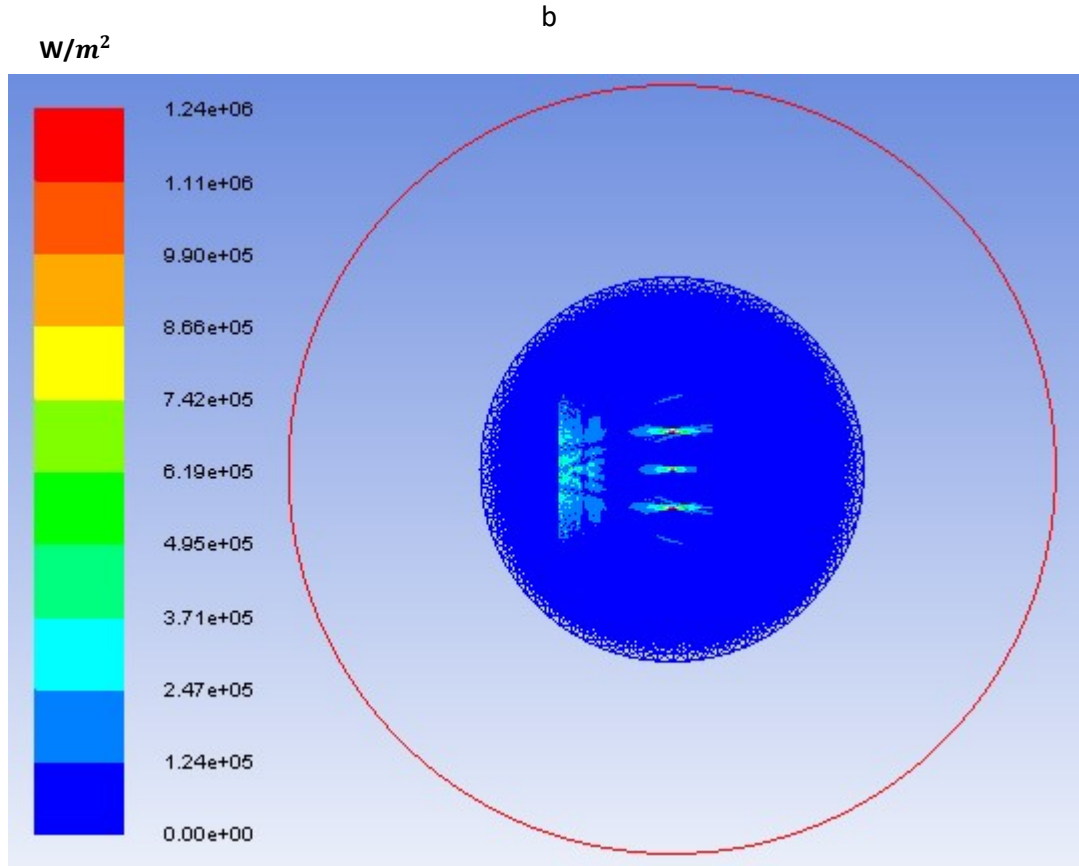


Figure 6. 4: Acoustic power field at tumour site with (a) two focal points, and (b) three focal points.

Figure 6.5 (a) and (b) show the SHZ with the two focal points and three focal points, respectively. It is obvious that more uniform heating is achieved with multiple focal points compared to that with a single focal point. The fractions of the SHZ relative to the tumour area are 40.3% and 36% for the two and three focal cases, respectively. It is surprising that the sufficient heating area obtained with the two focal points is higher than that of the three focal case. This is unexpected and suggests that the size of the SHZ may not necessarily increase with the number of focal points. When the number of focal points is altered, the acoustic power field changes accordingly. For a specific ultrasound transducer array with a fixed frequency, the number of focal points and acoustic power depend on each other, making it difficult to control each variable independently. However, some general trend can be found from the simulation results.

It is interesting to note that the acoustic power at the focal point is much lower in the double focal case compared to the single and triple focal cases. The FUS is so powerful that the target area near the focal points reaches 42°C rapidly. The feedback controller detects the temperature rise immediately and stops the power input from the ultrasound transducer array. However, when the heating is terminated, areas with low or no acoustic power input remain less heated or unheated. While the controller ensures that the temperature within the tumour

region is within a safety threshold, but it prevents areas away from the focal points from being heated. The results suggest that for multifocal hyperthermia, the acoustic power at the focal point should be smaller in order to achieve a more uniform temperature profile.

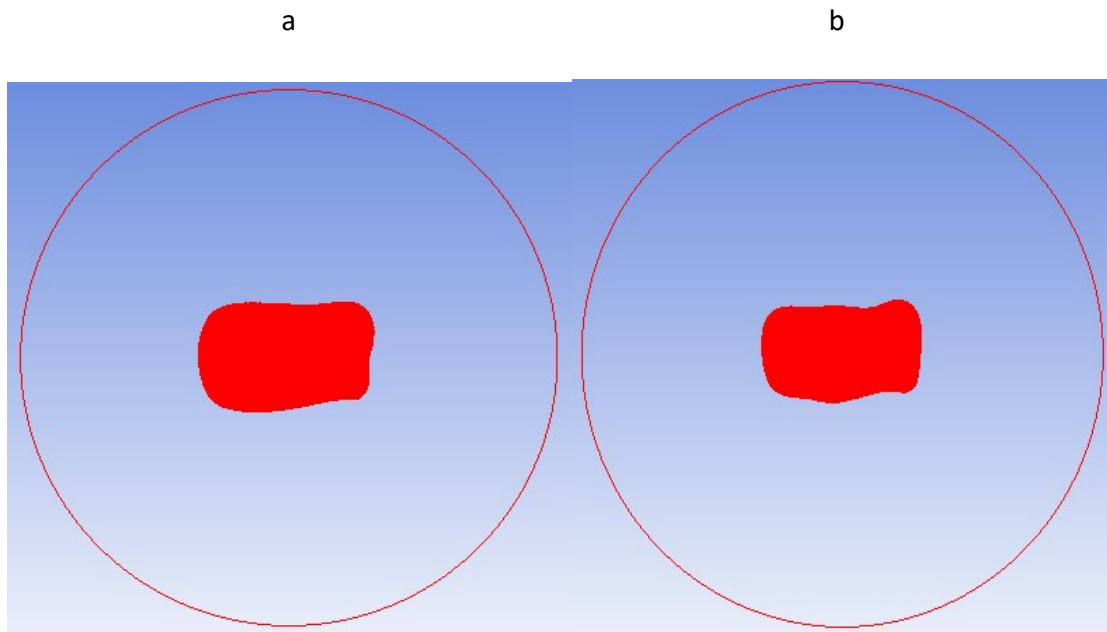


Figure 6. 5: Sufficient heating zone (SHZ) with multifocal heating in the circular tumour model with (a) two focal points, (b) three focal points.

6.4.3 Effects of tumour geometry and the location of focal points

Real tumours have irregular shapes, an example of which has been given in chapter 5. The shape of the tumour could have impacts on the sufficient heating area, even with the same acoustic power input. Figure 6.7 compares the predicted SHZ for different idealised tumour models with the same acoustic pressure input as the circular geometry discussed in the preceding section.

Figure 6.6 shows the locations of the focal points, which are at the geometric centre, the foci and a combination of both, for the single, double and triple focal cases respectively. Similarly, to findings from the circular geometry, a single focal is insufficient to heat a broad area of the tumour. Figure 6.7 (a1) and (b1) show that the SHZ for both ellipse A and B consists of two regions. The discontinuous and unevenly distributed high temperature would result in a patchy drug distribution in the tumour region. Increasing the number of focal points to two (Figure 6.7 (a2) and (b2)) and three (Figure 6.7 (a3) and (b3)) allows the SHZ to expand and cover more tumour area. A quantitative comparison of the fraction of SHZ between different tumour geometries is summarised in Table 6.2. It shows that for all three geometries, double focal offers the highest SHZ fraction, which supports the argument that the lower the power input at the focal, the more uniform the heating.

Another trend illustrated in Table 6.2 is that for the same number of focal points, the geometry with a higher aspect ratio achieves more uniform heating as measured by the fraction of SHZ. This can be explained by the location of the focal points. In the simulations, all focal points are distributed along the major axis. For ellipse A and B, the foci are selected as the focal points, which are further apart from each other covering more area of the tumour. The well spread focal points lead to a higher fraction of SHZ. With a single focal point located at the geometric centre, which is the same for all three models, the predicted SHZ fractions are similar. . Therefore, the fraction of SHZ is not affected by the geometry shape when the focal point is located at the geometric centre.

Since the size of the focal is much smaller than that of the tumour, the tumour geometry has a minor influence on the fraction of SHZ. Based on these results, we can deduce that the closer the focal points, the smaller the sufficient heating fraction.

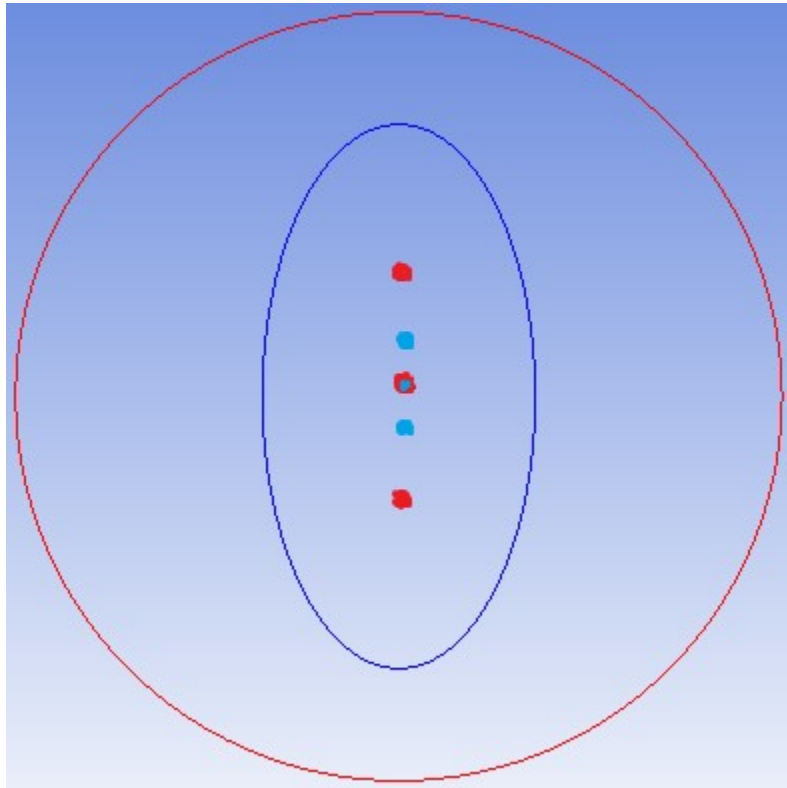
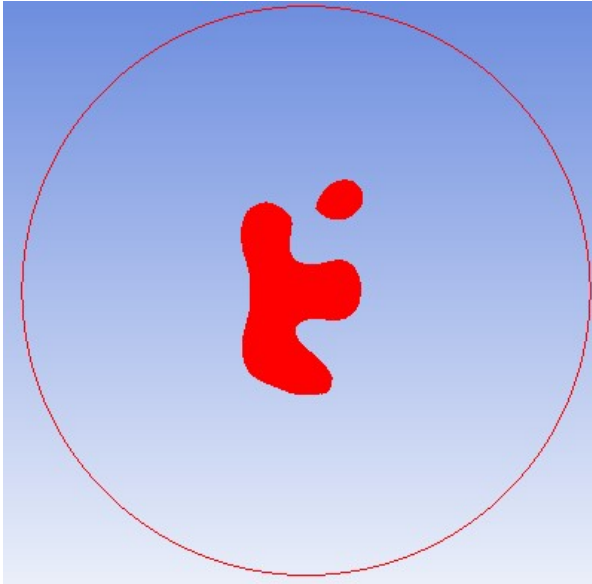
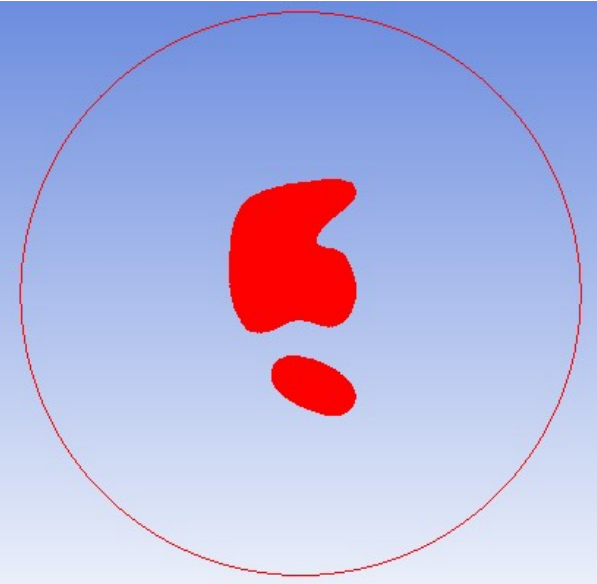


Figure 6. 6: Locations of the focal points: focal points at the foci (red dot) and focal points close to the geometry centre (blue dot)

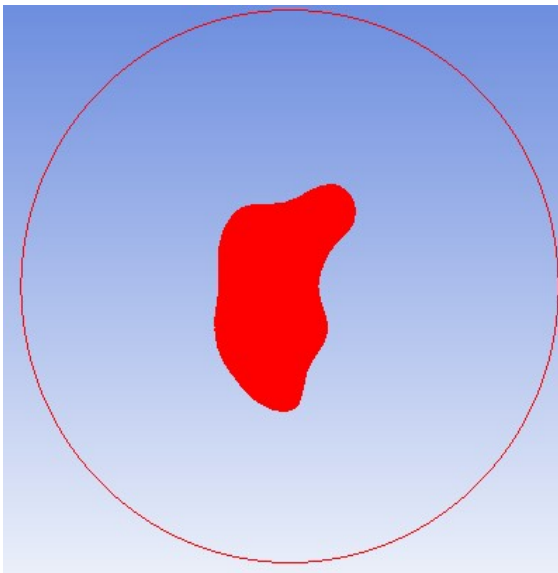
a1



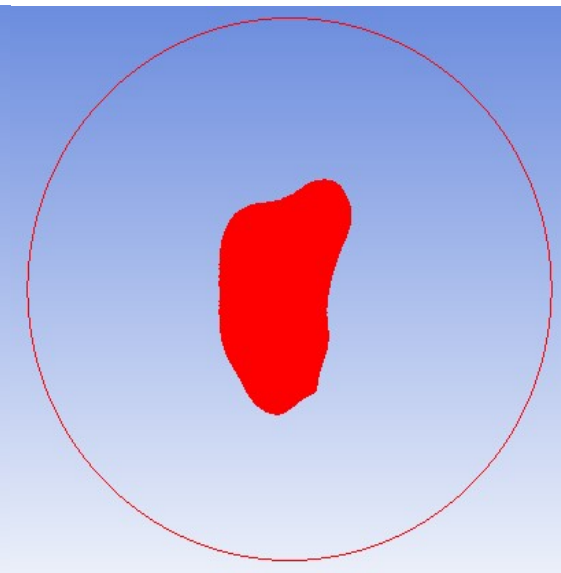
b1



a2



b2



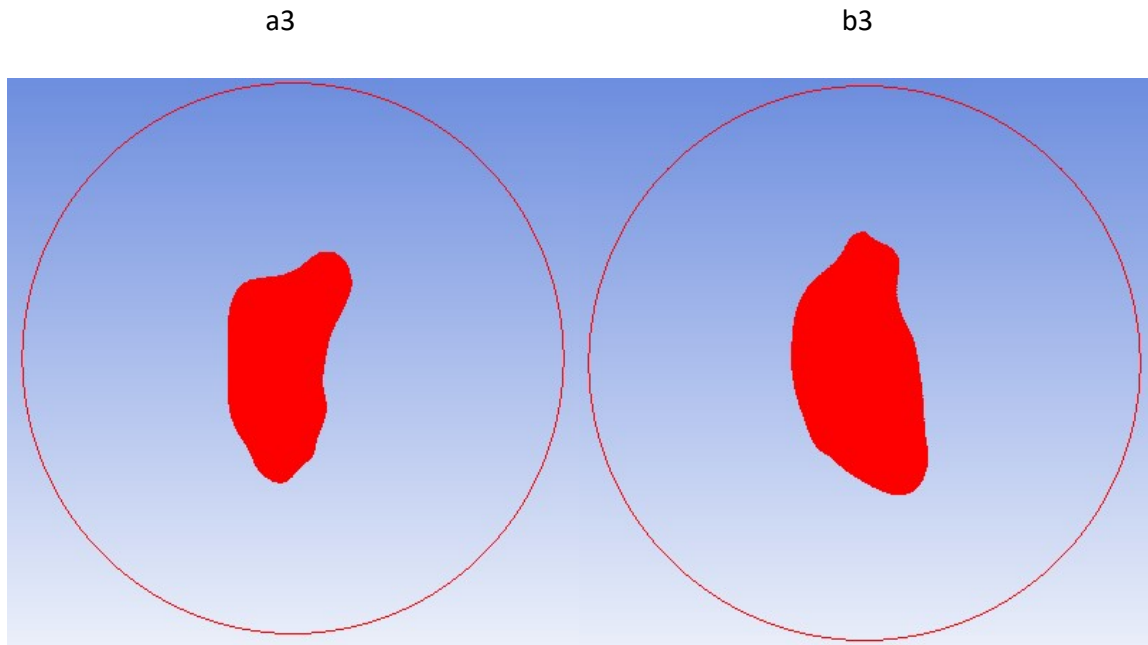


Figure 6. 7: Sufficient heating zone for different tumour geometry and numbers of focal points (a1) ellipse A with single focal (a2) ellipse A with double focal (a3) ellipse A with triple focal (b1) ellipse B with single focal (b2) ellipse B with double focal (b3) ellipse B with triple focal.

Table 6. 2: Summary of the predicted fraction of sufficient heating zone for different cases

	Single focal	Double focal	Triple focal
Regular circle	33%	40.3%	36%
Ellipse A	34%	49%	46%
Ellipse B	35.5%	53%	50%

The importance of the location of focal points can be appreciated by looking at the spatial temperature distribution. Figure 6.8 shows the acoustic power input at the tumour site with three focal points located at the foci (a) and closer to the geometric centre (b). Figure 6.9 displays the SHZ generated by the acoustic power field. The case with focal points located at the foci leads to a 53% sufficient heating fraction, which is 6% higher than the case with focal points closer to the centre.

As discussed in the preceding section, high power input at the focal point leads to an instantaneous temperature rise, which would trigger the FUS system to switch off. Therefore, high acoustic power at the focal will lead to a less uniform temperature profile, which is undesirable. Since a single focal is not powerful enough to achieve sufficient heating of its surrounding area, a more spatially distributed arrangement of focal points would be preferred in terms of achieving wider and more uniform heating. The transducer array limits the position of the focal. The rectangle piston employed in this simulation produces focal points along the same line, which is not convenient for irregular geometry of the tumour.

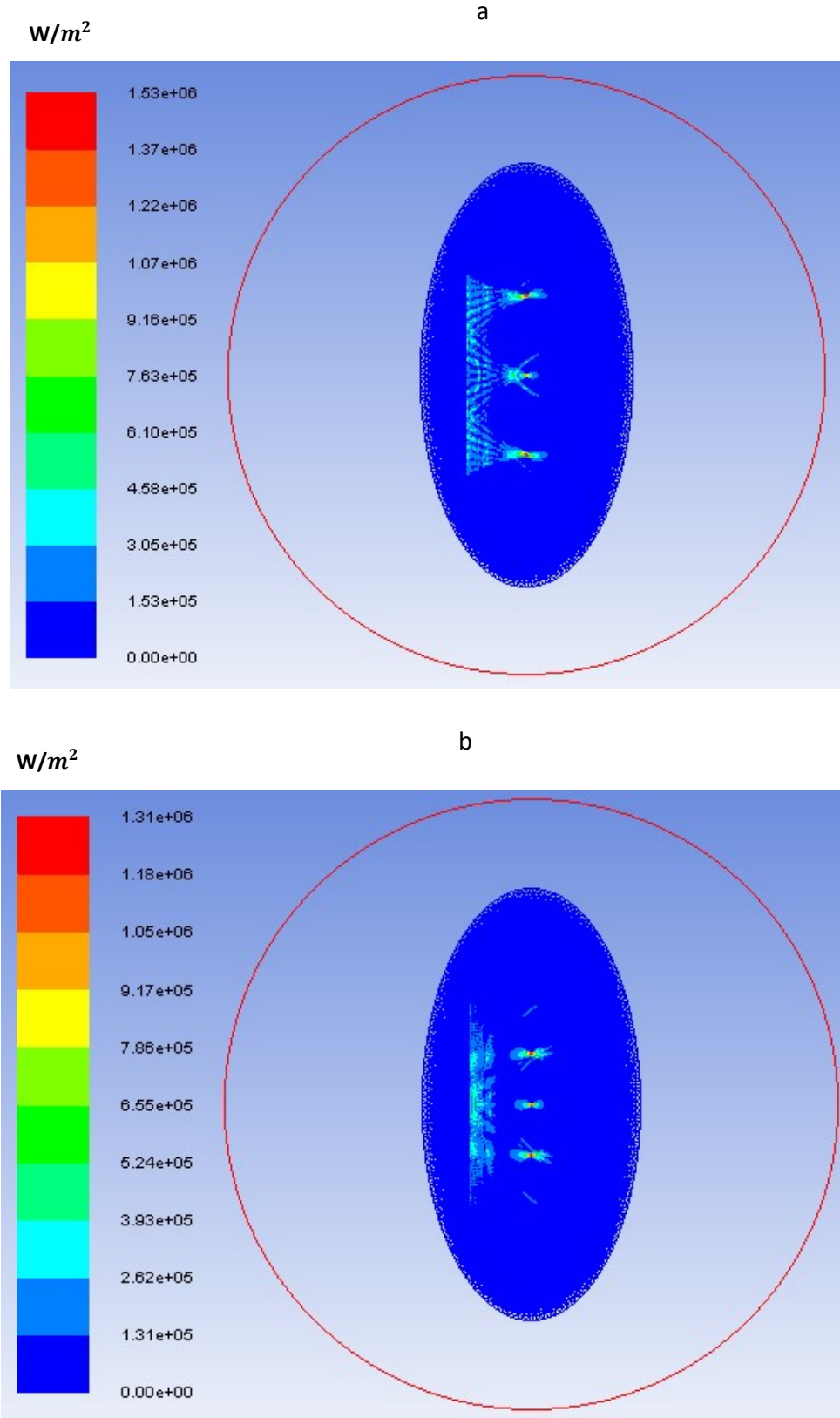


Figure 6. 8: Acoustic power field applied at tumour site (a) focal at the foci of the ellipse (b) focal concentrated at the centre of the ellipse.

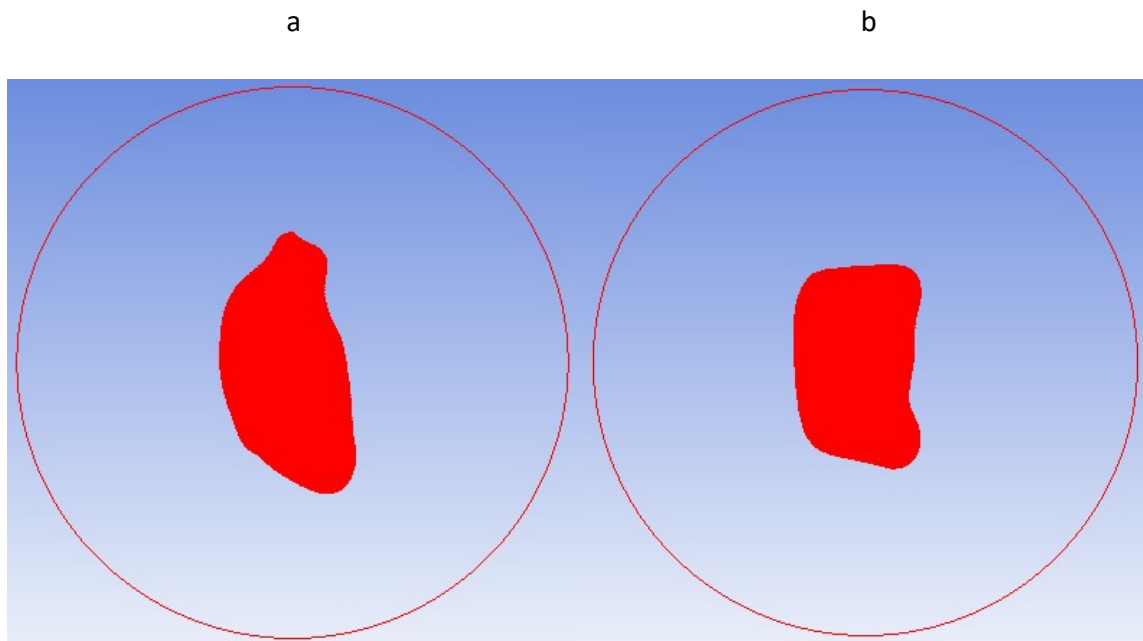


Figure 6. 9: Sufficient heating zone generated of ellipse B (a) focal at the foci of the ellipse (b) focal concentrated at the centre of the ellipse

6.5 Summary

FUS provides a promising way to heat tumour tissue noninvasively, which can be employed to trigger drug release from TSL under the guidance of MRI. However, due to the size of the focal and safety consideration, it is difficult to achieve sufficient heating for the entire tumour. Since drug distribution is strongly dependent on local temperature, understanding how the application of FUS may influence the temperature profile in the targeted tumour is important. Our model predicts the spatial temperature distribution at the tumour site by coupling the FUS simulation and the bio-heat transfer equation.

In this chapter, several factors that influence the temperature profile are investigated; these include the number of focal point, tumour geometric shape and the location of the focal points. An important parameter is defined, namely the fraction of sufficient heating zone (SHZ), which is used to measure and compare the efficiency of heating. Results show that increasing the number of focal points improves the fraction of SHZ. However, by changing the number of focal points, the acoustic power at the focal changes accordingly. Further analysis reveals that a lower power input at the focal would help produce a more uniform temperature profile, with more area of the tumour receiving sufficient heating. This finding is explained by the function of temperature controller.

With the same acoustic power input, the more spread the focal location, the better the hyperthermia efficacy is achieved.

The study presented in this chapter represents an initial effort towards optimising the FUS activated TSL drug delivery system for improved therapeutic efficacy. It has several limitations. Firstly, idealised geometry is employed to simplify the problem, whereas real tumour geometry constructed from MR images should be applied in further simulations. Secondly, when investigating the impacts of the number of focal points on the temperature profile, it is difficult to isolate this factor by keeping the same acoustic power input at the focal. Future effort should be made on controlling the acoustic power at the focal so that results would be independent of this factor. Thirdly, although the results suggest that a relatively low acoustic power at the focal is preferred, no rigorous testing is performed to determine the lower limit for a given setting. The acoustic power at the focal point can be optimised to achieve the highest SHZ. Fourthly, the temperature controller can be redesigned. The current controller limits the temperature at the tumour site to 42°. However, for tumours with a necrotic core, the temperature allowance at the centre of the tumour could be higher. Also, one possible design is to employ multiple ultrasound transducer arrays that are controlled by a different temperature controller. Finally, simple ultrasound transducer geometry is simulated here. This should be extended to include more complex FUS transducer geometries.

Chapter 7: Conclusion and recommendation for future work

In this thesis, two mathematical models are developed for the simulation of thermosensitive liposome (TSL)-mediated delivery of chemotherapy drugs: (i) a multi-compartmental model without including ultrasound acoustics and heat transfer, and (ii) a continuum-based multi-dimensional drug transport model coupled with ultrasound acoustics and bio-heat transfer. Both models are capable of predicting the temporal profiles of drug concentration in blood plasma, tumour extracellular space and tumour intracellular space. The therapeutic efficacy and side effect of the TSL delivery system are evaluated based on a pharmacodynamics model and the level of drug exposure in the systemic circulation, respectively. The physiological and biological parameters employed in the models are derived from experimental data in the literature. Although both models produce results that are potentially useful for the design and optimisation of therapeutic protocols, they focus on different aspects. The multi-compartmental model is used to understand the drug transport mechanism and interactions among different steps. When applied to a tumour cord model, it also provides details on spatial distribution of drug near the vessel wall on a micro scale. The continuum model is applicable to real tumour geometry reconstructed from patient images, giving a more realistic prediction of the treatment efficacy and spatial distributions of drug on a macro scale. Since non-uniform temperature profile and heterogeneous drug distribution at the tumour site are identified as limiting factors, initial attempts are also made to investigate how the number and location of ultrasound focal points may affect temperature distribution in the tumour. The main findings are summarised below.

7.1 The compartmental model

The multi-compartmental model employs idealised tumour core geometry consisting of a capillary vessel surrounded by tumour tissue of a uniform thickness. The model does not include bio-heat transfer and assumes that the temperature of the tumour reaches the hyperthermia temperature required to trigger drug release immediately upon the application of FUS. Simulation results show that in order to improve the therapeutic efficacy and reduce the level of drug in systemic plasma, liposomes with a low release rate at body temperature and a high release rate at the phase transition temperature would be preferred. . Another key factor that influences the predicted therapeutic outcome is heating duration. Increasing hyperthermia duration leads to a higher cancer cell killing fraction due to more free drug being available at the tumour site. This is because temperature controls not only the rate of drug release but also the transport parameters, such as drug diffusivity and permeability. These parameters increase with temperature, thereby enhancing the drug transport across

the endothelium and into the interstitial space. Therefore, maintaining hyperthermia at the tumour site would aid the delivery of chemotherapy drug to tumour cells.

Different chemotherapy drugs have different transport properties, which would affect the distribution of drug at the tumour site. Two TSL delivery systems, TSL-DOX and iTSL-TOP are compared. Results show that iTSL-TOP produces higher intracellular drug concentration than the conventional TSL-DOX delivery system. However, TOP concentration in blood plasma is also higher compared to DOX, indicating that the distinct advantage of TSL delivery system in reducing drug exposure at the systemic level may be slightly compromised with the use of iTSL-TOP.

7.2 The continuum drug transport model

The continuum-based drug transport model is applied to real tumour geometry and coupled with the bio-heat transfer equation. It is also integrated with the FOCUS code, allowing the simulation of the acoustic power field generated by FUS and the resultant heating effect at the tumour site. The influences of hyperthermia duration, drug release rate at the phase transition temperature, two different anticancer drugs and TSL formulations, are investigated.

The key findings are as follows:

(1) When compared with the free drug infusion without TSL and FUS, the TSL-DOX delivery system achieves much more effective cancer cell killing as measured by the predicted cancer cell survival fraction - 70% with TSL-DOX and 90% with free DOX, showing a 20% improvement. The better therapeutic efficacy achieved by the TSL delivery system can be attributed to the following: (a) TSL has a much lower blood clearance rate than that of the free drug, allowing more drug-loaded TSL to accumulate at the tumour site; (b) ultrasound hyperthermia improves the transport properties of the drug. The TSL-DOX delivery system not only improves the therapeutic efficacy but also reduces the level of drug exposure in systemic plasma. This is attributed to the fact that drug release from TSL is triggered by hyperthermia which is only applied at the tumour site, thereby minimising drug release outside the targeted tumour. (2) Extending the hyperthermia duration improves the therapeutic efficacy marginally. Longer heating duration helps to maintain a high temperature at the tumour site, which increases drug diffusivity and permeability, so that more free drug can accumulate in the tumour leading enhanced tumour cell killing. However, the predicted improvement is limited since most of the encapsulated drug is released in the first 10 minutes of heating. Extending the heating duration only has a marginal effect on reducing cancer cell

survival fraction at the centre of the tumour which receives sufficient heating. The temperature profiles with 10 min and 60 min heating are identical, suggesting that prolonged heating has no effect on improving the spatial distribution of temperature in the tumour region. In terms of potential side effect, extending the heating duration does not show any difference to the drug concentration in normal tissue, which means no impact on the side effect.

(3) Increasing the drug release rate at the liposome phase transition temperature improves the therapeutic efficacy significantly. The burst release of drug substantially increases the amount of bioavailable drug in the tumour interstitial space, so that more drug can enter the tumour cells. Higher intracellular drug concentration in tumour results in more effective cancer cell killing. Increasing the drug release rate has a more pronounced effect on cell death in the tumour core which receives sufficient heating, compared to the edge of the tumour which is under-heated. The negative impact of increasing the drug release rate is that faster drug release also increases the level of free drug in normal tissue. Nevertheless, the amount of increase is modest compared to the increase in therapeutic efficacy.

(4) Results obtained with iTSL-TOP reveal that it can achieve far more effective cancer cell killing than the conventional TSL-DOX - the predicted cell survival fraction is 30% with iTSL-TOP compared to 70% with TSL-DOX. This substantial enhancement in cell killing can be attributed to two reasons. Firstly, TOP has a much weaker binding with the protein in plasma, which means that more free drug is available for cancer cell killing. Secondly, TOP is more cytotoxic than DOX, meaning that a lower concentration of TOP is required to achieve the same cancer cell killing fraction as DOX. However, TOP concentration in normal tissue is much higher than that of DOX, which could increase the risk of side effect.

The findings from the continuum model are compared with those from the compartmental model. Both models predict that higher drug release rate at the liposome phase transition temperature would lead to a better treatment outcome at the cost of elevated risk of side effects. Regarding the effects of hyperthermia duration, although both models show that longer hyperthermia duration is beneficial, the compartmental model is more sensitive to this factor and predicts a more pronounced improvement in therapeutic efficacy than the continuum model. The reason for the observed difference is that the compartmental model does not include the bioheat transfer model and it assumes a uniform temperature rise for the entire tumour upon the application of FUS. However, the continuum model simulates the heating more realistically and considers the spatial distribution of temperature. It reveals that not all the tumour region receives sufficient heating. Therefore, attention should be turned to the factors that control the temperature distribution in tumour.

7.3 Selection of the number and location of focal points

The drug concentration distribution and treatment outcome are strongly related to the temperature profile generated by FUS hyperthermia. It is vital to achieving a more uniformly distributed temperature profile. The results of the continuum model show that increasing the hyperthermia duration does not improve the spatial temperature distribution. Therefore, other factors must be investigated. For a given ultrasound transducer type and geometry, the effectiveness of FUS heating is directly influenced by the selection of focal points, among other factors. In Chapter 6, an initial attempt is made to compare the effectiveness of heating, measured in terms of sufficient heating zone (SHZ), by changing the number and location of focal points. The main findings are summarised below.

(1) Lower power input at the focal point favours more uniform heating, thereby increasing the fraction of SHZ. This is mainly dictated by way the temperature controller works. Reducing the power input at the focal point would allow the FUS to stay on for longer, increasing the chance for more uniform heating.

(2) Increasing the number of focal improves the fraction of SHZ. By increasing the focal from one to two or three, the fraction of SHZ improves. More focal means the acoustic power is distributed more evenly in space, consequentially leading to a more evenly distributed temperature profile.

(3) For multifocal heating, the further apart the focal points, the more uniform the temperature profile. Since each focal point is powerful enough to heat its surrounding area, a wider distribution of the focal points gives a better dispersion of the heat.

Nevertheless, the above findings must be considered with caution as the temperature profile is affected not only by the location and number of the focal points, but also by the acoustic power at the focal. These factors interact with each other, for example, increasing the number of focal points would cause the acoustic power input to change. The location of the focal points also affects the power input. Therefore, the findings described above are subject to the influence of these confounding factors which could not be isolated.

7.4 Limitations and recommendations for future work

The geometry employed in the compartmental model is an idealised tumour cord, which is further reduced to a one-dimensional problem without considering changes along the vessel length. The bio-heat transfer equation is not included and the temperature profile is assumed to be uniform in different compartments. Ultrasound-induced hyperthermia can be incorporated into the model in the future. In this study, only two anticancer drugs

are simulated and compared, namely DOX and TOP. With the development of new drugs, more drugs can be simulated to compare their treatment efficacy and side effect. The pharmacodynamics model is decoupled from the model since the tumour cell killing function varies with tumour type. Therefore, a specific type of tumour can be selected and simulated with a specific pharmacodynamics model to predict the cancer cell survival fraction after treatment.

A major assumption in the continuum drug transport model is the uniform distribution of blood vessels. It is known that as tumour cells grow, the structure and morphology of blood vessels change and become more irregular, resulting in a necrotic core in most tumours. The presence of necrotic core is not considered in the present model, which can be incorporated and it is expected to affect both the temperature distribution and drug concentration profile. In terms of transport properties, the tumour extracellular space is assumed to be homogeneous. However, these properties may vary within the tumour and between different tumours. The heterogeneity of tumour and tumour-specific properties should be considered in the future. The work presented in Chapter 6 is preliminary. Although the impacts on the temperature profile by changing the number of focal points are investigated, the acoustic power at the focal is not controlled. FOCUS needs to be modified to maintain the same acoustic power at the focal for different numbers of focal. The lower limit of the acoustic intensity should be tested since the results show that lower power input at the focal point favours more uniform heating. The temperature controller is too simplistic and should be modified to allow for more flexible control of the power input rather than through a simple switch function. , Also, the PI control parameters can be adjusted to give more uniformly distributed temperature profile. The current model employs a single transducer array to produce multiple focal points. A new model with a multiple-element transducer should be implemented. Each transducer should be controlled by an individual controller scanning different areas of the tumour. The new set-up may maximise the sufficient heating zone, while keeping the temperature at the tumour site below 42°C. Different heating strategies can also be tested, for example by using multiple heating steps with short intervals in between.

In conclusion, two mathematics models have been developed to simulate TSL-mediated delivery of chemotherapy drugs in combination with FUS induced hyperthermia. The therapeutic efficacy and risk of side effect are evaluated based on the predicted cancer cell survival fraction and drug concentration in systemic plasma, respectively. Model predictions are qualitatively validated against limited experimental data available in the literature. The temperature distribution and hyperthermia effectiveness, which are essential for the release of drug from TSL, are investigated further by changing the FUS setting in an attempt to achieve the highest

fraction of sufficient heating. Although the computational models employed in the present study have a number of limitations, they are capable of providing detailed spatiotemporal information that cannot be obtained in vivo. The modelling framework can serve as a useful tool for evaluation and optimisation of therapeutic efficacy of FUS-TSL drug delivery systems.

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Appendix: Publications during the project

Journal papers

Yu Huang, Boram Gu, Cong Liu, Justin Stebbing, Wladyslaw Gedroyc, Maya Thanou and Xiao Yun Xu, 2019, Thermosensitive Liposome-Mediated Drug Delivery in Chemotherapy: Mathematical Modelling for Spatio-temporal Drug Distribution and Model-Based Optimisation, *Pharmaceutics* 2019, 11(12), 637; doi:10.3390/pharmaceutics11120637

Conference presentation

Winter School on Therapeutic Ultrasound, Les Houches, March 26th - 31st, 2017

One week training programme that involved lecturing and a short student presentation

Yu Huang, Wenbo zhan, Xiaoyun Xu, A multiphysics model for focused ultrasound-mediated drug delivery to solid tumour ThUNDDAR network, September 7th, 2019

Oral presentation: A multiphysics model for focused ultrasound-mediated drug delivery to solid tumour

Appendix: Nomenclature

A_f	frequency factor
b	chord from the ultrasound transducer centre to the radiator boundary
c	specific heat
C_{be}	bound doxorubicin concentration in interstitial fluid
C_{bp}	bound doxorubicin concentration in blood plasma
C_{fe}	Free doxorubicin concentration in the interstitial fluid
C_{fp}	free doxorubicin concentration in blood plasma
C_i	intracellular doxorubicin concentration
CL_{bp}	plasma clearance of bound doxorubicin
C_{le}	liposome encapsulated drug concentration in the interstitial fluid
CL_{fp}	plasma clearance of free doxorubicin
C_v	doxorubicin concentration in blood plasma
d	distance from a point on the radiator to a field point
D_{be}	diffusion coefficient of the bound doxorubicin-protein
D_c	tumour cell density
D_d	drug dose
D_{fe}	diffusion coefficient of free doxorubicin
D_l	diffusion coefficient of liposomes
DS	degree of vascular stasis
D_w	diffusion coefficient in water
EC_{50}	drug concentration producing 50% of f_{max}
F_{be}	bound doxorubic gain from blood vessels

F_{fl}	doxorubicin loss of doxorubicin to the lymphatic vessels per unit volume of tissue
F_{fp}	doxorubicin gained from the blood capillaries in tumour and normal tissues
F_{pf}	free doxorubicin gained from the capillaries in tumour and normal tissues
F_{ll}	loss of liposomes through the lymphatic vessels per unit volume of tissue
F_{lp}	liposomes gained from the capillaries in tumour and normal tissues
F_{ly}	interstitial fluid absorption rate by the lymphatics per unit volume of tumour tissue
f_{max}	cell-kill rate constant
F_v	interstitial fluid loss from blood vessels per unit volume of tumour tissue
h	depth of the concave surface
I_a	ultrasound intensity
k	thermal conductivity
k_a	doxorubicin-protein binding rate
k_{ac}	acoustic velocity potential
k_b	Boltzmann's constant
k_{ci}	PI control parameter
k_{cp}	PI control parameter
k_d	doxorubicin-protein dissociation rate
k_e	michaelis constant for transmembrane transport
k_g	cell physiological degradation rate
k_i	michaelis constant for transmembrane transport
k_{ly}	hydraulic conductivity of the lymphatic wall
k_p	cell proliferation rate constant
k_{rel}	release rate of doxorubicin from liposome
k_v	hydraulic conductivity of the microvascular wall
k_p	Transfer constant from systemic plasma to tissue
m_d	increased fold of diffusivity
m_p	increased fold of permeability
m_{tv}	increased fold of transmembrane rate

MW	molecular weight
P	permeability
p_a	acoustic pressure
p_{be}	permeability of vessel wall to bound doxorubicin
P_{ef}	trans-capillary Peclet number
p_{fe}	permeability of vasculature wall to free doxorubicin
p_i	interstitial fluid pressures
p_{ly}	intra-lymphatic pressure
p_v	vascular pressures
q	ultrasound power deposition
r	radius of spherical particle
R	universal gas constant
s	percentage of bound doxorubicin
S/V	surface area of blood vessels per unit volume of tumour tissue
$S/V *$	relative value of surface area of blood vessel per tumour size
s_b	net rate of doxorubicin gained from association/dissociation with bound doxorubicin-protein
s_i	net rate of doxorubicin gained from the surrounding environment
s_l	net rate of liposomes gained from the surrounding environment
s_{lp}	liposomes gained from plasma
s_{ly}/V	surface area of lymphatic vessels per unit volume of tumour tissue
s_r	released drug in the interstitial fluid
s_u	influx/efflux from tumour cells
s_v	net rate of doxorubicin gained from the blood/lymphatic vessels
T	temperature
T^*	absolute temperature
T_0	body temperature
t	time
t_d	infusion duration
T_n	normal body temperature
u_0	velocity constant
v_a	speed of ultrasound

V_B	plasma volume
V_{max}	rate of trans-membrane transport
V_T	tumour volume
W	diagonal matrix
w	perfusion rate of blood flow
w_0	perfusion rate of blood flow at body temperature
ΔE	activation energy

Tensor

C	matrix of the inertial loss term
F	Darcian resistance to fluid flow through porous media
I	unit tensor
u	normal velocity of a slightly curved radiating surface
v	velocity of the interstitial fluid

Greek Letter

α	ultrasound absorption coefficient
$\alpha 1$	compartment clearance rate of doxorubicin
$\alpha 2$	compartment clearance rate of doxorubicin
$\alpha 3$	compartment clearance rate of doxorubicin
ε	cellular efflux function
ζ	cellular uptake function
κ	permeability of the interstitial space
λ	wave length of ultrasound
μ	dynamic viscosity of interstitial fluid
πi	osmotic pressure of interstitial fluid
πv	osmotic pressure of the plasma
ρ	density
σ	osmotic reflection coefficient
σd	osmotic reflection coefficient for the drug molecules
σT	average osmotic reflection coefficient for plasma protein
τ	stress tensor
φ	tumour fraction extracellular space
Ψ	acoustic velocity potential
ω	angular frequency of ultrasound transducer

Abbreviation

BSA	body surface area
BSNC	bis-chloroethylnitrosourea
CFD	computational fluid dynamics
FVM	finite volume method
Gd-DTPA	gadopentetate dimeglumine
HIFU	high intensity focused ultrasound
IFP	interstitial fluid pressure
IFV	interstitial fluid velocity
MRI	magnetic resonance image
MW	molecular weight
PEG	polyethylene glycol
PDT	photodynamic therapy
P-gp	P-Glycoprotein
TSL	thermo-sensitive liposome
UDS	user defined scalar
SHZ	Sufficient heat zone

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