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**Development of a Multi-Physics and Multi-Scale Model
of Thrombolysis for Patient-Specific Applications**

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August 8, 2017

Andris Piebalgs

Abstract

Thromboembolic diseases such as myocardial infarction and ischemic stroke are one of the main causes of death in the world. These pathologies occur when an abnormal blood clot develops in the human body and travels throughout the circulatory system as an embolus. If the occlusion takes place in the cerebral arteries, it can lead to acute ischemic stroke which, if not treated rapidly, will cause morbidity and death. One of the forms of treatment for ischemic stroke is thrombolytic therapy whereby a drug known as alteplase is provided intravenously to the patient so as to dissolve the occluding thrombus. This treatment, however, can provoke life-threatening side-effects such as intracerebral haemorrhage (ICH) and therefore is limited to only a subset of patients that are free of contraindications. Furthermore, thrombolytic therapy can sometimes be ineffective in treating certain types of occlusions. As such, there is considerable interest in the medical community in determining the scenarios where thrombolytic therapy can be ineffective and how existing treatment can be optimised.

To this end, a multi-physics and multi-scale computational model has been developed in an attempt to simulate the treatment of ischemic stroke with thrombolytic therapy. Blood flow and drug transport are described by partial differential equations that are solved with the aid of computational fluid dynamics (CFD) software. Fibrinolysis is described using a microscale model whereby changes in the clot microstructure give rise to changes in macroscopic properties of the clot such as clot resistance and composition. Arterial geometries include idealised models using CAD software and patient-specific models extracted from computed tomography angiography (CTA) images. Physiological flow boundary conditions are implemented by using 3 element Windkessel models while drug transport boundary conditions are determined with the aid of a compartmental model. Clots of different shapes, lengths and locations are studied and their dissolution patterns are examined under different thrombolysis regimens.

Predicted changes in the intra-arterial flowrate and pressure are consistent with data in the literature. The results show that under the assumption of homogeneous clot properties, clot lysis patterns are strongly dependent on clot position, whereas clot dissolution time is merely determined by clot length. Numerical simulations of different thrombolysis regimens indicate that treatment dose and duration can be personalised according to clot length and clot composition in order to lower the risk of ICH (intracerebral haemorrhage) and ensure successful vessel recanalization. It is demonstrated that the computational model developed in this study can be used to investigate clot dissolution in patient-specific geometries. Future work will attempt to test the use of this computational model in clinical settings and as an aid in the development of new therapies.

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Nomenclature

Abbreviations

$\alpha 1 - PI$ Proteinase inhibitor

ACA Anterior cerebral artery

ADP Adenosine diphosphate

AIS Acute ischemic stroke

AP $\alpha 2$ -anti-plasmin

CAD Computer aided design

CFD Computational fluid dynamics

CTA Computed tomography angiography

DSPA Desmoteplase

DT Distance to thrombus

FDP Fibrin degradation product

GP Glycoprotein

ICA Internal carotid artery

ICH Intracerebral haemorrhage

IV Intravenous

MCA Middle cerebral artery

MG $\alpha 2$ -macroglobulin

mRS Modified Rankin scale

NE Neutrophil elastase

NET Neutrophil extracellular trap

ODE Ordinary differential equation

PAI Plasminogen activator inhibitor

<i>PDE</i>	Partial differential equation
<i>PLG</i>	Plasminogen
<i>PLS</i>	Plasmin
<i>RBC</i>	Red blood cell
<i>SEM</i>	Scanning electron microscope
<i>TAFI</i>	Thrombin-activatable fibrinolysis inhibitor
<i>TIMI</i>	Thrombolysis in myocardial infarction
<i>TNK</i>	Tenecteplase
<i>tPA</i>	Tissue plasminogen activator
<i>UK</i>	Urokinase
<i>VA</i>	Vertebral artery

Roman Symbols

$[ENDO]$	Concentration of endothelial cells at the wall
$\bar{u}$	Average phase velocity
A	Area
a	Propensity function
a_0	Sum of propensity function (equation 7.14) or amount of monomers per fibre length (equation B.1)
A_α	Concentration of adsorbed species α
C	Capacitance
C_α or $\bar{C}_\alpha$	Intrinsic phase average concentration of species α
C_L	Coefficient under laminar flow
C_T	Coefficient under turbulent flow
D	Diameter
$D_{\alpha,ij}$	Diffusion coefficient of species α in the direction perpendicular to the ij plane
$D_{\alpha,ij}^*$	Dispersion coefficient of species α in the direction perpendicular to the ij plane
E_L	Extent of lysis
E_{crit}	Extent of lysis at which the clot resistance collapses
F_1	Number of fibres 1 unit long ($L_M/2$)

F_b	Body force
G_α	Rate of generation/consumption of species α in the bound phase (on fibrin surface)
H	Height of the channel
I	Reaction index as determined by stochastic process
i	Imaginary unit
I_α	Rate of infusion of species α
J_0	0th order solution to the Bessel function
j_α	Diffusive mass flux of α
k	Clot permeability
k_2	Bound phase catalytic rate of PLS generation
K_α	Rate of degradation of species α
K_M	Bound phase Michaelis-Menten coefficient for PLS generation
$k_{2,f}$	Catalytic rate of PLS generation in the free phase
$k_{a,\alpha}$	Adsorption coefficient of species α
k_{AP}	Rate of PLS inactivation with AP
k_{cat}	Catalytic rate of binding site degradation
$K_{E,\beta}$	Escape coefficient of cellular element of type β
k_{HC}	Rate coefficient of hepatic clearance
$K_{M,f}$	Michaelis-Menten coefficient for PLS generation in the free phase
$K_{M,n}$	Michaelis-Menten coefficient for PLS generation in a stochastic system
$k_{r,\alpha}$	Desorption coefficient of species α
$k_{tPA,rls}^C$	Release coefficient of tPA from endothelial cells
$k_{tPA,rls}^{Fbn}$	Release coefficient of tPA from endothelial cells due to the presence of fibrin
$k_{tPA,rls}^{Thr}$	Release coefficient of tPA from endothelial cells due to the presence of thrombin
$k_{tPA-PLG}$	Formation coefficient of tPA-PLG complex
L	Amount of fibrin lysed (equation 3.6) or length (equation 2.19)
L_M	Length of fibrin monomer
L_{pf}	Length of protofibril
L_{PLS}	Concentration of degraded species with PLS adsorbed

M	Dissolution constant
M_{PLG}	Total number of free phase PLG molecules
MW	Molecular weight
n	Cryptic binding site concentration (equation 2.16) or mode (equation 2.5)
n_0	Initial binding site concentration
N_α	Number of binding sites of species α
n_α	Concentration of bound species α
N_{AV}	Avogadro's number
$N_{BS,L}$	Number of binding sites at protofibril layer L
N_{BS}	Number of binding sites
N_{CS}	Total number of cross-sections
n_{free}	Concentration of free binding sites
N_{layer}	Number of protofibril layers
N_{rxns}	Total number of reactions for stochastic system
n_{tot}	Total concentration of non-cryptic binding sites
nt	Number of timesteps
nx	Number of spatial steps
O_β	Number of cellular elements of type β
p or $\bar{p}^f$	Average pressure
$P(S, t)$	Probability of state S arising at time t
p_0	Density of protofibrils per surface area
P_{out}	Pressure at outlet
Q	Flowrate
q_α	Number of binding sites for each fibrin monomer
R	Radius of channel
r	Radial distance from channel centre
r_0	Radius of protofibril
R_1	Inertial resistance
r_1	Random number 1

R_2	Peripheral resistance
r_2	Random number 2
R_α	Rate of generation or consumption for species α in the free phase
R_f	Radius of fibrin fibre
R_T	Total resistance
R_{clot}	Hydrodynamic resistance of the clot
r_{clot}	Radius of mural clot
$R_{f,L}$	Radius of fibre at protofibril layer L
S	Source
t	Time
t_d	Dissolution time
$t_{1/2}$	Half-life
U_0	Volume of control envelope
u_i or $\bar{u}^f$	Average intrinsic phase average velocity
u_α	Velocity of α
v	Stoichiometry matrix
V_β	Volume of cellular element of type β
v_{front}	Velocity of lysis front
V_{plasma}	Volume of plasma compartment
w	Weight of patient
X	State array
x	Co-ordinate in x, y or z
z	Co-ordinate or length along the vessel

Greek Symbols

α	Angle
α_β	Release coefficient of cellular element of type β
α_{tPA}	tPA dose
β	Permeability coefficient
$\delta\theta$	Angular intra-protofibril distance

ΔP	Pressure drop
δr	Radial intra-protofibril distance
δ	Boundary length
ϵ	Voidage (volume fraction of empty space)
η	Similarity variable
γ	Amount of PLS cuts needed to dissolve a fibrin monomer
κ	Solubilisation constant (equation 3.7) or a generic kinetic constant (equation 2.12)
μ	Dynamic viscosity
μ_f	Mean value for fibre radius
ω	Angular velocity
ϕ_c	Volume fraction of cellular components
ϕ_f	Volume fraction of fibrin
π	Pi
ρ	Density of blood
ρ_f	Fibrin density in clot
ρ_{fibre}	Density of fibrin in fibre
σ_f	Standard deviation for fibre radius
τ	Timestep as determined by a Monte-Carlo process
θ	Angle of curvature
θ_α	Concentration of binding sites for species α
ζ	Number of protofibrils in a cross-section

Subscripts

0	Initial
α	Species
∞	Streamline
Fbg	Fibrinogen
Fbn	Fibrin
$free$	Free binding sites
max	Maximum

<i>mom</i>	Momentum
<i>out</i>	Outlet
<i>sol</i>	Solubilisation
<i>Thr</i>	Thrombin
<i>tot</i>	All binding sites

Superscripts

<i>b</i>	Bound phase
<i>f</i>	Fluid phase
<i>n</i>	Relating to the exposure of new binding sites

Dimensionless Symbols

<i>Pe</i>	Peclet number
<i>Re</i>	Reynolds number
<i>Wo</i>	Womersely number

Chapter 1

Introduction

1.1 A Stroke of Insight

On the morning of the 10th of December 1996, a talented neuroanatomist by the name of Dr. Jill Bolte Taylor awoke to find herself a victim of a stroke. A hereditary deformity in her cerebral blood vessels had burst, flooding her brain tissue with blood. In her story “My Stroke of Insight”, she expanded upon the horrific situation she found herself in.

“I watched my mind completely deteriorate in its ability to process information. By the end of that morning, I could not walk, talk, read, write, or recall any of my life. Curled up into a little fetal ball, I felt my spirit surrender to my death, and it certainly never dawned on me that I would ever be capable of sharing my story with anyone.” - [Taylor \(2009\)](#)

The rupture prevented blood from reaching the surrounding areas of her brain causing gradual tissue ischemia (lack of oxygen) and consequently necrosis (cell death). Every year in the UK alone, over 150,000 individuals find themselves suffering from strokes. A quarter of these are fatal, and of those victims that do survive, over a third are dependent on others for support ([Stroke Association; 2016](#)). Unlike the haemorrhagic stroke suffered by Dr. Taylor, over 80% of all strokes are ischemic. This occurs when an abnormal blood clot develops in the human body and travels throughout the arterial system until it is lodged in an artery that ends up blocking blood supply (otherwise known as a thromboembolism) ([Mohr et al.; 2011](#)).

The occurrence of thromboembolism can cause a myriad of cardiovascular diseases other than ischemic stroke. For instance, thromboembolism in a coronary artery may cause deprivation of blood to the heart thereby triggering a myocardial infarction. Alternatively, during periods of low physical activity, an embolus may develop and obstruct the efflux of blood from the legs otherwise known as deep vein thrombosis. If the embolus travels to the pulmonary arteries, it may cause pulmonary embolism ([Askari et al.; 2008](#)). These diseases are known as thromboembolic diseases and cumulatively in 2015 alone caused over 20% of global deaths ([WHO; 2017](#)). Figure 1.1 shows for instance how ischemic heart disease and stroke were the top two causes of worldwide mortality in 2015.

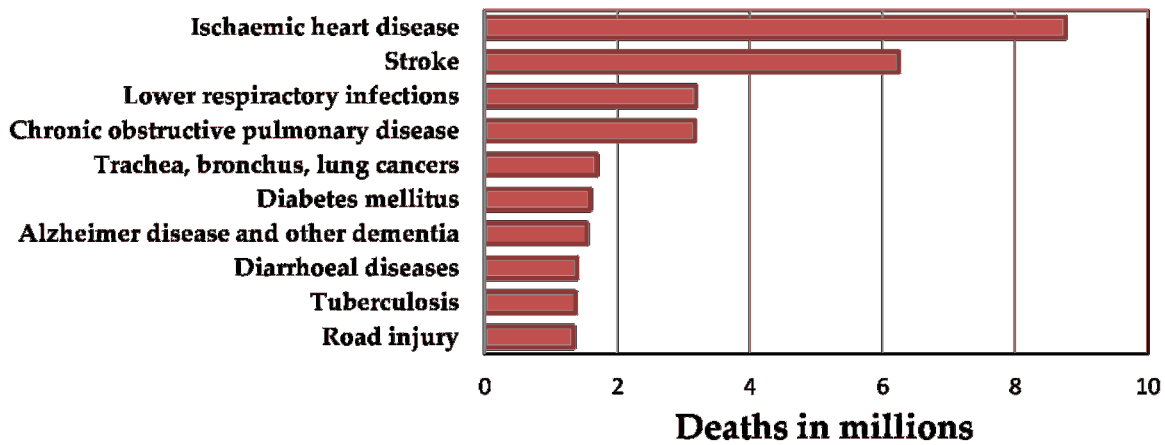


Figure 1.1: Leading causes of death in 2015 out of a total of 56.4 million deaths. Data retrieved from [WHO \(2017\)](#).

1.2 Causes of Thrombosis

To better treat and prevent thrombosis, it is important to ascertain its key causes. The pathologies and clot structures of venous thrombosis and arterial thrombosis are quite distinct. Venous thrombosis, as its name suggests, is characterised by the formation of fibrin dense clots in veins where blood flow is slower than in arteries. A type of venous thrombosis, known as deep vein thrombosis, was shown by Virchow to be a key cause of pulmonary embolism in one of his *in-vivo* animal studies ([Bagot and Arya; 2008](#)). Arterial thrombosis is characterised by a more platelet rich clot formed in high velocity environments and is typically responsible for ischemic stroke and myocardial infarction. The typical causes behind both types of thrombosis can be best explained by Virchow's triad as shown in Figure 1.2.

The three main factors that contribute to thrombosis are blood stasis, damage to the endothelial wall and hypercoagulability. Firstly, blood stasis is a typical cause of deep vein thrombosis and arises in areas of low fluid shear stress. This is particularly prevalent in regions of flow separation such as aneurysms and venous valves, and typically occurs at times of low physical activity. Secondly, injury to the endothelial wall is a natural trigger for the development of blood clots in the human body. As such the presence of foreign substances and internal trauma to blood vessels may lead to the development of thrombosis. The formation of a low density lipoprotein calcified plaque on the arterial wall, due to a disease known as atherosclerosis, may trigger the formation of a clot on the atherosclerotic plaque. As the vessel gets constricted over time, the pressure due to blood flow on the plaque builds until a certain threshold is reached and the plaque ruptures thereby dislodging the thrombus and forming an embolus ([Viles-Gonzalez et al.; 2004](#)). The third cause of thrombosis in Virchow's triad is a predisposition to clotting otherwise known as hypercoagulability. This is typically linked to genetic factors that cause an imbalance in the normal haemostatic coagulation cycle. Genetic traits such as Factor V Leiden, prothrombin G20210A mutation and deficiency in or resistance to proteins C and S have been shown to encourage the development of venous thrombosis ([Bertina et al.; 1994](#); [Martinelli et al.; 2010](#)).

The mechanism of blood clotting is a complex and delicate process that involves the balance of many different pro-clotting factors and their inhibitors. Any imbalance in this cycle may lead either to thrombosis or extensive bleeding. The wall of a blood vessel is lined with endothelial cells that inhibit

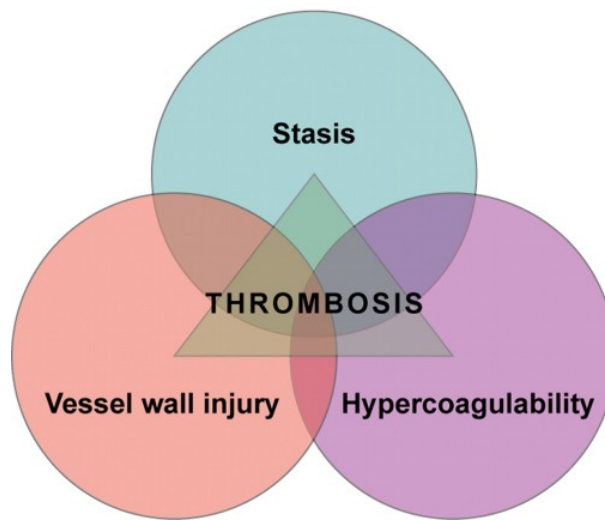


Figure 1.2: Virchow's triad (Kyrle and Eichinger; 2009).

the attachment of disk-shaped cell structures known as platelets. Injury at the endothelial or sub-endothelial level exposes collagen, thromboxane and other factors that promote their activation and aggregation. These adhesive platelets lead to a premature blocking of the injured vessel wall, which is later consolidated with a type of 'glue' known as fibrin (Kaplan and Jackson; 2011). The coagulation cycle is what leads to the formation of fibrin from fibrinogen via a combination of different pro-clotting factors. The blood clot can be dissolved by breaking up the cross-linked fibrin that holds the platelets together by using the enzyme plasmin (PLS). PLS is derived from its precursor plasminogen (PLG) through the activity of a plasminogen activator (PA). In the human body, tissue plasminogen activator (tPA) is excreted by the endothelial cells and cleaves PLG to form PLS. PLS then degrades fibrin into FDPs (Fibrin Degradation Products) and promotes the dissolution of the blood clot.

1.3 Prophylaxis and Current Treatments

Both Virchow's triad and the nature of blood clot formation have been extensively studied as part of efforts to create drugs that inhibit thrombus formation and lyse occluding clots. Anti-coagulant therapies aim to prevent thrombosis by inhibiting the clotting factors that are needed to make fibrin from fibrinogen. These can be vitamin-K antagonists such as Warfarin which inhibit the recycling of vitamin K that is needed for the formation of various pro-coagulant factors. Another form of anti-coagulant therapy could be through the use of heparinoids such as heparin which forms a complex with anti-thrombin that enhances thrombin inhibition (Lip and Blann; 2003).

These drugs however, can cause bleeding by inhibiting the formation of normal haemostatic clots or alternatively they can cause the development of thrombosis. For instance, heparin can cause heparin induced thrombocytopenia, which is a result of excessive platelet activation leading to the development of thrombus and bringing about a low platelet count. Alternatively, warfarin can cause warfarin necrosis whereby a lack of vitamin K can stifle the development of anti-clotting factors and hence promote the development of thrombosis.

Surgery and stent placement are available for patients at severe risk of thromboembolism. For instance, carotid endarterectomy is a procedure which aims to remove the atherosclerotic plaque formed in the carotid arteries that could rupture and potentially cause a stroke. Alternatively, a

balloon inflated stent can be placed to unblock the narrowed artery and restore normal blood flow. However, both treatments carry a chance of stroke and instant death if the blood clot is dislodged during the procedure. Another treatment method, mechanical prophylaxis, can be used for patients who may be at risk of developing venous thrombosis. This typically involves the use of compression stockings and other mechanical instruments to prevent haemostasis and subsequent clot formation. Despite minimal bleeding, it is yet to be proved effective in the prevention of thrombosis (Lippi et al.; 2011).

As mentioned earlier, clots can be lysed through the use of a plasminogen activator such as tPA in a process known as thrombolytic therapy. Although a variety of plasminogen activators can be used to dissolve the embolus in patients suffering from thromboembolism, only the recombinant counterpart of tPA (alteplase) has been approved for use by the FDA. Streptokinase is a cheaper, non-fibrin specific fibrinolytic that is derived from *Streptococci* but can provoke an antigenic response. Thrombolytic therapy is used to treat severe cases of pulmonary embolism and myocardial infarction, and is considered one of the main forms of treatment for ischemic stroke. It can actively reverse thrombus formation and bring about arterial recanalisation thus making it an important tool for the treatment of thromboembolic diseases.

1.4 Innovations in Thrombolysis

Thrombolytic therapies, particularly when used for the treatment of ischemic stroke, can cause serious life-threatening side-effects. Alteplase has a very low half-life in the human body and therefore is needed in high concentrations to effectively lyse occluding clots. The use of these drugs carries a serious risk of bleeding because the thrombolytic agent cannot discriminate between normal haemostatic clots and thrombus. As such, patients are put at risk of acute internal bleeding and may develop life-threatening side-effects such as intracerebral haemorrhage (ICH). This has been found to occur in over 5% of patients (NINDS; 1995). The administration of FDA approved fibrinolytics is so stringent that only a small minority of patients suffering from thrombosis are eligible for thrombolytic treatment. Furthermore, since emboli can occur in many different locations in the cerebral vasculature and have varying compositions, thrombolytic therapy can be ineffective in certain scenarios. This is particularly the case for large vessel occlusions, where poor recanalisation rates are causing clinicians to search for alternatives such as mechanical retrieval.

For the aforementioned reasons, there is a strong clinical interest in using imaging modalities to determine the probability of success and the risk of side-effects in thrombolytic therapy on a case-by-case basis. Parameters such as clot length, position and composition as determined from clinical scans have been identified as important predictors of the probability of recanalisation (Riedel et al.; 2011; Friedrich et al.; 2015; Santos et al.; 2016). Some studies have advocated using a methodology such as the clot burden score to determine the probability of success with thrombolytic therapy (Puetz et al.; 2008). In addition to this, novel technologies are being developed that attempt to lower the risk of adverse side-effects from thrombolytic therapy and aim to enhance drug penetration into the occluding thrombus. This can be accomplished by increasing the half-life of the plasminogen activator and releasing the drug only at the clot surface through the use of a nano-carrier. For instance, Korin et al. (2012) manufactured PLGA microparticles that upon reaching a critical shear stress value, would break up into nanoparticle fragments and release the encapsulated fibrinolytic drug into the

blood stream (shown in Figure 1.3). Similarly, other studies have attempted to encapsulate tPA into echogenic liposomes that release their contents when subject to ultrasound induced cavitation (Tiukinhoy-Laing et al.; 2007). Research has also been undertaken in developing novel thrombolytic agents such as Tenecteplase (TNK) and Desmoteplase (DSPA) as alternatives to tPA due to their longer half-life, decreased neurotoxicity and simpler dosing regime (Bivard et al.; 2013).

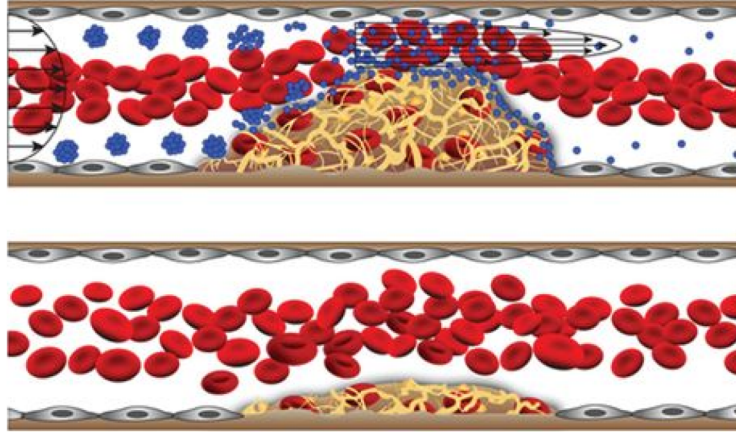


Figure 1.3: Schematic of shear activated microparticles. Yellow strands represent fibrin, red cells are erythrocytes and the blue bundles are microparticles that break apart as they pass through the constriction. The microparticles release tPA and allow for vessel recanalisation. Image taken from Korin et al. (2012).

Although there is currently a strong interest in ameliorating the rate of survival and functional independence for stroke patients, a computational modelling framework is still missing. Computational modelling can serve as a useful tool for the medical community in predicting disease progression and the efficacy of new procedures. For instance, Kandail et al. (2016) used CFD (computational fluid dynamics) modelling to determine the drag force exerted on stent-grafts in the treatment of abdominal aortic aneurysms. In this case, by predicting the risk of stent-graft migration, modelling can be used to ensure the success of endovascular aneurysm repair (EVAR). Another study also used CFD modelling to evaluate the development of thrombus in aortic dissections (Menichini et al.; 2016). The prediction of thrombus development can be useful for clinicians in determining the subsequent actions to take in enhancing a patient's recovery from disease. In this case, a mathematical model of thrombolysis can help in determining the effect that certain parameters have on thrombolysis. Furthermore, it can test the efficacy of new therapies *in-silico* before they are applied to animal models and clinical trials. Although a number of researchers have modelled thrombolysis in idealised settings (Diamond and Anand; 1993; Wootton et al.; 2002; Bajd and Sersa; 2013; Bannish et al.; 2014b), there is no report thus far in the literature that has investigated thrombolysis in patient-specific geometries. The development of a computational framework that can model clot dissolution in patient models would be a valuable tool for optimising current thrombolytic treatment and assisting the development of novel therapies.

1.5 Research Objectives

The aim of this study is to develop a computational model to investigate the dissolution of blood clots in acute ischemic stroke. The model can be used as an investigatory tool to gain insights into the lysis

of clots in clinically relevant geometries and can be used to evaluate and optimise the development of novel therapies *in-silico*. Current clinical research is attempting to evaluate how certain parameters affect the rate of clot dissolution and why certain patients respond more favourably to thrombolytic therapy than others. The development of a computational model of thrombolysis for patient-specific applications may help in addressing these research questions. Thus the objectives of this research project is to:

- Develop a computational model of thrombolysis that can describe clot dissolution in macroscopic geometries.
- Apply the computational model to physiologically relevant 3D idealised and patient-specific arterial geometries.
- Investigate the effect of key parameters on the efficacy of thrombolysis such as clot length, clot position and drug dose.

Thrombolysis is a difficult process to model as it involves the interplay of different physical phenomena such as protein kinetics, blood flow and drug transport. The tPA drug is injected intravenously into the patient and is carried around the circulatory system until it reaches the site of the clot. Upon contact with fibrin, tPA and PLG react to form the protease enzyme PLS that then cleaves the binding sites on the fibre network. As the fibrin fibres are degraded, the blood clot is gradually dissolved until fluid flow is re-established in the occluded artery. The presence of lysis inhibitors such as anti-plasmin (AP) and plasminogen activator inhibitor (PAI) slow down the rate of clot lysis by altering the activity of plasmin and tPA correspondingly.

As such, a complete model of thrombolysis would need to consider, among other things, the change in blood flow throughout the dissolution process, the intravenous infusion of the tPA drug and its interaction with proteins in the plasma, and a mathematical description of fibrinolysis. The latter is a multi-scale process that involves the microscopic dissolution of fibrin fibres which has important ramifications on the macroscale properties of the clot. Furthermore, fibrinolysis can be influenced by many different microscopic factors such as the presence of cellular components and changes in the fibrin architecture (Collet et al.; 2000, 2002). Thus, the resulting computational model will need to, in addition to describing macroscale phenomena such as blood flow, provide an accurate description of fibrinolysis. Therefore, an additional objective of this thesis is to provide a scalable and accurate model of fibrinolysis that can account for microscopic phenomena. The outline of the thesis is presented in the next section.

1.6 Thesis Structure

Chapter 2 is a literature review that provides an outline of the clinical, biochemical and mathematical background required to develop a patient-specific model of thrombolysis. The clinical background examines the current procedures involved in treating ischemic stroke and investigations into clinical parameters that may affect the efficacy of thrombolytic therapy. Furthermore, it reviews the current technological trends that are taking place to ameliorate the clinical outcome for patients suffering from acute ischemic stroke. The biochemical background outlines the mechanism of fibrinolysis and provides an overview of the effect that the fibrin architecture and cellular components have on the rate

of lysis. Lastly, the mathematical background examines the existing models for blood flow, thrombus formation and thrombolysis and how they may be applied to investigate clot lysis in patient-specific geometries.

Chapter 3 presents the work done on developing a model of thrombolysis to simulate the dissolution of a 2D clot under different pressure drops. The results showed how the lysis of a fully occluding thrombus led to the formation of mural clots that cause a local increase in the fluid velocity due to arterial constriction. The rapid lysis of the complete occlusion was followed by a slower dissolution of the remaining mural clots. At higher pressure drops, the flow became disturbed and led to asymmetric clot lysis patterns.

Chapter 4 evaluated the dissolution of a clot in a 3D idealised geometry that attempts to replicate a scenario encountered in ischemic stroke. A bifurcation that arises from the internal carotid artery (ICA) into the middle cerebral (MCA) and anterior cerebral (ACA) arteries was created with a fully occluding 3 mm and 8 mm clot in the MCA. Flow at the inlet was specified as either steady or pulsatile and the pressure at the outlets was determined using a 3-element Windkessel model. Fibrinolysis was determined as the cleavage of fibrin binding sites rather than homogeneous shrinking of the fibrin fibre radius. The model showed that after an initial period of clot dissolution, the lysis front moved through the clot at different rates for the 3 mm and 8 mm clot respectively.

Chapter 5 used the previous modelling framework to evaluate the lysis of a fully occluding clot in the middle cerebral artery for a patient-specific geometry extracted from CT images. The dissolution of clots of different lengths and positions along the middle cerebral artery was modelled to investigate the effect of these parameters on clot lysis. It was found that the increasing clot length led to a substantial increase in the breakthrough time while the clot position was important in determining the clot lysis pattern.

Chapter 6 examined the effect of tPA dose on the efficacy of thrombolysis. In order to model the dynamic concentration of lysis proteins in the plasma, a compartment model was developed to account for different thrombolysis regimens. This was integrated with the computational model described in chapter 5 so as to provide the dynamic inlet conditions for the free phase concentrations of all lysis proteins during the dissolution of a 3 mm fibrin clot in the middle cerebral artery. It was found that high tPA doses (>0.9 mg/kg) led to a modest decrease in the clot lysis time for fibrin clots but substantially increased the risk of ICH (as indicated by low fibrinogen levels).

Chapter 7 develops a new microscopic model of fibrinolysis that accounts for the influence of the fibrin architecture on the rate of thrombolysis. This was achieved by creating a hybrid continuous-stochastic model of fibrinolysis that monitored the cleavage of cross-sections along the fibrin fibre. The difference in the lysis between coarse and fine fibrin clots can be explained by the exponential increase in the number of cleavable cross sections for low fibrin fibre radii.

Chapter 8 summarises the key findings of this thesis and concludes by discussing the limitations of the computational model and how they can be addressed.

Chapter 2

Literature Review

2.1 Introduction

This chapter is composed of 3 parts. The first part provides a comprehensive overview of the cerebral vasculature, current clinical practice for the treatment of ischemic stroke and novel developments in thrombolytic therapy that aim to improve patient outcomes. The second part covers the biochemistry behind thrombolysis and the microscopic parameters that control clot dissolution. This includes evaluating the influence of clot structure, cellular components and shear stress on fibrinolysis. The final part provides a summary of the work done on mathematical and computational modelling of blood flow and clot dissolution.

2.2 Clinical Background

2.2.1 Cerebral Vasculature

The vascular system allows for the transport of blood around the human body to provide tissue with the oxygen needed for cell metabolism. Blood is oxygenated in the lungs and then pumped via the left ventricle of the heart into the aorta which is the largest artery in the human body. The aorta branches off into several smaller arteries that provide blood to all the major organs such as the liver, the kidneys and the brain. The brain alone is responsible for about 20% of the total energy consumption and depends on a highly complex network of arteries to supply it with blood. The main arteries that provide blood to the brain are the left and right sections of the vertebral arteries (VA) and the internal carotid arteries (ICA). The latter arise from a split in the common carotid artery (more commonly known as the carotid bifurcation) and supply around 80% of blood to the brain. The left and right VAs originate from the subclavian arteries and join up to form the basilar artery. The basilar artery and the common carotid arteries feed a circulatory anastomosis known as the circle of Willis (shown in Figure 2.1) that supplies blood to the cerebellum.

This structure is composed of the ACAs, anterior communicating artery (ACoA), ICAs and the posterior cerebral arteries (PCAs). The ACAs supply blood to the frontal lobes of the brain and are connected by the ACoA. The left and right PCA branches out from the basilar artery (which itself arises from the fusion of the left and right VA) to provide blood to the occipital lobes. The posterior communicating artery (PCoA) connects these arteries to the ICA which then goes on to bifurcate into

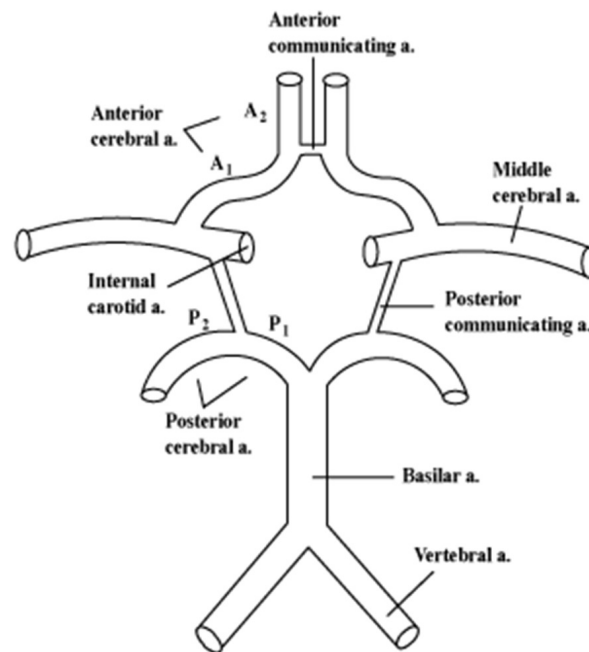


Figure 2.1: Schematic of the cerebral vasculature (from [Borgdorff and Tangelder \(2014\)](#)). ‘Artery’ is denoted as ‘a.’

the ACA and the middle cerebral artery (MCA). Although the MCA is not traditionally part of the circle of Willis, it plays an important role in supplying blood to a major part of the cerebrum. The MCA can be divided into several parts known as the M1, M2 and M3 sections. The M1 MCA segment stretches from the ICA bifurcation to the first branch in the insular segment. The subsequent portion of the artery from this branch until the Sylvian fissure is the M2 segment while the section from the Sylvian fissure until the cortical surface of the brain is the M3 segment.

The highly integrated vascular architecture of the circle of Willis makes the brain resistant to injury and trauma and allows for heterogeneity in structure from patient to patient. For instance, it has been found that around 50% of healthy individuals have one or several missing cerebral arteries. One of the most common sources of damage is ischemic stroke, which involves an embolic occlusion that cuts off blood supply to brain tissue. The majority of occlusions are located in the M1 and M2 segments of the MCA, the ICA bifurcation and to a lesser extent the basilar artery ([Marder et al.; 2006](#)). The next section outlines the procedures involved in treating ischemic stroke.

2.2.2 Clinical Management of Ischemic Stroke

Early symptoms of stroke include numbness of a side of the body, difficulty moving and slurred speech. When someone is showing these signs they should be admitted into hospital as soon as possible to prevent excessive damage to brain tissue ([Stroke Association; 2016](#)). Upon admission, the patient will undergo a CT scan to determine whether their stroke is ischemic or haemorrhagic. If the patient is suffering from an ischemic stroke, then they will be provided with treatment that removes the occluding embolism. Normally aspirin and clopidogrel (anti-platelet drugs) are provided to prevent the early onset of coagulation. Sometimes the patient is provided with anti-coagulants such as heparin to minimise clot formation. If the stroke is acute and certain criteria are met, thrombolytic therapy can be given.

Thrombolytic therapy involves intravenous infusion of alteplase that travels throughout the circulatory system until it reaches the site of occlusion. Alteplase reacts with other proteins in the body to create the enzyme PLS that then dissolves the fibrin strands that bind the offending clot together. If thrombolytic therapy fails, then the patient may be eligible for endovascular therapy which is a surgical intervention to remove the blood clot (Campbell; 2016). This can be accomplished via dissolution (using catheter delivered tPA), aspiration or direct removal using a stent retriever (thrombectomy). Although, the latter is yet to be approved by the FDA, it has so far shown great promise in clinical trials. Currently, thrombolytic therapy is only available to around 5% of patients partly due to the enormous number of contraindications associated with this treatment (Adeoye et al.; 2011). In order for the patient to receive thrombolytic therapy, amongst other things, they need to be less than 80 years of age, have no previous history of haemorrhagic stroke and must not suffer from hypertension. These contraindications are enforced to minimise the occurrence of ICH (Antonios and Silliman; 2013). The next section examines the historical development of thrombolytic therapy.

2.2.3 Thrombolytic Therapy

History

Ischemic stroke was originally treated by using a thrombolytic agent known as streptokinase (SK) in the 1950s (Röther et al.; 2002). SK is an enzyme produced by *Streptococcus* bacteria and has been shown to be effective at treating acute myocardial infarction. However, its repeated use can trigger an inflammatory response from the immune system. The enzyme tPA was first purified in 1979, and its recombinant version was produced in 1983 (Collen and Lijnen; 2004). Unlike SK and another thrombolytic agent known as urokinase (UK), tPA is fibrin specific and as such, is only effective within the immediate vicinity of fibrin. It is one of the main forms of treatment for ischemic stroke after a clinical trial found that patients who received it were more likely to achieve favourable neurological outcomes (NINDS; 1997). However, due to the risk of adverse side-effects, treatment is only available to a small subset of patients. As such, there have been notable efforts to expand the use of tPA in thrombolytic therapy to a wider patient population. This includes creating a network of medical care that can allow for the rapid diagnosis and treatment of stroke as well as narrowing the time window available for treatment after the onset of symptoms (Cheng and Kim; 2015). A summary of the relevant clinical trials is given below.

Expanding the use of tPA

The NINDS clinical trial provided evidence that tPA could enhance the outcome of patients suffering from acute ischemic stroke (AIS) (NINDS; 1995). A total of 291 patients were given either a placebo or an intravenous tPA infusion of 0.9 mg/kg within a 3 hour time window (length of time from the onset of symptoms). The success in a clinical trial can be determined by using a modified Rankin scale (mRS) which is a measure of the extent of a patient's neurological disability. The trial showed an increase of 13% in good functional outcome (mRS score of 0-1) and a decrease of 4% in the mortality rate after 90 days for patients treated with tPA. However, in addition to this improvement in clinical outcome, there was a significant increase in the rate of ICH at 5.8%.

Following this trial, the FDA authorised the use of tPA to treat patients suffering from AIS within

a 3 hour window. Another trial showed that the time window could be successfully extended to 4.5 hours without affecting the patient recovery rate ([Hacke et al.; 2008](#)). In this clinical trial, out of 821 patients who could be treated with tPA within a 3 to 4.5 hour time window, a dose of 0.9 mg/kg of alteplase was delivered intravenously to 419 patients. Despite the high increase of ICH (9.4%) in the thrombolysis group and similar rates of mortality with the placebo group, there was a 7% increase in functional outcome. The results of this trial led many European hospitals to approve thrombolytic therapy for patients within a 4.5 hour time window.

Another clinical trial aimed to examine whether older patients (>80 years old) could benefit from thrombolytic therapy ([IST-3 Group; 2012](#)). A group of 3035 patients, among whom 53% were over 80 years old were randomised and treated with 0.9 mg/kg of tPA within a 6 hour time window. The trial found that despite the age contraindications, there was no significant increase in mortality between the 2 groups. Furthermore, there was a modest increase of 2% in functional outcome for the tPA group. Despite a rise in the rate of ICH, the study claimed that it would be effective to treat patients over the age of 80 with thrombolytic therapy.

The use of thrombolytic therapy within certain contraindications can provide effective treatment to a wider variety of patients. However, other studies have also shown that providing treatment outside the recommended protocol can cause an increase in mortality rates.

Risks of Thrombolytic Therapy

One of the first clinical trials to evaluate the use of tPA for the treatment of ischemic stroke was the European Cooperative Acute Stroke Study (ECASS) ([Hacke et al.; 1995](#)). It enrolled 620 patients who were randomly selected to receive either a placebo or 1.1mg/kg of alteplase within 6 hours of symptom onset. Although it was found that there was no significant benefit for the tPA treated group, it was acknowledged that over 100 patients were outside the recommended protocol (e.g. treated with heparin or showed signs of major infarct on the CT scan). Of those patients that fell within the restrictions, there was a minor benefit in the tPA treated group. The ECASS II clinical trial enrolled around 800 patients with no signs of major infarction to be treated with placebo or with a lower tPA dose of 0.9mg/kg within a 6 hour time window ([Hacke et al.; 1998](#)). There was an 8.3% increase in functional outcome for the tPA treated patients and a higher risk of ICH (5.4%). There were no significant differences in 90 day mortality between the placebo group and the tPA treated group.

The ATLANTIS clinical trial attempted to investigate the safety of using tPA for the treatment of AIS outside the 3 hour time window ([Clark et al.; 1999](#)). A total of 613 patients were included and were randomly treated either with placebo or with 0.9 mg/kg of rt-PA over a 1 hour infusion. The study had to be discontinued as there seemed to be no benefit in outcome for the tPA treated group (34% to 32% in favour of placebo). Furthermore there was an elevated risk of ICH (increase of 6%) and a higher risk of mortality at 90 days (4.1%). The study suggested the use of advanced imaging tools to determine groups of patients that may be more likely to respond favourably to thrombolytic therapy outside the 3 hour time window.

These clinical trials show that although providing thrombolytic therapy within contraindications may extend the treatment availability to a larger range of patients, there is a higher risk of ICH and low potential for clinical improvement.

Future of Thrombolytic Therapy

Almost all of the clinical trials have accentuated the need for quicker treatment to prevent excessive tissue necrosis and promote higher survival rates ([Barreto; 2011](#)). Since its inception, the use of thrombolytic therapy has been increasing in recent years. A study confirmed that the use of tPA therapy to treat AIS has approximately doubled from 2005 to 2009 as found by the MEDPAR and Premier databases ([Adeoye et al.; 2011](#)). This is shown in Figure 2.2 and is suspected to be due to increased awareness and recognition that stroke should be treated immediately.

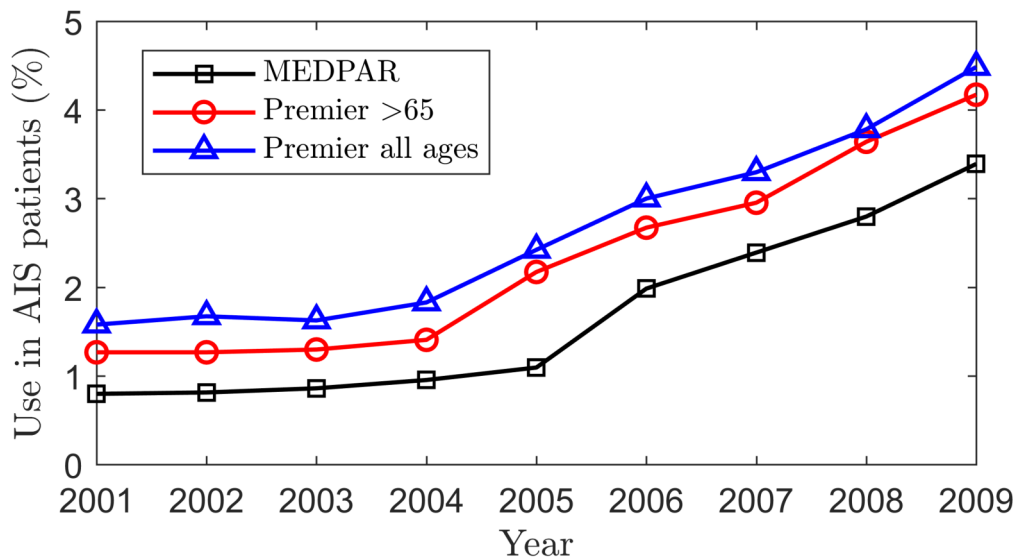


Figure 2.2: History of the use of tPA from 2001 to 2009 in the United States according to MEDPAR (Medicare Provider and Analysis Review) and Premier pharmacy records. Data retrieved from [Adeoye et al. \(2011\)](#).

It is likely that tPA use for the treatment of AIS will increase over time. A recent review of 27 clinical trials comprising 10,187 participants that aimed to evaluate the efficacy of thrombolytic therapy found that there is benefit in extending the treatment window to 6 hours ([Wardlaw et al.; 2014](#)). However the study also emphasized that any patient treated within a 3 hour time window has a more significant likelihood of favourable neurological outcome. Studies speculate that by ameliorating factors such as ambulance response time, stroke awareness among patients and diagnostic efficacy in hospital protocol, the likelihood of good clinical outcome can be dramatically improved ([CASPR; 2005](#); [Saver et al.; 2013](#)).

Future trials will aim to evaluate the effectiveness in further extending the treatment time window and mechanisms to reduce the occurrence of ICH. An exciting area of research looks at the potential of using imaging modalities to identify which patients are more likely to respond favourably to thrombolytic therapy. These include tools such as ultrasound, CTA, MRA and multimodal CT and MRI to determine characteristics such as clot location, size and composition that may help in predicting treatment outcome ([Gasparian et al.; 2015](#)). Despite these exciting developments, there is still uncertainty regarding how these parameters actually affect the likelihood of recanalization. Aside from uses in borderline cases, the delay in acquiring extra information from clinical images may have deleterious effects ([Campbell; 2016](#)). As such, the influence of clot parameters on the efficacy of thrombolytic therapy would need to be established before attempts are made to include them in

identifying subgroups of patients most suitable for treatment.

2.2.4 Important Parameters in Thrombolytic Therapy

Unsurprisingly, recanalization (reopening) of an occluded artery has been shown to correlate with good neurological outcome for ischemic stroke ([Rha and Saver; 2007](#)). Treatment has also been shown to be affected by clot size, length, location and porosity. Other factors such as the shape of the cerebral vasculature and the capacity of collateral circulation to provide adequate blood perfusion for the brain during injury and trauma are also implicated in the efficacy of thrombolytic therapy ([Menon and Goyal; 2016](#)).

Clot Length

The length of a clot has been found to have an important influence on recanalization rates for MCA occlusions. In a study involving 138 patients treated with IV tPA who had their clot lengths measured using non-enhanced CT images, 62 achieved successful recanalization and all of them had an occlusion length less than 8 mm ([Riedel et al.; 2011](#)). [Rohan et al. \(2014\)](#) found different results when measuring the clot length of 80 patients (using a technique known as maximum intensity projection) suffering from MCA occlusions who were treated with thrombolytic therapy. Although the length of the occluding thrombus was shown to have a strong influence on the probability of recanalization, there was no clear cut off point. Recanalization was still achieved for clots longer than 20 mm, albeit rarely, and a clot length of 11 mm was found to have around a 50% chance of recanalization. Furthermore, it was found that clot extension into the M2 segment of the MCA was a detriment to patient recovery. Using multi-modal MRI, [Yan et al. \(2016\)](#) examined 74 patients with an MCA occlusion and they found that the the average length of the occlusion was around 8 mm with a variation of 4.5 mm. Good outcome with thrombolytic therapy was typically achieved for clot lengths less than 6.77 mm as indicated by Figure 2.3. Almost all studies agree that very long clots (>10 mm in length) show poor signs of recanalization and recovery. However, the correlation between clot length and recanalization does not necessarily imply that the clot length is the only factor in determining treatment outcome.

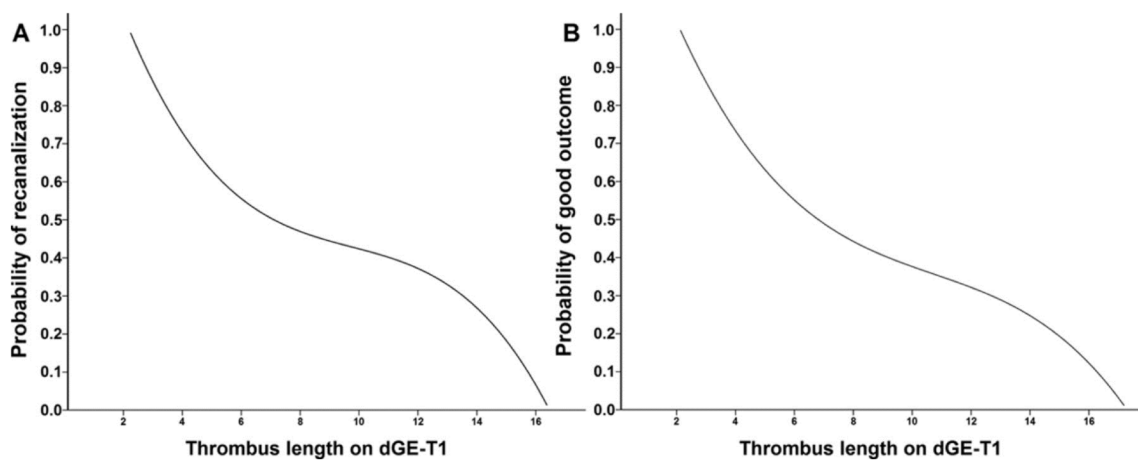


Figure 2.3: An estimate on how clot length (mm) impacts (A) the probability of vessel recanalisation and (B) the likelihood of a good outcome. Image taken from [Yan et al. \(2016\)](#).

Clot Position

A study looking at the treatment of 335 patients with intravenous tPA found that the location of occlusion was associated with clinical outcome ([Saqqur et al.; 2007](#)). Distal MCA occlusions had a significantly higher rate of recanalization (44%) as compared to proximal MCA occlusions (30%) and were characterised by a 7% decrease in mortality at 3 months. Terminal ICA had the lowest recanalization rates (6%) and a high rate of mortality (45%) while basilar arteries showed high rates of recanalization (33%) and elevated levels of mortality (75%). Another study analysed the relationship between clot size and location in ischemic stroke by using non-contrast enhanced CT imaging to determine clot length ([Kamalian et al.; 2013](#)). Out of 53 patients, it was found that 94% of patients with an ICA terminal occlusion and 73% of patients with an M1 occlusion had a clot length equal to or greater than 8 mm. However, 78% of patients with an M2 occlusion tended to have clots shorter than 8 mm suggesting that long clots were likely to be found close to the ICA bifurcation. [Friedrich et al. \(2015\)](#) investigated how the distance of the thrombus from the ICA bifurcation (DT) affects the efficacy of thrombolytic therapy on 136 patients with an MCA occlusion. They found that patients with distal occlusions had a better chance for treatment success. In fact for DT>16 mm, the probability of a good clinical outcome was greater than 50%. Similar to previous studies, longer thrombi (>8mm) were found more frequently at DT<20 mm. The authors suggest that treatment outcome is predominately affected by clot location and that the correlation of clot length with poor treatment outcome as found by [Riedel et al. \(2011\)](#) is due to larger clots occurring in proximal arteries.

Collateral Circulation

One of the reasons why clot location can have an important influence on the outcome of thrombolytic therapy may be related to the status of collateral circulation. Collateral circulation refers to the perfusion of blood by smaller vessels when a major artery has been occluded. The status of the collaterals has shown to be an important predictor of the success of recanalisation therapies as good circulation can minimize infarct growth and sustain the tissue surrounding the occlusion. A study found that in a group of 222 patients, a favourable outcome was achieved for 41.5%, 25.5% and 14.1% of those with excellent, good and poor collaterals respectively ([Bang et al.; 2011](#)). Furthermore, the study found that collateral status was a good indication of treatment outcome independent of the location of the occlusion or the type of treatment. Another study confirmed that the collaterals are important in predicting therapeutic outcome for endovascular therapies ([Liebeskind et al.; 2014](#)). Of those with a collateral grade of 2, 3 or 4, recanalisation was achieved in over 80% of cases. Those with a collateral grade of 1 or 0 had around a 50% chance of recanalisation. [Qazi et al. \(2015\)](#) hypothesized that the location of an occlusion can lead to areas of large flow stasis which promote the growth of the occlusion. The development of flow stasis may be due to a poorly structured cerebral vasculature and insufficient collaterals as shown in Figure 2.4. In the study, 104 patients with M1 MCA occlusions with or without corresponding ICA occlusions were imaged with MRI and non-contrast CT. It was found that patients with poor collateral circulation had longer clots (average of 50 mm) against those with good circulation (average of 20.5 mm). Thus individuals with good collaterals are expected to have a better response to thrombolytic and endovascular therapies due to the occurrence of shorter clots.

Collateral Status	Original Clot Composition	Angio-Architecture	Total Clot	IV RECAN	IA RECAN
				Very High	Very High
				Intermediate	High
				Low	Intermediate
				Low	Intermediate
				Low	Intermediate
				Low	Low
Good Intermediate Poor	RBC Rich Fibrin Rich	Efficient Inefficient			

Figure 2.4: Effect of collateral structure on an ischemic occlusion and consequences for treatment. The columns with IA recan and IV recan indicate the likelihood of recanalisation with thrombolysis and thrombectomy, respectively. Image taken from [Qazi et al. \(2015\)](#).

Clot Composition

The structure of the clot has also been found to be an important parameter that may affect the success of thrombolytic therapy. A study examined 508 patients with ultrasound before and 1 hour after administration of tPA and found that the overall probability of recanalization decreased over time ([Muchada et al.; 2014](#)). Individuals treated at 90 minutes had an exceptionally high recanalization rate of 45.5% compared to the 16% for those who received treatment after 270 minutes. The authors hypothesized that the association between poor outcome and late treatment might be due to older clots being more difficult to treat. Clots that show a high degree of perfusion are associated with a favourable treatment outcome. One study found that out of 66 patients who received IV tPA, 66.6% of those with occult anterograde flow (blood perfusion through the clot as measured with CT-perfusion) achieved recanalization ([Ahn et al.; 2015](#)). On the other hand, those with retrograde flow had a substantially lower rate of recanalization (29.7%). The permeability of the clot also affects the likelihood of thrombolytic success. A study of 308 patients with CT angiography found that more permeable clots have a higher rate of recanalization than non-permeable ones. The permeability of the clot was measured with a parameter known as the thrombus attenuation increase (TAI) on CT images where the authors define any thrombus with a TAI greater than 23 HU as permeable. Around 48% of those patients who achieved a favourable outcome had an average TAI of 25.51 HU while those with a bad outcome have an average TAI of 15.11 HU. The average TUI of all patients is around 20.1 with only 42% of patients having permeable thrombi.

Including Multiple Factors

When a patient is found to have a stroke, a CT scan will be performed to determine whether their stroke is ischemic or haemorrhagic. Data from the scan can provide valuable information regarding certain attributes of the blockage that can help determine the severity of an ischemic stroke. The severity can be evaluated using the clot burden score (CBS) based on the degree of contrast opacification in CT images (Puetz et al.; 2008). A maximum CBS of 10 for a patient is given before points are deducted by analysing the results from the clinical scan. Presence of thrombus in the proximal M1 or ICA bifurcation deducts 2 points from the CBS while 1 point is deducted if the thrombus is spotted in the distal M1 or ACA. A score of 0 corresponds to a large vessel occlusion that is in practice difficult to treat while a score of 10 indicates that there are no signs of a major occlusion. Results show that CBS values higher than 6 have good rates of recanalisation for patients treated with tPA (Tan et al.; 2009). Some studies have tried to combine all the factors together in search for an easily implementable algorithm to determine the rate of success for thrombolytic therapy. One study used CTA characteristics (such as clot length location and permeability) to determine a patient's suitability for thrombolytic therapy (Mishra et al.; 2014). Out of a total of 228 patients, around 70% with a clot length smaller than 15 mm achieved reperfusion. Terminal ICA occlusions were associated with a poor clinical outcome (23%) as compared to M2 occlusions (75%) and M1 occlusions (58.1%). Those patients with a distal M1 occlusion and good collaterals, and who showed no residual flow had a higher chance of recanalisation (around 37%). The authors conclude that using CTA to determine a patient's likelihood of success in thrombolytic therapy may be an effective way to enhance existing treatment. Many factors have an important effect on the efficacy of thrombolytic therapy. Clot length, location and composition (as measured by perfusion) as well as the extent of collateral flow affect clinical outcome. Current research is focused on finding an imaging modality that is quick, easy and safe to use and can help identify the patients who are most likely to respond favourably to thrombolytic therapy. This will aid clinicians in personalising treatment and minimising the occurrence of side-effects.

2.2.5 Novel Developments

There has been considerable effort in the scientific community to develop new technologies and therapies that can radically improve the treatment of AIS in a simple, efficient and safe manner. In this section, the progress and development of these new approaches are summarised to evaluate the immediate future of thrombolytic therapy.

New Thrombolytic Drugs

Although alteplase can be effective at treating ischemic stroke, there are several issues with its administration. Alteplase has a relatively short half-life (<5 mins) and a high neurotoxicity. Recently, two new thrombolytic drugs have been developed in an attempt to overcome these limitations: Tenecteplase (TNK) and Desmoteplase (DSPA) (Logallo et al.; 2016).

The TNK enzyme is a modified version of tPA that has shown to be 14 times more fibrin selective, have a notably longer half-life (~20 mins) and greater resistance to fibrinolytic inhibitors. Recent clinical trials have been undertaken to determine its clinical efficacy in comparison to alteplase. The ATTEST clinical trial randomly assigned 52 patients suffering from AIS to receive 0.25 mg/kg of

TNK and 52 to receive 0.9 mg/kg of alteplase within a 6 hour window (Huang et al.; 2015a) and the results showed that functional outcome evaluated at 90 days was higher for TNK (28%) than for the alteplase group (20%). Further analysis of the venous blood samples extracted from 30 patients who took part in the trial revealed that TNK had a noticeably smaller impact on the coagulation system than alteplase thereby decreasing the likelihood of hypofibrinogenaemia (Huang et al.; 2015b). This is due to TNK's exceptional fibrin specificity, which the authors argue is consistent with the lower levels of ICH observed for TNK patients in the ATTEST clinical trial. The TEMPO-1 trial was a dose-escalation study that provided 2 groups of 25 patients with 0.1 mg/kg and 0.25 mg/kg of TNK (Coutts et al.; 2015). Only a single incidence of ICH was reported along with high recanalization rates for both groups (39% for 0.1 mg/kg and 52% for 0.25 mg/kg). The successful results from these studies have spurred on the development of large scale clinical trials to evaluate the efficacy of TNK such as NOR-TEST, TASTE and TEMPO-2 (Logallo et al.; 2016). NOR-TEST is a phase-3 clinical trial that aims to evaluate the efficacy of a single bolus shot of TNK (0.4 mg/kg) against IV administration of alteplase (0.9 mg/kg with 10% delivered as a bolus and the rest via a 1 hour continuous infusion) (Logallo et al.; 2014). The results of these clinical trials are yet to be published but are eagerly anticipated.

DSPA is a thrombolytic agent that is derived from the saliva of *Desmodus rotundus*, the vampire bat. Early studies showed that it was highly fibrin selective and more importantly had a low neuro-toxicity. An early dose study examined its safety and efficacy when administered to patients within 3 to 9 hours of ischemic stroke onset (Hacke et al.; 2005). The DIAS clinical trial found that high levels of ICH occurred for doses greater than 37.5 mg/kg. A readjusted dose of 125 μ g/kg significantly decreased ICH rates to levels only slightly higher than those of a placebo group. Over 60% of patients achieved a favourable 90 day outcome at the highest DSPA dose as compared to 22.2% of patients who received a placebo.

The subsequent DIAS-2 clinical trial compared the efficacy of treating 186 patients suffering from ischemic stroke with either a 90 μ g/kg or 125 μ g/kg dose of DSPA, or a placebo (Hacke et al.; 2009). There was a significant increase in ICH for the DSPA groups as compared to placebo (increase of 4.5% and 3% for 125 μ g/kg and 90 μ g/kg respectively). Furthermore, functional outcome at 90 days was the lowest for the highest DSPA dose (36%) and 1% higher than placebo for the lower DSPA dose (47%). The authors speculate that the relatively poor performance of DSPA may have been due to the high recovery rate of the placebo group who were suffering from mild stroke. As such the DIAS-3 clinical trial was conducted to investigate whether DSPA was effective in treating patients with a severe occlusion within 3-9 hours of symptom onset (Albers et al.; 2015). Both the placebo and DSPA treated groups achieved similar rates of good functional outcome ($\sim$ 50%) and low levels of ICH ($\sim$ 2%).

The disappointing results of the DIAS-3 clinical trial caused the development of DSPA to be discontinued. Its reported low performance could potentially be due to the application of DSPA to a longer time-window than what is typically applied in thrombolysis (<3 hours) as this might have altered the clot composition and made the occlusion more resistant to thrombolysis. At the current moment, TNK is the only new thrombolytic agent under serious investigation as a potential replacement for alteplase.

Ultrasound and Microbubbles

The use of ultrasound, particularly low frequency ultrasound, has been shown to be effective at enhancing clot lysis in both animal ilio-femoral arteries and human coronary arteries (Suchkova et al.; 2000; Cohen et al.; 2003). There is a hope that these treatments may be extended to the treatment of strokes caused by blood clots in cerebral arteries (a diagram of ultrasound imaging in the brain is shown in Figure 2.5 a). Alexandrov et al. (2004) performed a phase 2 clinical study after their previous investigations showed promise in enhancing clot lysis with a 1 MHz Doppler ultrasound (Alexandrov et al.; 2000). With a sample size of 126 patients, they found that there was a statistically non-significant trend towards an increasing rate of thrombolysis with ultrasound. However, they noted the trial could not be double blinded and the skill of each individual operator was highly variable.

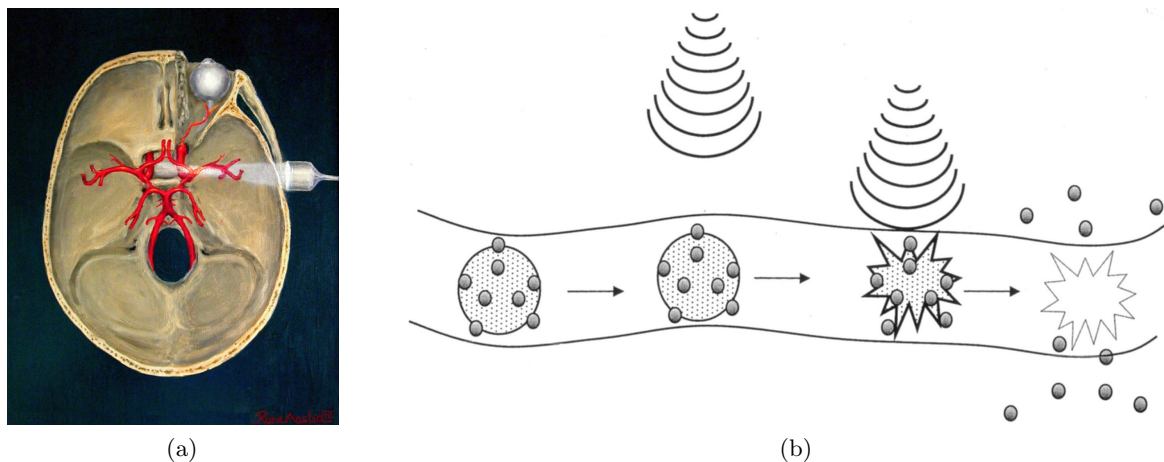


Figure 2.5: (a) Illustration of ultrasound imaging in the human skull (from Runeaslid (2007)) and (b) diagram of drug delivery through micro bubble destruction (from Bekerredjian et al. (2005)).

A device has recently been developed that allows for an operator-independent application of ultrasound in an effort to combat the difficulties found in the previous study. This device is applied to the patient and delivers 2 MHz of pulsed ultrasound at low power (Barlinn et al.; 2013). By evaluating the outcome of 20 stroke patients treated with sono-thrombolysis with this device, Barreto et al. (2013) found that none of the patients had any adverse complications while 10 of them achieved partial or complete recanalization. The study concluded that this device shows promise and a comparison of its lytic efficiency with tPA administration alone should be performed.

Daffertshofer et al. (2005) conducted a phase 2 clinical trial to test the efficacy of using low frequency ultrasound in promoting thrombolysis in ischemic stroke. The clinical trial had to be discontinued because 13 out of 14 patients in the treatment group suffered from haemorrhagic complications while only half the control group had similar complications. The presence of subarachnoid haemorrhaging and the formation of hot spots outside the focus point indicate that it was the ultrasound, which caused the complications. The failure of the trial has been linked to many different causes. Azuma et al. (2005) argued that the formation of a standing wave in the skull could lead to a lower threshold for cavitation. They confirmed the presence of standing waves and cavitation effects by sonicating a human skull submerged in water with the same ultrasound parameters as used by Daffertshofer et al. (2005). They argued that any negative side-effects by the ultrasound could be mitigated by using shorter pulse duration. Reinhard et al. (2006) concluded that the haemorrhagic complications could have resulted from an increased permeability of the blood-brain barrier induced by the ultrasound.

This explains how some of the complications occurred without symptoms and were at some distance from the focused region. Wang et al. (2008) postulated that the parameters used in the Daffersthoffer trial were inadequate as they gave a very wide beam length, a large mechanical index and a large pulse length. Through the combination of both high and low frequency ultrasound, they created a “Dual Mode Ultrasonic Thrombolysis” (DMUT) transducer that reduces the beam width and shortens the pulse length. Therefore although low frequency ultrasound in thrombolytic stroke treatment caused severe complications, it may be due to poorly chosen ultrasound parameters.

Aside from using ultrasound as an adjunct to thrombolysis, it can be combined with microbubbles to encourage clot dissolution. Microbubbles are contrast agents that are encapsulated by a polymer or lipid barrier and are commonly used in ultrasound to enhance imaging of biological media. Recently, they have been found to lower the cavitation threshold in blood and act as suitable drug carriers that release their contents when triggered by ultrasound insonation (Figure 2.5 b). Tachibana and Tachibana (1995) showed in an idealised in-vitro study that the combination of albumin microbubbles, tPA and ultrasound can lead to increased fibrinolysis. They believe this is predominately caused by inertial cavitation that induces higher shear rates and hence leads to increased thrombus porosity and allows for better drug penetration.

Microbubbles can come in a variety of compositions and forms and can even be engineered to have a particular affinity for thrombotic clots. Schumann et al. (2002) showed that microbubbles can target the GPIIb/IIIc receptors on platelets and therefore enhance the diagnostic imaging of newly formed blood clots. Various researchers have followed up on this to investigate a potential enhancement of clot lysis with targeted microbubbles. Brown et al. (2011) performed a large scale in-vitro and in-vivo study on the internal carotid artery of rabbits with different contrast agents and tPA concentrations. Using 2 different contrast agents, Definity and adMB (albumin dextrose microbubble), they found that not only the type of contrast agent is important for lytic efficiency, but also its tPA concentration. The combined treatment of microbubbles with tPA and ultrasound, was shown to be better than the administration of free tPA and this, the authors believe, is primarily caused by ultrasound. No evidence of any adverse effects of the treatment such as ICH was found although there was no examination of any long term complications that might arise.

High frequency ultrasound studies indicate a non-statistically significant improvement in recanalization whereas low-frequency has been shown to cause serious secondary complications. This may be due to the strong attenuation at the temporal bone and the formation of standing waves. However, a change in the parameters used for low frequency ultrasound may lead to its clinically safe use for treating ischemic stroke in the future. Alternatively, the use of microbubbles may be able to accelerate the rate of thrombolysis but still remains to be tested in a clinical environment.

Polymeric and Metal Nanoparticles

The use of metal nanoparticles offers another way of encapsulating and guiding the thrombolytic drug to the thrombotic embolus. Kaminski et al. (2008) postulated that it may be possible to magnetically guide magnetite coupled with PLA-PEG (polylactic acid-poly ethylene glycol) nanoparticles and trigger the release of drug through the use of ultrasound. They showed this was possible by exceeding clinical levels of tPA concentration (1-4 $\mu\text{g/ml}$) needed for thrombolysis in a saline bath at room temperature. Torno et al. (2008) explored these ideas by conducting an in-vitro study to test the ability

of magnetic nanoparticles to improve clot lysis. They found that clot lysis was enhanced by 2.7 fold for white blood cell clots and 1.7 fold for red blood cells when compared to the free administration of tPA. They maintain that this improvement is due to deeper thrombus penetration made possible through magnetic guidance. This effect is particularly marked for white blood clots as they have a denser fibrin mesh that is harder to penetrate for free tPA. [Korin et al. \(2012\)](#) showed that drug carriers can be triggered without the use of ultrasound by recanalising a rat pulmonary embolism model through the use of shear stress triggered polymer microparticles. Once these spray dried PLGA (poly lactic co-glycolic acid) microparticles reach a critical shear stress value induced by a severe narrowing of the vessel, they break apart into smaller tPA conjugated nanoparticles that then penetrate the thrombus. These particles are easily cleared from the system within a short time period and hence avoid accumulation of drug in healthy tissue. Remarkably they used only 1/100th the amount of tPA used in free administration to recanalise a rat thrombus in the mesenteric artery. The authors argue that the absence of an external trigger needed to release the tPA means that these particles can be used to treat patients immediately after symptom onset. However, the inability of these particles to reach completely occluded arteries where there is no flow remains a problem.

Echogenic Liposomes

As mentioned before, contrast agents are typically used with ultrasound to enhance the imaging of biological media. They can also be encapsulated in liposomes to maintain longer echogenicity in the blood stream. The use of echogenic liposomes for thrombolytic therapy was tested by [Tiukinhoy-Laing et al. \(2007\)](#) who successfully encapsulated both tPA and the contrast agent Optison in a liposome. Half of the tPA was encapsulated, echogenicity suitable for imaging was achieved and an in-vitro clot was successfully dissolved with the aid of ultrasound. The latter was achieved through the induction of high shear stresses that were able to disrupt the liposome and release the tPA. The authors claim that their work provides a suitable proof that echogenic liposomes can indeed be used in thrombolytic therapy.

An in-vivo study was done by [Laing et al. \(2012\)](#) who examined the dissolution of thrombotic clots in the femoral arteries of rabbits. They found similar results to [Shaw et al. \(2009\)](#) in that ultrasound enhanced the lytic efficiency of echogenic liposomes whereas blank liposomes with ultrasound showed poor or no clot lysis. Therefore, it is clear that there is a binding affinity of the tPA-ELIP to the blood clots and this is enhanced by ultrasound. By comparing ELIP-tPA, Optison-tPA (microbubble) and the administration of free tPA, they found that none of them had a statistically significant superior lytic efficiency over the other regimes. Furthermore, two different ultrasound regimes were used to account for the two different cavitation regimes that may take place in the blood stream. These include lysis by acoustically enhanced diffusion and rapid fragmentation of the blood clot through high shear stress. The authors concluded that more research needs to be done to investigate which treatment has a better thrombolytic efficacy. Furthermore, they stress the need for the optimisation of the ELIP technique.

Thrombectomy

Thrombectomy is an endovascular treatment that has been shown to be successful in recanalising large artery occlusions that are resistant to thrombolysis. It is essentially a minimally invasive procedure

that is able to mechanically retrieve clots through the use of a catheter, a guide wire and a stent-retriever device as shown in Figure 2.6. Initially, several clinical trials done in 2013 failed to show any benefit in the use of endovascular therapy following tPA administration when compared with thrombolytic therapy alone (Ciccone et al.; 2013; Kidwell et al.; 2013; Broderick et al.; 2013).

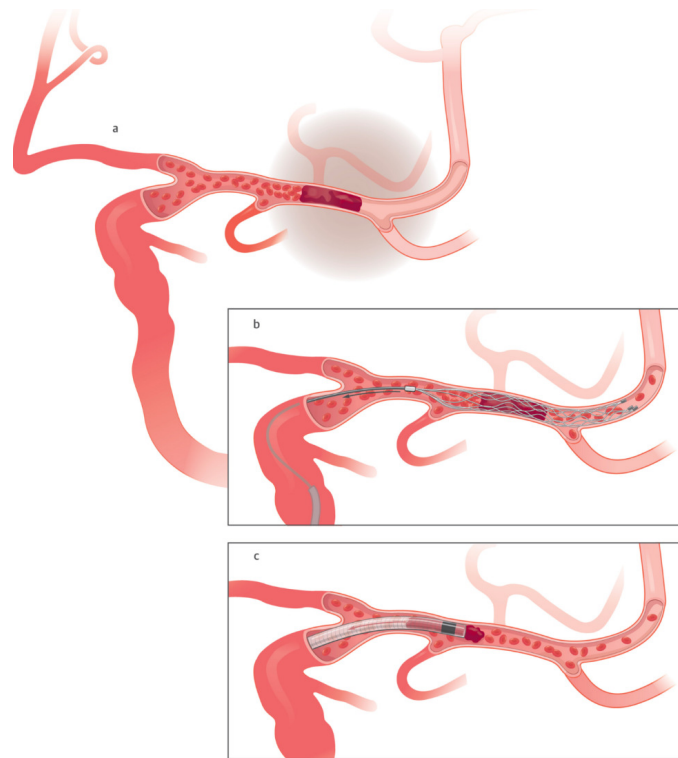


Figure 2.6: (a) Occlusion in the M1 division of the MCA that can be removed via (b) thrombectomy or (c) catheter aspiration. Image taken from Papanagiotou and White (2016).

However, a string of recent clinical trials indicated a potential improvement in the use of thrombectomy over thrombolytic therapy. The MR CLEAN clinical trial found that intra-arterial treatment (catheter based thrombolysis or mechanical thrombectomy) done within 6 hours of the onset of symptoms had a 13.5% increase in the functional independence of the patients combined with intravenous alteplase alone (Berkhemer et al.; 2015). The SWIFT PRIME clinical trial was interrupted due to superior clinical outcome in patients undergoing thrombectomy with 60% achieving functional independence against 35% who received tPA alone (Saver et al.; 2015). The REVASCAT clinical trial was also discontinued after clot removal with the Solitarie stent-retriever allowed for a 15.5% increase in functional outcome over tPA treatment alone in a total pool of 290 patients (Jovin et al.; 2015). The EXTENT-IA clinical trial involved treating 70 patients with 0.9mg/kg alteplase within 4.5 hours of stroke symptoms with some receiving further thrombectomy (Campbell et al.; 2015). The trial was halted due to the enhanced recovery of patients who received thrombectomy with a 30% increase in patients with an mRS score of 0-2. The ESCAPE trial was also stopped early after it reported significant rates of recovery (53% to 29.3%) against those who received alteplase treatment alone (Goyal et al.; 2015).

From the latest clinical trials, it is clear that thrombectomy used in conjunction with thrombolytic therapy is effective for treating large vessel occlusions that are particularly resistant to thrombolysis. The success of the latest clinical trials have led some to suggest that thromboectomy should replace thrombolysis as the first line of treatment for ischemic stroke in the context of large vessel

occlusions ([Antonenko and Caso; 2016](#)). There are, however, still some reservations regarding replacing thrombolysis completely with thrombectomy. The clinical trials only evaluated success of the treatment in conjunction with tPA rather than as a standalone treatment. Furthermore, there are concerns regarding the scarcity of the necessary infrastructure and experience to successfully undertake thrombectomy procedures ([Muir; 2016](#)). In order to maximise benefit from thrombectomy, researchers stress the need for an optimised system to deliver patients efficiently to specialist stroke centres ([Klourfeld et al.; 2016](#)). At the current moment, it is likely that endovascular procedures will be combined with thrombolysis to maximise care and improve patient recovery.

2.2.6 Summary

Ischemic stroke occurs when there is a blockage of a cerebral artery by a blood clot that can prevent blood flow from reaching the brain. Treatment usually involves medication (aspirin and clopidogrel) and thrombolytic therapy with alteplase (if the patient qualifies). Due to alteplase's low half-life and neurotoxicity, it can cause adverse side-effects limiting thrombolytic treatment to only around 5% of ischemic stroke patients. Successful efforts have been undertaken to extend the time window for treatment (4.5 hours after the occurrence of symptoms) and to allow treatment to a wider patient population (e.g. those over 80 years of age). Other considerable efforts have been made to find predictors that can indicate when thrombolytic therapy will be successful. Some exciting new progress has been made in the development of alternative treatment methods such as new thrombolytics (TNK), encapsulation and targeted delivery with nanoparticles, and endovascular therapy. The latter is especially promising due to reported high rates of recovery but faces issues with the large-scale distribution of treatment. As such, thrombolytic therapy is expected to be still used in the near future for the treatment of ischemic stroke.

2.3 Biochemical Background

2.3.1 Clot Formation and Thrombolysis

Overview of Clot Formation

The mechanism of blood clotting is a complex process that involves the balance of many different pro-clotting factors and their inhibitors. Imbalances in this cycle may lead to thrombosis or to extensive bleeding. The wall of a blood vessel is lined with endothelial cells that inhibit the attachment of disk-shaped cell structures known as platelets. Injury at the endothelial or sub-endothelial level exposes collagen and leads to the production of thromboxane and other factors that promote platelet activation and aggregation. These adhesive platelets lead to a premature blocking of the injured vessel wall, which is later on consolidated with a type of 'glue' known as fibrin ([Kaplan and Jackson; 2011](#)). The coagulation cycle, discussed in the section below, is what leads to the formation of fibrin from fibrinogen via a combination of different clotting factors.

Description of the Coagulation Cascade

The activation cascade of clotting factors can occur via two different pathways, known as the ‘intrinsic’ and ‘extrinsic’ pathways as shown in Figure 2.7. These eventually lead to the formation of fibrin via the ‘common’ pathway. The whole cycle is made up of more than 20 different proteins each of which plays a specific role in the formation of fibrin from fibrinogen (Lip and Blann; 2003). The intrinsic pathway, as the name suggests, can trigger the formation of a blood clot without any influence external to the blood plasma itself. In brief, the interplay of factors IX, XI, XII and VIII leads to the activation of factor X. The extrinsic pathway, on the other hand, is triggered by any damage to the sub-endothelial as this brings about the presence of factor III (otherwise known as tissue factor, TF). TF then forms a complex with factor VIIa and calcium that subsequently also leads to the activation of factor X. The common pathway uses activated factors V and X (along with phospholipids and calcium) to create a prothombinase complex that then converts prothrombin to thrombin, which is a serine protease that leads to the formation of fibrin from fibrinogen (Palta et al.; 2014). Thrombin plays a multi-faceted role in coagulation through the activation of platelets and factor XIII; the latter forms cross-links across the fibrin mesh.

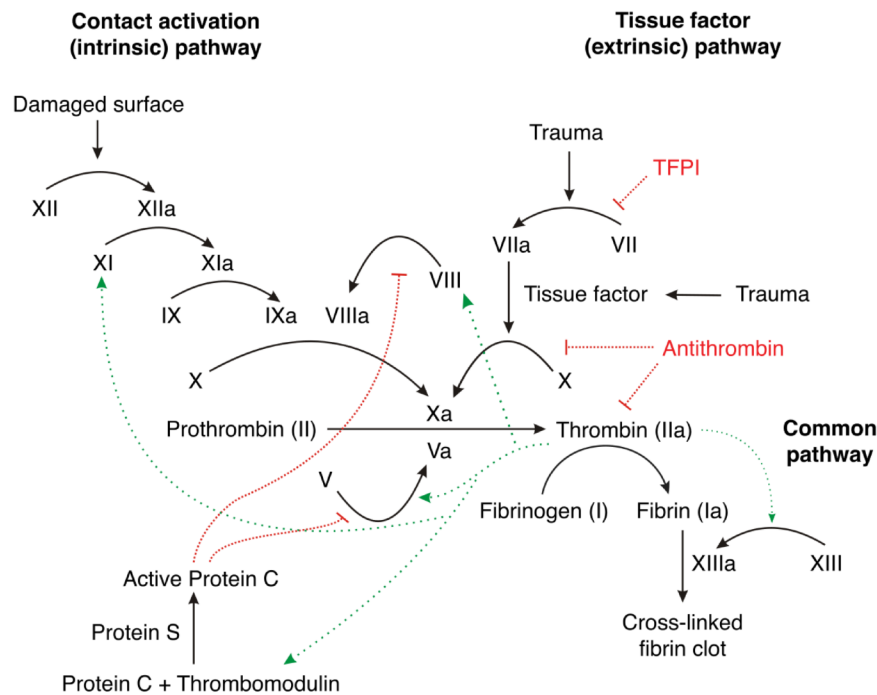


Figure 2.7: Overview of the coagulation cascade (from Steinsky (2007)).

Formation of the Fibrin Network

The protein thrombin is very important as it allows for the formation of fibrin monomers from the ubiquitous fibrinogen molecules circulating in the blood stream. Fibrinogen is a glycoprotein that is made up of 2 A α , 2 B β and 2 γ protein chains connected to each other by disulphide bonds. The N terminal of these polypeptides all join up to make the central E globular domain of the protein while the C terminal ends stretch out to create 2 separate D domains. The A α chains protrude slightly outside of the D domains and are important for fibrin cross-linking. Thrombin cleaves fibrinopeptides A and B in the central E domain from fibrinogen to create a fibrin monomer. The removal of these

fibrinopeptides creates ‘knobs’ A and B that can bind to the ‘holes’ of the γ and β nodules of another fibrin monomer (Weisel and Litvinov; 2013). These interactions allow the fibrin monomers to connect to each other creating long half-staggered structures known as protofibrils. The protofibrils can then laterally aggregate to form large fibrin fibres that make up the fibrin mesh (Weisel and Nagaswami; 1992). The fibrin mesh can be further stabilised by crosslinking, which is when factor XIIIa (transglutaminase) connects neighbouring α chains and γ nodules together (Duval et al.; 2014). The process of fibrin polymerisation is shown in Figure 2.8.

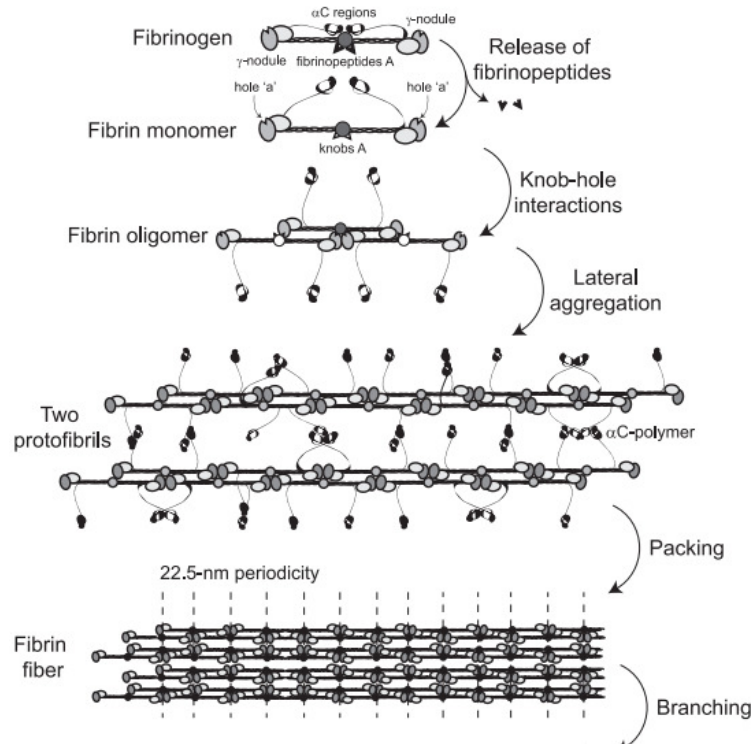


Figure 2.8: Schematic of fibrin polymerisation (from Weisel and Litvinov (2013)).

The development of the fibrin network is highly sensitive to the amount of fibrinogen, thrombin and calcium ions that are present. For instance, high thrombin levels favour the development of a tight fibrin network composed of thin fibres (Gabriel et al.; 1992). Individuals suffering from low plasma levels of fibrinogen (hypofibrinogenemia) can suffer from haemorrhage due to a lack in clot stability. Calcium ions are located around the site of a wound and bind with prothrombin to localise the generation of thrombin. As such, they also play an important role in determining the final fibrin network structure. During the process of fibrin polymerisation, cellular elements such as platelets, red blood cells and leukocytes can get trapped inside the newly formed fibrin mesh. Over time, the blood clot tends to shrink due to the contraction of the platelet intracellular cytoskeleton (a process otherwise known as clot retraction). The fibrin architecture of the clot as described by parameters such as porosity, fibre diameter and degree of branching can significantly vary depending on the biochemical environment.

Description of Fibrinolysis

Fibrinolysis is a process by which a protease enzyme dissolves the fibrin mesh that binds the blood clot together. The presence of thrombin during the coagulation cascade triggers the release of tPA

from the endothelial cells. tPA then forms a ternary complex with PLG and fibrin to generate the fibrin cleaving protease PLS. This enzyme goes on to cleave the fibrin fibres of the clot into fibrin degradation products (FDPs) which allows for the gradual dissolution of the clot.

There are numerous inhibitors that regulate the rate of fibrinolysis. Some inhibitors, such as plasminogen activator inhibitor 1 (PAI-1), form an inert complex with tPA thereby inhibiting its activity, while other inhibitors bind with PLS e.g. α 2-antiplasmin (AP) and α 2-macroglobulin (MG). Furthermore, there are other proteins that actively prevent the adhesion of lysis proteins onto fibrin binding sites thereby limiting fibrinolysis. These can be substances such as lipoprotein(a) that share binding sites with PLG due to their chemical similarities. Others such as thrombin-activatable fibrinolysis inhibitor (TAFI) degrade the C-terminal lysines on fibrin that act to provide additional binding sites for PLG activation. A schematic of the process of fibrinolysis is given in Figure 2.9. The level of tPA in haemostasis is relatively low with a concentration of around 4 ng/mL. During thrombolytic therapy the concentration of tPA is increased by 1000 fold (Weisel and Litvinov; 2014) thereby reducing the time till lysis from a matter of days to just a few hours.

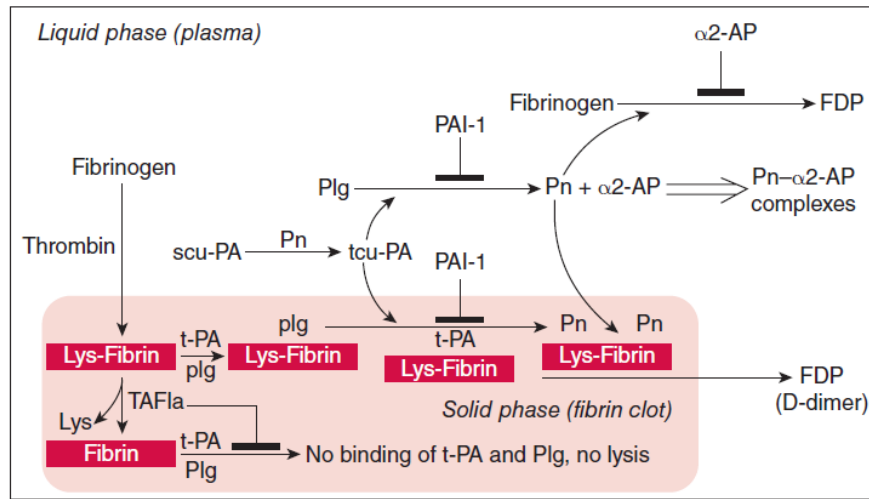


Figure 2.9: Schematic of fibrinolysis where tPA is tissue plasminogen activator, Plg is plasminogen, Pn is plasmin, TAFIa is activated TAFI, Lys-fibrin represents fibrin with lysine residues that allow for selective tPA and PLG binding ,tcu-PA is two chain urokinase and scu-PA is single-chain urokinase. Arrows denote reactions and T-like symbols show the activity of inhibitors. Image from Weisel and Litvinov (2014).

The efficacy of fibrinolysis can be influenced by the structure of the fibrin network, the presence of cellular components and other factors such as mechanical stress. The effect that each component has on fibrinolysis is discussed in the subsequent sections.

2.3.2 Effect of Clot Structure on Fibrinolysis

As mentioned before, the properties of a fibrin network can be altered by changing the thrombin, fibrinogen and calcium concentrations. Furthermore, the shape of the fibrinogen molecule can also cause changes in the fibre architecture of the clot. When parameters such as fibre length, diameter and branching density are changed, the binding of lysis proteins and lytic rates can be drastically affected (Weisel and Litvinov; 2008; Longstaff et al.; 2011). This section looks at the influence that fibrin fibre size and the shape of the fibrinogen molecule have on fibrinolysis.

Influence of Fibrin Fibre Size

Early studies of the effect of clot structure on fibrinolysis found that changing the fibrin fibre size affected lysis rates. [Gabriel et al. \(1992\)](#) varied fibrin fibre diameters by changing thrombin/fibrinogen ratios in clotting samples, and they found that decreasing the fibre diameter (decreasing mass/length ratio) by 5.4 times gave rise to an 8 fold increase in the rate of fibrinolysis. The authors speculated that this was due to a slower generation of PLS on the fibrin surface as well as greater resistance to PLS dissolution for thinner fibres. Another study corroborated this result by varying fibre diameter through changing thrombin or ionic concentrations ([Carr Jr and Alving; 1995](#)), and they found that the increase in fibrinolytic rate obtained with thicker fibres was independent of the method used to alter the fibre diameter.

[Collet et al. \(2000\)](#), on the other hand, found that although a fibrin network of thin fibres in a tight conformation dissolved more slowly than a network of thicker fibres, individual thin fibres were actually cleaved more rapidly than thicker ones (shown in Figure 2.10). More importantly, the authors found that the cleavage of fibrin fibres during fibrinolysis occurred by transverse cutting rather than a homogeneous shrinking of the fibre radius. The authors speculate that it is the network conformation that determines the fibrinolytic speed rather than the fibre diameter itself and that thin fibre networks have a larger fibrin fibre density thereby making the mesh more difficult to lyse.

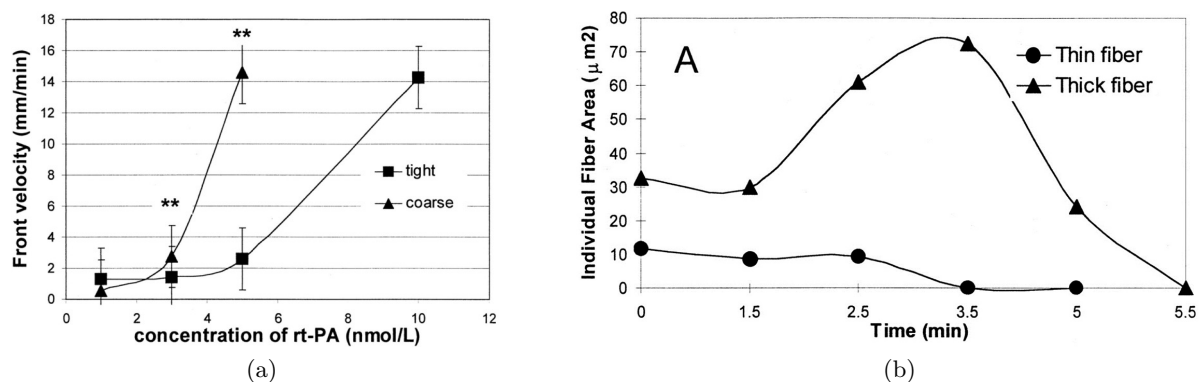


Figure 2.10: (a) Lysis front velocity against concentration of rt-PA for tight and coarse fibrin networks and (b) change in fibrin fibre area over time for thick and thin fibres. Images taken from [Collet et al. \(2000\)](#).

Another study examined the influence of mechanical properties on the lysis speed of single fibres and found that mechanical stress is important in describing fibrinolysis ([Bucay et al.; 2015](#)). In a fibrin network, fibres are under an initial tension that is gradually lost as the fibres are cleaved. The authors found that thin fibres lyse quickly until cleavage by lateral transection causes them to lose their pre-strain. Thicker fibres on the other hand undergo elongation during the early phase of fibre lysis that provokes a loss in tension and a drop in the fibrinolytic rate as shown in Figure 2.11. The study concludes that the biomechanics of the fibrin structure need to be considered in order to create accurate models of clot lysis.

Structure of Fibrinogen Molecule

The structure of the fibrinogen molecule can affect fibrin polymerisation and radically change the fibrin architecture. An important part of the fibrinogen molecule is the α C region that can allow for the

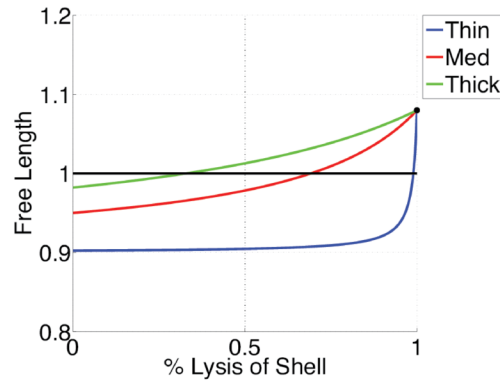


Figure 2.11: Simulation results for the lysis of a single fibre with a thin (40 nm), medium (80 nm) and thick (120 nm) diameter. Figure shows the change in the normalised free length of the fibre as the fibrin shell is lysed. Thin fibres elongate only towards the latter stages of lysis while the pre-strain for thicker fibres is lost early on in the dissolution process. Image taken from [Bucay et al. \(2015\)](#).

formation of cross-links and thus enhance the degree of protofibril aggregation. Controlling the rate of this process will create fibres with larger diameters and correspondingly bigger pores. Studies have been performed that have attempted to examine the effect that the α C region has on clot stability. [Weisel and Medved \(2001\)](#) compared fibrin clots formed from normal fibrinogen against those made with truncated A α chains. The study found that the abnormal clots had a lower permeability (reduced by 1/2) and a higher resistance to fibrinolysis than normal fibrin clots. This was corroborated by [Collet et al. \(2005\)](#) who found that fibrinogen molecules with an impaired α C domain had a significant effect on the biochemical and mechanical properties of the fibrin clot. The reduction in the rate of protofibril aggregation allowed for the development of a highly branched fibrin structure with thin fibres. This consequently leads to fewer binding sites available for PLS cleavage.

Fibrinogen molecules can have a variety of heterogeneities, the most important of which is known as fibrinogen γ' . This modification involves an extension of 20 amino acids in the γ chain of the D domain and is consequently negatively charged ([Macrae et al.; 2016](#)). Its presence has a substantial effect on the structure of the fibrin network thereby altering its lysability. This heterogeneity is thought to occur in all individuals and can account for between 7% to 15% of all fibrinogen in the body ([Pieters et al.; 2013](#)). [Falls and Farrell \(1997\)](#) quantified the resistance of clots in the presence of factor XIII and found that clots with fibrinogen γ' were more resistant to lysis (took 72 mins to achieve 50% lysis of normal clots but 179 mins for clots with fibrinogen γ'). However, in the absence of factor XIII, the difference in the lysis time was dramatically shortened to just a few minutes. The authors speculate that this is due to the localization of FXIII in fibrinogen γ' clots that allows for the development of more γ cross links. [Collet et al. \(2004\)](#) found that γ'/γ' clots in comparison to $\gamma A/\gamma A$ clots had a 20% drop in permeability, were three times stiffer and had a lysis front velocity that was 8 times slower. The lack of substantial modifications in the fibrin structure led the authors to conclude that cross-linking is the main driver of increased fibrinolytic resistance of γ' clots.

Another study took blood samples from a group of patients who had varying levels of fibrinogen γ' and compared the lysis rate of the corresponding clots that were formed from them ([Pieters et al.; 2013](#)). They found that the ratio of γ' against total fibrinogen decreased at higher fibrinogen concentrations and was the major contributor to variances in the clot lysis times (around 12%). [Kim et al. \(2014\)](#), on the other hand, found that the difference in lysis time may be due to the delayed release of fibrinopeptide B from γ' clots that in turn delays the appearance of cryptic binding sites that

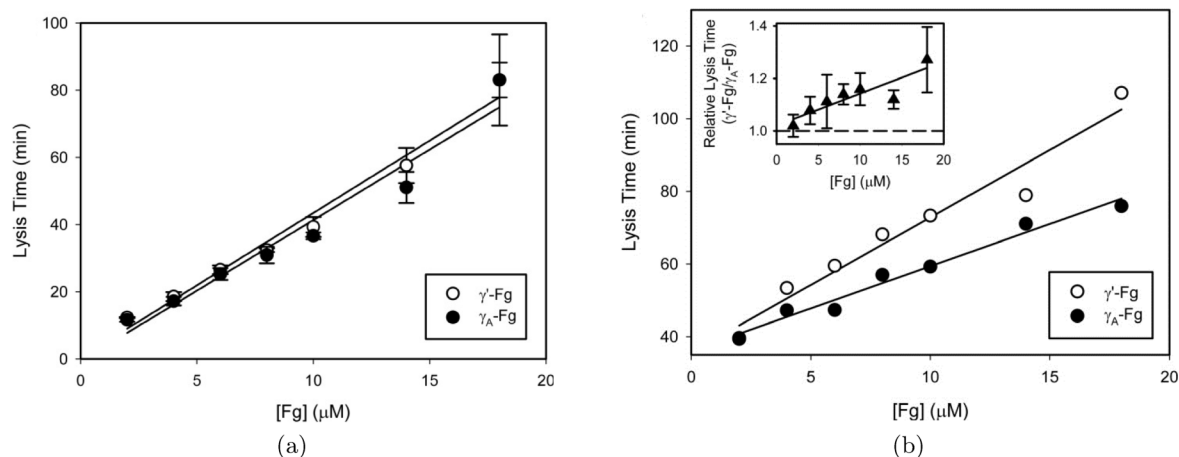


Figure 2.12: (a) Lysis of γ' and normal clots using 5 nM PLS and (b) lysis using 0.25 nM tPA. Images are taken from [Kim et al. \(2014\)](#).

allow for enhanced PLG activation. This speculation was observed when the difference in clot lysis times between two different clots at different fibrinogen concentrations disappeared upon initiation of fibrinolysis with PLS instead of tPA (shown in Figure 2.12).

These studies demonstrate how modest changes in the structure of fibrinogen can generate large discrepancies in the lytic susceptibility of blood clots. This can be due to the altered fibrin network that impedes the binding of tPA and PLG, decreases the extent of cross-linking of the γ and α chains or provokes a delayed release of the cryptic binding sites that allow for additional PLG activation.

2.3.3 Effect of Cellular Components on Thrombolysis

Thrombi are composed of many different cellular elements in addition to the fibre structure formed from fibrin polymerisation. Components such as platelets, leukocytes and erythrocytes can affect the structure and properties of a clot in many different ways thereby altering their susceptibility to fibrinolysis ([Wohner; 2008](#)). Blood clots recovered from patients suffering from AIS during endovascular therapy show differences in clot composition ([Ahn et al.; 2016](#)). Of the 22 patients who were suffering from a cardioembolism, fibrin (39.5%) was the most common constituent of the clot followed by platelets (25.6%) and red blood cells (30%). Of the 8 patients who were suffering from atherosclerosis, erythrocytes were the most abundant (56.9%) with the rest being fibrin (22.8%), platelets (16.5%) and white blood cells (3.9%). Another interesting observation was the heterogeneity present in some of the clots with arteriogenic subtypes featuring red blood cells clustered in the centre while platelets were spread towards the edges of the thrombus. Thrombogenic clots had platelets spread throughout the clot that were engulfed by the fibrin mesh. The presence of these cellular components has been shown to radically affect the efficacy of thrombolysis.

Platelets

Platelets are important for creating a preliminary plug that acts as a first response in the case of trauma. Fibrin then begins to form into fibres and create a network that consolidates the platelet plug into a clot. Platelets contain GPIIb/IIIa receptors that can bind to the fibrin network and affect the lytic stability of the clot. [Kunitada et al. \(1992\)](#) found that platelets caused a decrease

in the rate of fibrinolysis when lysed with tPA (200-800 U/ml)¹. The authors speculate that this is primarily due to platelet induced clot retraction bringing about a denser clot which impairs the binding of tPA onto the fibrin fibres. Another study found a similar delay in the fibrinolysis of platelet rich clots but speculated that this was mainly due to the presence of inhibitors such as PAI that are released from platelet α granules (Fay et al.; 1994). The study emphasized that the role of these inhibitors is important for lysis at low tPA levels (0.6 U/ml) but is unlikely to have a noticeable effect at pharmacological levels.

Collet et al. (2002) investigated the movement of the lysis front in platelet rich clots using fluorescent microscopy. A key observation was that the lysis of these clots was markedly heterogeneous with regions close to platelets dissolving at much slower rates (Figure 2.13 a). Microscopy shows that in the presence of platelets, a tight network of thin fibres surrounds the cellular components thereby making these clot segments difficult to lyse. However, the use of GPIIb/IIIa receptor antagonist such as abciximab before clotting removed the disparity and heterogeneity in lysis front velocities between platelet rich and fibrin rich clots (Figure 2.13 b).

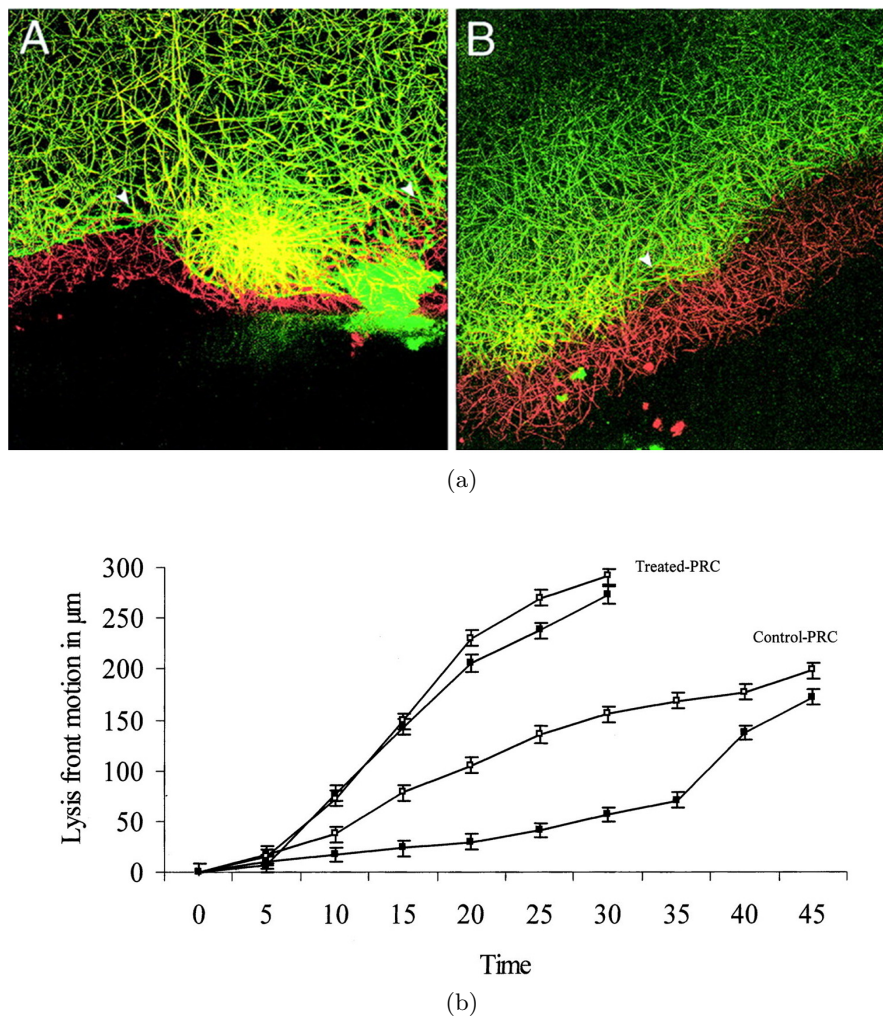


Figure 2.13: (a) Fluorescent microscopy images of clot lysis in (A) a platelet rich clot and (B) a fibrin clot; (b) Lysis front motion over time for a clot treated with abciximab and a control. Open and closed squares refer to platelet-rich and platelet-poor areas, respectively. Images taken from Collet et al. (2002).

¹For tPA, 1 U/ml is around 2 $\mu\text{g/l}$

Mutch et al. (2010) investigated whether polyphosphate released from platelets would affect the lysis time for platelet rich clots. The addition of polyphosphate before clot formation caused the development of fine fibres in certain segments of the fibrin network. Overall, clots with polyphosphate caused a reduction in the binding of PLG and tPA to the fibres and a drop in the binding of PLS to cryptic binding sites. Furthermore, the change in fibrin architecture with the addition of polyphosphate was similar to that seen in platelet rich clots. Another finding stresses that the influence of PAI-1 in platelets may be more important than previously thought (Brogren et al.; 2011). The study found that the preparation of clots for experimental studies (such as sonification) may deteriorate a significant portion of PAI in platelet rich clots. Previous studies found that only a small fraction of PAI-1 is active (up to 5%) whereas the authors found that over 50% of it may still be functional. This suggests that the role of PAI-1 in platelets may have been previously underestimated.

The role of platelets in clots can have profound implications for fibrinolysis. This has been found in studies that aimed to enhance clot lysis through the use of microbubble enhanced sonothrombolysis. For instance, Sutton et al. (2013) found that in the absence of retraction, microbubble-enhanced thrombolysis is better at clot dissolution than tPA administration alone. However, this improvement becomes non-significant in dealing with retracted clots due to the tightening of the fibrin network. Similar conclusions were reached in a rat embolic stroke model where platelet clots were difficult to lyse with microbubble-enhanced sonothrombolysis (Tomkins et al.; 2015).

Leukocytes

Leukocytes circulate in the blood stream and offer protection against foreign agents. Scanning electron microscopy (SEM) images show that these cells can be incorporated into a blood clot during the generation of the fibrin fibre network. Their influence on fibrinolysis is mediated through an agent known as neutrophil elastase (NE) that is secreted by neutrophils and macrophages.

Machovich and Owen (1989) were among the first researchers to find that NE can have an effect on the fibrinolytic system. They found that NE can convert PLG to mini-PLG thereby making it 10 times more prone to activation. Furthermore, its activated counterpart mini-PLS is 10 times less sensitive to inactivation by AP than normal PLS. In addition to NE's ability to enhance the rate of PLG activation, it can also cleave fibrin fibres. Komorowicz et al. (1998) studied the fibrinolysis of a non-occlusive clot under different flow rates (shear rates from 25 s^{-1} to 500 s^{-1}) using PLS, mini-PLS and NE. The study found that the rate of cleavage was enhanced at the highest flow rate by 4, 2.9 and 2.8 for NE, mini-PLS and PLS respectively. More importantly, the degradation of fibrin by NE gave rise to markedly different FDPs as compared to PLS and mini-PLS thereby altering the structure of the clot during fibrinolysis. The authors found that NE did not result in a sudden disassembly of the fibrin mesh in contrast to PLS and mini-PLS which favoured the development of small thromboemboli.

Another study attempted to examine the effect of NE by focusing on the concentration of its primary inhibitor alpha1-proteinase inhibitor ($\alpha 1$ -PI) (Gombás et al.; 2004). Blood was extracted from 56 patients, examined and subject to tPA and PLS lysis. As expected, samples with higher inhibitor levels had a decreased time till clot lysis when subject to tPA. However, lysis time was independent of PLS induced lysis indicating that the primary contribution of NE to lysis was due to the conversion of PLG to mini-PLG. Alternatively, the inhibition of thrombin by $\alpha 1$ -PI can cause changes in fibrin structure thereby affecting its resistance to fibrinolysis.

Red Blood Cells

Red blood cells (RBCs) are a key component of certain clots but their role in fibrinolysis has until recently been ignored. Recent work has shown that the presence of RBCs can significantly alter the fibrinolytic rate by changing the fibrin structure to create a more tightly packed fibrin mesh especially after contraction (thereby decreasing permeability and increasing fibrinolytic resistance). On the other hand, RBCs can cause heterogeneities in the clot with a mesh of tight thin fibres surrounding the cellular component (Litvinov and Weisel; 2016). Wohner et al. (2011) found that the presence of erythrocytes can radically alter the structure of the fibrin network through GP IIb/IIIa receptors. For instance, a 40% RBC sample (by volume) can change the average fibre diameter from 150 nm to 96 nm. Further to this, there was a 22.7% increase in the fibrin lysis time, primarily due to the decrease in the available binding sites for tPA induced PLG activation. These changes were not observed in the presence of GP IIb/IIIa inhibitors such as eptifibatide. Another study found that RBCs in close proximity do not solely interact with the clot through the fibrin fibres but also interact with one another and change their disk-shaped morphology to bind with the filopodia of other cells (van der Spuy and Pretorius; 2013). This capability of extending and interdigitating with one another is suspected of increasing the clot's stability.

Further to this, the presence of RBCs and platelets during blood clotting allows for increased thrombin generation due to the presence of their pro-coagulant phospholipids. A larger concentration of thrombin can significantly alter the fibre architecture of a clot by encouraging the development of a network of thin fibres. For instance, Varin et al. (2013) found that the lytic stability of various clot types responded differently to Rivaroxaban, a factor X inhibitor. The addition of Rivaroxaban to plasma blood clots reduced clot lysis times by 5.2 fold while its addition to whole blood clots allowed for a 100 fold decrease. Furthermore, the drug allowed for the porosity of whole blood clots to approach that of plasma clots with the former going from being 6.7 times less porous to 1.5 times. Therefore RBCs seem to affect the rate of fibrinolysis primarily by modifying the fibrin structure (encouraging the development of a network of thin fibres) or taking up volume in the clot thereby reducing the clot permeability.

Other Components

Other components that can affect the rate of fibrinolysis are known as neutrophil extracellular traps (NETs) which are primarily composed of DNA and histones. Work done by Varjú (2015) found that the major components of NETs in blood clots are at levels substantial enough to affect fibrinolysis. Histones can counteract thrombin inactivation but at a reduced rate in the presence of DNA. These components can also influence the fibrin architecture during clotting. Furthermore, NETs can interact with FDPs and work against the dissolution of the fibrin network by hindering PLS formation.

Another study analysed the effect that some components typically found in the blood stream (such as chondroitin sulphate, type I collagen, decorin, etc.) can have on fibrinolysis (Rottenberger et al.; 2013). They found that the presence of these components can modify the fibrin structure and compromise the mechanical and biochemical stability of the clot as shown in Figure 2.14. For instance, the presence of glycosylated decorin changed the average fibrin diameter from 85 nm to 187 nm. Furthermore, almost all of the components halved the lysis time with the exception of decorin. The authors speculate that the decrease in lysis time is in part due to a change in the fibrin network

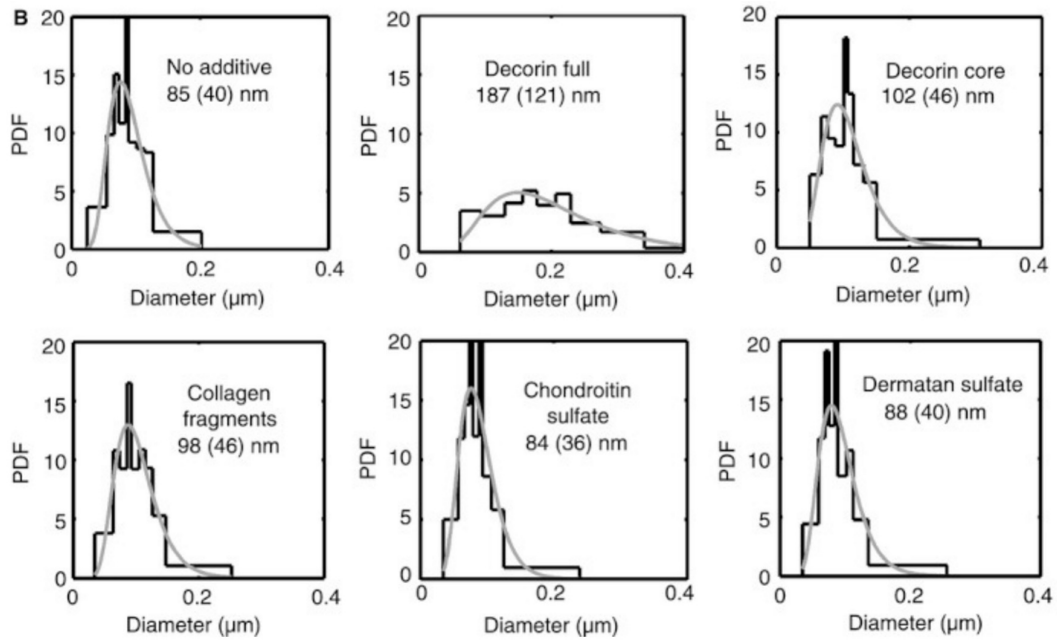


Figure 2.14: Effect of various components on distribution of the fibre diameter (image taken from [Rottenberger et al. \(2013\)](#)).

that caused the development of coarse and easier to lyse fibrin fibres. However, changes in the clot lysis time in the absence of any changes in the fibrin network indicate unknown interactions between cellular elements and fibrin fibres.

It has also been found that myosin, a key protein that makes up 5% of all proteins in platelets, could affect the rate of fibrinolysis ([Kolev and Machovich; 2003](#)). Myosin is normally released by platelets following their activation and can be observed in older blood clots that have a resistance to proteolytic degradation by PLS. Myosin has a duality in its interaction with the fibrinolytic system. In the free form, it can act as a cofactor of tPA thereby enhancing the rate of PLG activation. On the other hand, when it is bound to fibrin, myosin no longer encourages PLG activation and makes the fibrin fibre more resistant to degradation. Thus at low concentrations of myosin, tPA induced fibrinolysis is accelerated while at high concentrations it is retarded. These effects are not seen in the fibrinolytic degradation of myosin clots with UK.

2.3.4 Influence of Macroscale Effects on Thrombolysis

Other parameters such as mechanical stress can also influence fibrin structure and impair plasminogen activation ([Weisel; 2004](#)). Fibrin has a special molecular attribute that can cause fibres to extend yet maintain their permeability. Under mechanical stress, thrombus has a dramatic drop in clot volume (due the removal of water) and a plateau in compressibility ([Brown et al.; 2009](#)). The mechanical properties of fibrin can bring about interesting changes in the properties of thrombus when under strain. [Campbell et al. \(2010\)](#) analysed the influence of flow on fibrin structure and found that under stasis, clots had a uniform mean fibre diameter of around 80 nm whereas under flow the diameter increased to 220 nm and could vary by 180 nm. Another study found that fibrin fibres tended to align themselves in the direction of blood flow (as shown in Figure 2.15 at shear rates of 5 s^{-1} and 20 s^{-1}) ([Gersh et al.; 2010](#)). The study corroborated that there is a heterogeneity found in clots formed under flow with thicker fibres parallel and thin fibres perpendicular to the direction of flow.

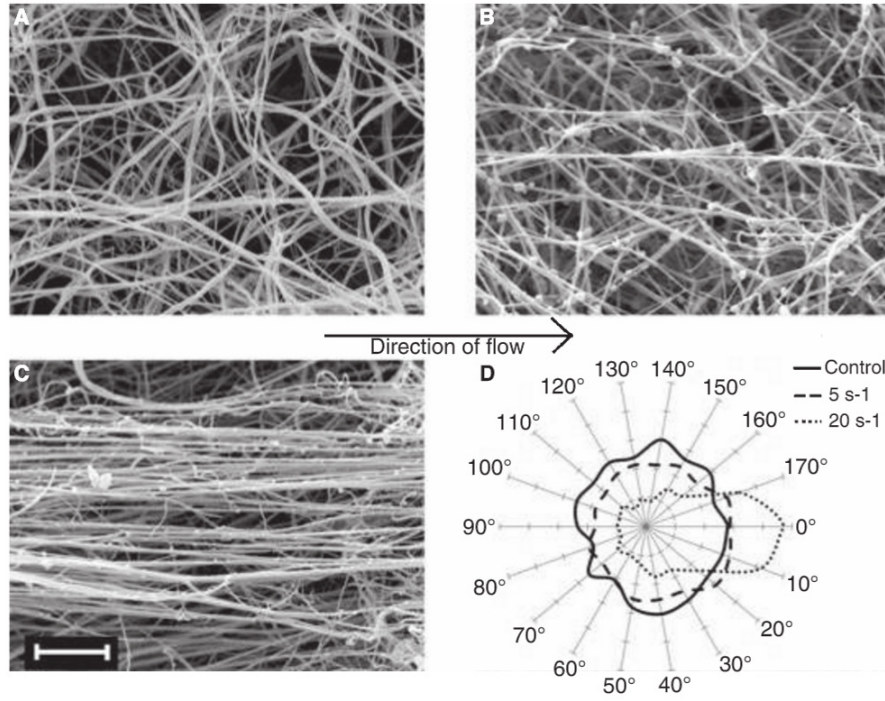


Figure 2.15: SEM images of fibrin structure under (A) stasis, (B) 5 s⁻¹ and (C) 20 s⁻¹. Bottom right image shows the fibre alignment for each regime (image taken from [Gersh et al. \(2010\)](#)).

The effect of mechanical stress on fibrinolysis was investigated by [Varjú et al. \(2011\)](#), who found that mechanical strain caused a decrease in fibrinolytic efficacy of a fibrin network and changes in the fibrin structure as shown in Figure 2.16. A clot under stress caused an alignment in the orientation of fibrin fibres and a reduction in fibrin volume. Furthermore, PLS activation was halved when subject to mechanical stress indicating a reduction in its fibrinolytic ability. This lytic resistance could potentially explain why clot dissolution in retracted clots is impaired.

2.3.5 Summary

It is clear that fibrinolysis is a very complex process that depends on many different factors. First, the structure of the fibrin network can be a major determinant of a clot's lytic susceptibility. Blood clots formed under high thrombin concentrations give rise to tight networks of thin fibres that degrade more slowly than coarse, loosely packed fibres. Second, the fibrinogen molecule itself can cause important changes in clot structure as seen by the presence of fibrinogen γ' . Third, the presence of cellular components such as platelets and RBCs can radically affect the rate of fibrinolysis via changes in the fibrin network and adsorption efficacy. Lastly, macroscale forces such as mechanical stress can affect fibrinolysis by altering fibrin structure. A realistic mathematical model of clot lysis will need to consider how the influence of each of the above components may affect the overall rate of fibrinolysis.

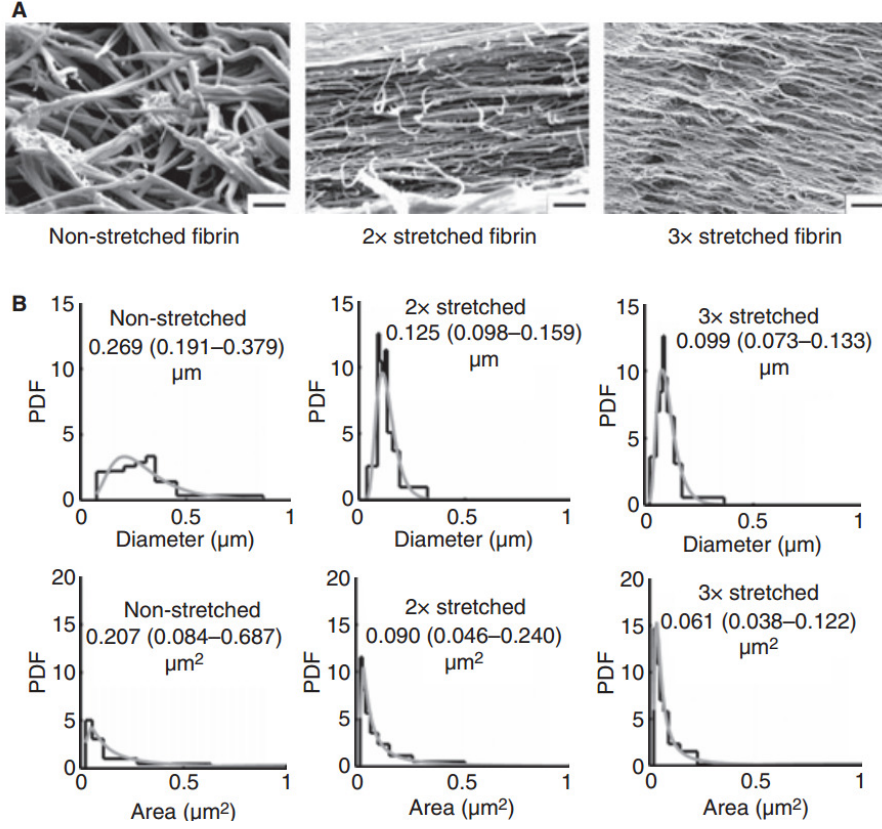


Figure 2.16: Distribution of fibre diameters under varying levels of shear stress. (A) SEM images of fibrin network; (B) Distribution of fibre radii for different levels of strain. PDF represents the population distribution function. Image taken from [Varjú et al. \(2011\)](#).

2.4 Mathematical Background

2.4.1 Blood Flow

Transport Equations

Blood is composed of plasma and many different cellular elements. In large arteries blood can be approximated as a Newtonian fluid whose viscosity is independent of the shear rate ([Nichols et al.; 2011](#)). The main equations that have been used to describe blood flow include equations based on the conservation of mass and the conservation of momentum ([Wood; 1999](#)). For a system involving flow through a porous region, the conservation equations take a different form to account for the presence of additional phases ([Bear and Bachmat; 1991](#)). A porous medium can be subdivided into small control volumes as shown in Figure 2.17 (a) to determine the equations that govern fluid flow in each compartment. Using the control volume shown in Figure 2.17 (b) and applying the method of volume averaging, the mass and momentum conservations equations can be written as:

$$\frac{\partial \epsilon \rho}{\partial t} = -\nabla \cdot (\rho \bar{u}^f \epsilon) \quad (2.1)$$

$$\rho \epsilon \left(\frac{\partial \bar{u}^f}{\partial t} + \bar{u}^f \nabla \cdot (\bar{u}^f) \right) = -\epsilon \nabla \bar{p}^f + \mu \nabla^2 \bar{u}^f \epsilon - \frac{\mu \epsilon^2}{k} (\bar{u}^f) \quad (2.2)$$

where ρ represents the density of the fluid, $\bar{u}^f$ is the intrinsic phase average velocity, t is the time,

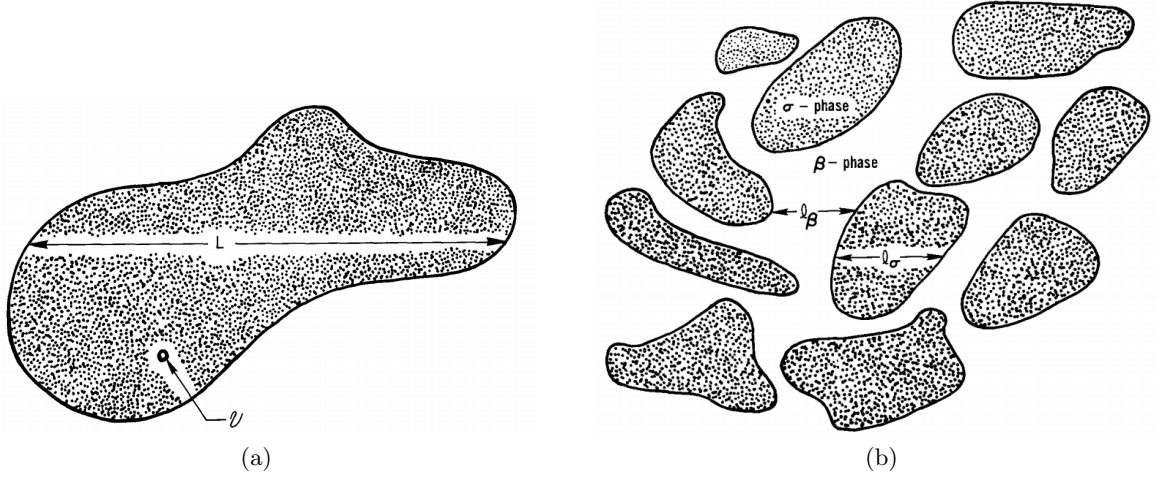


Figure 2.17: (a) Macroscopic overview of porous domain and (b) microscopic control envelope. Image taken from [Whitaker \(1986\)](#).

$\bar{p}$ is the average phase pressure, ϵ is the voidage (the fraction of fluid in a given control volume), μ is the dynamic viscosity and k is the permeability (measure of the resistance of the blood clot to fluid flow). Using the same volume averaging approach, the movement of a component in the fluid can be derived with a component balance to give equation 2.3 where D_α is the diffusion tensor of species α , D_α^* is the dispersion tensor of species α , $\bar{C}_\alpha^f$ is the phase average concentration, ΣR_α is the net generation/consumption in the fluid phase and A is the net concentration adsorbed onto the porous medium. The component balance can also be expressed for the bound component according to equation 2.4 where it is assumed that the bound component ($\bar{C}_\alpha^b$) cannot diffuse or advect but can react in the bound phase (G_α). The rigorous derivation of these equations is given in the Appendix.

$$\frac{\partial \epsilon \bar{C}_\alpha^f}{\partial t} + \nabla(\epsilon \bar{u}^f \bar{C}_\alpha^f) = \nabla(\epsilon(D_\alpha^* + D_\alpha)\nabla \bar{C}_\alpha^f) - \frac{\partial(1-\epsilon)A}{\partial t} + \epsilon \Sigma R_\alpha \quad (2.3)$$

$$\frac{\partial(1-\epsilon)\bar{C}_\alpha^b}{\partial t} = \frac{\partial(1-\epsilon)A_\alpha}{\partial t} + (1-\epsilon)\Sigma G_\alpha \quad (2.4)$$

Physiological Boundary Conditions

When modelling blood flow, it is important to assign accurate boundary conditions to ensure physiologically relevant results. The conditions specified at the inlet usually include a flowrate with a certain velocity distribution. For a steady inlet flowrate, a uniform velocity (plug flow) or parabolic profile can be specified ([Nichols et al.; 2011](#)). For pulsatile flow (due to the cyclical pulse of the heart), a Womersley velocity distribution is commonly used as shown in equation 2.5.

$$u_n = \frac{\pi R^2}{\rho} \frac{1}{in} \left[1 - \frac{J_0(Wo \, ri^{3/2})}{J_0(Wo \, i^{3/2})} \right] e^{int} \quad (2.5)$$

where J_0 is the 0^{th} order solution to the Bessel function, i is imaginary unit, R is the total radius of the vessel, n is the mode, r is the radial distance from the centre and Wo is the Womersley number

defined in equation 2.6 with the angular velocity (ω).

$$Wo = R\sqrt{\frac{\omega\rho}{\mu}} \quad (2.6)$$

Outlet boundary conditions are especially important for flow in branched vessels as they determine the division of flow among the branches. If the division of flow for each branch is known beforehand, then a flowsplit can be imposed on all outlets. However, this is usually unknown and the flowsplit between each branch is likely to change over time; resistance boundary conditions in such cases can be used to determine the appropriate flow division. This is done by assigning the pressure at each branch outlet to equal the product of the outgoing flowrate and the resistance of the downstream vasculature as given in equation 2.7 (Grinberg and Karniadakis; 2008). The resistance can be found by dividing the average intra-arterial pressure by the average flowrate obtained from clinical studies. Another more accurate method of obtaining pressure values at the outlet is by using Windkessel boundary conditions (Westerhof et al.; 2009). The pressure at the outlet is determined not only by the resistance of the downstream vasculature, but also by the arterial compliance (equation 2.8).

$$P_{out} = Q_{out}R_T \quad (2.7)$$

$$Q_{out}\left(1 + \frac{R_1}{R_2}\right) + CR_1\frac{dQ_{out}}{dt} = C\frac{dP_{out}}{dt} + \frac{P_{out}}{R_2} \quad (2.8)$$

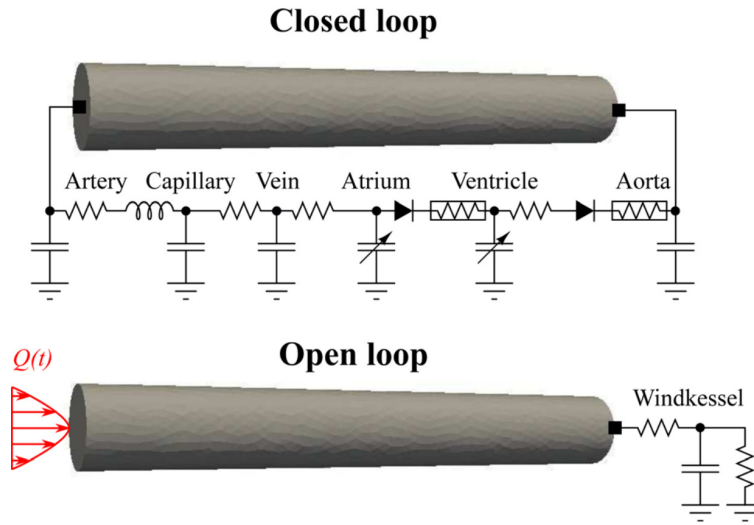


Figure 2.18: Schematic of an open and closed loop system of boundary conditions (image taken from Marsden and Esmaily-Moghadam (2015)).

where Q_{out} is the outlet flowrate, R_1 and R_2 are vasculature resistances, R_T is the total resistance, C is the capacitance and P_{out} is the arterial pressure at the outlet. The total resistance can be found using the following expression $R_T = R_1 + R_2$. A number of studies have combined 3D CFD models with a comprehensive 0D vascular network that accounts for the influence of other arteries on the inlet and outlet boundary conditions. This can be further extended to create a closed loop model where the outlet boundary conditions are connected to the inlet conditions through a lumped parameter model (shown in Figure 2.18) (Marsden and Esmaily-Moghadam; 2015).

Numerical Studies of Blood Flow

The equations for blood flow and drug transport can be solved using numerical techniques such as CFD. This methodology has been extensively used as a tool to investigate the haemodynamics of different arteries such as the aorta and its branches. For instance, [Kandail et al. \(2016\)](#) modelled stent-graft performance under different conditions such as resting, exercise and hypertension to estimate the risk of thrombosis and stent-graft migration. [Cheng et al. \(2014\)](#) modeled the flow of blood for an aortic dissection and found that it matched well with PC-MRI images. [Kouser et al. \(2013\)](#) showed that the Reynolds-averaged Navier-Stokes (RANS) based shear stress transport transitional model was promising in determining turbulent flow in an aorta. Other studies have employed 1D blood flow models to simulate the entire human vasculature ([Blanco et al.; 2015](#)). For instance, [Strocchi et al. \(2017\)](#) created a model consisting of 55 arteries modelled with 1D models and 0D models to represent the major human organs such as the heart, lungs and microcirculation. [Alastruey et al. \(2007\)](#), on the other hand, studied exclusively the blood circulation in the cerebral arteries and the influence of anatomy on blood perfusion. Starting from the left ventricle, the authors simulated blood flow in the entire circle of Willis, and they found that the presence of communicating arteries are important in ensuring sufficient blood supply to the brain in the case of trauma. Another study looked at modelling blood flow in the circle of Willis whilst also using a viscoelastic model to describe the behaviour of the wall ([DeVault et al.; 2008](#)).

CFD studies have also been used to study the movement of an embolus that may cause ischemic stroke. For example, a recent study examined flow in the aortic arch and the internal carotid arteries, and then using a particle dynamics solver, determined how the size of the embolus may affect its final location in the vasculature ([Khodae et al.; 2016](#)). The study found that a significant portion of emboli go into the larger arteries of the cerebral vasculature such as the MCA. The effects of embolus size, point of release and material properties on the end location of an occlusion were studied by [Mukherjee and Shadden \(2016\)](#). These computational models show the diverse use of CFD to investigate arterial haemodynamics in patient-specific vasculature. Important vessels can be modelled in a 3D environment whereas the influence of smaller vessels can be approximated using reduced order models. Furthermore, the movement of discrete objects such as emboli can be tracked using particle dynamics models.

2.4.2 Modelling Thrombolysis

Thrombolysis involves the cleavage of PLG to form PLS that later cleaves the fibrin scaffold of a blood clot. The kinetics of this process can be quite complex as there are many proteins in the blood that exert an influence on the lysis process. The following sections are concerned with work reported in the literature on the mathematical modelling of thrombolysis.

Kinetics of Thrombolysis

[Hoylaerts et al. \(1982\)](#) investigated the activation of PLG to PLS and found that the reaction followed Michaelis-Menten like kinetics. PLS formation was strongly enhanced by the presence of fibrin that acts as a suitable binding site for the protease suggesting that both tPA and PLG adsorb onto the surface of the fibrin to generate PLS. Their investigations show that tPA is effective at generating PLS

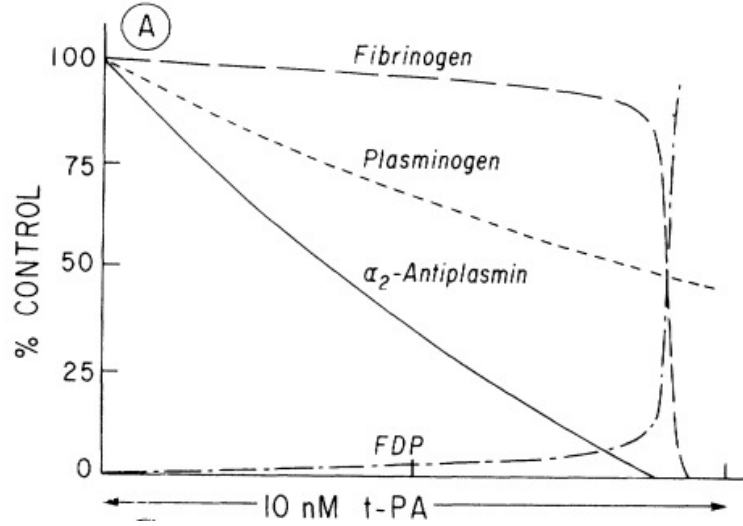


Figure 2.19: Plasma concentrations of fibrinogen, PLG, AP and FDP as predicted by Tiefenbrunn et al. (1986) over a 10nm tPA infusion. Image taken from (Tiefenbrunn et al.; 1986).

on clot surfaces but ineffective at doing the same in the blood plasma. This is highly desirable in the human body for normal haemostatic clot regulation as it actively prevents haemophilia. Tiefenbrunn et al. (1986) developed a kinetics model for tPA induced fibrinolysis. The model consisted of 7 different reactions and involved the interplay between PLG, tPA, PLS, AP, fibrinogen and MG and was found to be in good agreement with experimental results where a range of different patients were given tPA infusions for intervals as long as 7 hours. The predicted change in plasma concentrations is shown in Figure 2.19.

Anand and Diamond (1996) developed a compartment model to calculate the systemic concentrations of lytic proteins in plasma. The entire system was composed of 12 species and included the effect of inhibitors such as AP, PAI and MG. Their results were in good agreement with measured plasma concentrations of tPA and PLG after 50-mg bolus infusion as shown in Figure 2.20. The concentration of each species was calculated using equation 2.9 where V_{plasma} represents a plasma volume of 3.5 L, I_{α} represents the infusion rate of species α (with bolus infusions occurring over a 5 sec period) and K_{α} represents the half-life due to hepatic clearance (as determined using equation 2.10 where k_{HC} is the rate coefficient of hepatic clearance).

$$V_{plasma} \frac{dC_{\alpha}}{dt} = V_{plasma} \sum_j \sum_k R_{\alpha}(C_j, C_k) + I_{\alpha}(t) - K_{\alpha}(t) \quad (2.9)$$

$$K_{\alpha} = k_{HC} C_{\alpha} V_{plasma} \quad (2.10)$$

Other pharmacodynamics models reported in the literature include the 2-compartment model by Chandler et al. (1997) where in addition to the plasma, there is a second compartment that accounts for the take-up of drug by tissue. This model has been used to study the effect of delays between the bolus and continuous drug infusions (Brodoehl et al.; 2015). The additional complexity of the model accounts for a modest improvement in the fit with experimental data.

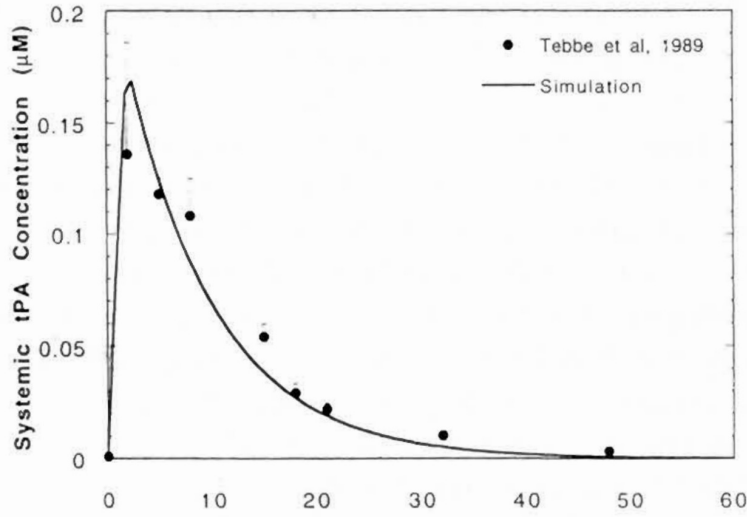


Figure 2.20: Comparison of tPA plasma levels as predicted by the simulation of [Anand and Diamond \(1996\)](#) against those found from clinical studies after a 50 mg bolus infusion. (Image taken from [Anand and Diamond \(1996\)](#)).

Diffusion Limited Thrombolysis

To fully understand the process of clot dissolution, it is of paramount importance to take into account the influence of mass transport. A number of models have been found in the literature that have included the movement of lytic proteins in diffusion-controlled transport. [Zidansek and Blinc \(1991\)](#) modelled clot dissolution with UK under diffusion-controlled and perfusion enhanced conditions. A set of governing transport equations relating to the movement and reaction of a plasminogen activator, PLG, PLS and a PLS inhibitor were solved analytically to determine the steady-state velocity of thrombolysis. The study found that increasing the concentration of a plasminogen activator is unlikely to enhance the rate of clot lysis for diffusion dominated transport. On the other hand, pressure induced permeation was found to significantly increase the movement of the lysis front for a homogeneous clot by almost 2 orders of magnitude. The degradation of PLS over time and the dissolution velocity for quasi steady-state clot lysis (v_{front}) is given in equations 2.11 and 2.12 respectively, where C_{Fbn} is the concentration of fibrin, D is the diffusion coefficient, $k_{tPA-PLG}$ is a kinetic constant for the formation of the PLG-tPA complex, k_{AP} and k_{cat} are the kinetics constants for PLS inhibition by AP and fibrin degradation correspondingly and M is the dissolution constant defined in equation 2.13 with the dissolution time (t_d).

$$\frac{dC_{Fbn}}{dt} = -k_{cat}C_{PLS}C_{Fbn} \quad (2.11)$$

$$v_{front} = \sqrt{\frac{D\sqrt{\kappa_2[PA]_0}}{\sqrt{M}(1 + k_{tPA-PLG}k_{AP}\sqrt{\frac{M}{\kappa_2[PA]_0}})}} \quad (2.12)$$

$$M = \int_{-\infty}^{t_d} C_{PLS}(t)dt \quad (2.13)$$

[Anand et al. \(2008\)](#) reported a computational model consisting of a system of 23 reaction-diffusion equations to simulate clot formation (initiated by the release of tissue factor) and subsequent dissolution with tPA secreted by endothelial cells. PLG activation and fibrin degradation were modelled

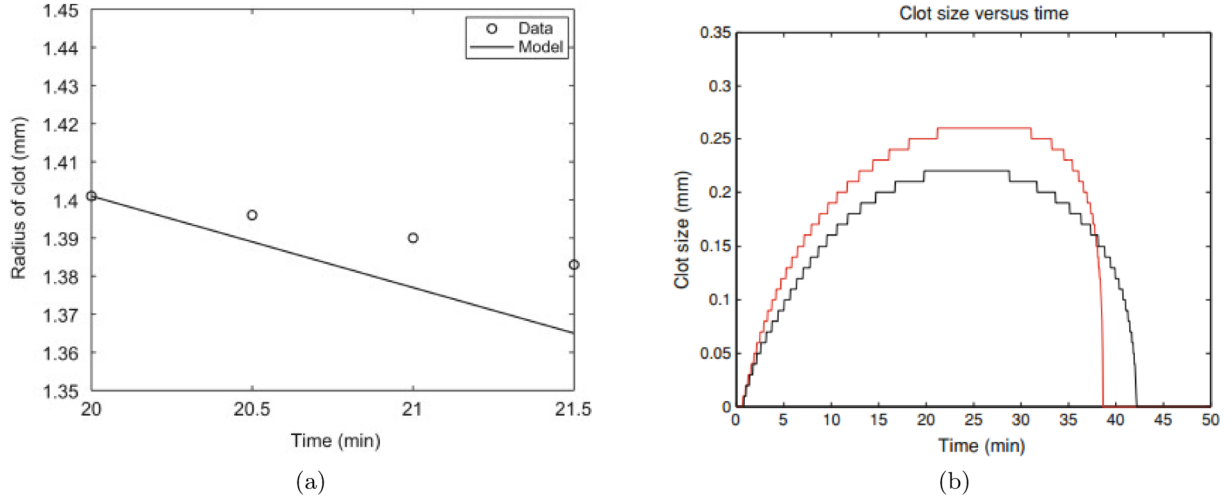


Figure 2.21: (a) Comparison of thrombus size as predicted by the model of Anand et al. (2008) against experimental data from Sakharov et al. (1996) and (b) clot size over time when including the intrinsic pathway (red line). Images are taken from Anand et al. (2008) and LaCroix and Anand (2011), respectively.

with Michaelis-Menten kinetics (shown in equation 2.14) and fibrinolysis was inhibited by the presence of AP. Boundary conditions at the wall specified the standalone and induced secretion of tPA by thrombin and fibrin (shown in equation 2.15). The movement of the clot was determined by examining the location where the fibrin concentration exceeded 350 nM. This model was subsequently extended to a system of 28 reaction-diffusion transport equations in order to model the effect of the intrinsic pathway on clot formation and dissolution (LaCroix and Anand; 2011). The computational results showed good agreement with experimental data (Figure 2.21 a) and found that the intrinsic pathway not only led to a larger clot, but also one that would dissolve more quickly (Figure 2.21 b).

$$G_{Fbn} = -\frac{\kappa_1 C_{PLS} C_{Fbn}}{\kappa_2 + C_{Fbn}} \quad (2.14)$$

$$\left. \frac{\partial C_{tPA}}{\partial x} \right|_{x=0} = -\frac{(k_{tPA,rls}^c + k_{tPA,rls}^{Thr} C_{Thr}|_{x=0} + k_{tPA,rls}^{Fbn} C_{Fbn}|_{x=0}) [Endo]}{D_{tPA}/L} \quad (2.15)$$

where κ_1 and κ_2 are kinetic constants for fibrin degradation, G_{Fbn} represents the generation of fibrin, $k_{tPA,rls}$ is the rate of tPA release from endothelial cells due to thrombin, fibrin or standalone, L is the length of the wall and $[Endo]$ is the quantity of endothelial cells per area along the vessel wall.

In an attempt to model fibrinolysis as the transverse cutting of fibres using a continuous model, Bannish et al. (2014b) created a 1D reaction-diffusion model. The fibrin fibre is represented by binding site doublets (binding sites that are in close proximity to one another) whereby only tPA and PLG on the same doublet will allow for PLS activation. Initially all binding site doublets are specific in that only a tPA molecule can bind to one site and PLG or PLS can bind to the other. As lysis proceeds, new non-specific binding sites become available that allow for either tPA or PLG binding. The exposure of new binding sites is given by equation 2.16, while the total degradation of exposed binding sites is given in equation 2.17.

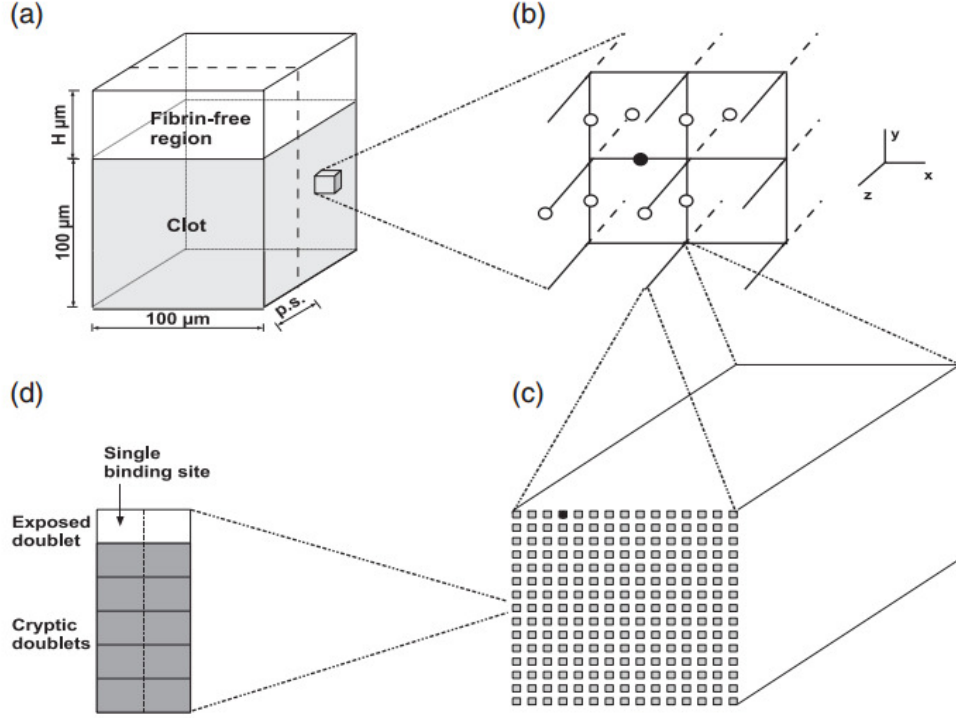


Figure 2.22: Schematic overview of the [Bannish et al. \(2014a\)](#) model. The macroscale geometry (a) is divided up into cells where each edge corresponds to a single fibre (b). The fibre itself is represented on the microscale by binding locations (c) that are further subdivided into specific and non-specific binding doublets (d). (Image taken from [Bannish et al. \(2014a\)](#)).

$$\frac{dn}{dt} = -\frac{k_{cat}^n n n_{PLS}}{K_M^n + n + (K_M^n/K_M) n_{tot}} \quad (2.16)$$

$$\frac{dn_{tot}}{dt} = -\frac{k_{cat} n_{tot} n_{PLS}}{K_M + n_{tot} + (K_M/K_M^n) n} \quad (2.17)$$

where n refers to the total concentration of unexposed binding sites, n_{tot} is the concentration of all exposed binding sites, n_{PLS} is the total concentration of bound PLS, and K_M^n and K_M are the Michaelis-Menten constants for the exposure and degradation of binding doublets respectively. With a total clot length of around $100 \mu\text{m}$, the fibrin distribution was modelled as spatially heterogeneous to take into account the effect that coarse and fine fibres may have on fibrinolysis. The authors found that continuum models were incapable of detailing the difference in lysis times between coarse and fine clots. An alternative approach to using continuous equations to describe clot lysis was developed in [Bannish et al. \(2014a\)](#) where fibrinolysis was modelled as a stochastic process to properly take into account the low levels of tPA that occur during haemostatic fibrinolysis. Based on a microscale model for the cleavage of a single fibre cross-section, single fibre lysis times were determined using Gillespie's stochastic method. The results were then fed into a macroscale model consisting of a lattice grid with each edge being a single fibre (shown in Figure 2.22). The lysis front velocity was determined according to the movement of the lysis front in the macroscale model. The study found that by adjusting the relevant simulation parameters (e.g. amount of tPA molecules for a given clot cross-section), the differences in the lysis of fine clots and coarse clots can be captured.

Convection Enhanced Thrombolysis

Fluid flow can significantly impact the transport of proteins within a thrombolytic environment. Using a Hele-Shaw random walk model, [Zidansek et al. \(1995\)](#) showed that the dissolution of blood clots follows a finger-like lysing pattern. The authors confirmed the occurrence of these dissolution patterns using nuclear magnetic resonance (NMR) imaging and speculated that it was due to the random heterogeneous distribution of the clot's voidage. Diamond's group conducted a series of studies on the modelling of fibrinolysis in 1D and 2D geometries. In [Diamond and Anand \(1993\)](#), the authors constructed a 1D mathematical model for the lysis of a porous clot. Darcy's law was used to model porous flow through the clot (see equation 2.18) and the transport of PLG, PLS, UK and tPA was modelled with a set of convection-diffusion-reaction equations. The changing porosity and permeability were modelled as functions of the fibrin fibre radius that became smaller as lysis progressed (see equation 2.19). These variables were then fed back to the transport and fluid equations to solve for the new flow field where R_f is the radius of the fibre. The model predicted that pressure driven permeation was important in enhancing lysis and that the fibrin specificity of tPA might have slowed down the lytic rate. Therefore in certain cases, a drug cocktail of non-specific UK and fibrin specific tPA may be used to improve lytic efficacy.

$$u = -\frac{k(t)}{\mu} \frac{\Delta P}{L} \quad (2.18)$$

$$k = k(\epsilon, R_f) \quad (2.19)$$

where R_f is the radius of the fibrin fibre, ΔP is the pressure difference across the clot and L is the clot length.

[Anand and Diamond \(1996\)](#) reported an improved mathematical model by accounting for the influence of pro-coagulants such as AP, PAI and MG. The model predicted lysis times that were comparable to experimental results (Figure 2.23 a). Furthermore, the compartmental model described in section 2.4.2 was used to determine the temporal inlet boundary conditions following a tPA infusion. [Anand et al. \(1997\)](#) modified the model to examine 2D lysis front patterns following the addition of PLS to a clot with a heterogeneous fibrin composition. The authors concluded that the random distribution of fibrin can account for the finger-like dissolution patterns (shown in Figure 2.23 (b)) typically seen in experimental studies.

In order to account for the effect of convection, [Wootton et al. \(2002\)](#) extended the previous models by solving the Navier-Stokes equations where the resistance of the clot to blood flow was incorporated using a Darcian momentum source term. To reduce computational complexity, the transport equations were solved in 1D. The authors applied the computational model to investigate the dissolution of mural thrombi and found that the model predictions agreed well with experimental results. However, at high shear rates, discrepancies were noticed, potentially due to the acceleration of clot lysis by fluid shear forces. [Pleydell et al. \(2002\)](#) developed a quasi-steady state mathematical model to evaluate the temporal evolution of lytic proteins in a mural thrombus. Through the use of a similarity variable, species transport equations were reduced to a single dimension (shown in equation 2.20) allowing for the concentration of species to be solved along the clot length.

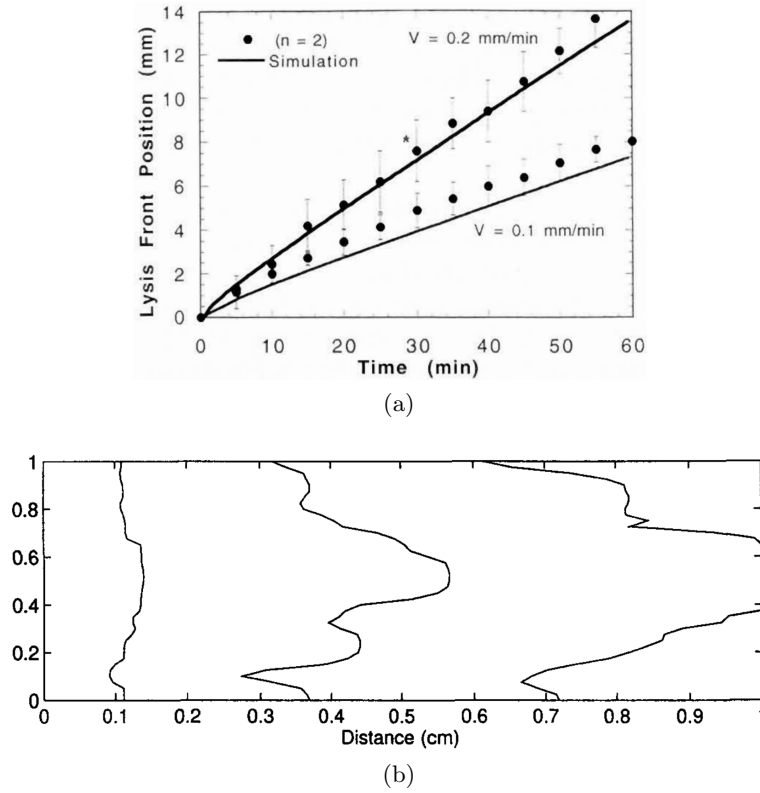


Figure 2.23: Experimental results of (a) lysis front position (black circles) compared against simulation results (solid lines) for a constant perfusion velocities of 0.1 mm/min and 0.2 mm/min (from [Anand and Diamond \(1996\)](#)) and (b) 2D lysis front position for a heterogeneous fibrin clot at 3 different time points (from [Anand et al. \(1997\)](#)).

$$\frac{d^2 C_\alpha}{d\eta^2} + 3\eta^2 \frac{dC_\alpha}{d\eta} = \Sigma R(Pe, u_\infty) \quad (2.20)$$

where η is the similarity variable, Pe is the Peclet number and u_∞ is the streamline velocity. Previous computational models have attempted to treat the blood clot as a porous medium whereby lysis can be expressed as changes in the clot's physical properties. Another approach is to model the blood clot as a separate fluid as done by [Anand et al. \(2003, 2006\)](#). These studies used transport reactions to describe the coagulation cascade and fibrinolysis in a similar manner to [LaCroix and Anand \(2011\)](#). Any location where the fibrin concentration exceeds 600 nM was used as an indication of clot formation. Blood and clot were modelled as viscoelastic fluids with the clot having a viscosity several times higher than that of the blood. The predicted change in the fibrin concentration is given in Figure 2.24.

[Sersa et al. \(2007a\)](#) constructed an analytical fluid dynamics model to examine the effect of flow on clot dissolution for both laminar and turbulent regimes. Their model geometry consisted of a cylinder split into 2 separate parts with an empty part followed by a partially occluded section. Clot lysis was modelled with a proportionality constant and assumed to proceed radially. They found that lysis was proportional to u^2 for laminar flow (shown in equation 2.21) and u^3 for turbulent flow (shown in equation 2.22). The study confirmed the model predictions with MRI images and found that fast flow accelerated lysis due to greater mechanical disruption of the blood clot.

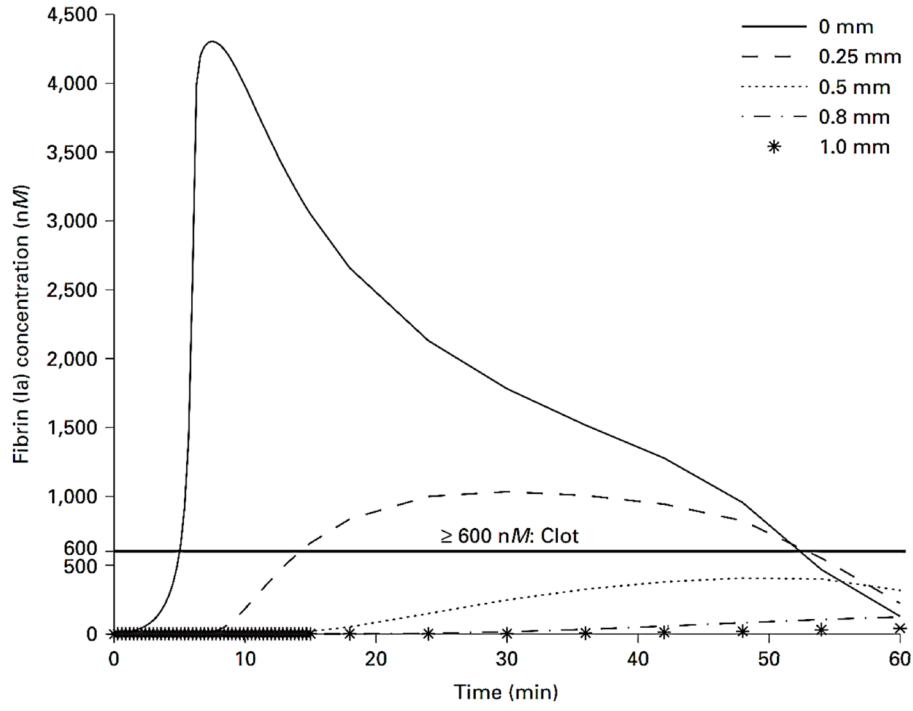


Figure 2.24: Temporal evolution of the fibrin concentration showing clot formation and subsequent clot dissolution at different lengths. Image taken from (Anand et al.; 2006).

$$\frac{dr_{clot}}{dt} = \frac{C_L u^2}{A} \quad (2.21)$$

$$\frac{dr_{clot}}{dt} = \frac{C_T u^3}{\sqrt{A}} \quad (2.22)$$

where r_{clot} is the radius of the mural clot, u is the velocity, C_L and C_T refer to a coefficient under laminar and turbulent blood flow respectively, and A is the cross-section of the channel. This model was extended by Sersa et al. (2007b) to evaluate the effect of entrance length on the dissolution of mural thrombi. The velocity was evaluated as a function of radius and entrance length as shown in equation 2.23. The authors highlighted the importance of flow in influencing the efficacy of thrombolysis not only through quicker permeation, but also through mechanical shearing of the clot by the flow.

$$u(r, z) = \begin{cases} \frac{2uR^2}{(R^2 + R - \delta(z))^2}, & \text{if } r < R - \delta(z). \\ \frac{2uR^2(R^2 - r^2)}{(R^4 + R - \delta(z))^4}, & \text{if } r \geq R - \delta(z). \end{cases} \quad (2.23)$$

where δ refers to the boundary length and z is the length along the mural clot. Bajd and Sersa (2013) constructed a coarse grained model of microscale blood clot fragmentation inspired by the nature of clot removal seen from optical microscopy. Applying Newton's laws of motion to spherical objects attached by up to 20 elastic bonds, the authors showed that higher plasma flow gave rise to increased clot fragmentation and the generation of clot fragments of different sizes. It was highlighted that blood clot lysis was a corrosion-erosion process with both biochemical kinetics and hydrodynamics playing important roles in bond cleavage and bond overstretching. The separation of clot fragments from the original thrombus has the potential to lead to predictions concerning embolisation.

2.4.3 Summary

The use of computational models for predicting blood flow in patient-specific arteries is well established and can provide valuable information to researchers and clinicians. Computational modelling of thrombolysis on the other hand, has received less attention. There are a number of analytical and numerical models available that solve for clot lysis in simplified geometries, but none of these has been applied to clot dissolution in patient-specific geometries. Modelling thrombolysis in a realistic setting would help elucidate the role of patient vasculature and different thrombolytic drugs and therapies in determining the efficacy of thrombolytic therapy.

2.5 Conclusion

This chapter covered an extensive literature review of the main fields of interest to thrombolytic therapy. The clinical background shows that the implementation of thrombolysis for the treatment of ischemic stroke is still fraught with difficulties. Due to the fact that a large number of AIS patients are ineligible for thrombolytic therapy, research is underway to find new drugs or therapies that can improve recanalisation rates. The biochemical background shows that part of the difficulty lies in the inherent complexity of fibrinolysis as lytic efficacy depends on many different factors. Finally, the mathematical background shows that although computational models have been developed to predict thrombolysis, they have been confined to simplified geometries. The development of a computational tool that can simulate clot dissolution in patient-specific geometries under physiological conditions may aid clinicians in determining favourable scenarios for thrombolysis. Furthermore, this tool may also be used to determine the suitability of new therapies that may ameliorate patient recovery rates.

Chapter 3

Developing a 2D Model of Thrombolysis

3.1 Introduction

Clot lysis is a very complex process that is influenced by blood flow, protein reaction kinetics and clot structure. It occurs over a wide span of length and time scales thus making it a very challenging problem to model. A complete model of clot lysis would have to take into account the effect of blood flow (macroscopic effects) and the dissolution of fibrin fibres (microscopic effects). Among the existing computational models of thrombolysis, the kinetics and fibrin microstructure model of [Diamond and Anand \(1993\)](#) is one of the most promising in terms of its ease of scalability and applicability to large vessel geometries. In this case, fibrinolysis is modelled as the uniform shrinking of the fibre radius, whereby changes in the microstructure cause alterations in the macroscale properties of the occlusion. In a similar fashion to [Wootton et al. \(2002\)](#), blood flow can be described with continuity and momentum equations where a Darcian source term is used to describe the viscous resistance of the clot to blood flow. The transport and reaction of the tPA drug with proteins in the plasma can be determined through the use of convection-diffusion-reaction transport equations.

The overall aim of this chapter is to develop a multi-physics model for the dissolution of fibrin clots in scenarios representative of thrombolytic therapy. This requires a robust computational model that is capable of simulating clot lysis from the initial diffusion-limited transport stage to reestablishment of blood flow and subsequent mural clot lysis post-canalization. In this work, the original kinetics and fibrin microstructure model of [Diamond and Anand \(1993\)](#) has been modified to include the solubilization of bound species during clot lysis. The effect of permeation on clot lysis is incorporated through continuity and momentum equations for the blood with a Darcian term to describe the viscous resistance of the clot to blood flow. This is implemented in a different way from the work of [Wootton et al. \(2002\)](#) in that our model couples the full convection–diffusion transport equations with equations describing the reaction kinetics and blood flow. Furthermore, unlike previous studies, we examine the entire lysis process, from the dissolution of a fully occluding clot to the lysis of the mural clots that remain after canalization. The capability and robustness of the clot lysis model are demonstrated through application to a simplified model of an artery with an occlusive clot under varying pressure drops ¹.

¹The content of this chapter has been published in [Piebalgs and Xu \(2015\)](#)

The predicted lytic front velocities during the initial stage of lysis agree well with experimental and computational results reported by others. The initial slow flowrate causes a parabolic profile of tPA to develop that lyses the centre of the clot first. Following canalisation, lysis patterns of the remaining mural clots are strongly influenced by local flow patterns which are symmetric at low pressure drops, but asymmetric at higher pressure drops and give rise to larger recirculation regions and extended areas of intense drug accumulation. By incorporating the effect of convective blood flow, the evolution of lysing patterns and the shape of remnant clots that typically arise in thrombolytic therapy can be better understood.

3.2 Methods

In the following section, the kinetic equations that govern a well-mixed model of fibrinolysis are presented first. This is followed by an extension of the model to account for convection and spatial heterogeneity.

3.2.1 Adsorption and Reaction Kinetics of Fibrinolysis

The kinetic pathway for fibrinolysis can be described by the interplay of 3 different proteins: PLS, PLG and tPA. Upon introduction of tPA, which is commonly used in fibrinolysis (Zamarron et al.; 1984), it reacts with the PLG to form the enzyme PLS. The PLS protease then goes on to dissolve the blood clot by cleaving its fibrin fibres. Since tPA is fibrin specific, it needs to be adsorbed onto the fibrin surface with PLG in order to generate PLS as illustrated in Figure 3.1.

The rate of adsorption of a species onto a fibrin surface can be described by the following equation:

$$\frac{dA_\alpha}{dt} = k_{a,\alpha}C_\alpha(\theta_\alpha - n_\alpha) - k_{r,\alpha}n_\alpha \quad (3.1)$$

where n_α and C_α are the adsorbed and free phase concentrations of a species α respectively, θ_α is the concentration of total binding sites available for adsorption on the fibrin surface, A_α refers to the rate of adsorption of a species α and $k_{a,\alpha}$ and $k_{r,\alpha}$ are the reaction rate coefficients for adsorption and desorption respectively. The species α can refer to either PLS, PLG or tPA. For a well-mixed system, the rate of change of the free phase and bound phase concentrations of a species can be modelled as

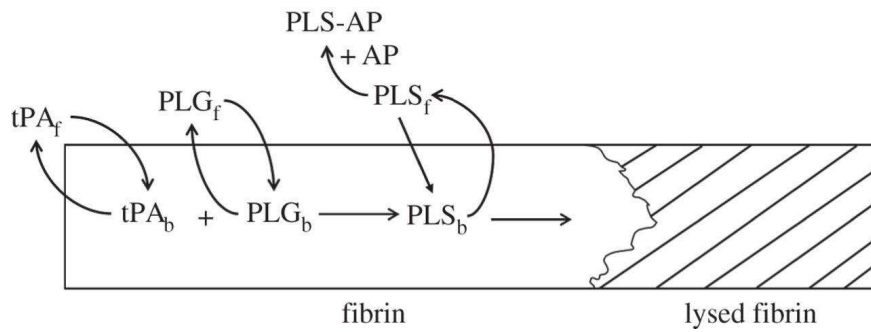


Figure 3.1: Kinetic pathway of fibrinolysis.

$$\frac{d(C_\alpha \epsilon)}{dt} = -(1 - \epsilon) \frac{dA_\alpha}{dt} + \sum R_\alpha + n_\alpha \frac{d\epsilon}{dt} \quad (3.2)$$

$$\frac{dn_\alpha}{dt} = \frac{dA_\alpha}{dt} + \sum G_\alpha \quad (3.3)$$

where ϵ is the voidage, R_α is the rate of consumption of species α in the free phase and G_α is the rate of generation of species α in the bound phase. The voidage represents the volume fraction of the clot occupied by the fluid. Equation 3.2 can be used to obtain the free phase concentration by solving for the superficial concentration $C_\alpha \epsilon$. The two main bound phase reactions occurring in this study are the proteolytic cleavage of the plasminogen protein to form the plasmin protease and the fibrinolytic degradation of fibrin fibres. Plasmin in the adsorbed phase is produced by the reaction between bound plasminogen and bound tPA. Plasmin generation follows Michaelis-Menten kinetics (Hoylaerts et al.; 1982) and can be expressed as follows.

$$G_{PLS} = -G_{PLG} = \frac{k_2 n_{PLG} n_{tPA}}{K_M + n_{PLG}} \quad (3.4)$$

where k_2 and K_M are the Michaelis-Menten coefficients required to determine the rate of plasminogen consumption and plasmin production. A variety of clot lysis inhibitors are present in the blood stream that can reduce the rate of fibrinolysis. The main ones are PAI, $\alpha 2$ -antiplasmin (AP) and $\alpha 2$ -macroglobulin (MG) (Tiefenbrunn et al.; 1986). However, since MG is only very weakly effective at removing plasmin from the free phase (Tiefenbrunn et al.; 1986) and PAI only occurs in the blood circulation at low concentrations (Booth et al.; 1988), their effect on clot lysis has been neglected. The free phase plasmin is inhibited by AP and can be expressed as:

$$R_{PLS} = -\epsilon k_{AP} C_{AP} C_{PLS} \quad (3.5)$$

3.2.2 Quantifying Clot Lysis

Although it is recognised that fibrin fibres degrade through transverse cutting as observed by Collet et al. (2000) rather than a homogeneous shrinking of the fibre radius, the latter is assumed in the present study and is based on Diamond and Anand's microstructure model for fibrin fibre degradation. In order to check the validity of this assumption, results for a 1D diffusion limited transport case are compared with those from Bannish et al. (2014b) who constructed a 1-D continuous model that takes into account the lateral transection of fibrin fibres by plasmin. The full equations of the fibrin microstructure model have been described in detail by Diamond and Anand (1993) and are presented again in the Appendix for completeness. Lysis is assumed to proceed via a homogeneous shrinking of the fibrin fibre radius, which then goes on to change the properties of the porous medium such as its voidage, the concentration of binding sites and the permeability of the clot. The extent of clot lysis can be described by the following:

$$\frac{dL}{dt} = k_{cat} n_{PLS} \gamma \quad (3.6)$$

where L is the amount of fibrin lysed, k_{cat} is the reaction rate constant associated with clot lysis

and γ is the solubilisation rate (number of cuts required by PLS to cleave one unit of fibrin). The concentration of fibrin lysed can then be used to express the change in fibrin fibre radius $R_f(L)$, voidage $\epsilon(R_f)$, binding site density $\theta_\alpha(R_f)$, and clot permeability $k(\epsilon)$. These variables are then used in the macroscale transport as well as the mass and momentum balance equations. As the clot is gradually lysed, the bound terms on the fibrin fibre will be solubilised and return to the free phase. The solubilisation term takes the form

$$G_{\alpha,sol} = -\frac{\kappa}{(1-\epsilon)} n_\alpha \frac{d\epsilon}{dt} \quad (3.7)$$

$$R_{\alpha,sol} = -\kappa n_\alpha \frac{d\epsilon}{dt} \quad (3.8)$$

where κ is the solubilisation constant. These two terms ensure that there are no bound phase concentrations present when the clot has been completely lysed.

3.2.3 Including the Effects of Convection and Transport outside the Clot

In order to describe the movement of blood through a porous medium, a mass and momentum balance over an arbitrary control volume needs to be taken. Since the configuration of the porous-fluid interface can be very complex, a volume averaging method to derive the equations of fluid flow was used (Bear and Bachmat; 1991; Whitaker; 1999). The fluid is assumed to be incompressible, Newtonian (as given by equations 2.1 and 2.2) and the clot is assumed to be homogeneous and isentropic. The well-mixed kinetics described in section 3.2.1 can then be extended to include spatial variations in concentration. The following expression, written in Einstein summation notation, evaluates the temporal and spatial changes in concentration for any species in the free phase:

$$\frac{\partial C_{\alpha\epsilon}}{\partial t} + \frac{\partial u_i C_{\alpha\epsilon}}{\partial x_i} = \frac{\partial}{\partial x_j} (D_{\alpha,ij} \frac{C_{\alpha\epsilon}}{\partial x_j}) + \sum R_\alpha \quad (3.9)$$

where $D_{\alpha,ij}$ refers to the hydrodynamic dispersion and $\sum R_\alpha$ represents the well mixed kinetic terms defined on the right hand side of equation 3.2. In this study, the diffusion has been assumed to be isotropic and constant throughout the domain. Furthermore, since the free phase species is spatially varying, the bound phase species (represented by equation 3.3) are a function of x , y and t .

3.2.4 Numerical Procedures and Model Details

First, the well-mixed kinetics described by equations 3.1 to 3.8 were solved to evaluate the temporal change in the concentrations of tPA, PLG, PLS, and AP. The system of ordinary differential equations (ODEs) was solved in Matlab using ode45 that employs a fourth order Runge-Kutta solver. Values for all the kinetic constants used in the simulation can be found in the Appendix. The well-mixed kinetics model was then extended to evaluate the spatial distribution of protein concentrations and clot properties for a 1D diffusion-limited transport system. This was implemented by performing an explicit finite difference discretisation of a simplified version of the transport equation described by equation 3.9. The second order diffusion term was discretised using a second order central difference scheme while the transient term was discretised using a first order forward difference approach.

Finally, the complete model including the effects of convection was implemented in ANSYS Fluent via user-defined functions (UDFs) written in the C programming language. To reduce the computational demand, only the three main components of lysis (PLG, PLS and tPA) were included. The role of AP was deemed negligible as the well-mixed kinetics model shows that in its absence, only a small amount of PLS in the free phase is present in the blood stream and this has a negligible effect on the lysis time. The model was applied to a rectangular occlusive clot in a 2D channel as illustrated in Figure 3.2. The channel height of 5 mm corresponds to an average diameter of a cerebral artery in the brain (Alastruey et al.; 2007), while the clot width of 2.5 mm is representative of clots typically found in ischemic stroke (Marder et al.; 2006). The length of the channel was chosen to ensure that the model inlet and outlet were sufficiently far so as to minimise the influence of artificial boundary conditions on predicted flow patterns and concentration profiles near the clot. A structured mesh was generated and mesh sensitivity tests showed that over 200,000 elements would be required to achieve mesh independent solutions.

Regarding initial conditions, starting with the well-mixed model a physiological PLG concentration of $2 \mu\text{M}$ (Anand and Diamond; 1996) was first introduced into the system and allowed to reach a dynamic equilibrium. The fibrin clot had an initial fibrin density of 0.28 mg/ml (Weisel; 2005) and an initial fibrin radius of 250 nm (Blinic et al.; 2000). The resulting free phase and bound phase PLG concentration was 1.65 and $34.9 \mu\text{M}$ respectively. After this, 50 nM of tPA (Anand and Diamond; 1996) was added to the system and monitored until the clot had been completely lysed. For the 1D diffusion-limited transport model and 2D convection-included model, the following boundary conditions were applied: at the inlet a tPA concentration of 50 nM and a PLG concentration of $2 \mu\text{M}$ were specified, while the outlet was located sufficiently far such that the concentration gradient is zero. In addition, the 2D model had pressure values imposed at the inlet and outlet to give a constant pressure drop across the channel, with pressure drops ranging from 1 Pa to 20 Pa based on previous experimental work (Wu et al.; 1994). Walls were specified as no-slip and non-permeable. The 2D clot lysis model was initially solved for steady fluid flow with a completely occluding clot before tPA was introduced into the system. The concentration of bound PLG was set to $34.9 \mu\text{M}$ as found from the well-mixed kinetics simulation. The same kinetics parameters (specified in the Appendix) were adopted in all simulations unless otherwise specified.

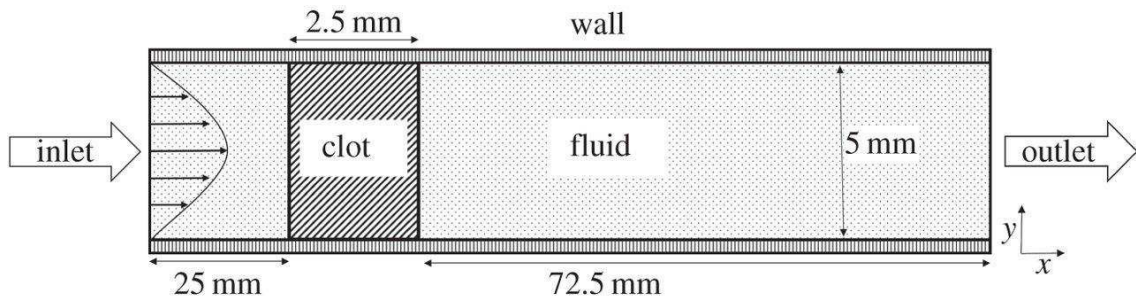


Figure 3.2: Geometry of a fully occluded artery used in the computational simulations.

3.3 Results

In this section, the results for the well-mixed kinetics model are given first followed by those of the 1D diffusion limited transport model. After this, results obtained from the extended transport model for clot lysis in a fully-occluded channel are presented for a variety of pressure drops.

3.3.1 Well-Mixed Kinetics Model

Figure 3.3 shows the concentration of each component as well as changes in the properties of the clot over time. It shows that clot lysis is completed at around 90 seconds upon addition of 50 nM of tPA to the system. It should be noted that the concentration of free tPA and that of free PLG are not constant, but change by a small amount over time. As a part of a validation exercise, simulations were repeated to replicate the experimental and computational conditions found in the literature (Rijken et al.; 1982; Anand et al.; 1995). Figure 3.4 (a) shows the comparison of minimum time required to achieve 95% lysis in a well-mixed system for a clot of a fibrin concentration of $5.88 \mu\text{M}$ for different tPA concentrations. The values of K_M and k_2 were changed to $2.42 \mu\text{M}$ and 0.22 s^{-1} to match the experimental conditions (Rijken et al.; 1982). Figure 3.4 (b) shows the comparison of change in fibrin radius between our prediction and experimental data, for which an extra consumption term was introduced to account for the loss of plasmin activity over time.

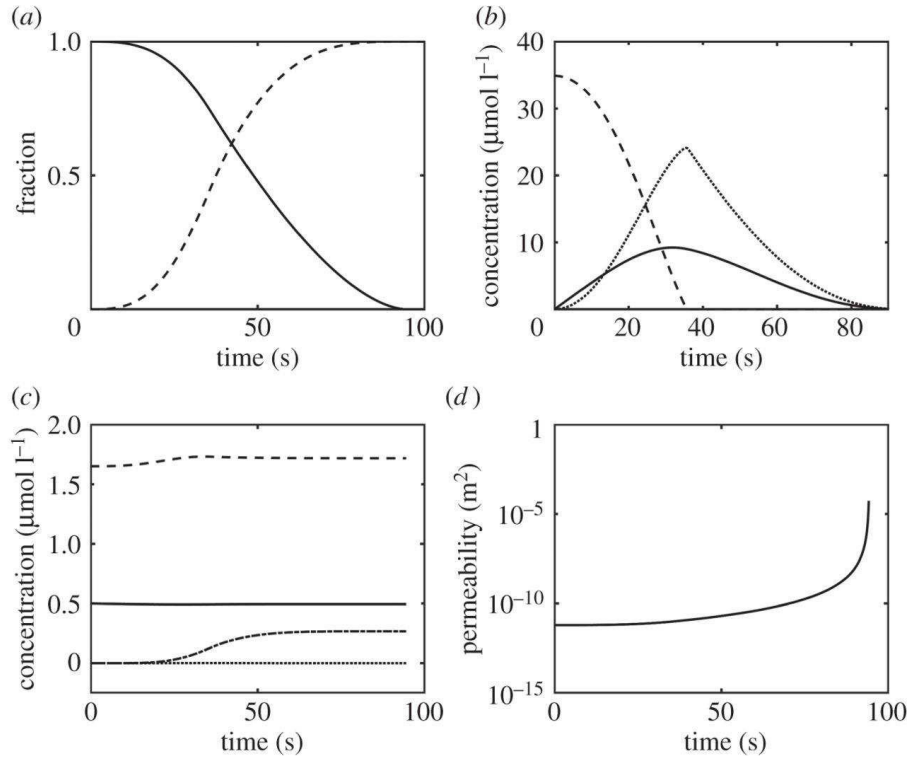


Figure 3.3: Temporal variations obtained from the well-mixed kinetics model. (a) Normalized fibre radius and the fraction of clot lysed. Solid line refers to the ratio R_f/R_{f0} and the dashed line to the fraction of clot lysed; (b) concentrations of bound tPA x100 (solid), PLG (dashed) and PLS (dotted); (c) concentrations of free tPA x10 (solid), free PLG (dashed), free PLS (dotted) and free AP-PLS complex (dotted and dashed) and (d) permeability of the system that tends to infinity as the clot is completely lysed.

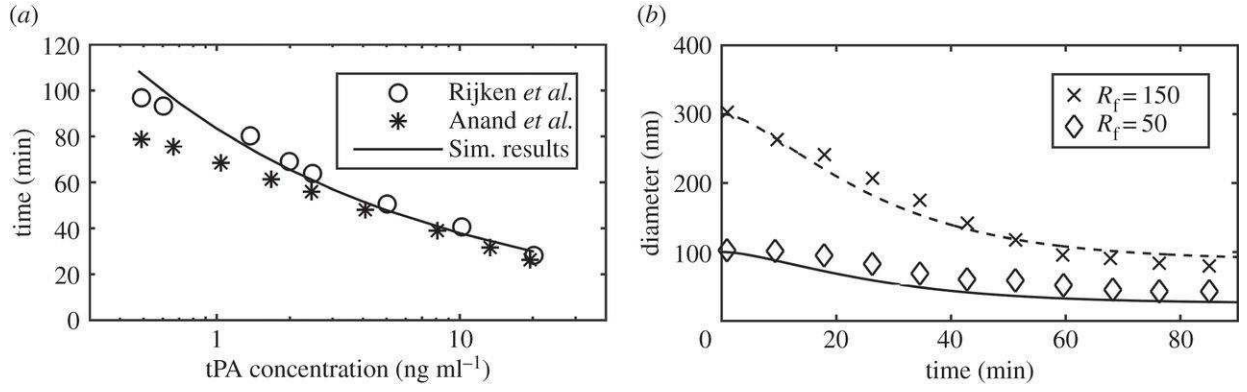


Figure 3.4: (a) Time taken to achieve 95% lysis in a well-mixed clot model for different tPA concentrations. Simulation results (solid line) are compared with experimental results (circles) and simulation results in the literature (asterisks) (Anand *et al.*; 1995; Rijken *et al.*; 1982) and (b) change in fibrin fibre radius compared with experimental results for an initial fibrin radius of 150 nm (crosses) and 50 nm (diamonds) (Blinic *et al.*; 2000).

3.3.2 1D Diffusion Model

Figure 3.5 shows spatial variations of clot properties and concentrations of the bound and free phase lysis proteins. The lysis front seems to accelerate in the initial period before traveling at a constant speed through the clot medium as shown in Figure 3.5 (a). Figures 3.5 (b) and (c) show the movement of the bound tPA and PLS over time respectively. The initial concentration profile is narrow with a sharp peak which rapidly falls off and the profile becomes wider over time. Figure 3.5 (d) shows the free tPA gradually diffusing through the system and approaching a uniform concentration over time. Figure 3.6 shows the influence of tPA concentration on the lysis front position (point of 95% lysis or greater) and average front velocity. It can be seen that after an initial acceleration period, the lysis front velocity tends to remain relatively constant. Gradually increasing the initial tPA concentration at the inlet seems to give rise to modest increases in the lysis front velocity, as demonstrated by the logarithmic plot in Figure 3.6 (b).

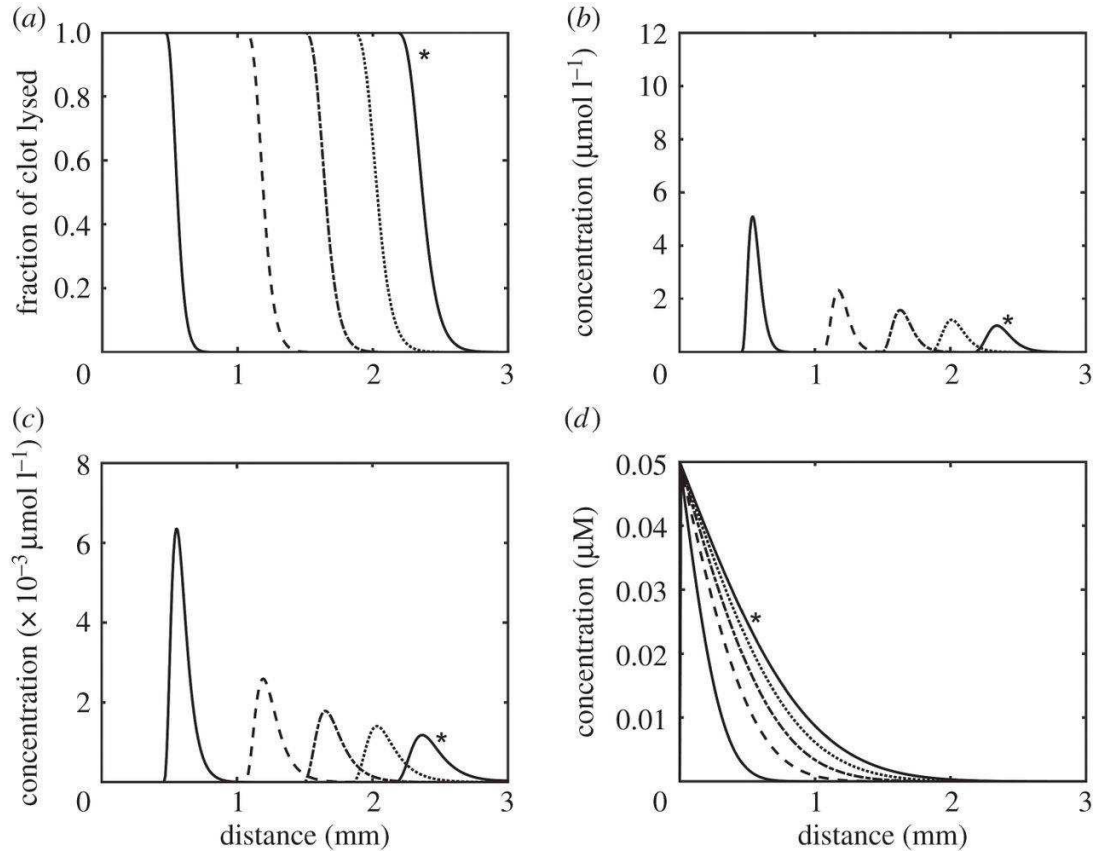


Figure 3.5: (a) Movement of the lysis front, (b) concentration of bound PLS, (c) concentration of bound tPA and (d) concentration of free tPA. The solid, dashed, dashed with dot, dotted and solid with asterisk lines for each plot correspond to the value of the variables in each plot at 10, 30, 50, 70 and 90 min, respectively.

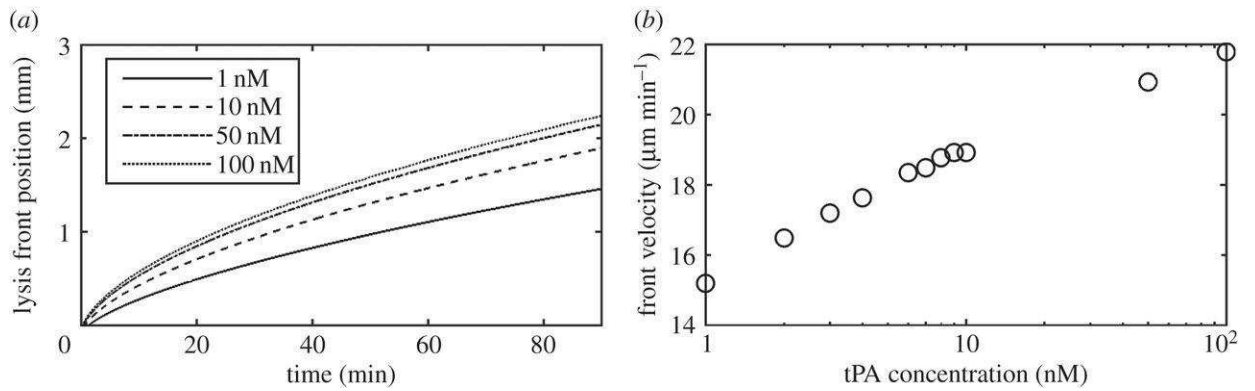


Figure 3.6: (a) Lysis front position over time for different tPA inlet concentrations. The lysis front position was calculated as the farthest x node that has reached 95% lysis and (b) change in the lysis front velocity for different tPA concentrations. The average velocity was taken as the positive change in the lysis front position from 10 to 80 min.

3.3.3 2D Simulations of Clot Lysis

Figure 3.7 shows the evolution of lysis contours superimposed on velocity vectors for an inlet pressure of 1 Pa and outlet pressure of 0 Pa. A dimensionless quantity, termed the breakthrough ratio (BTR), is used to investigate clot lysis similarities across different pressure drops. It is defined as the simulation time over the breakthrough time, with zero corresponding to the time when the first cell has been lysed by 1.0 % and a breakthrough time being assigned when one of the cells at the clot exit reaches over 95.0% lysis. This simulation was repeated for pressure drops of 5 Pa and 10 Pa. Figure 3.8 shows the free tPA concentration contours while Figure 3.9 shows the concentration of free tPA distributed throughout the system for different pressure drops. Each row of images is for a certain pressure drop and each column of images is for a chosen BTR. To analyse the association between flow and lysis patterns, instantaneous streamlines at selected pressure drops and time points are given in Figure 3.10.

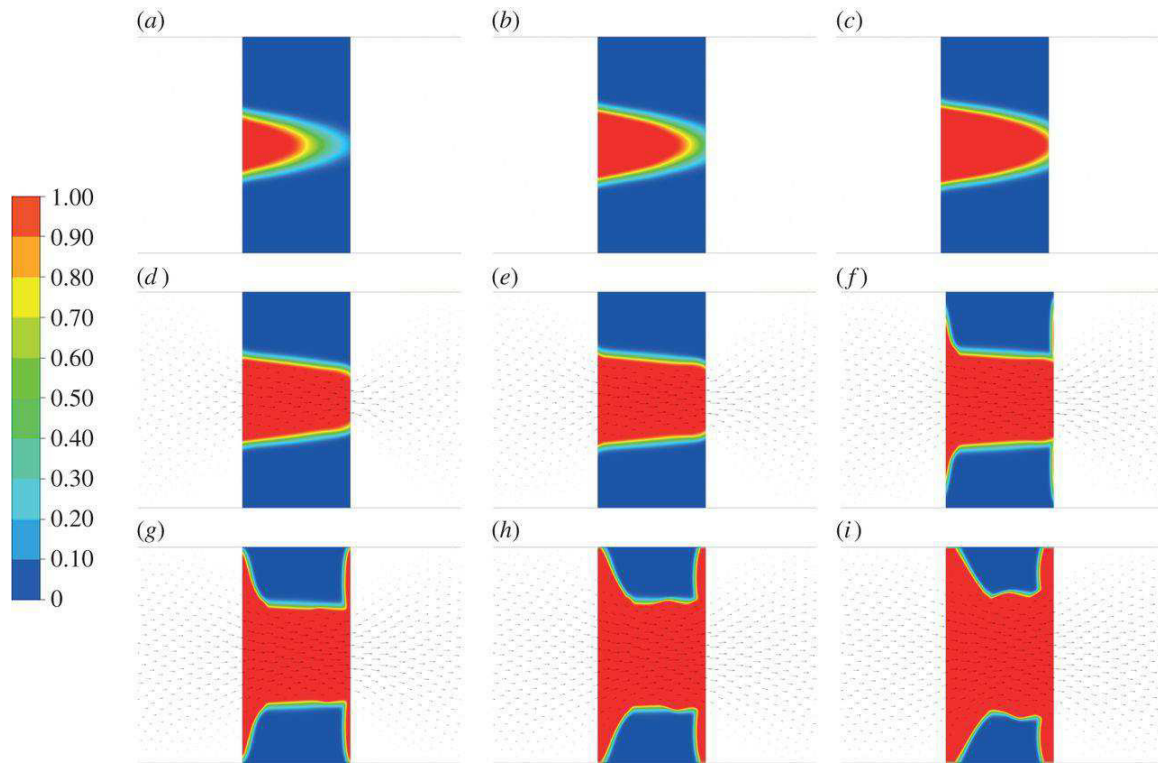


Figure 3.7: Clot lysis contours over time for a 1 Pa pressure drop. The legend on the left refers to the fraction of clot that has been lysed with red corresponding to complete lysis and blue corresponding to no lysis. (a) BTR = 0.50, (b) BTR = 0.75, (c) BTR = 1.00, (d) BTR = 1.25, (e) BTR = 1.50, (f) BTR = 1.75, (g) BTR = 2.00, (h) BTR = 2.25 and (i) BTR = 2.50.

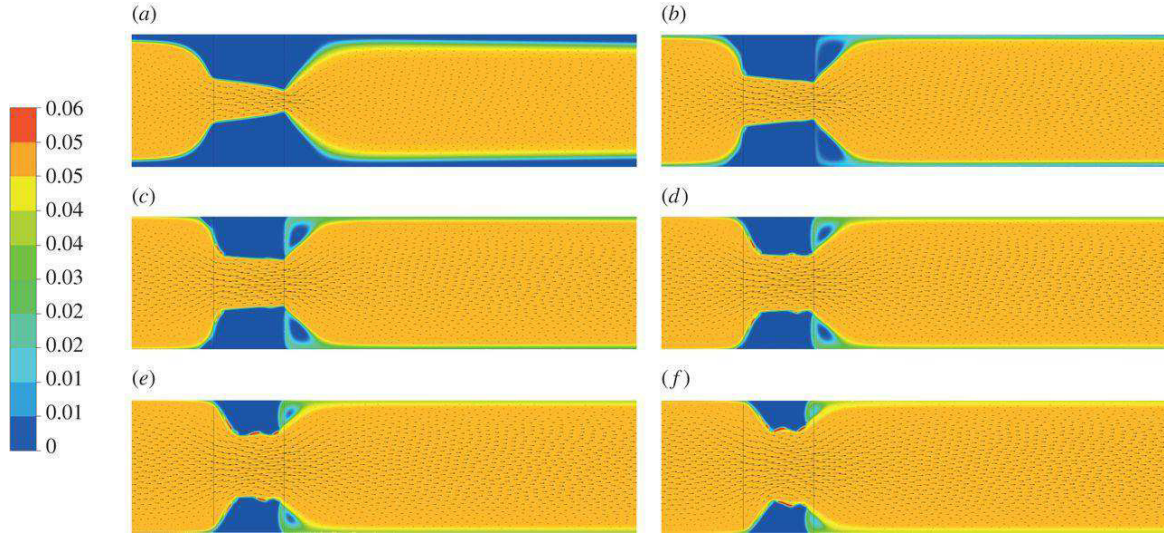


Figure 3.8: Concentration of free tPA contours (in μM) for a 1 Pa pressure drop. The red regions denote areas of a concentration of $0.06 \mu M$ or higher, while the blue regions show areas with almost no tPA. (a) BTR = 1.25, (b) BTR = 1.50, (c) BTR = 1.75, (d) BTR = 2.00, (e) BTR = 2.25 and (f) BTR = 2.50.

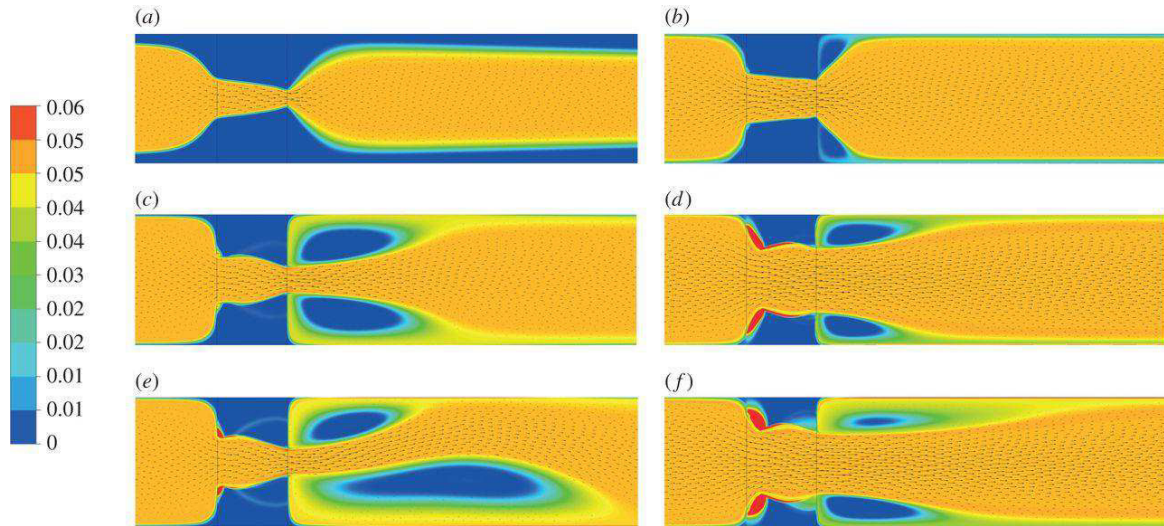


Figure 3.9: Concentration of free tPA contours (in μM) for different pressure drops. (a) 1 Pa pressure drop, BTR = 1.20; (b) 1 Pa, BTR = 1.50; (c) 10 Pa, BTR = 1.20; (d) 10 Pa, BTR = 1.50; (e) 20 Pa, BTR = 1.20 and (f) 20 Pa, BTR = 1.50.

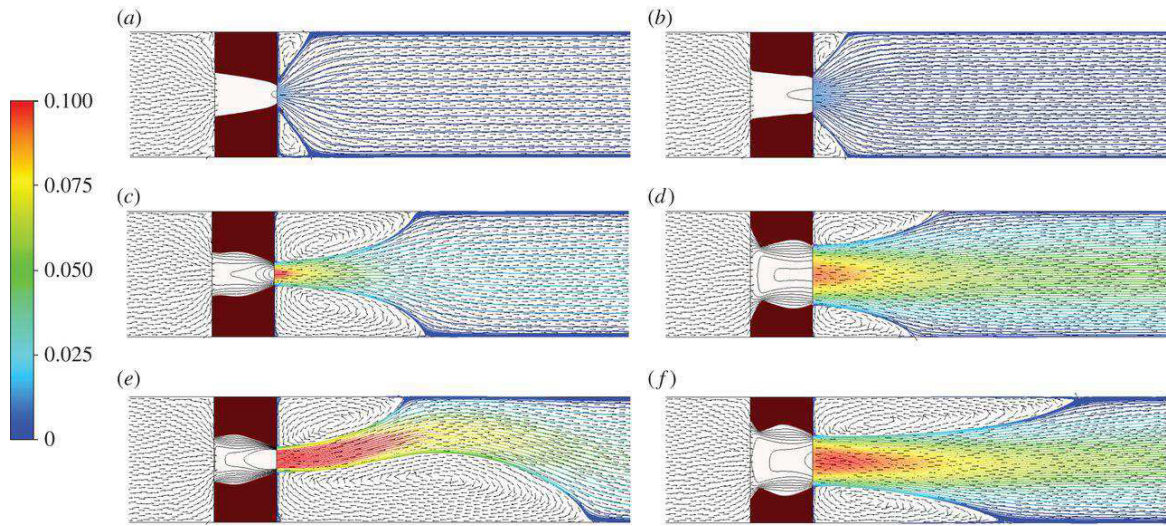


Figure 3.10: Velocity streamlines and velocity vectors for different pressure drops. The dark red region represents the amount of clot that has not been lysed. (a) 1 Pa pressure drop, BTR = 1.20; (b) 1 Pa, BTR = 1.50; (c) 10 Pa, BTR = 1.20; (d) 10 Pa, BTR = 1.50; (e) 20 Pa, BTR = 1.20 and (f) 20 Pa, BTR = 1.50.

3.4 Discussion

In this chapter a multi-physics model is presented that is capable of simulating blood clot lysis in a macroscale artery upon introduction of tPA. The occluding blood clot was modelled as a porous medium with its properties varying as a function of the bound PLS concentration. The transport of tPA and other proteins was modelled with a set of convection-diffusion-reaction equations while the blood flow was solved based on fluid mass and momentum balances. The coupled transport and fluid flow model was implemented in ANSYS Fluent and can be applied to realistic arterial geometries. The results of the well-mixed model show that lysis reaches completion at around 90 seconds, which is consistent with data in the literature ([Anand et al.; 1995](#)) except that the lysis curve gradually tends to 100% in our model. This is due to the modified source term describing the solubilisation of the bound plasmin to the free phase following clot lysis. The well-mixed simulation results have been compared with experimental data ([Rijken et al.; 1982](#)) as shown in Figure 3.4 (a) and (b), demonstrating the validity of the kinetics model.

The 1D diffusion model shows clearly that there is an accumulation of bound lytic proteins at the lysis front (Figures 3.5 b and c) which falls off as the clot is gradually lysed. This moving lysis front is consistent with experimental observations in the literature ([Sakharov and Rijken; 1995](#)) where lysis was found to be preceded by the accumulation of lytic proteins. These results are also very similar to those reported by [Bannish et al. \(2014b\)](#) who constructed a continuous 1D model of clot lysis taking into account the transverse cutting of fibrin fibres. The same trend of the lysis front moving gradually through the clot can be seen as well as the shape and change of bound species concentration profiles, the latter displaying a similar trend in becoming shorter and wider over time due to enhanced uniformity of free tPA spreading throughout the domain. The change in peak value is more marked in our simulation because of the larger clot width (3 mm as opposed to 0.3 mm modelled by [Bannish et al. \(2014b\)](#)). The similarity in the results confirms the validity of using the [Diamond and Anand \(1993\)](#) fibrin microstructure model for macroscopic simulations of clot lysis.

The lysis front position and average front velocity shown in Figure 3.6 suggest that in the low concentration range (1-10 nM) increasing tPA concentration would lead to a large increase in the lysis front velocity as reported in the literature ([Collet et al.; 2000](#)). At high concentrations, however, the gradient of the lysis front velocity appears to decrease exponentially as shown in the logarithmic plot in Figure 3.6 (b). The values for the lysis front velocity in diffusion limited transport lie within the same range as those given in the literature. For instance, [Wootton et al. \(2002\)](#) found typical lysis front velocities at around 30 $\mu\text{m}/\text{min}$, [Anand and Diamond \(1996\)](#) at 50 $\mu\text{m}/\text{min}$ and [Bannish et al. \(2014b\)](#) at 20 $\mu\text{m}/\text{min}$ for diffusion limited transport.

Having established the validity of the kinetics model and parameter values adopted in the model, we can now focus on examining the factors that influence the lysing pattern of a single occlusive blood clot. By simulating the dissolution of a fully occluding clot in a 2D channel, clot lysis patterns and their evolution over time can be obtained. Figure 3.8 shows clearly that lysis starts at the centre of the clot and creates a funnel shaped space that allows for lysis to proceed radially. This is consistent with the observations of [Anand et al. \(1997\)](#) and [Zidansek et al. \(1995\)](#) in the case of diffusion-limited transport, except for the finger-like lysing pattern which is not predicted by our model owing to the assumption of uniform initial clot properties. Upon recanalization (BTR=1.0), lysis of the remnant clot occurs not just radially but also at the front and the back of the clot. There also appears to be a

dent in the middle of the clot surface which tends to become more noticeable over time (Figures 3.7 h and i). The mechanisms responsible for these observations can be explained by examining the free tPA concentration contours given in Figure 3.8 and 3.9, in conjunction with the streamline plots in Figure 3.10.

It can be seen that following recanalization, blood flow through the centre of the clot is established. Typically, there is a high velocity jet through the canal along the centre, but the sudden expansion of flow channel at the end of the clot causes flow to decelerate and consequently allow for the formation of flow recirculation distal to the clot. Flow recirculation helps recycle the free phase tPA to the back of the clot and allows the lysis front to develop behind the obstruction. Furthermore, there appears to be a build-up of tPA concentration at the surface in the front and in the middle of the remnant clot, especially in areas where the surface caves in thus trapping tPA in a slow moving region.

Figure 3.9 shows the free phase tPA concentration contours for 3 different pressure drops: 1 Pa, 10 Pa and 20 Pa. It can be seen that the value of the pressure drop has a strong influence on the distribution of free tPA throughout the domain. First of all, a higher pressure drop causes an elevation of free tPA concentration towards the front of the clot. This causes a strong lysis front pushing the dissolution of the clot forward in the direction of blood flow. Secondly, a higher pressure drop promotes the development of larger recirculation regions distal to the clot that improves mixing and accelerates the development of the lysis front distal to the clot. Lastly, at very high pressure drops, the flow becomes more disturbed as can be seen by the formation of asymmetric recirculation regions (Figure 3.10). Naturally these asymmetric regions cause non-uniform lysis of the remnant clot with a larger recirculation region corresponding to faster clot dissolution. How further disturbed flow may affect clot lysis patterns remains to be seen, but due to the improved mixing, it is suspected that it will accelerate clot lysis ([Sersa et al.; 2007a](#)) .

The present model has a number of limitations. Clot breakup due to breakage and erosion is not incorporated due to its high computational demand ([Bajd and Sersa; 2013](#)). A combination of stochastic and continuous processes may help to understand the effect of clot breakage at different pressure drops. Furthermore, clot dissolution is described by fibrinolysis while in reality clots are composed of many different cells, including red blood cells and platelets. The exposure of new binding sites due to proteolytic cleavage of fibrin by plasmin and the effect of endothelial cells on clot dissolution have also been neglected. The clot dissolution kinetics only describes the dynamics of three proteins and hence does not account for clot regeneration due to thrombin activity. Lastly, fibrinolysis is described using a model based on the homogeneous shrinking of the fibrin fibre radius rather than lateral transection ([Collet et al.; 2000](#)). A continuum model of this was attempted but found to be lacking in describing the differences in the lysis time between fine and coarse blood clots ([Bannish et al.; 2014b](#)). A hybrid stochastic and continuous model may be required to implement this feature ([Bannish et al.; 2014a](#)).

However, despite the assumptions involved, the model provides a multiphysics framework for the simulation of a clot lytic process in physiologically realistic geometries. The model shows that higher pressure drops across the clot can lead to secondary complications whilst enhancing the lysis of the clot through better mixing. Clinically, it is important to achieve arterial recanalization as soon as possible (within a few hours) of the diagnosis of a thromboembolic disease such as stroke. Treatment with thrombolytic therapy can sometimes be discarded if the patient has increased susceptibility to side effects due to certain physiological risk factors such as high blood pressure. The model results show

that although lysis can proceed more rapidly at elevated pressures due to faster drug penetration, the development of disturbed flow may increase the chances of small clot fragments breaking off from the original clot. These fragments may cause secondary occlusions and can also bring about thrombosis in other areas. This may have important implications for hypertensive patients or patients who have an elevated resistance in their downstream vasculature. In the next chapters, this model will be used to examine lysis patterns that occur in physiologically realistic geometries in the hope of evaluating the effectiveness of thrombolytic therapy in treating ischemic stroke based on patient-specific data.

3.5 Conclusions

A continuum approach multi-physics model has been developed to simulate the entire lytic process of an idealised fully occlusive blood clot. In this model the kinetics presented by [Diamond and Anand \(1993\)](#) has been modified by including the solubilisation of bound species during clot lysis and the work of [Wootton et al. \(2002\)](#) was extended to allow for multi-dimensional transport. The model predictions agree well with experimental data ([Rijken et al.; 1982](#); [Blinck et al.; 2000](#)) and computational results reported by others ([Bannish et al.; 2014a](#); [Anand et al.; 1995](#)). By using the modified continuity and fluid momentum equations, the effects of permeation on clot lysis could be accounted for and the effect of fluid flow on clot lysis can be evaluated. It has been found that pressure drop across the clot has a strong influence on the patterns of clot lysis and the clot lysis time. High pressure drops allow the drug to break through the clot at a much faster rate, resulting in the formation of a thinner funnel shaped canal at the centre of the clot. At the same time, high pressure drops also lead to the development of recirculation regions distal to the clot that promote faster clot lysis, resulting in thinner and taller remnant clots. It is possible that at higher pressure drops, the development of these recirculation regions may cause the lysis fronts at the upstream and downstream end of the clot to break off into small clot fragments.

Chapter 4

3D Model of Thrombolysis

4.1 Introduction

Clinical studies have shown that different types of occlusions in ischemic stroke can have radically different probabilities of recanalisation. [Riedel et al. \(2011\)](#) suspect that clot length is an important determinant of the efficacy of thrombolysis, whereas [Friedrich et al. \(2015\)](#) believe that the clot location plays a more substantial role. Understanding the parameters that significantly affect the likelihood of treatment success will help clinicians in understanding why certain patients are more responsive to thrombolytic therapy. Currently, there are no computational models that have examined the dissolution of a clot in clinically relevant geometries. As such, the following chapter looks at the extension of the previous model of thrombolysis towards examining thrombus dissolution in 3D geometries with physiological boundary conditions.

More specifically, the aim of the work presented in this chapter is to elucidate the process of clot lysis in an occluded M1 segment of the middle cerebral artery (MCA), one of the main causes of ischemic stroke ([Marder et al.; 2006](#)). Towards this end, a 3D idealised geometry has been created to represent the internal carotid artery (ICA) bifurcation where the M1 segment of the MCA is completely occluded by a 3 mm short clot or a 8 mm long clot. The kinetics of fibrinolysis is described using convection-diffusion-reaction transport equations, while the degradation of fibrin is modelled according to the degradation of fibrin binding sites. Physiological boundary conditions have been incorporated by accounting for physiologically relevant steady and pulsatile flow at the inlet and a lumped parameter Windkessel model at the outlets. Due to the enormous computational cost involved in modelling thrombolysis in a realistic time frame, a steady inlet flow was used to determine clot lysis patterns and average arterial pressures and flowrates. Modelling thrombolysis under pulsatile flow was achieved by accelerating the kinetics of thrombolysis so as to determine instantaneous changes in arterial pressures and flowrates throughout the course of clot lysis. This computational model represents the first attempt to simulate thrombolysis in a 3D environment representative of ischemic stroke.

4.2 Methods

4.2.1 Kinetics of Fibrinolysis

The kinetics of fibrinolysis can be described as the interplay of 3 proteins: tPA, PLG and PLS (Tiefenbrunn et al.; 1986). When tPA and PLG reach the site of fibrin fibre, they adsorb onto the available binding sites on the fibre's surface. These 2 bound components go on to generate bound PLS, which lyses the fibrin fibre as illustrated in Figure 4.1. PLS then returns to the free phase after the binding site has been solubilised. Although the bound species do not advect or diffuse through the system, they vary in each control volume according to the following equations:

$$\frac{\partial n_{tPA}}{\partial t} = k_{a,tPA} C_{tPA} n_{free} - k_{r,tPA} n_{tPA} \quad (4.1)$$

$$\frac{\partial n_{PLG}}{\partial t} = k_{a,PLG} C_{PLG} n_{free} - k_{r,PLG} n_{PLG} - \frac{k_2 n_{tPA} n_{PLG}}{K_M(1 - \epsilon) + n_{PLG}} \quad (4.2)$$

$$\frac{\partial n_{PLS}}{\partial t} = k_{a,PLS} C_{PLS} n_{free} - k_{r,PLS} n_{PLS} + \frac{k_2 n_{tPA} n_{PLG}}{K_M(1 - \epsilon) + n_{PLG}} - k_{cat} \gamma n_{PLS} \quad (4.3)$$

$$\frac{\partial n_{tot}}{\partial t} = -k_{cat} \gamma n_{PLS} \quad (4.4)$$

$$\frac{\partial L_{PLS}}{\partial t} = k_{cat} \gamma n_{PLS} - k_{r,PLS} L_{PLS} \quad (4.5)$$

$$n_{free} = n_{tot} - (n_{tPA} + n_{PLG} + n_{PLS}) \quad (4.6)$$

where n_α is the bound phase superficial concentration of species α , C_α is the concentration of species α in blood, n_{free} is the superficial concentration of free binding sites, k_2 and K_M are the Michaelis-Menten reaction rate coefficients for PLG activation, k_a and k_r are the adsorption/desorption rate coefficients, ϵ is the voidage (defined as the volume fraction of fluid in the clot), n_{tot} is the total binding site concentration, k_{cat} is the reaction rate coefficient for catalytic degradation of the binding site, γ is the solubilisation factor and L_{PLS} is the concentration of lysed binding sites with PLS adsorbed onto them. These equations depend on the free phase concentration of proteins such as tPA and PLG which in turn vary spatially as solved by the transport equations that determine their movement.

The role of PAI-1 (plasminogen activator inhibitor) has been neglected as its low concentration in plasma ($\sim 0.0003 \mu\text{M}$) and the high concentration of tPA during thrombolytic therapy ($\sim 0.04 \mu\text{M}$)

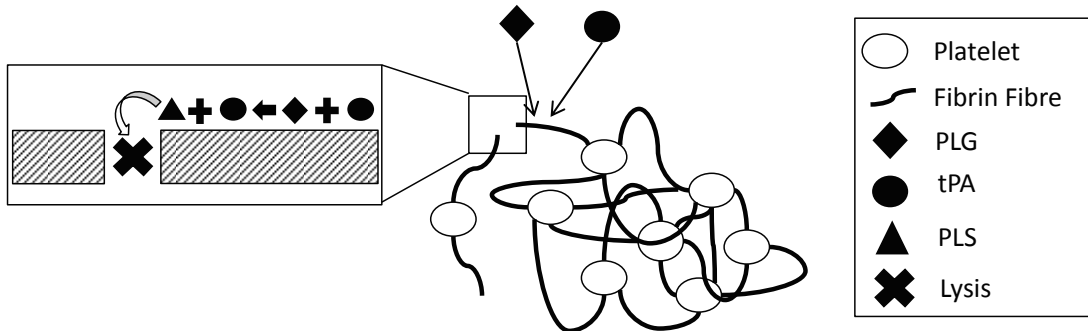


Figure 4.1: Mechanisms of fibrinolysis. The proteins tPA and PLG adsorb onto the fibrin fibre and react to form PLS. PLS then lyses the fibrin fibre thereby degrading the clot.

make its effect negligible (Anand and Diamond; 1996). The role of anti-plasmin, on the other hand, is more important due to its high concentration in the circulation ($\sim 1 \mu\text{M}$). A coarse fibrin clot has a large pore size ($\sim 10 \mu\text{M}$) and its dissolution under high pressure has been shown to be a permeation controlled process (Wu et al.; 1994; Diamond; 1999). As such all fibrin binding sites have been assumed to be available for adsorption in a similar manner to Wootton et al. (2002) and Anand et al. (1995). Despite these limitations, the kinetic model agrees well with experimental findings of well-mixed and convection enhanced lysis of fibrin clots.

4.2.2 Protein Transport and Fluid Flow

The movement of free phase tPA, PLG and PLS can be described by convection-diffusion transport equations (given in equation 3.9). The net rate of consumption/generation of free phase proteins is defined according to the following equations:

$$\sum R_{tPA} = -(k_{a,tPA}C_{tPA}n_{free} - k_{r,tPA}n_{tPA}) \quad (4.7)$$

$$\sum R_{PLG} = -(k_{a,PLG}C_{PLG}n_{free} - k_{r,PLG}n_{PLG}) \quad (4.8)$$

$$\begin{aligned} \sum R_{PLS} = & -(k_{a,PLS}C_{PLS}n_{free} - k_{r,PLS}n_{PLS}) \\ & + k_{r,PLS}L_{PLS} - k_{AP}C_{AP}C_{PLS} \end{aligned} \quad (4.9)$$

where C_{AP} is the free phase concentration of anti-plasmin and k_{AP} is the rate constant of PLS inhibition by AP. The velocity can be calculated by solving the mass and momentum equations of the fluid expressed in equations 2.1 and 2.2. The resistance of the (λ_{clot}) is defined by the following equations (Wufsus et al.; 2013):

$$\lambda_{clot} = \begin{cases} \frac{16\phi_f^{1.5}(1 + 56\phi_f^3)}{\beta R_{f0}^2}, & \text{for } t < t_{crit} \\ 0, & \text{for } t \geq t_{crit}. \end{cases} \quad (4.10)$$

where R_{f0} is the initial fibre radius and β is a proportionality coefficient. t_{crit} is determined as the time when the extent of lysis E_L reaches a critical value E_{crit} . This function is typically used for the prediction of permeability in fibrin gels and is valid for fibrin volume fractions of less than 0.3.

4.2.3 Microscopic Fibrinolysis Model

Clot properties are determined by the fibrin microstructure of the thrombus. Fibrin monomers join together in a half-staggered manner to form protofibrils that aggregate laterally to form fibrin fibres (Weisel; 2005). The resulting fibre strand can be discretised into a number of cross-sections that contain a distribution of binding sites available for the adsorption of lysis proteins (Walker and Nesheim; 1999; Weisel and Litvinov; 2013). The voidage (fraction of fluid in the clot) and the initial total number of binding sites (n_0) can be determined by specifying the initial fibrin density of the clot (3mg/ml), initial fibrin fibre radius (85 nm) and the density of fibrin in a fibre (0.28 g/ml) (Wootton et al.; 2002; Undas et al.; 2009). The extent of lysis is modelled via the degradation of binding sites as:

$$E_L(t) = 1 - \frac{n_{tot}(t)}{n_0} \quad (4.11)$$

where E_L is the extent of lysis, n_0 is the initial binding site concentration and n_{tot} is the total binding site concentration at time t . Thus the fibrin fibre volume fraction can be expressed as

$$\phi_f(t) = \phi_{f,0}(1 - E_L(t)) \quad (4.12)$$

where ϕ_f is the volume fraction of fibrin.

4.2.4 Model Geometry

The arterial geometry used in the computational simulation is an idealised model of the internal carotid bifurcation with the presence of a completely occluding clot in the MCA branch as commonly seen in clinical studies of ischemic stroke (Marder et al.; 2006). Figure 4.2 (a) is a schematic illustration of the 3D model (not drawn to scale) which has physiologically relevant dimensions. The diameters are 3.35 mm for the ICA, 3.0 mm for the MCA and 2.20 mm for the anterior cerebral artery (ACA). The branching angles for the MCA and ACA branches to the ICA centreline are 90° and 60° respectively. A blueprint of the geometry is given in Figure 4.2 (b) where the value of each constant is specified in Table 4.1. The geometry was created and meshed in ICEM CFD 14.0. Ansys Fluent 14.0 was chosen as the computational solver with the source terms and transport equations being coded in the C programming language and implemented via user defined functions (UDFs). CFD-Post v14.0 was used for the post-processing of the results. A mesh sensitivity test was conducted to ensure that the simulation results were independent of the computational grid. It was found that a mesh with over 0.8 million elements was sufficient to achieve grid independence for velocity values and tPA concentrations.

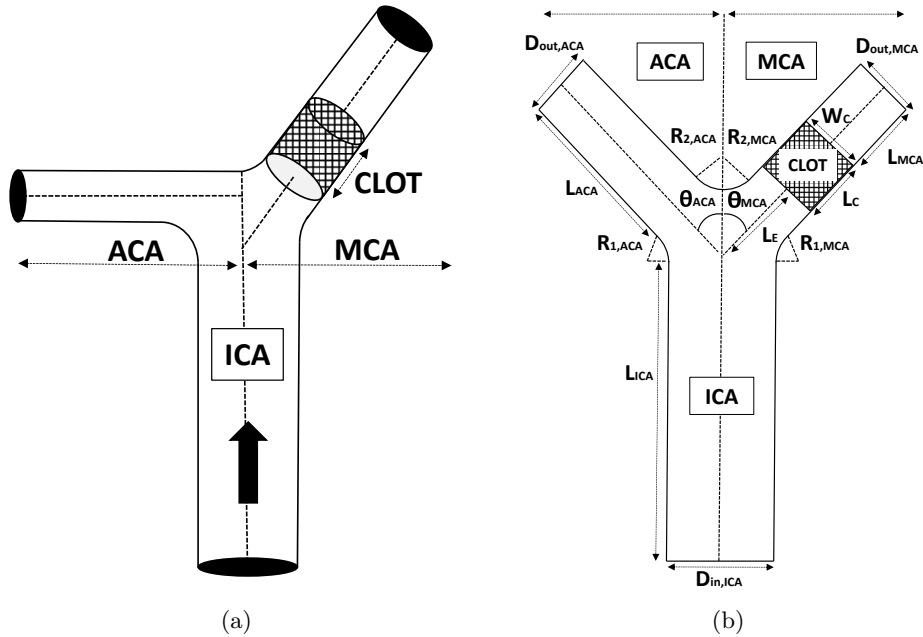


Figure 4.2: (a) Schematic of a 3D idealised ICA-MCA-ACA bifurcation using dimensions given in Table 4.1. Arrow corresponds to the direction of flow and (b) 2D schematic of the ICA bifurcation.

Table 4.1: Parameters for an idealised geometry representative of the ICA bifurcation

Symbol	Description	Value	Units	Source
D_{ICA}	Diameter of ICA inlet	3.35	mm	Calculated using Rossitti and Löfgren (1993)
D_{ACA}	Diameter of ACA outlet	2.20	mm	Ingebrigtsen et al. (2004)
D_{MCA}	Diameter of MCA outlet	3.00	mm	Ingebrigtsen et al. (2004)
L_{ACA}	Length of ACA	$5 \times D_{ACA}$	mm	-
L_C	Length of the clot	3.00	mm	Marder et al. (2006)
L_E	Distance from the bifurcation centre	D_{MCA}	mm	Marder et al. (2006)
L_{ICA}	Length of ICA	$5 \times D_{ICA}$	mm	Wood (1999)
L_{MCA}	Length of distal MCA	40	mm	-
$R_{1,ACA}$	Radius of curvature for ICA to ACA	0.5	mm	-
$R_{1,MCA}$	Radius of curvature for ICA to MCA	0.5	mm	-
$R_{2,ACA}$	Radius of curvature for ACA to centreline	0.5	mm	-
$R_{2,MCA}$	Radius of curvature for MCA to centreline	0.5	mm	-
W_C	Width of the clot	D_{MCA}	mm	-
θ_{ACA}	Angle of curvature between ACA and centreline	90	-	Ingebrigtsen et al. (2004)
θ_{MCA}	Angle of curvature between MCA and centreline	60	-	Ingebrigtsen et al. (2004)

4.2.5 Boundary Conditions

In order to solve the system of partial differential equations, boundary conditions need to be specified at the ICA inlet and the MCA and ACA outlets. A pulsatile flow rate with Womersley velocity profiles was applied at the model inlet. The ICA flowrate was obtained from [Blanco et al. \(2015\)](#) and fitted to a trigonometric Fourier series (shown in Figure 4.3 a), from which the corresponding Womersley velocity profiles were calculated ([Nichols et al.; 2011](#)) (shown in Figure 4.3 b). A steady flowrate of 4.31 ml/s was obtained by averaging one cycle of the pulsatile flowrate.

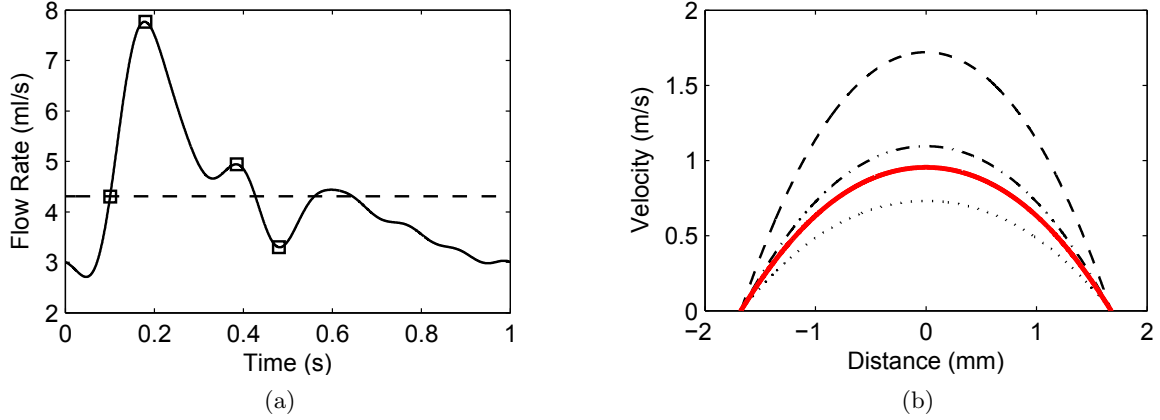


Figure 4.3: Inlet boundary conditions. (a) Pulsatile flow cycle as taken from [Blanco et al. \(2015\)](#). Horizontal line corresponds to the cycle averaged flowrate and squares show the flowrates taken to analyse the velocity distribution in (b); (b) Velocity distribution as a function of radial distance from the inlet centre. Bold red line corresponds to velocity distribution at the cycle averaged flow rate. The flowrate used to determine the velocity distribution is given by the squares in (a).

Physiological pressure outflow boundary conditions are required to attain relevant and valid simulation results. Since the pressure is expected to change as the clot is gradually lysed and this is not known a priori, a 3-element Windkessel model was employed for the outlet boundary conditions ([Westerhof et al.; 2009](#)). The pressure and flow at each outlet are related by:

$$Q_{out,ACA}(1 + \frac{R_{1,ACA}}{R_{2,ACA}}) + C_{ACA}R_{1,ACA}\frac{dQ_{out,ACA}}{dt} = C_{ACA}\frac{dP_{out,ACA}}{dt} + \frac{P_{out,ACA}}{R_{2,ACA}} \quad (4.13)$$

$$Q_{out,MCA}(1 + \frac{R_{1,MCA}}{R_{2,MCA}}) + C_{MCA}R_{1,MCA}\frac{dQ_{out,MCA}}{dt} = C_{MCA}\frac{dP_{out,MCA}}{dt} + \frac{P_{out,MCA}}{R_{2,MCA}} \quad (4.14)$$

where Q_{out} is the flowrate at the model outlet, R_1 is the inertial resistance, R_2 is the peripheral resistance, C is the capacitance and P_{out} is the pressure at the outlet. Values for the Windkessel parameters namely C , R_1 and R_2 are given in Table 4.2.

4.2.6 Kinetics Constants

Values for additional kinetics and transport parameters used in the simulation are given in Table 4.3. The incorporation of pulsatile blood flow has introduced a different time scale from that of clot lysis: blood flow has a pulse cycle of around 1 second, whereas the thrombolytic process is expected to take place over several minutes ([Alexandrov et al.; 2001](#)). This sets a limit on the largest time-step size

Table 4.2: Windkessel parameters for each outlet

Boundary Patch	Symbol	Value	Units	Source
MCA	$R_{T,MCA}$	3.2×10^9	Pas/m^3	Calculated as $R_T = P_{mean}/Q_{mean} = (60 \text{ mmHg})/0.6 \text{ } Q_{ICA}$ using Ogoh et al. (2005) ; Matano et al. (2016) for pressure and Enzmann et al. (1994) for flow split
	$R_{1,MCA}$	1.792×10^8	Pas/m^3	$R_1 = 0.056R_T$ (Xiao et al.; 2013)
	$R_{2,MCA}$	3.0208×10^9	Pas/m^3	$R_2 = 0.944R_T$ Xiao et al. (2013)
	C_{MCA}	4.66×10^{-11}	m^3/Pa	Xiao et al. (2013)
ACA	$R_{T,ACA}$	4.8×10^9	Pas/m^3	Calculated according to a flow split (Enzmann et al.; 1994) with the aid of (Grinberg and Karniadakis; 2008)
	$R_{1,ACA}$	3.3322×10^8	Pas/m^3	Calculated by assuming the same wave speed in all branches
	$R_{2,ACA}$	4.4668×10^9	Pas/m^3	Calculated as $R_{T,ACA} - R_{1,ACA}$
	C_{ACA}	2.33×10^{-11}	m^3/Pa	Xiao et al. (2013)

that can be used to ensure numerical stability. In order to ascertain the effects of blood flow pulsatility on the slower clot lysis process, two simulations have been performed: one for steady inflow at 4.31 ml/s with a time-step of 0.01 s, and another for pulsatile inflow with a smaller time-step of 0.001 s. In order to render the pulsatile flow simulation computationally tractable, the kinetics of fibrinolysis had to be accelerated and this was achieved by multiplying the following constants by 10: k_{cat} , k_2 and k_a and k_r for tPA, PLG and PLS, respectively.

4.3 Results

4.3.1 Validation of Kinetics Model

The mathematical model was compared against literature data to validate the predicted lysis time. First, the kinetics model was tested in a well-mixed environment by comparing predicted lysis curves with those found by [Kunitada et al. \(1992\)](#) for different initial tPA concentrations. The model was solved with the Runge-Kutta 4th order method using a timestep of 0.756 s and the kinetic constants listed in Table 4.3. The value for k_{cat} was fitted to the experimental data by using the Nelder-Mead optimisation algorithm. As shown in Figure 4.4 (a), the degree of lysis predicted by the model agreed well with the experimental results of [Kunitada et al. \(1992\)](#) for different initial tPA concentrations.

The predicted lysis front position during convection enhanced thrombolysis was compared against the experimental results found in [Anand and Diamond \(1996\)](#). The transport equations were discretised using the finite difference methods whereas the kinetic sources terms were solved with the 4th order Runge-Kutta method. As shown in Figure 4.4 (b) predicted results seemed to match

Table 4.3: Kinetics and transport parameters used in the simulation

Symbol	Description	Value	Units	Source
$C_{PLG,0}$	Initial concentration of PLG	2.2	μM	Diamond (1999)
$C_{tPA,0}$	Initial concentration of tPA	40	nM	Anand and Diamond (1996)
D	Dispersion coefficient for tPA. PLG and PLS	5×10^{-11}	m^2/s	Weigandt et al. (2012)
E_{crit}	Extent of lysis at which clot resistance collapses	0.95	-	Using data from Wu et al. (1994)
k_2	Michaelis reaction rate coefficient	0.3	s^{-1}	Zamarron et al. (1984)
$k_{a,PLG}$	Adsorption coefficient for PLG	0.1	$\mu M^{-1} s^{-1}$	Wootton et al. (2002)
$k_{a,PLS}$	Adsorption coefficient for PLS	0.1	$\mu M^{-1} s^{-1}$	Wootton et al. (2002)
$k_{a,tPA}$	Adsorption coefficient for tPA	0.01	$\mu M^{-1} s^{-1}$	Husain et al. (1989)
k_{cat}	Lysis coefficient	2.178	s^{-1}	Using data from Kunitada et al. (1992)
K_M	Michaelis constant	0.16	μM	Hoylaerts et al. (1982)
$k_{r,PLG}$	Desorption coefficient for PLG	3.8	s^{-1}	Lucas et al. (1983)
$k_{r,PLS}$	Desorption coefficient for PLS	0.05	s^{-1}	Wootton et al. (2002)
$k_{r,tPA}$	Desorption coefficient for tPA	0.0058	s^{-1}	Husain et al. (1989)
n_0	Initial binding site concentration	0.492	μM	-
β	Permeability Coefficient	0.286	-	Wu et al. (1994)
γ	Cuts needed for PLS to cut 1 fibrin unit	10	-	Wootton et al. (2002)
μ	Viscosity of blood	3.5×10^{-3}	m^2/s	Lowe et al. (1993)
ρ	Density of blood	1060	kg/m^3	Kenner (1989)

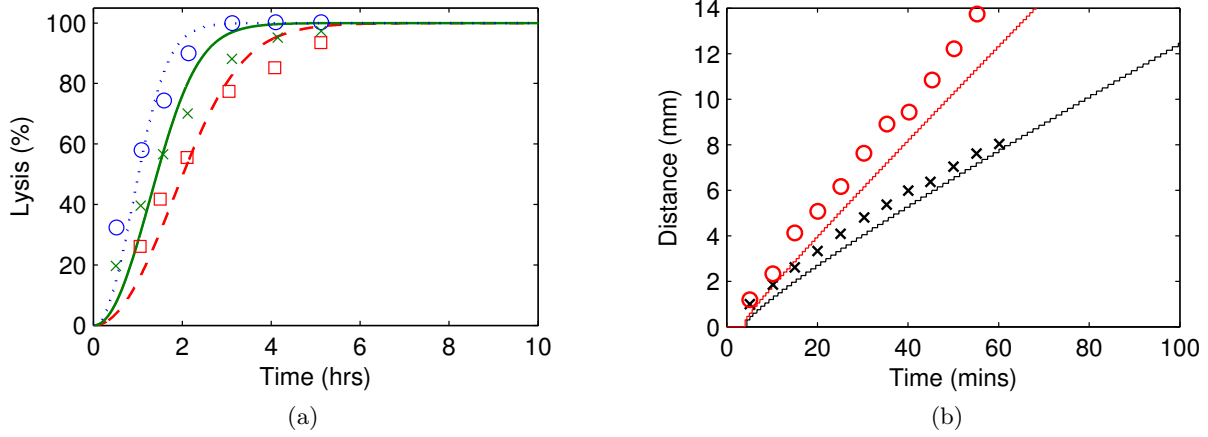


Figure 4.4: Comparison of simulation results with experimental data from (a) Kunitada et al. (1992) and (b) Anand and Diamond (1996). The experimental results of Kunitada et al. (1992) for the degree of lysis are shown in circles (blue), crosses (green) and squares (red) for a free phase tPA concentration of 2×10^{-5} , 1×10^{-5} and 5×10^{-6} μM , respectively, while the corresponding simulation results are shown in the dotted (red), solid (green) and dashed (red) lines. A comparison of predicted lysis front position with experimental data (Anand and Diamond; 1996) at perfusion rate of 0.1mm/min (red) and 0.2mm/min (black). Y-axis corresponds to the lysis front position while the X-axis refers to the time. Symbols represent lysis progression found experimentally while the solid lines refer to the corresponding simulation results.

well with the experimental data of Anand and Diamond (1996).

4.3.2 Simulations of the ICA Bifurcation under Steady Inlet Flow

Simulations of thrombolysis in a 3D geometry were performed by using the finite volume discretisation available in Ansys Fluent. Due to the small timescale required to resolve pulatile flow (timestep of 0.001 s) and the large timescale required to simulate thrombolysis (several minutes), steady inlet flow simulations were initially performed to evaluate the breakthrough time and patterns of clot lysis. Two different clot lengths were selected to compare the dissolution of a short clot (3 mm) and a long clot (8 mm). The breakthrough time (the point at which over 95% of blood flow is restored to the occluded artery) obtained with a constant inlet flow rate for the 3 mm and 8 mm clot is 233 s and 323 s respectively. The computational time required for the steady inflow simulation was 5 days on an Intel Xeon E5-2697 workstation with 24 cores and 96GB RAM. Figure 4.5 shows the instantaneous velocity streamlines at 6 selected time points during clot lysis for an initial thrombus length of 3 mm. It is clear that the presence of the clot (red section) brings about a large recirculation region that gradually advances as the clot is being degraded (a, b and c). In the latter stages of lysis, the recirculation region expands as the clot becomes smaller (d and e). Due to the significant reduction in clot length, flow begins to return to the occluded MCA (f). The dissolution pattern and the velocity streamlines of an 8 mm clot during thrombolysis are shown in Figure 4.6. The occluding clot prevents blood flow from reaching the MCA (a). A uniform lysis front pushes through the clot (b, c, d and e) until flow is restored in the occluded artery (f). Although the pattern of dissolution is similar, the time taken to dissolve an 8 mm clot is in a much longer timescale than that for a 3 mm clot.

The clot lysis pattern can be visualised in 3D by generating isovolumes (regions of computational cells that contain a value within a certain range) of $\lambda_{clot} > 1 \text{ m}^{-2}$ as shown in Figure 4.7. Various

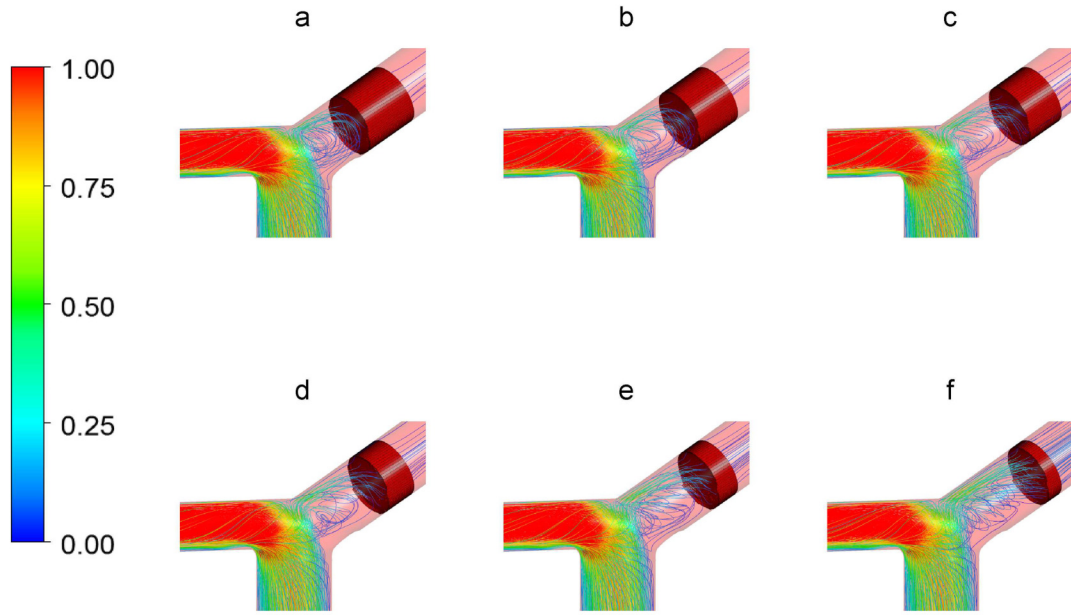


Figure 4.5: Instantaneous velocity streamlines at different time points during the lysis of a 3mm clot. Colour corresponds to the magnitude of velocity as shown by the legend in m/s. The red region shows the presence of the clot where $\lambda_{clot} > 1 \text{ m}^{-2}$. (a) $t = 220 \text{ s}$; (b) $t = 222 \text{ s}$; (c) $t = 224 \text{ s}$; (d) $t = 226 \text{ s}$; (e) $t = 228 \text{ s}$; (f) $t = 230 \text{ s}$.

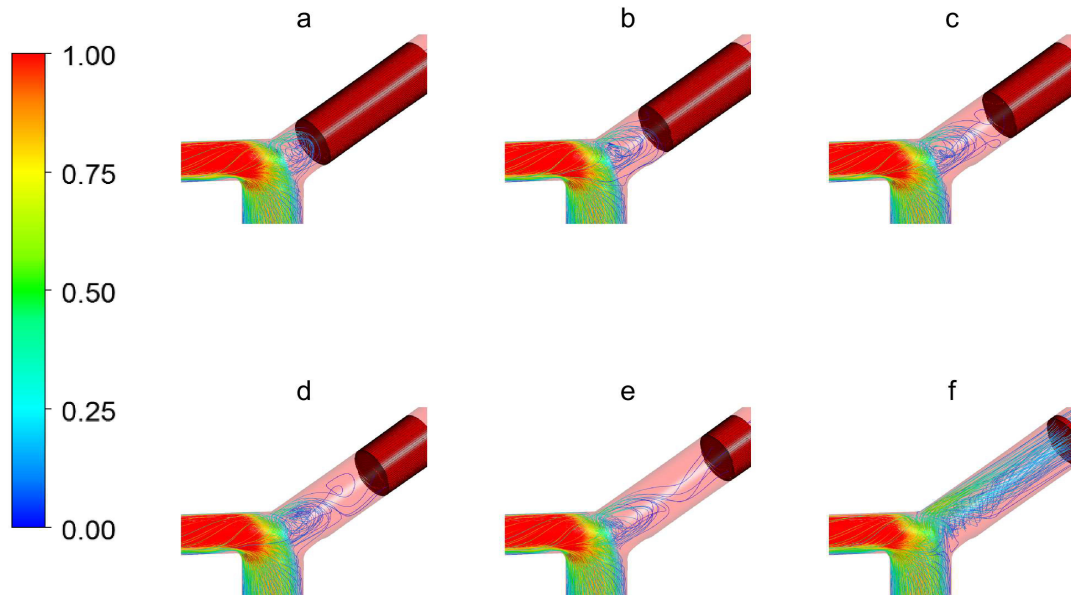


Figure 4.6: Instantaneous velocity streamlines at different time points during the lysis of a 8mm clot. Colour corresponds to the magnitude of velocity as shown by the legend in m/s. The red region shows the presence of the clot whereby $\lambda_{clot} > 1 \text{ m}^{-2}$. (a) $t = 220 \text{ s}$; (b) $t = 240 \text{ s}$; (c) $t = 260 \text{ s}$; (d) $t = 280 \text{ s}$; (e) $t = 300 \text{ s}$; (f) $t = 320 \text{ s}$.

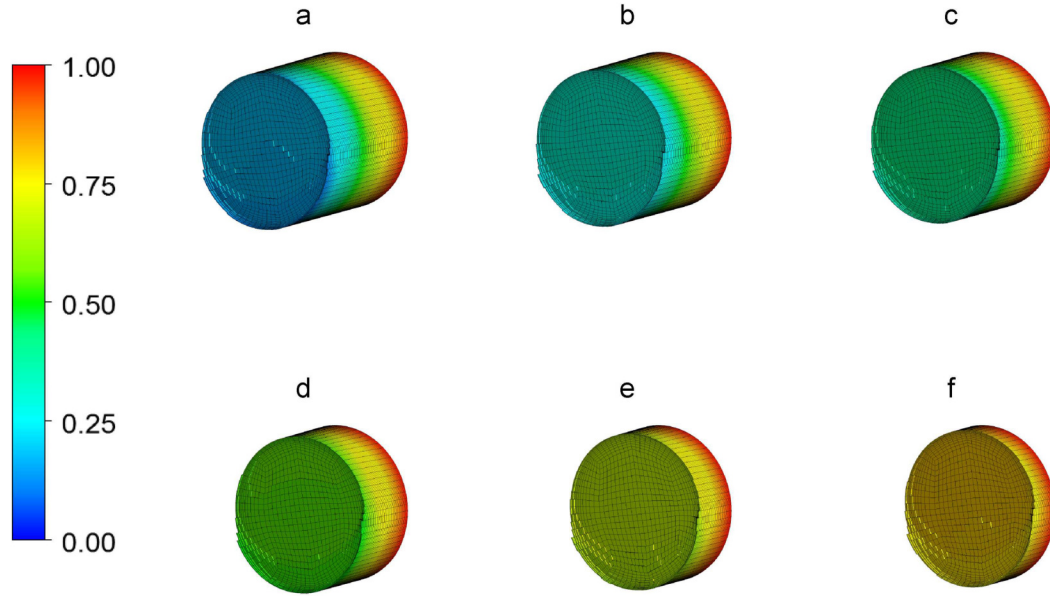


Figure 4.7: Isovolumes depicting the pattern of clot degradation over time. Each visible cell has a value of $\lambda_{clot} > 1 \text{ m}^{-2}$. The colour legend shows the normalised depth of each cell in the clot from the clot inlet with blue representing the upstream end and red representing the downstream end of the clot. (a) $t = 220 \text{ s}$; (b) $t = 222 \text{ s}$; (c) $t = 224 \text{ s}$; (d) $t = 226 \text{ s}$; (e) $t = 228 \text{ s}$; (f) $t = 230 \text{ s}$.

time points were chosen to illustrate the initial clot geometry (a), midway through clot lysis (b, c and d) and clot dissolution towards the latter stages of lysis (e and f). The colour corresponds to the normalised depth of the clot from the upstream end of the clot (where 0 refers to the upstream end and 1 refers to the downstream end of the clot domain). It can be seen that the lysis front for an initial homogeneous clot advances in a uniform manner.

The recirculation region seen in Figure 4.5 can be better visualised on the symmetric plane as shown in Figure 4.8 where the 2D streamlines are coloured according to the magnitude of velocity. The initial position of the clot is shown by 2 thick red lines that mark the entrance and exit of the thrombus. Initially, there is a recirculation region at the proximal face of the clot (a, b) that gradually expands as the clot is being lysed (c, d and e). When breakthrough is reached, the recirculation region eventually disappears as flow is restored in the occluded MCA artery (f).

The average extent of lysis and changes in the overall resistance of the clot to blood flow can be seen in Figure 4.9 a. For the 3 mm clot, the average extent of lysis slowly accelerates (0 s - 75 s) until it increases at a steady rate (100 s - 200 s). When clot breakthrough is approached (233 s), the extent of lysis begins to slow down dramatically as blood flow is re-established. The average resistance of the clot to flow over time (Figure 4.9 b) is maintained in the early stages of lysis (0 - 150 s). However, as the degree of clot lysis reaches 50% there is a sharp decrease in the clot resistance that eventually tends to 0 as the clot has been completely lysed.

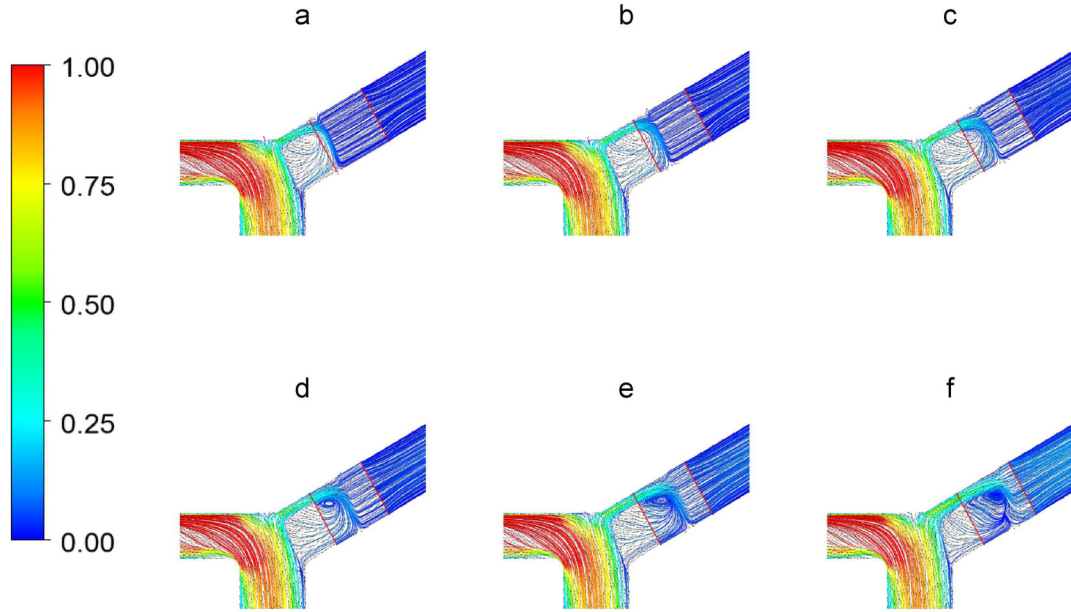


Figure 4.8: 2D streamlines on the plane during clot lysis. Velocity streamlines are coloured according to the legend that has units of m/s. (a) $t = 220$ s; (b) $t = 222$ s; (c) $t = 224$ s; (d) $t = 226$ s; (e) $t = 228$ s; (f) $t = 230$ s.

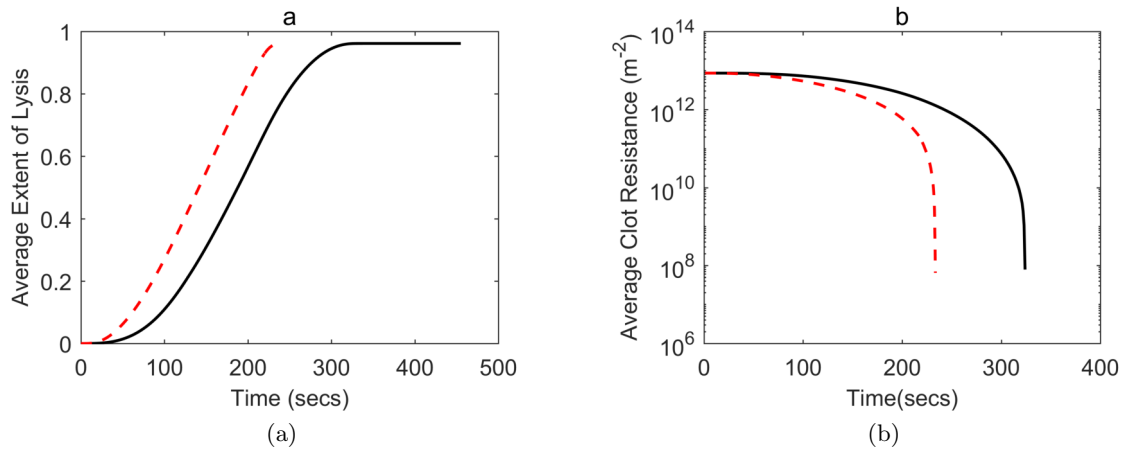


Figure 4.9: Variations of clot lysis over time for an initial clot length of 8 mm (solid black) and 3 mm (dashed red). (a) Average extent of lysis in the clot domain against time; (b) Average clot resistance in the clot domain over time.

4.3.3 Simulations of the ICA Bifurcation under Pulsatile Flow

In order to determine instantaneous changes in the pressure and flowrate during clot lysis, an additional pulsatile inlet condition was assigned. Since a cardiac cycle has a period of 1 s, a small time-step has to be used (1 ms) which, if solved in a realistic thrombolysis timeframe, would take several weeks. For this reason, the kinetics of clot lysis were accelerated by a multiple of 10. To save on computational cost, the role of anti-plasmin has been neglected and as such the lysis time predicted by our model should be interpreted in light of its absence. The predicted breakthrough time was 31 s and the computational time required for the pulsatile inflow simulation was 2 times that of the steady inflow simulation. Figure 4.10 shows the flowrate and pressure at the ACA and MCA outlets over time for both steady and pulsatile inflow simulations. The abscissa has been normalised with respect to the corresponding breakthrough times ($\hat{t} = \text{time}/(\text{breakthrough time})$) to allow for direct comparisons. Clearly, the presence of the clot blocks blood flow to the MCA (Figure 4.10 b) and diverts it to the ACA (Figure 4.10 a) before the breakthrough time is reached. As tPA enters the system and reaches the clot surface, the blood clot dissolves gradually until blood flow is re-established in the MCA, causing a sudden increase in the MCA flowrate and a sharp fall in the ACA flowrate. The corresponding changes in intra-arterial pressure can be seen from Figure 4.10 (c) and (d) where the ACA pressure falls and the MCA pressure rises to physiologically normal values. It can also be observed that results from the steady flow simulation represent closely the cycle-averaged values for the pulsatile flow simulation, except during the period approaching the breakthrough time.

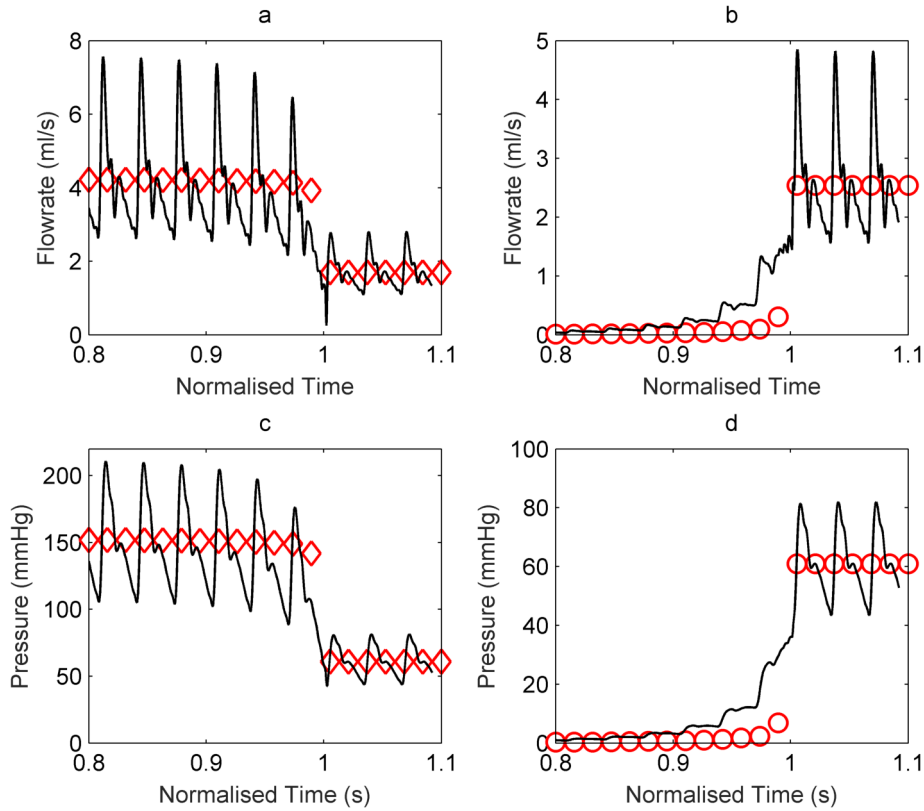


Figure 4.10: Intra-arterial flowrates and pressures over time. Solid lines represent results obtained with pulsatile flow at the inlet while red symbols show results with steady flow at the inlet. (a) Volumetric flow rate at the ACA outlet; (b) volumetric flowrate at the MCA outlet; (c) intra-arterial pressure at the ACA outlet; (d) intra-arterial pressure at the MCA outlet.

4.4 Discussion

The aim of thrombolytic therapy is to restore blood supply to a blocked artery by dissolving an occluding blood clot. To simulate this process, a computational model was developed to examine the clot lysis patterns that may occur in ischemic stroke. All the kinetics parameters used in the computational simulation were found or derived from the literature as shown in Table 4.3, with the exception for k_{cat} which was reported to vary between 1 s^{-1} (Wootton et al.; 2002) and 5 s^{-1} (Anand and Diamond; 1996). In order to obtain a realistic value of k_{cat} , the results of the mathematical model was fitted to the experimental data of Kunitada et al. (1992) who determined the degree of lysis in a buffer containing 3 mg/ml of fibrinogen and $2\text{ }\mu\text{M}$ of PLG for different concentrations of tPA. Using the Nelder-Mead algorithm, a k_{cat} value of 2.2 s^{-1} was obtained.

Using the derived k_{cat} value, the computational results were compared to the experimental data found by Anand and Diamond (1996) for clot lysis under perfusion enhanced transport (shown in Figure 4.4 b). The lysis front of a clot (that was created from platelet poor plasma) was followed upon infusion of $1\text{ }\mu\text{M}$ of PLG and $0.1\text{ }\mu\text{M}$ of tPA with a constant permeation velocity of 0.1 mm/min and 0.2 mm/min . Without fitting any of the kinetics constants, a good agreement was found between the simulation results and the experimental data.

In order to model the lysis of a fibrin clot in a 3D environment and in a realistic timeframe, a steady inlet flow was applied. The predicted breakthrough time is much shorter compared to the average time required for clot lysis with intravenous thrombolysis, which has been reported to be around 23 ± 16 minutes based on a study of 43 patients (Alexandrov et al.; 2001). This is thought to be primarily due to 2 reasons: not taking into account the time required for the tPA to reach the site of the clot via intravenous delivery and the highly heterogeneous nature of the clot. Clots retrieved from patients suffering from acute ischemic stroke contain different cellular components (such as red blood cells and platelets) that can cause a severe delay in fibrinolysis (Ahn et al.; 2016). For instance, the presence of platelets alone can slow down the lysis of a clot by around a multiple of 3 (Collet et al.; 2002). Furthermore, tPA delivered through intravenous infusion can react with plasminogen in the blood stream thereby lowering the availability of the substrate for plasmin generation (Noe and Bell; 1987). Taking these factors into account, the predicted time of around 4-6 mins for thrombolysis of a fibrin clot seems plausible.

The dissolution of the clot allows for flow to be re-established in the MCA. Initially a great proportion of the flow is diverted from the MCA to the ACA branch (Figure 4.5, 4.6 a). There is a recirculation region proximal to the face of the clot that enhances local mixing and helps tPA to be distributed more uniformly. As the clot is gradually degraded the clot front is pushed back and the recirculation region extends (Figure 4.5, 4.6 b, c and d). The diminishing length of the clot allows for more flow to pass through the MCA and causes an increase in the MCA velocity and a corresponding reduction in the ACA velocity as shown in Figure 4.5 and 4.6 (e and f). The time for the lysis front to sweep through the clot and restore blood flow in the MCA took around 10 s and 1.5 mins after almost 4 minutes of tPA injection for a 3 mm clot and a 8 mm clot respectively. This sudden recanalization has been observed in clinical studies where transcranial Doppler ultrasound scans of patients suffering from an MCA occlusion showed that an abrupt recanalization took place over 35 seconds after 31 minutes of tPA bolus (Alexandrov et al.; 2001). Further to this, a rapid collapse of the clot has been seen in fibrinolysis studies where the time till lysis is determined by a ball sedimentation assay

(Wohner et al.; 2011).

The clot lysis pattern in Figure 4.7 indicates that the progression of the lysis front occurs uniformly during the entire lytic process in a similar manner to experimental observations of diffusion limited and convection enhanced clot lysis (Wu et al.; 1994). Confocal microscopy (Collet et al.; 2002) and magnetic resonance imaging (Walvick et al.; 2011) show that for a homogeneous fibrin clot, the lysis front maintains a flat shape with few irregularities. The absence of fingering dissolution patterns observed by Zidansek et al. (1995) is likely due to the initial homogeneous properties of the occluding clot (Anand et al.; 1997) and to the uniform tPA distribution as a result of enhanced mixing at the clot front.

The initial length of the occlusion has a considerable influence on the breakthrough time (<4 mins and >5 mins for the 3 mm and 8 mm clot respectively) although the movement of the lysis front starts at the same time for both clot lengths at around 220 s. This is consistent with Zidansek and Blinc (1991) who found that the dissolution time is uniquely a function of the parameters of fibrinolysis such as the initial tPA concentration and the value of kinetics constants. The lysis front velocity however is noticeably different between the short and long clots. This is due to the change in perfusion velocity which according to Darcy's law, is inversely related to the length. The lysis front velocity is highly dependent on the perfusion velocity (Zidansek and Blinc; 1991; Wu et al.; 1994) thereby accounting for the longer breakthrough time seen in the dissolution of the 8 mm clot.

The recirculation region and changes in blood velocity can be seen more readily in Figure 4.8 resulting in streamlines that evolve over time as a direct result of changes in clot permeability (hence flow resistance) during clot lysis. Initially there is a recirculation region proximal to the surface of the clot (Figure 4.8 a); this gradually pushes into the clot domain as the clot is being lysed (Figure 4.8 b, c and d). The change in flow patterns during clot lysis is consistent with findings by Alexandrov et al. (1994) where transcranial Doppler scans of a patient suffering from an MCA occlusion showed a 25% decrease in the peak velocity of the ACA following recanalization (from 1.60 m/s to 1.20 m/s). They also found that the MCA velocity jumped from 0 to 1.00 m/s while the ICA velocity decreased from 1.76 m/s to 1.48 m/s. Results obtained in the present study show a similar jump in the peak velocity of the MCA following recanalisation with a 44 % decrease in the ACA peak velocity (from 1.59 m/s to 0.89 m/s). The change in the ICA velocity however could not be predicted as the velocity is constrained by the constant inlet boundary condition. Future studies may need to include more realistic boundary conditions to predict any changes in the inlet flowrate during thrombolysis.

The average extent of clot lysis for the 3 mm and 8 mm clot (Figure 4.9 a) has a similar sigmoid like shape, although the 8 mm clot takes a longer time to dissolve and therefore is shifted slightly to the right. This is consistent with experimental studies of clot lysis where there is an initial acceleration in the rate of lysis that gradually slows down as the whole clot is dissolved (Kunitada et al.; 1992). The predicted change in the in average clot resistance (Figure 4.9 b) show a similar trend as the findings by Zidansek and Blinc (1991), in that an initial period of slow clot dissolution is followed by a more rapid movement of the clot lysis front.

While simulations under steady inlet flow are useful for determining clot lysis patterns and breakthrough times, simulations under pulsatile inlet flow can provide information on the instantaneous response of intra-arterial pressures and flowrates throughout the lytic process. Since blood flow has a pulse cycle of around 1 second and clot lysis occurs over several minutes, the kinetics of clot lysis was accelerated by a multiple of 10 for the pulsatile inflow simulation. The predicted lysis time for a

3 mm clot under pulsatile flow with accelerated kinetics is 31 s (~ 0.5 mins)

The lack of blood flow into the MCA due to the presence of a large blood clot has been observed clinically with transcranial Doppler sonography (TCD) (Burgin et al.; 2000; Alexandrov et al.; 1994). As the clot is subsequently dissolved, there is a sudden increase of flow into the MCA and this causes a change in flow split between the MCA and ACA (Figure 4.10 a and b). The MCA to ACA flow ratio after clot dissolution rises to a physiologically reasonable value of 1.5 (as seen by Enzmann et al. (1994)), with the corresponding mean flowrates being 2.5 ml/s and 1.8 ml/s for the MCA and ACA respectively, which are in the range reported by Blanco et al. (2015). These values are slightly higher than those found in clinical studies where the mean flowrates of the MCA and ACA were reported to be 2.1 ml/s and 1.5 ml/s respectively (Stock et al.; 2000; Enzmann et al.; 1994). This discrepancy can be attributed to large individual variations of mean flow going into the ICA which can range from 3.4 ml/s to 5.4 ml/s (Bogren et al.; 1994). Before clot lysis, the flowrate in the ACA oscillates between 7 ml/s to slightly less than 3 ml/s. After recanalization, the change in ACA flowrate within a cardiac cycle decreases to around 1 ml/s. On the other hand, the change in the MCA flowrate during a cardiac cycle increases with a peak flowrate of slightly under 5 ml/s and a minimum flow rate of just under 2 ml/s.

Regarding changes in pressure, the mean pressure at the ACA outlet is 150 mmHg, which then decreases to a mean value of 60 mmHg after clot lysis (Figure 4.10 c and d). The mean pressure at the MCA outlet in the early stages of clot lysis is almost 0 mmHg before rising abruptly to a physiologically realistic value of 60 mmHg following clot dissolution. The average pressure drop from the clot (which can be calculated as the difference between the ACA and MCA pressures) is around 150 mmHg before clot lysis and is in the range typically expected for acute occlusions (Konala et al.; 2011). By applying a pulsatile flowrate at the domain inlet, the corresponding pulsatile pressure within each cardiac cycle can be obtained. Before clot lysis, there is a large pressure difference of 100 mmHg in the ACA between the diastolic and systolic phases. Following clot dissolution, the pulse pressure in both the ACA and the MCA is reduced to around 50 mmHg.

4.5 Conclusion

In this chapter, a multiscale computational model of 3D clot lysis is presented and applied to an M1 occlusion of the MCA at the ICA-MCA-ACA bifurcation which represents a common scenario seen in thrombolytic therapy. The mathematical model has been validated against experimental data for well-mixed and perfusion enhanced 1-D systems. The application of a pulsatile inlet flowrate allows instantaneous changes in pressure and flow to be captured while the 3-element Windkessel pressure boundary conditions allowed for the flow-split to be determined throughout the dissolution process. Results show that the clot lysis pattern is characterised by a uniform lysis front for both the 3 mm and 8 mm occlusion. Pressure values in the ACA oscillate around a mean of 150 mmHg before recanalisation and return to a physiological mean of 60 mmHg thereafter. Furthermore, the pulse pressure in the ACA is reduced from around 100 mmHg to 50 mmHg before and after clot lysis respectively. Similarly, the flowrate in the ACA had a range of 5 ml/s and 1 ml/s before and after clot lysis, respectively. This chapter presents the first application of a thrombolysis computational model in a 3D physiologically relevant geometry that gave predicted changes in pressure and flowrate consistent with those seen in experimental and clinical studies.

Chapter 5

3D Patient Specific Model of Thrombolysis

5.1 Introduction

Although IV thrombolysis is considered the treatment of choice for acute ischemic stroke, the procedure can sometimes be ineffective and cause haemorrhage. Several clinical studies have been conducted to investigate which parameters may be important for determining treatment outcome. A potential important predictor is clot length as long clots are often associated with a low probability of recanalisation. [Riedel et al. \(2011\)](#) observed that out of 138 patients who received IV thrombolysis, none of them with a clot length greater than 8 mm achieved successful recanalisation. [Rohan et al. \(2014\)](#), on the other hand, found that longer clots (>20 mm in length) could be recanalised, albeit with a much lower probability of success, while shorter clots with a length less than 12 mm had a greater than 50% chance of attaining recanalisation. [Yan et al. \(2016\)](#) found that the average clot length in the MCA was 8 mm and clots shorter than 6.77 mm were more likely to achieve recanalisation.

The clot location in the cerebral vasculature has also been identified as an important predictor of treatment success. Ischemic stroke can occur at different locations such as the ICA bifurcation, the vertebral artery and different locations along the MCA. [Saqquar et al. \(2007\)](#) found that occlusions located at the ICA bifurcation had a high rate of mortality (45%) and only a 6% chance of achieving recanalisation. In contrast, MCA occlusions had a greater than 30% rate of attaining breakthrough whereas those located distal to the ICA bifurcation had a 14% higher likelihood of recanalisation as compared to their proximal counterparts. Studies have found that this might be due to the tendency for longer clots to be present closer to the ICA bifurcation. For instance, [Kamalian et al. \(2013\)](#) and [Friedrich et al. \(2015\)](#) found that clot lengths greater than 8 mm were typically observed in the proximal arteries (less than 20 mm away from the ICA bifurcation). [Qazi et al. \(2015\)](#) suggested that the tendency for longer clots to develop at certain locations could be due to zones of flow stasis proximal to the clot that encourage thrombus formation.

Therefore, it is important to investigate how geometric parameters, such as the associated arterial geometry, thrombus length and clot position can affect the likelihood of recanalisation in thrombolytic therapy. In this chapter, the influence of clot position and length on the efficacy of thrombolysis is evaluated using the computational model described in the previous chapter and applied to a realistic

ICA-MCA-ACA bifurcation. The geometry was extracted from CTA scans of a healthy patient and a clot of varying lengths and at different positions was imposed in the MCA. The lysis of a proximal MCA occlusion for 4 different clot lengths (3 mm, 6 mm, 9 mm and 12 mm) and the dissolution of a 3 mm clot at 4 different positions in the MCA (3 mm, 6 mm, 9 mm and 12 mm away from the ICA bifurcation) is investigated. Analysis of results is focused on clot lysis patterns, changes in clot lysis time and variations in the intra-arterial flowrates and pressures. This is done in an effort to understand the influence of clot length and position in the MCA on the efficacy of thrombolysis and recanalisation rates.

5.2 Methods

5.2.1 Creating a Patient-Specific Geometry

A patient-specific geometry was reconstructed from CTA scans (provided by Dr. Dylan Roi and Dr. Kyri Lobostesis) of the brain using the image processing software Mimics v18.0. Biological tissue can be segmented according to the scan pixel intensity or grey level. Due to the similarities in the pixel intensity between brain matter and the cerebral arteries, contrast is introduced to differentiate between them. The ICA bifurcation is reconstructed (Figure 5.1 a) and then isolated to focus on the M1 segment of the MCA and the A1 segment of the ACA (Figure 5.1 b). The centreline distance of ACA, MCA and ICA from the centre of the ICA bifurcation was cut at 15.2, 10.5 and 10.7 mm, respectively. The geometry was then imported into the software ICEM CFD to generate a structured mesh with a minimum cell quality greater than 0.2 and a minimum element angle greater than 18° . The main geometric parameters are summarised in Table 5.1. The length of the arteries, as shown in this table, were artificially extended so as to ensure an adequate distance between the boundary conditions and the bifurcation.

As mentioned before, the aim of this study was to evaluate the effect of clot length and position in the MCA on clot lysis patterns and its potential implications for success in thrombolytic therapy. An initial short (3 mm) clot was introduced at around 3 mm from the ICA bifurcation. This 3 mm clot was then placed at 3 mm intervals distally along the MCA branch (3 mm, 6 mm, 9 mm and 12 mm from the ICA bifurcation) to stay within the mean length of the M1 segment of the MCA (15

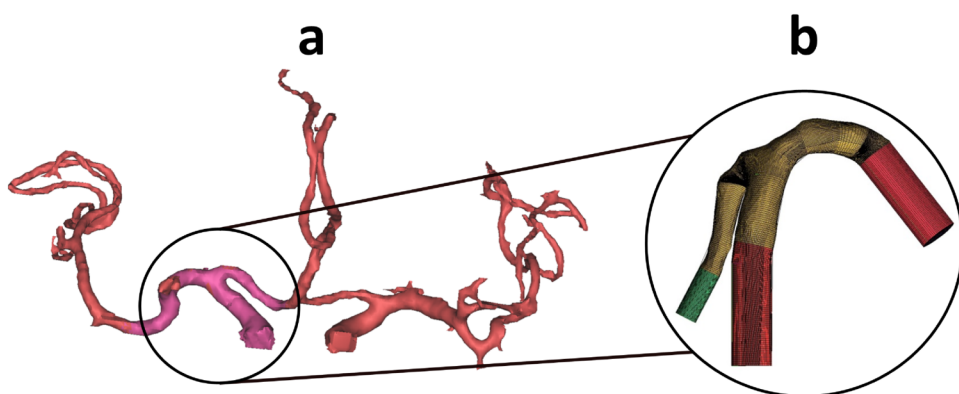


Figure 5.1: (a) Reconstructed arterial geometry from CTA scans of the ICA bifurcation and the ACA and MCA branches. (b) Magnification of a selected part of the geometry that is removed, meshed and translated into the computational domain for clot dissolution simulations.

Table 5.1: Geometric parameters of the patient-specific model

Symbol	Meaning	Value	Units
$D_{in,ICA}$	Diameter of ICA inlet	4.08	mm
$D_{out,ACA}$	Diameter of ACA inlet	2.05	mm
$D_{out,MCA}$	Diameter of MCA inlet	3.62	mm
L_{ICA}	Length of ICA	16	mm
L_{ACA}	Length of ACA	20	mm
L_{MCA}	Length of MCA	31.5	mm
θ_{ACA}	Angle of curvature between ACA and centreline	90	-
θ_{MCA}	Angle of curvature between MCA and centreline	30	-

mm) ([Žurada et al.; 2011](#)). For a fixed clot location at 3 mm from the ICA bifurcation, the length of the clot was changed to 3 mm, 6 mm, 9 mm and 12 mm. This was done so as to investigate the dissolution of clots longer and shorter than the critical 8 mm length reported by [Riedel et al. \(2011\)](#).

5.2.2 Model for Thrombolysis

The same 3D model of clot dissolution as discussed in the previous chapter was used to determine the clot lysis pattern and breakthrough time. The occlusion was assumed to be a fibrin clot containing a fibrin density of 3 mg/ml and an average fibre radius of 85 nm. The same kinetic constants as those listed in Table 4.3 were used along with the Windkessel parameters given in Table 4.2. The inlet flowrate was assumed to be constant at 4.31 ml/s, while an initial concentration of 40 nM and 2.2 μM was applied at the inlet for free phase tPA and PLG, respectively.

5.3 Results

5.3.1 Reference Case

So as to compare difference in lysis patterns between a patient-specific and an idealised geometry, results obtained with a 3 mm clot located at 3 mm from the ICA bifurcation are presented. Figure 5.2 shows the velocity streamlines throughout the thrombolytic process. Initially, the blood clot occludes the MCA and forces the blood flow into the ACA branch (a). As the tPA is introduced into the system, the clot is gradually dissolved (b and c) until blood flow is restored into the MCA branch (d). Unlike the clot lysis patterns in the idealised geometry, clot remnants remain attached to the wall that constrict the flow, causing a local increase in blood velocity (e and f).

The clot lysis patterns of a 3 mm fibrin clot in the patient-specific ICA bifurcation are presented in Figure 5.3. Although the length of the clot along the centreline is 3 mm, the curved nature of the MCA segment creates an irregular clot shape with non-uniform length (i.e. longer along the greater curvature and shorter along the lesser curvature) (a). The lysis front can be seen to have changed from its initial flat shape to an oblique surface as lysis occurs more rapidly along the lesser curvature (b and c) until the re-establishment of flow. At this point, the remnant clot has a thick side attached to the arterial wall along its greater curvature and a thin side facing the flow (d). The remaining mural clot is gradually dissolved (e and f).

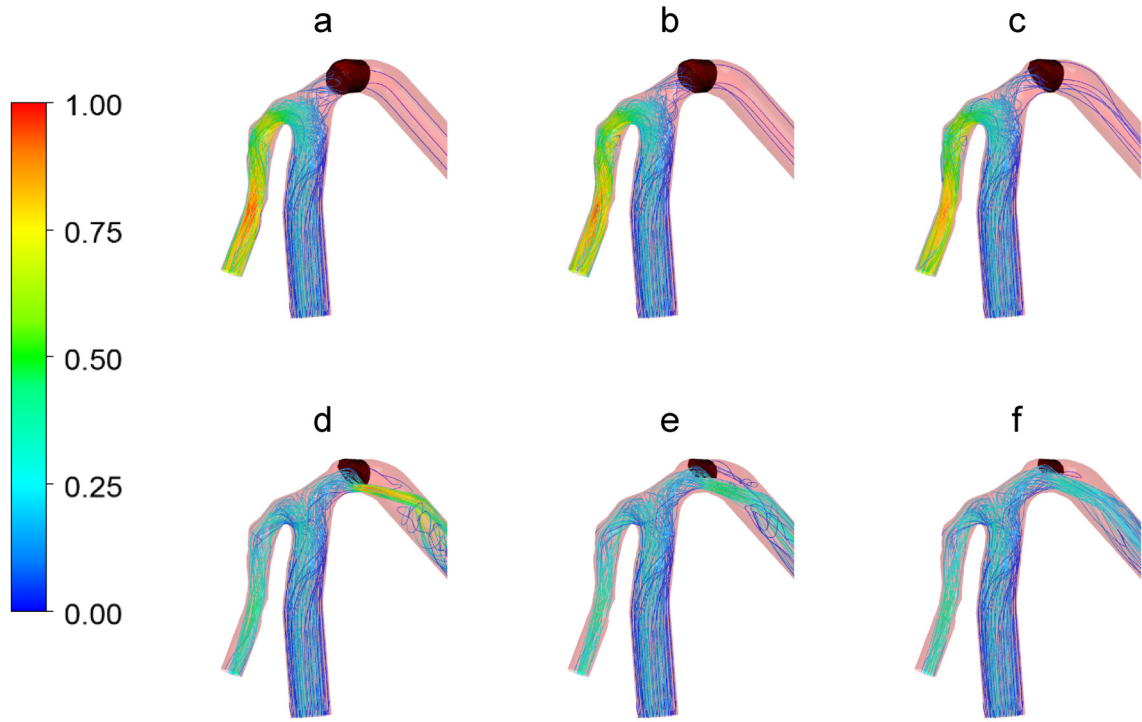


Figure 5.2: Velocity streamlines as the clot is dissolved over time. (a) 217 s, (b) 218 s, (c) 219 s, (d) 220 s, (e) 221 s, (f) 222 s. The clot is 3 mm in length and located at 3 mm from the ICA bifurcation.

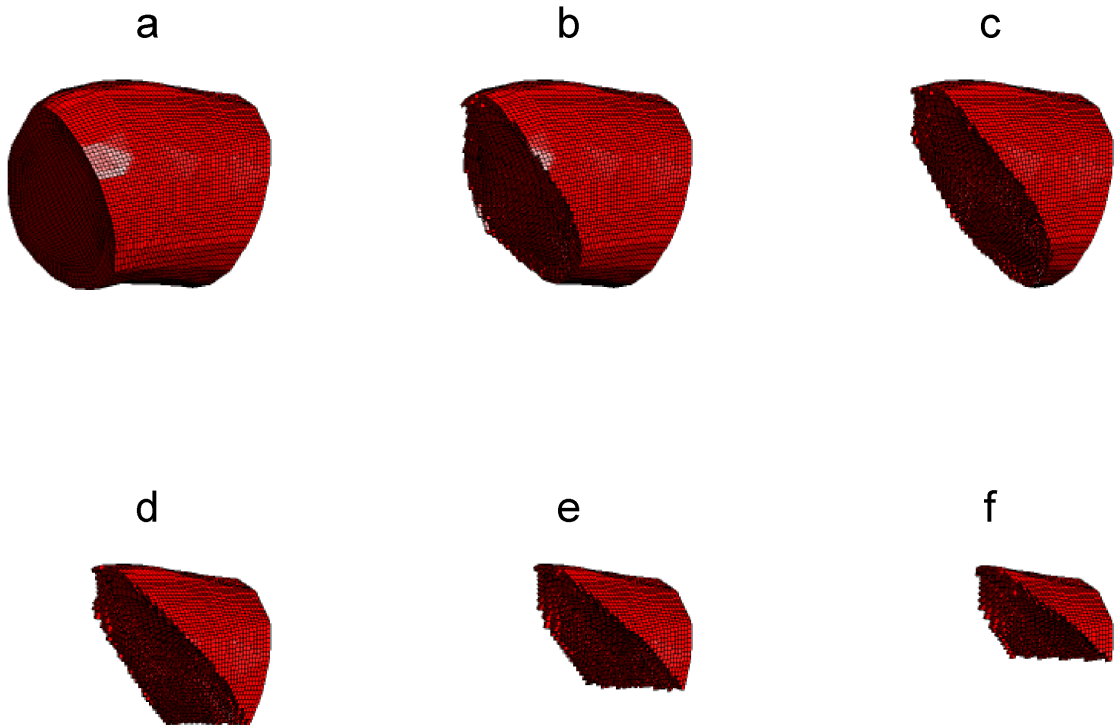


Figure 5.3: Clot lysis patterns as shown by isovolumes for $R_{clot} > 1 \text{ m}^{-2}$ for a 3 mm clot located at 3 mm from the ICA bifurcation. (a) 217 s, (b) 218 s, (c) 219 s, (d) 220 s, (e) 221 s, (f) 222 s.

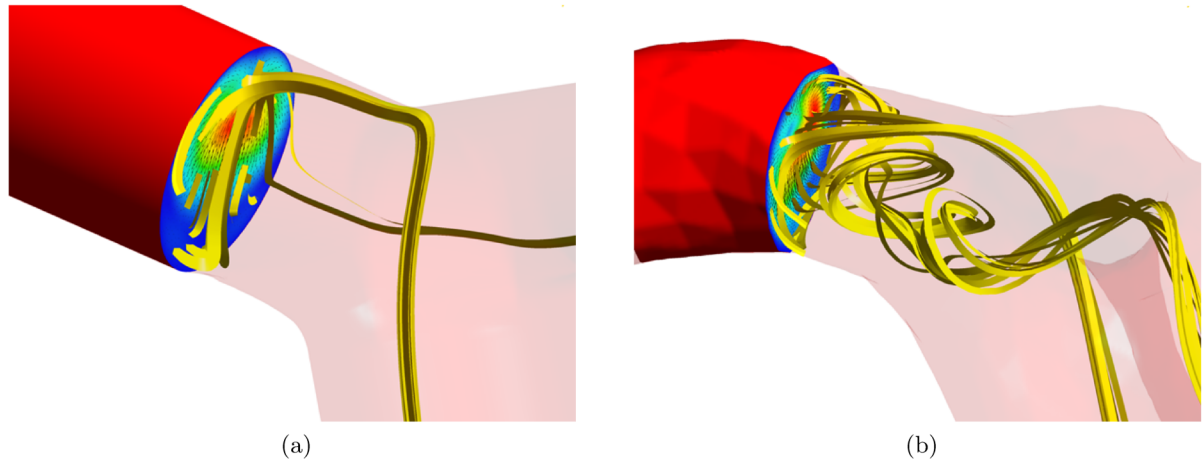


Figure 5.4: Velocity streamlines proximal to the clot surface for the idealised geometry (a) and the patient-specific geometry (b). Face of the clot is coloured according to velocity.

The difference in the clot lysis patterns may potentially be attributed to the shape of the clot and the geometry of the vasculature. The velocity streamlines close to the proximal face of the clot is presented in Figure 5.4 where particle paths are mapped out as yellow ribbons and their impact on the surface of the occlusion is described by local velocity values. The idealised geometry, shown in Figure 5.4 (a), shows very smooth and organised streamlines that hit the surface of the clot and are pushed back into the ACA segment while the patient-specific geometry, shown in Figure 5.4 (b), gives rise to more disturbed flow patterns proximal to the face of the clot.

5.3.2 Effect of Clot Length

The effect of clot length on the thrombolytic process is summarised in Figure 5.5. The ACA flowrate is at an initially high value of around 4.3 ml/s as it carries most of the flow from the inlet (Figure 5.5 a). The sudden drop in the ACA flowrate over the course of thrombolysis as clot breakthrough is achieved is mirrored by a corresponding increase in the flowrate at the MCA outlet (Figure 5.5 b). The changes in intra-arterial pressure at the ACA and MCA outlets follow a similar trend to their corresponding flowrates whereby an initially high ACA pressure of 150 mmHg drops to around 60 mmHg after clot dissolution (Figure 5.5 c). The pressure at the MCA increases from 0 mmHg before thrombolysis to over 60 mmHg following clot breakthrough (Figure 5.5 d). The results show that increasing the clot length has a profound effect on the time it takes for flowrate and pressure to return to physiologically normal levels.

The breakthrough time for different clot lengths is presented in Figure 5.6 (a). The breakthrough time for a 3 mm fibrin clot is around 3.6 mins which increases to 4 mins and 4.5 mins for a clot length of 6 mm and 9 mm, respectively. There is a substantial increase in the clot breakthrough time for a clot length of 12 mm (5.5 mins) which is more than 1.5 times that of the 3 mm clot. The extent of lysis (averaged over the entire clot domain) over time is given in Figure 5.6 (b). For a 3 mm clot the extent of lysis curve displays a sharp linear increase from 0 to 0.95, whereas those for longer clots begin to resemble a sigmoid function. For instance, the extent of lysis for a 12 mm clot accelerates at the start of thrombolysis and increases at a steady rate until it encounters a deceleration phase when clot breakthrough is approached.

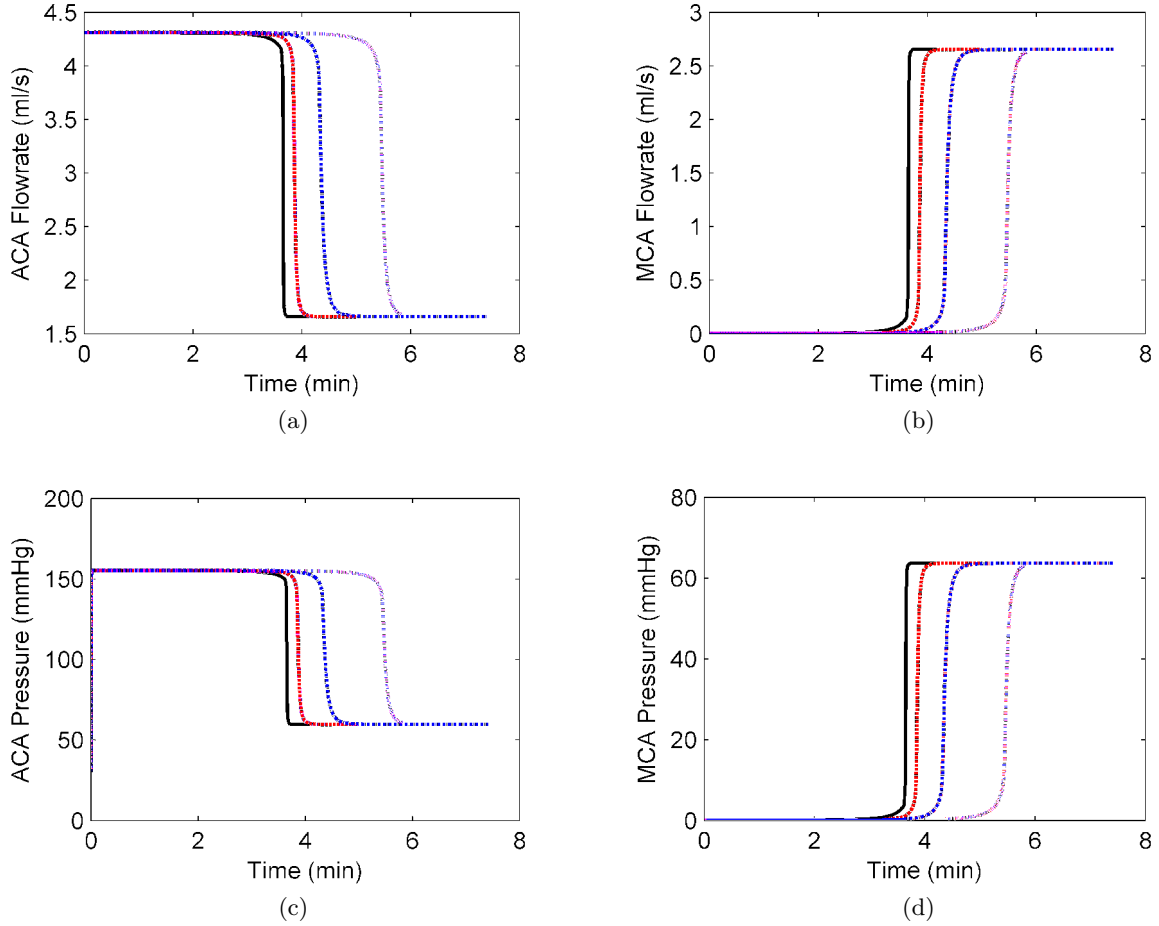


Figure 5.5: Flowrate and pressure over time for 3 mm (solid black), 6 mm (dash red), 9 mm (dash-dot blue) and 12 mm (dotted magenta) clot lengths. (a) ACA outlet flowrate, (b) MCA outlet flowrate, (c) ACA outlet pressure and (d) MCA outlet pressure.

3D velocity streamlines for the dissolution of each clot length (6 mm, 9 mm and 12 mm) are shown in Figure 5.7. Each row corresponds to lysis at different clot lengths and each column shows the velocity streamlines at the early, middle and late stages of thrombus dissolution. In each scenario, the clot blocks blood flow from entering the MCA during the early stages of clot lysis (a, d and g). As the blood clot is dissolved, more flow enters the MCA branch (b, e and h) until breakthrough is achieved and the flow in the MCA is re-established (c, f and i). The remaining clot attached to the arterial wall causes a localised increase in velocity due to flow constriction.

Clot lysis patterns at each scenario can be visualised in Figure 5.8. Initially, the clot has a rather blunt front (a, d and g). The lysis front becomes sharper as the clot is dissolved with its thicker side along the greater curvature and thinner side along the lesser curvature (b, e and h). After breakthrough is achieved, clot remnants are attached to the wall along the greater curvature until they are completely dissolved (c, f and i). The results indicate that although clot length has a strong influence on the breakthrough time, it seems to have a minor effect on the clot lysis pattern.

The normalised perfusion velocity in the clot is shown by velocity contours in Figure 5.9 for each initial thrombus length. The pattern is asymmetric, where a higher velocity occurs closer to the lesser curvature. The discrepancy between the perfusion velocity may account for the non-uniform lysis patterns seen in the patient-specific model. Furthermore, the maximum value of the perfusion

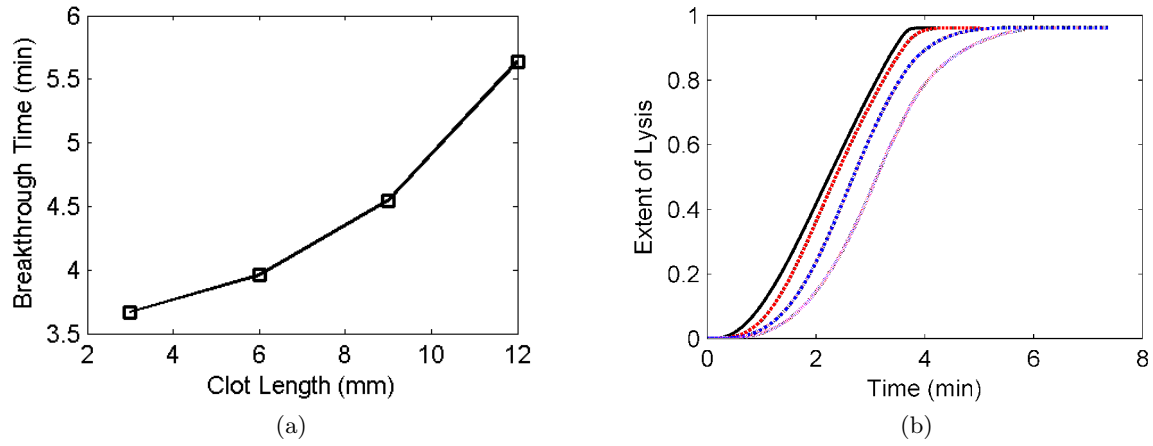


Figure 5.6: (a) Breakthrough time (as determined when flow in the MCA reaches 95% of final level) with respect to clot length and (b) the extent of lysis over time for clot lengths of 3 mm (solid black), 6 mm (dashed red), 9 mm (dash-dot blue) and 12 mm (dotted magenta).

velocity is noticeably different, with 0.27 mm/s, 0.17 mm/s and 0.13 mm/s for clot lengths of 6 mm, 9 mm and 12 mm, respectively. On the other hand, the distribution of the perfusion velocity for each clot length remains the same.

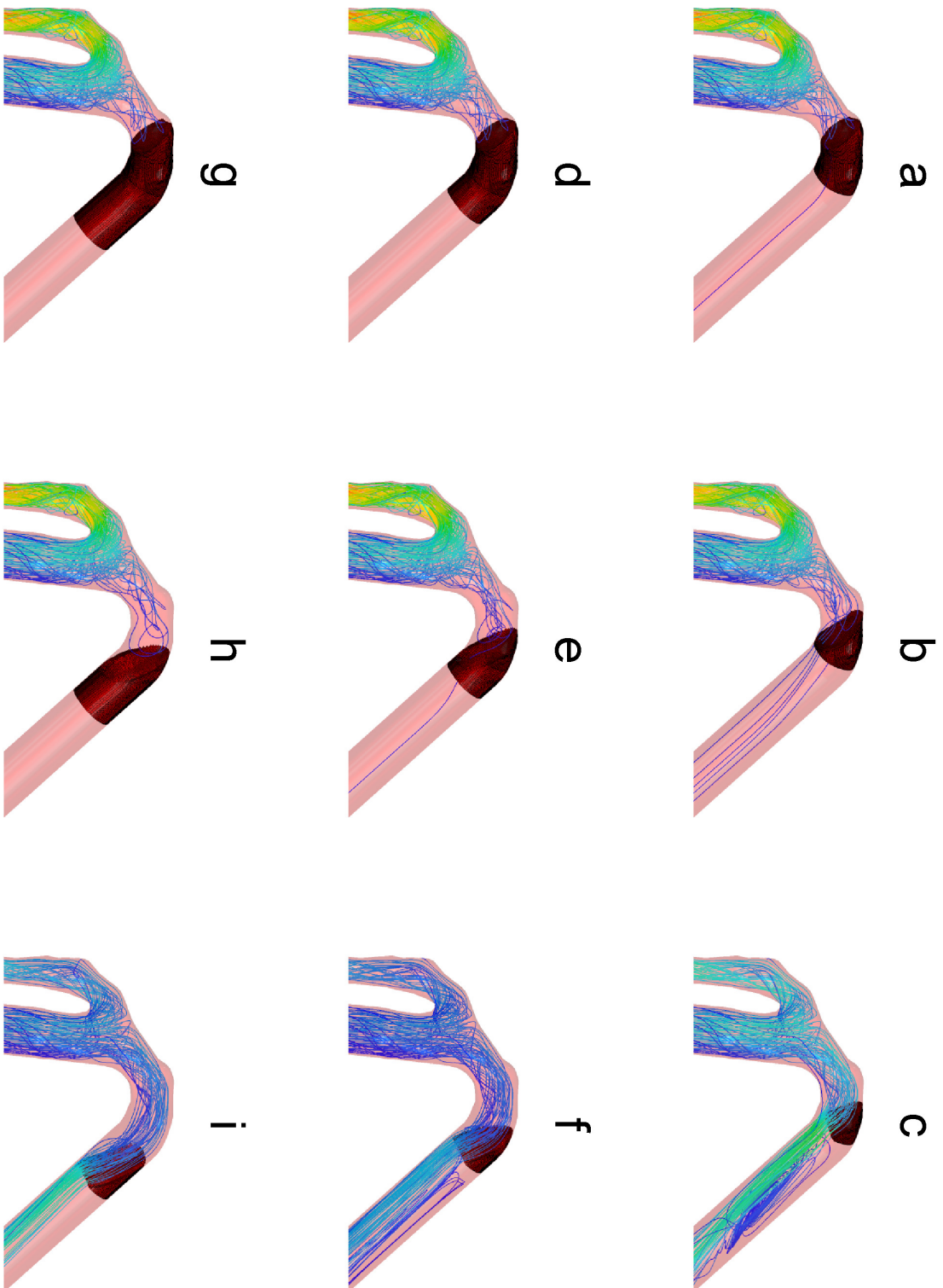


Figure 5.7: Velocity streamlines as the clot is dissolved over time for an initial clot length of 6 mm at (a) 220 s, (b) 230 s and (c) 240 s, an initial clot length of 9 mm at (d) 220 s, (e) 250 s and (f) 280 s and an initial clot length of 12 mm at 220 s (g), 280 s (h) and 340 s (i).

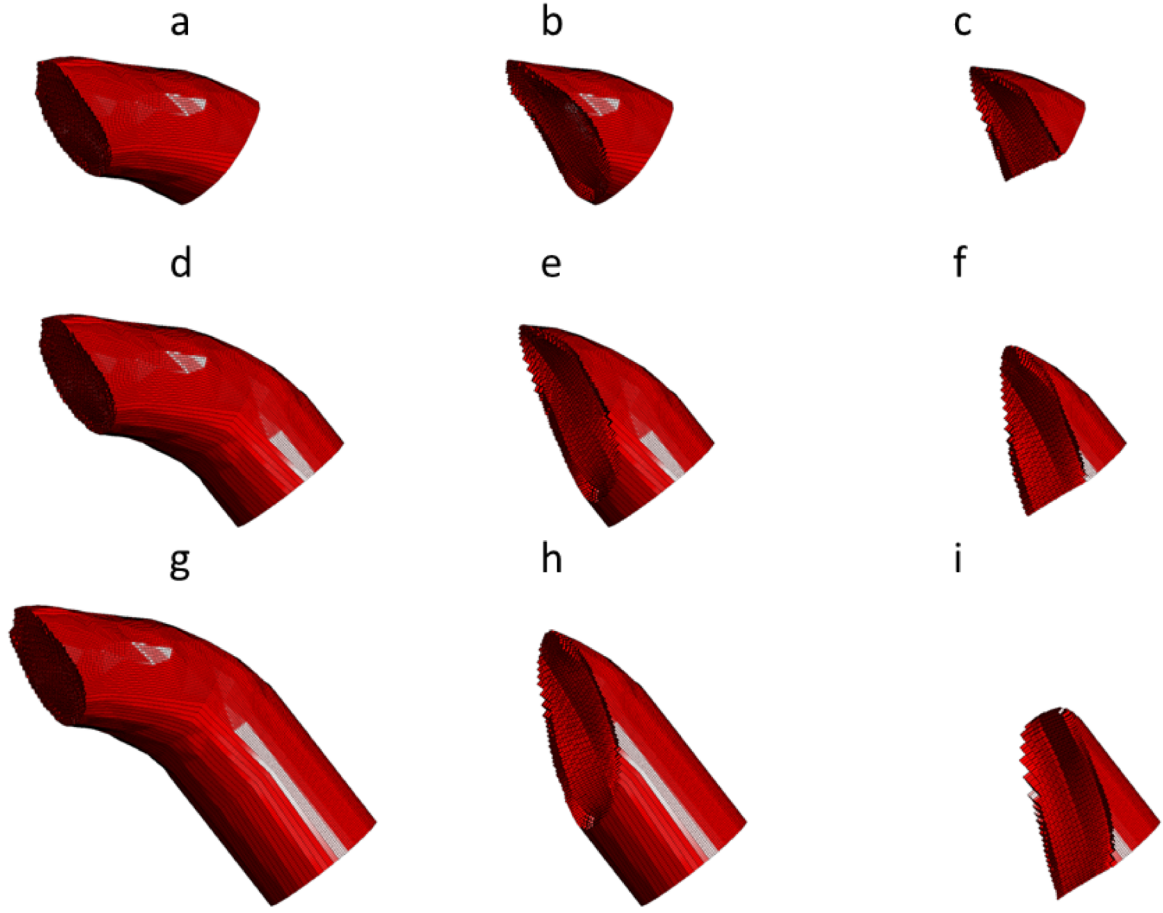


Figure 5.8: Clot lysis patterns as shown by isovolumes for $R_{clot} > 1$ for a clot at located at 3 mm from the ICA bifurcation with an initial length of 6 mm at (a) 220 s, (b) 230 s and (c) 240 s, an initial clot length of 9 mm at (d) 220 s, (e) 250 s and (f) 280 s and an initial clot length of 12 mm at 220 s (g), 280 s (h) and 340 s (i).

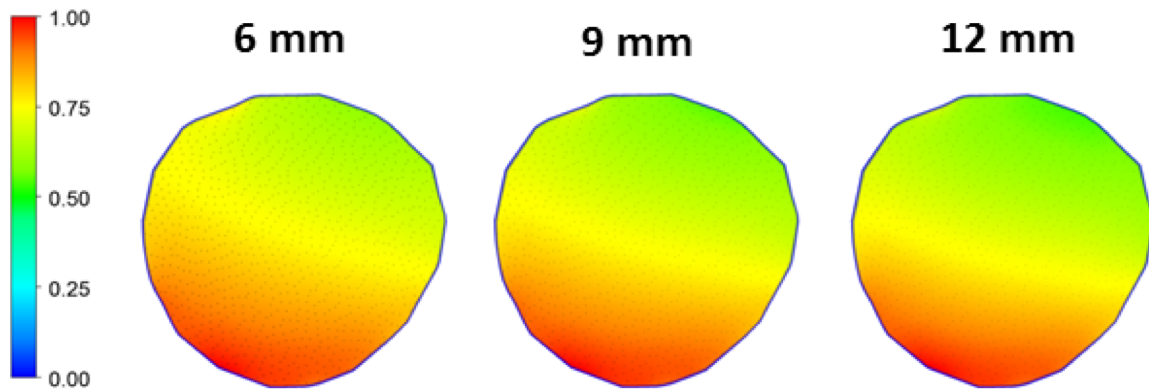


Figure 5.9: Local velocity values on plane perpendicular to the artery at 3 mm from the clot entrance for thrombi of different length. Contour values have been normalised according to the maximum velocity value for each case with 0.27 mm/s, 0.17 mm/s and 0.13 mm/s for initial clot lengths of 6 mm, 9 mm and 12 mm, respectively.

5.3.3 Effect of Clot Position

The effect of clot position on the lysis of a 3 mm fibrin clot can be seen in Figure 5.10. The initially high flowrate in the ACA decreases to a normal physiological level at around 3.7 mins as shown in Figure 5.10 (a). This is mirrored by a corresponding increase in the MCA flowrate (Figure 5.10 b). The position of the clot has a very limited effect on the rate of thrombolysis as shown by similar changes in the MCA and ACA flowrates. Figure 5.11 (a) shows that the breakthrough time is only slightly affected by the clot position along the MCA with a maximum difference of 0.05 min between the most proximal (3 mm from the ICA bifurcation) and the most distal (12 mm from the bifurcation) clot allowing for a more than 1% increase. The extent of lysis curves are almost identical for the different clot positions as shown in Figure 5.11 (b). All lysis curves show an initial acceleration in the degree of thrombolysis that then proceeds at a steady rate until the entire clot has been dissolved at just under 4 mins.

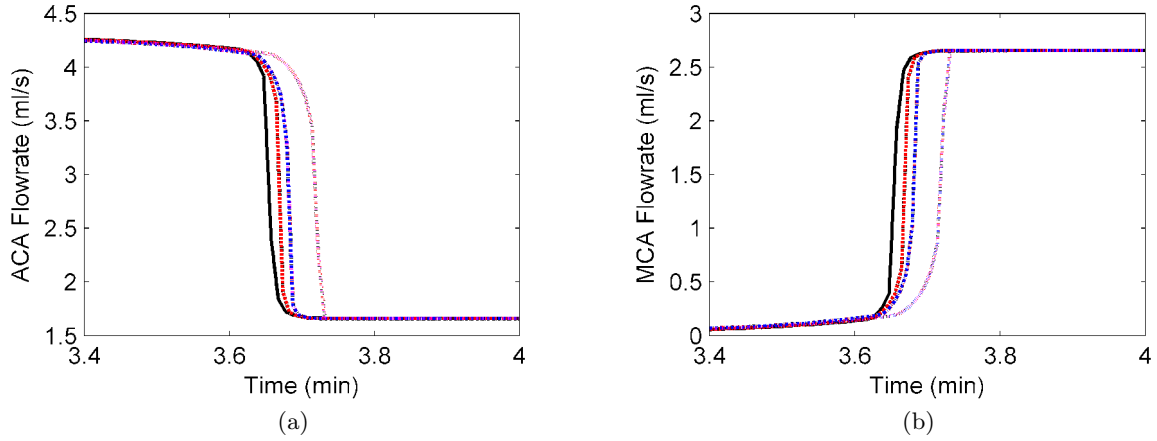


Figure 5.10: Variations of flowrate and pressure over time for different clot positions of 3 mm (solid black), 6 mm (dashed red), 9 mm (dash-dot blue) and 12 mm (dotted magenta) from the ICA bifurcation. (a) ACA outlet flowrate and (b) MCA outlet flowrate.

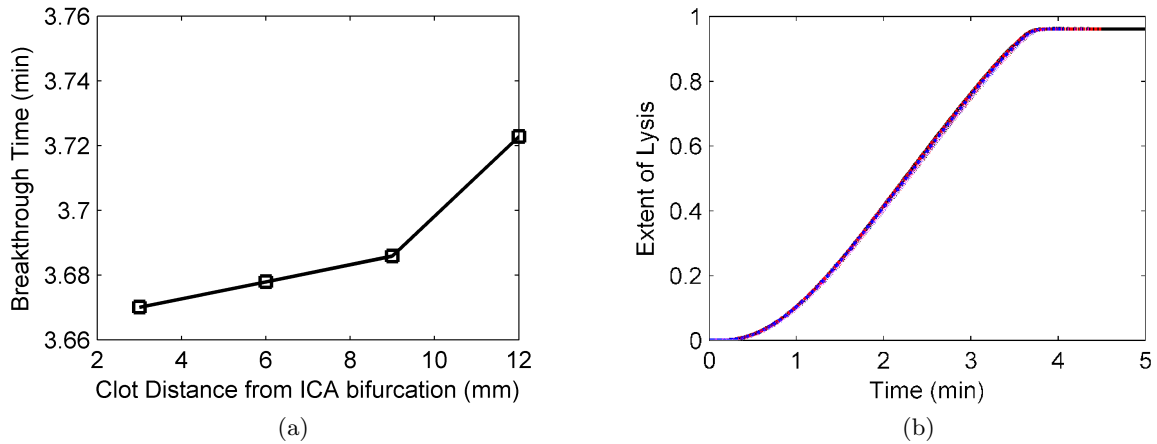


Figure 5.11: (a) Clot breakthrough time as a function of the distance of the clot from the ICA bifurcation and (b) the average extent of lysis over time for the dissolution of a 3 mm clot at different clot positions along the MCA segment of 3 mm (solid black), 6 mm (dashed red), 9 mm (dash-dot blue) and 12 mm (dotted magenta) from the ICA bifurcation.

Figure 5.12 shows the velocity streamlines during the dissolution of a 3 mm clot at different positions along the MCA. The first, second and third row show the lysis of a 3 mm clot that is 6 mm, 9 mm and 12 mm away from the ICA bifurcation during the early, mid and latter stages of thrombolysis (first, second and third column respectively). The results show that flow patterns, especially the size of the recirculation region proximal to the clot (a, d and g), are very sensitive to the position of the clot whereby a distance further away from the bifurcation allows for the development of a longer recirculation region. This change in the clot position can drastically affect the lysis patterns (b, e and h) and the shape of the remaining thrombus following clot breakthrough (c, g and i).

This effect can be observed more clearly in the clot lysis patterns presented in Figure 5.13. The lysis patterns for a 3 mm clot at 6 mm (a, b and c) and 9 mm (d, e and f) from the ICA bifurcation are similar in that the relatively blunt clot front becomes shaper as the clot is lysed, leaving a remaining clot partially attached to the wall following clot breakthrough. However, the clot that is 12 mm away from the ICA bifurcation has markedly different patterns of clot dissolution. In this scenario, the centre of the clot is the first zone to be lysed (g and h) and is carved out during thrombolysis until breakthrough is reached (i). The residual clot remains completely attached to the arterial wall and is lysed at a lower rate. The clot position has a limited effect on the breakthrough time but can cause significant changes in the clot lysis pattern.

5.4 Discussion

The previous chapter focused on the dissolution of a clot in an idealised geometry of the ICA bifurcation constructed using dimensions similar to those found in the literature. In this chapter the same computational model was used to investigate the dissolution of a homogeneous clot in a patient-specific geometry extracted from CTA scans. The reconstructed artery bears many geometric similarities to the idealised model in terms of arterial diameters and bifurcation angles (detailed in Table 5.1). The main geometric difference between the two models is the significant curvature of the ACA and MCA branches in the patient-specific model, which was neglected in the idealised geometry. As such, it is possible to determine the effect of realistic vessel curvatures on the process of thrombolysis by comparing results obtained with the patient-specific and idealised models. The time taken for clot breakthrough is similar in both the patient-specific and idealised models, occurring at approximately under 4 minutes. However, their clot lysis patterns are markedly different, with a distinctly non-uniform pattern in the patient-specific model. An initially blunt clot front is rapidly transformed into a sharp front as clot lysis proceeds (Figure 5.2 b and c) until flow is restored in the MCA segment (Figure 5.2 d, e and f). The idealised geometry showed that for an initial homogeneous fibrin clot, a uniform lysis front passes through the clot as reported in the literature (Wu et al.; 1994; Collet et al.; 2002). Non-uniform lysis patterns have been observed for heterogeneous clots where different fibrin concentrations (Anand et al.; 1997), or the presence of cellular components (Collet et al.; 2002) can alter lysis rates. In this study, we find that the curvature of the arterial geometry can also be responsible for the development of non-uniform lysis fronts.

The formation of a sharp lysis front in a patient-specific geometry could be due to 2 reasons. Firstly, the presence of the arterial curvature allows for the formation of a highly skewed velocity pattern that encourages the transport of the drug into a specific section of the clot. Figure 5.4 shows how the arterial geometry allows for a substantial variation in the velocity streamlines proximal to

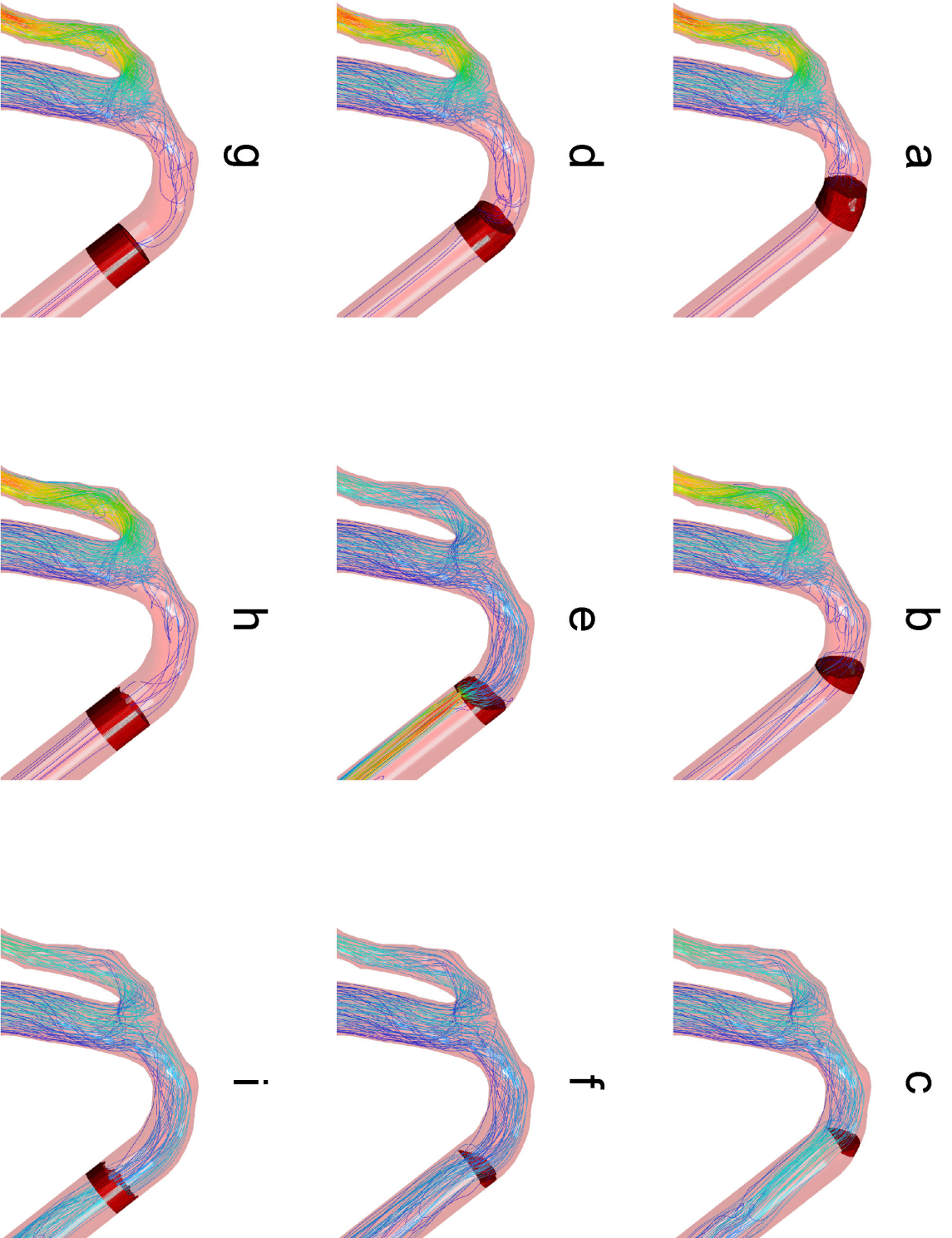


Figure 5.12: Velocity streamlines as the clot is dissolved over time for an initial clot length of 3 mm at a distance of 6 mm (a) 218 s, (b) 220 s and (c) 222 s, 9 mm at (d) 219 s, (e) 221 s and (f) 223 s and 12 mm at 220 s (g), 222 s (h) and 224 s (i) from the ICA bifurcation.

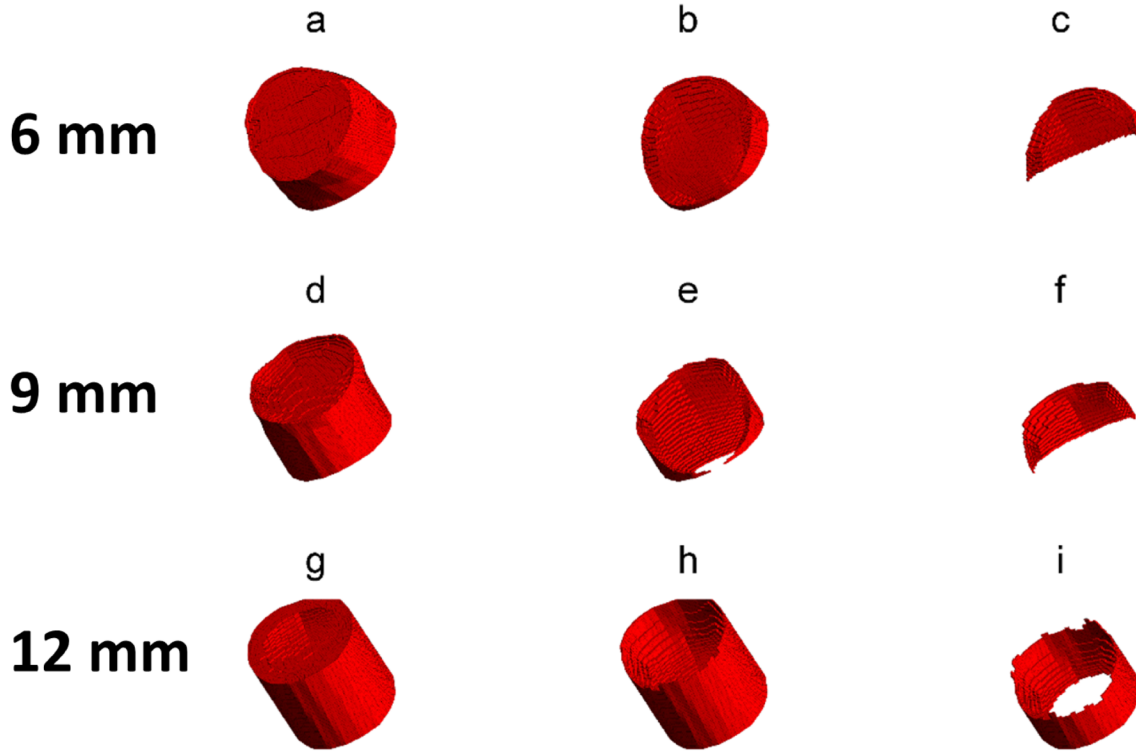


Figure 5.13: Clot lysis patterns as shown by isovolumes for $R_{clot} > 1$ for an initial clot length of 3 mm at a distance of 6 mm (a) 218 s, (b) 220 s and (c) 222 s, 9 mm at (d) 219 s, (e) 221 s and (f) 223 s and 12 mm at 220 s (g), 222 s (h) and 224 s (i) from the ICA bifurcation.

the clot surface which can allow for the build-up of tPA in certain regions. Secondly, although the length of the clot along the centreline is 3 mm, the curvature of the geometry allows for an initially inhomogeneous clot shape (Figure 5.3 a) where the length of one end is shorter than another. The pressure exerts a uniform force normal to the surface of the clot thereby causing an increase in the perfusion velocity through the shorter clot segment (close to the inner curvature of the arterial wall). This difference in the perfusion velocity (shown in Figure 5.9) consequently allows for the formation of a non-uniform lysis front.

The occurrence of mural thrombi have been visualised in clinical studies of ischemic stroke (Koroshetz and Ropper; 1987; Alexandrov et al.; 1994) and myocardial infarction (Vaitkus; 1995) and are considered risk factors for future embolisms. For instance, a study found that out of 28 patients who were suffering from cerebral infarction after thrombolytic treatment for myocardial infarction, 17 showed signs of cardiac abnormalities such as the presence of mural clots (Sloan et al.; 1997). The simulation results show that the occurrence of a mural clot following thrombolysis is affected by the geometry of the artery, and this may be an indicator of future complications. Mural clots can cause re-stenosis if not treated properly and may lead to distal embolisms. The presence of mural thrombi in the vasculature can also cause important changes in the flow whereby the residual clot that is partially attached to the wall constricts the artery and causes a local increase in the fluid velocity. This has been seen in clinical studies where Alexandrov et al. (1994) recorded an increase in the MCA velocity in the presence of a stenosis during ischemic stroke. Using transcranial Doppler sonography, the same authors found a peak systolic velocity of 3.3 m/s in the MCA and a corresponding velocity of 1.3 m/s in the ACA. Our results show a similar increase in the peak MCA velocity at around 2.5 times that

of the ACA (shown in Figure 5.2 d).

The effect of clot length on the rate of thrombolysis is significant as shown in Figure 5.5. An increase in clot length leads to a progressively longer time for the ACA and MCA to reach physiologically healthy values (Figure 5.5 a and b). The sudden drop in the ACA flowrate and the abrupt increase in the MCA flowrate after clot breakthrough are consistent with observations in clinical studies. For instance, [Groen et al. \(2010\)](#) evaluated the flow division between the internal carotid artery and the external carotid artery for patients suffering from carotid stenosis. The authors found that a stenosis of less than 65% didn't cause a significant change in the flow division between the 2 arteries. The abrupt opening of the MCA artery in the simulations of thrombolysis (Figure 5.3 d) allows a rapid increase in the MCA flowrate that restores the majority of the flow to the occluded artery. The presence of the mural clot after breakthrough is not large enough to severely restrict the flow of blood.

More importantly the pressure drop across the clot (as expressed by the difference in the MCA and ACA outlet pressures) remains the same for each clot length as shown in Figure 5.5 (c) and Figure 5.5 (d). Before thrombolysis, the pressure drop is around 150 mmHg and then drops to 0 after clot breakthrough has been achieved. This can explain why the time till clot breakthrough seems to increase as a function of clot length (Figure 5.6 a). Darcy's law states that the perfusion velocity through a porous domain is inversely proportional to its length. Since the pressure drop across the clot is not significantly affected by the clot length, the perfusion velocity decreases for longer clots (shown in Figure 5.9). The analytical model of [Zidansek and Blinc \(1991\)](#) showed that clot lysis occurs in 2 stages. Initially, the clot undergoes dissolution until it reaches a critical point that allows for the progression of a lysis front through the clot. This lysis front proceeds at a similar rate as the perfusion velocity; hence the slower rate of clot lysis at lower velocities. This also explains that the 'widening' of the lysis curve shown in Figure 5.6 (b) is caused by a slower lysis front velocity that takes a longer time to reach dissolution. The exponential increase in the dissolution time as a function of clot length (due to both the presence of a longer clot and the decrease in the perfusion time) can explain why longer clots (>10 mm) are typically associated with low recanalisation rates.

The effect of clot length on clot lysis patterns and the corresponding flow patterns (shown in Figure 5.5 and Figure 5.10) is more subtle. The early stages of clot lysis begin at around the same time for all clot lengths (220 s). This is expected as the early stages of dissolution are only dependent on kinetic parameters such as the initial tPA and PLG concentrations and the kinetics of PLG activation and clot dissolution ([Zidansek and Blinc; 1991](#)). Clot lysis seems to follow the same pattern regardless of the clot length albeit at considerably different rates for 3 mm, 6 mm, 9 mm and 12 mm clot dissolution (Figure 5.5). The rate of clot dissolution has been found to be highly variable in clinical studies whereby the restoration of flow (as measured by transcranial Doppler) has been found to occur either rapidly over 35 s or in a step wise manner over 11 minutes ([Alexandrov et al.; 2001](#)). The latter shows high intensity bursts potentially due to partial recanalisation of the embolus and a residual mural clot that causes high local velocities (as predicted by the simulation in Figure 5.5). Furthermore, a longer clot allows for the appearance of a larger mural thrombus after breakthrough has been reached (Figure 5.5 c, f and i). Thus the length of the clot not only plays an important role in determining the rate of clot dissolution but can also affect the size of the final mural clot that appears.

Simulation results obtained with varying clot positions along the MCA show no considerable difference in the breakthrough time. The flowrates at the ACA and MCA outlets decreased and increased at a similar rate respectively (shown in Figure 5.10 a and b). The breakthrough time

increases slightly as a function of clot distance from the ICA bifurcation up to 9 mm. This is expected since the further away a clot is from the inlet, the longer it takes for the drug to reach it. On the other hand, the recirculation region at the proximal face of the occlusion (Figure 5.12 a and d) increases the drug residence time there. However, the delay in clot dissolution due to the distance of the thrombus becomes more apparent when the clot is around 12 mm away from the ICA bifurcation. The position of the clot in this scenario allows for the formation of a large stagnant region proximal to the clot surface (shown in Figure 5.12 g) that begins to impede drug transport. Nevertheless, the difference in lysis time is still very small ($\sim 1\%$ between 3 mm and 12 mm from the bifurcation).

Although, the average extent of lysis appears to be identical for different clot positions (Figure 5.11 b), there is a significant difference in the clot lysis pattern. Clot dissolution at distances of 3 mm, 6 mm and 9 mm away from the ICA bifurcation appears to yield similar clot lysis patterns as shown in Figure 5.13. A non-uniform lysis front pushes the dissolution of the occluding clot until breakthrough is achieved and a mural thrombus remains. However, for the lysis of a clot that is 12 mm away from the ICA bifurcation, the lysis pattern is significantly altered (Figure 5.13 g, h and i). The central component of the clot is dissolved first, leaving a ring shaped mural clot following breakthrough. This scenario is similar to previous studies of clot lysis (Sersa et al.; 2007b) and was analysed in Chapter 3. The position of the clot affects the velocity distribution proximal to the face of the clot and this consequently is expected to cause alterations in the clot lysis patterns. We speculate that differences in the clot lysis patterns and the dissolution times will become more profound the further away the clot is from the ICA bifurcation.

Although the position of the clot has been found to have limited effects on the breakthrough time, the large region of flow stasis in the 12 mm case can give rise to complications. Regions of flow stasis can cause thrombosis and this consequently may cause the clot to grow in size before the administration of tPA (as speculated by Qazi et al. (2015)) thereby increasing the time till clot dissolution. However, clinical studies have shown that the more distal the clot is to the ICA, the more likely it is that the patient will have a favourable outcome after thrombolytic therapy (Kamalian et al.; 2013). This is suspected to be due to the prevalence of long clots in the proximal region of the MCA as compared to the distal regions (Friedrich et al.; 2015). Our studies show that the clot position alone has a negligible effect on the clot dissolution time and that the clot length can significantly alter recanalisation rates. Thus the dissolution of distal clots may be highly dependent on the state of collateral circulation that may help to dissipate the stagnant region (Qazi et al.; 2015).

Finally, it should be mentioned that results presented in this chapter are limited to the specific conditions adopted in the computational model such as for instance the assumption of a fibrin clot formed at physiological levels of fibrinogen (3mg/ml) (Collen et al.; 1972b). There exist a variety of clot compositions that can alter their resistance by up to 2 orders of magnitude (Diamond; 1999). Furthermore, the initial heterogeneous nature of the clot is expected to severely affect the clot lysis patterns as seen in the literature (Anand et al.; 1997). Retracted clots in particular have a very interesting architecture with tessellated erythrocytes localised in the centre of the clot and surrounded in the periphery by a network of fibrin fibres and platelets (Cines et al.; 2014). The impact of this on the clot lysis patterns and dissolution patterns should be examined.

5.5 Conclusion

In this chapter, a computational model of thrombolysis is applied to a patient-specific ICA-ACA-MCA bifurcation with an occluding clot in the M1 segment. It has been found that the geometric shape of the bifurcation, especially the nonplanar curvature of the branches can have a strong impact on clot lysis patterns. The results show that clot dissolution can produce a mural clot that causes a local increase in blood velocity as seen in clinical studies. The length of the clot in a M1 MCA occlusion is found to have important effects on the breakthrough time but not on the clot lysis pattern. The clot position, on the other hand, has a limited effect on the clot dissolution time for up to 9 mm from the ICA bifurcation. However, there is a modest increase in clot breakthrough time for a 3 mm clot located at 12 mm from the ICA bifurcation and a drastic change in clot lysis pattern. The finding that clot breakthrough time increases exponentially with the length of clot for longer clots support clinical observations that patients with longer clots past a certain threshold are less likely to receive benefit from thrombolytic therapy.

Chapter 6

Simulating Thrombolytic Therapy in Ischemic Stroke

6.1 Introduction

The treatment of ischemic stroke via thrombolytic therapy involves intravenous infusion of tPA. During this process, reactions take place in the plasma that alter the concentrations of lytic proteins such as PLG, AP and fibrinogen (Noe and Bell; 1987). The concentration of fibrinogen is particularly important in ascertaining the risk of bleeding as low levels of this protein have been implicated in the occurrence of ICH (Vandelli et al.; 2015). Skeik et al. (2013) for example found that patients with fibrinogen concentrations less than 1.5 mg/ml had increased susceptibility to bleeding complications. The occurrence of ICH is one of the major limiting factors in applying thrombolytic therapy to a wide variety of patients. As such, over the last 2 decades, there has been a drive to reduce the amount of drug needed to recanalise an occluded artery during ischemic stroke.

One of the major problems involved in reducing the tPA dose given to patients is concerned with its pharmacodynamics. The half-life of tPA is around 4 minutes (Tanswell et al.; 1989) and as such requires a continuous dose to maintain a sufficiently high concentration for clot dissolution. Currently, thrombolytic therapy involves the infusion of alteplase at 0.9 mg/kg with 10% given as a 1 minute bolus infusion and the remaining 90% given over a continuous 1 hour infusion. This is the procedure adopted in the NINDS clinical trial (NINDS; 1995) which led to the FDA approval and wide-spread adoption of thrombolytic therapy for ischemic stroke.

However, other clinical trials have used different tPA doses for the treatment of ischemic stroke. For instance, the ECASS clinical trial used a larger infusion dose of 1.1 mg/kg (Hacke et al.; 1995) while a string of other studies have used tPA doses as low as 0.3 mg/kg (Dong et al.; 2016). The J-MARS study evaluated the use of 0.6 mg/kg dose of tPA for the treatment of ischemic stroke and found low levels of ICH (<5%) and relatively high levels of functional outcome at 3 months (>30%) (Nakagawara et al.; 2010). As such, the optimal tPA dosing regimen remains contested and needs to be addressed in order to enhance the treatment of ischemic stroke with thrombolytic therapy.

In order to help assess the effectiveness of different tPA dosing regimens, a mathematical model has been developed that can evaluate clot lysis patterns and the risk of ICH for different therapies. This is achieved by integrating the computational model presented in the previous chapters with a new

compartmental model which describes the temporal evolution of tPA, PLG, PLS and AP in plasma. This will not only ensure that different infusion regimens are accounted for but will also help assess the rate of potential complications associated with high tPA and low fibrinogen concentrations.

In this study, the efficacy of thrombolysis is evaluated at low (0.6 mg/kg), normal (0.9 mg/kg) and high (1.2 mg/kg) alteplase doses. Numerical studies are based on an 80 kg patient with a 3 mm clot completely occluding the proximal MCA artery. The effectiveness of the treatment is determined by the breakthrough time, i.e. the time it takes for 95% of the flow to be returned in the occluded MCA artery.

6.2 Methods

6.2.1 Compartmental Model

In order to evaluate the temporal variations of lysis proteins during IV infusion of a plasminogen activator, a compartmental model is needed. Plasminogen activators such as tPA have relatively low half-lives compared to other proteins (such as PLG) and therefore rely on a steady infusion rate to maintain sufficiently high plasma concentrations. The major source of consumption of PLG is due to PLG activation by a plasminogen activator. This results in the production of PLS that then cleaves the fibrinogen molecules in plasma. PLS has a very low half-life and is quickly inhibited by AP. The role of PAI has been neglected as its low concentration in the plasma has a minor effect on changing the pharmacological level of tPA during thrombolytic therapy. PLG, AP and fibrinogen (Fbg) are present at high levels in the plasma (2.2 μM , 1 μM and 8 μM respectively) and are continuously generated. This system can be described by equations 6.1-6.5.

$$\frac{dC_{tPA}}{dt} = \frac{I(t)}{V_{plasma}} - k_{HC}C_{tPA} \quad (6.1)$$

$$\frac{dC_{PLG}}{dt} = -k_{HC,PLG}C_{PLG} - \frac{k_{2,f}C_{tPA}C_{PLG}}{K_{M,f} + C_{PLG}} + G_{PLG} \quad (6.2)$$

$$\frac{dC_{PLS}}{dt} = -k_{HC,PLS}C_{PLS} + \frac{k_{2,f}C_{tPA}C_{PLG}}{K_{M,f} + C_{PLG}} - k_{AP}C_{AP}C_{PLS} \quad (6.3)$$

$$\frac{dC_{Fbg}}{dt} = -k_{HC,Fbg}C_{Fbg} + G_{Fbg} - k_{cat,f}C_{Fbg}C_{PLS} \quad (6.4)$$

$$\frac{dC_{AP}}{dt} = -k_{HC,AP}C_{AP} - k_{AP}C_{PLS}C_{AP} \quad (6.5)$$

where V_{plasma} represents the volume of plasma circulating in the human body, $k_{HC,\alpha}$ is the rate of haptic clearance of species α , $k_{2,f}$ and $K_{M,f}$ are the Michaelis-Menten coefficients relating to the rate of PLG activation in the free phase, G_α refers to the rate of generation of species α in the blood stream and $I(t)$ is the rate of infusion of plasminogen activator into the system as shown in the conditional equation 6.6.

$$I(t) = \begin{cases} 0.1\alpha_{tPA}w/t_B, & \text{if } t \geq t_0 \text{ and } t < (t_0 + t_B) \\ 0.9\alpha_{tPA}w/t_C, & \text{if } t \geq (t_0 + t_B + t_D) \text{ and } t < (t_0 + t_B + t_D + t_C) \\ 0, & \text{otherwise} \end{cases} \quad (6.6)$$

Table 6.1: Kinetic constants for the compartment model

Symbol	Value	Units	Source
$C_{AP,0}$	1	μM	Cederholm-Williams (1981)
$C_{Fbg,0}$	5-13 (8)	μM	Noe and Bell (1987); Anand and Diamond (1996), Collen et al. (1972b)
$C_{PLG,0}$	2.0-2.5	μM	Anand and Diamond (1996), Collen et al. (1972a)
$C_{PLS,0}$	0	-	Anand and Diamond (1996)
$C_{tPA,0}$	~ 0	-	Booth and Bennett (1994)
G_{AP}	1.82×10^{-4}	$\mu M min^{-1}$	Using equation 6.8
G_{Fbg}	2.67×10^{-4}	$\mu M min^{-1}$	Using data from Noe and Bell (1987)
G_{PLG}	5.48×10^{-4}	$\mu M min^{-1}$	Using data from Noe and Bell (1987)
G_{PLS}	0	-	Using equation 6.8
G_{tPA}	0	-	Using equation 6.8
$k_{2,f}$	0.3	s^{-1}	Tiefenbrunn et al. (1986)
k_{AP}	10	$\mu M^{-1} s^{-1}$	Anand and Diamond (1996)
$k_{cat,f}$	6	$\mu M^{-1} s^{-1}$	Using data from Noe and Bell (1987)
$k_{HC,AP}$	1.82×10^{-4}	min^{-1}	Using equation 6.7
$k_{HC,Fbg}$	1.16×10^{-4}	min^{-1}	Using equation 6.7
$k_{HC,PLG}$	2.19×10^{-4}	min^{-1}	Using equation 6.7
$k_{HC,PLS}$	416	min^{-1}	Using equation 6.7
$k_{HC,tPA}$	0.183	min^{-1}	Using data from Tanswell et al. (1989)
K_M	28	μM	Tiefenbrunn et al. (1986)
$t_{1/2,AP}$	2.64	days	Collen and Wiman (1979)
$t_{1/2,Fbg}$	4.14	days	Collen et al. (1972b)
$t_{1/2,PLG}$	2.2	days	Collen et al. (1972a)
$t_{1/2,PLS}$	0.1	s	Lijnen and Collen (1985)
$t_{1/2,tPA}$	~ 4	mins	Noe and Bell (1987); Tanswell et al. (1989)
V_{plasma}	3.9	L	Max. volume for 80 kg patient from Collen et al. (1972b)

where w is the weight of the patient, α_{tPA} is the tPA dose (in mg/kg bodyweight), t_0 refers to the time at the start of the infusion, t_B is the duration of bolus infusion, t_D is the time delay between the bolus and continuous infusions and t_C is the duration of continuous infusion. The hepatic clearance coefficient $k_{HC,\alpha}$ can be calculated by using the half-life of species α ($t_{1/2,\alpha}$) as given in equation 6.7. The rate of generation of each species can be determined by assuming that it is counterbalanced by the rate of hepatic clearance at equilibrium. Assuming that the rate of generation is constant over time, it can be calculated using equation 6.8 where $C_{\alpha,0}$ is the initial concentration of species α . The values for all the kinetics constants can be found in Table 6.1.

$$k_{HC,\alpha} = \frac{\ln(2)}{t_{1/2,\alpha}} \quad (6.7)$$

$$G_{\alpha} = k_{HC,\alpha} C_{\alpha,0} \quad (6.8)$$

6.2.2 3D Model of Thrombolysis

In order to model thrombolysis in 3D patient-specific geometries, the same approach adapted in the previous chapter is used. The movement and reaction of lysis proteins is modelled using convection-diffusion-reaction transport equations while the degradation of the clot is related to the cleavage of binding sites on the fibrin fibres. Blood flow is described using the continuity and Navier-Stokes equations, where the resistance of blood clot to fluid flow is accounted for by a Darcian momentum source term. As the clot is dissolved, the permeability increases and the resistance correspondingly decreases to 0.

A steady flowrate is specified at the inlet and pressures at the outlets are defined using a 3-element lumped parameter Windkessel model. The compartmental model described in the previous section is used to provide temporal changes in the concentration of lysis proteins at the inlet for a given therapy. This model is solved with the 4th order Runge-Kutta method while the equations governing the fluid flow and transport equations are solved using the finite volume method implemented in Ansys Fluent. The parameters for the compartmental model are specified in Table 6.1, while the constants for the clot dissolution model are the same as those used in chapter 4.

A schematic diagram of the entire integrated model is shown in Figure 6.1. The model requires specification of the drug infusion regimen (e.g 7.2 mg initial bolus over one minute followed by 64.8 mg continuous over 60 minutes) and the initial concentrations of proteins in the plasma. The compartmental model solves for the temporal change of each free species at every time-step, which then provides the inlet concentration of each species for the 3D model. The clot dissolution model provides spatial and temporal variations in blood velocity, pressure and clot lysis patterns.

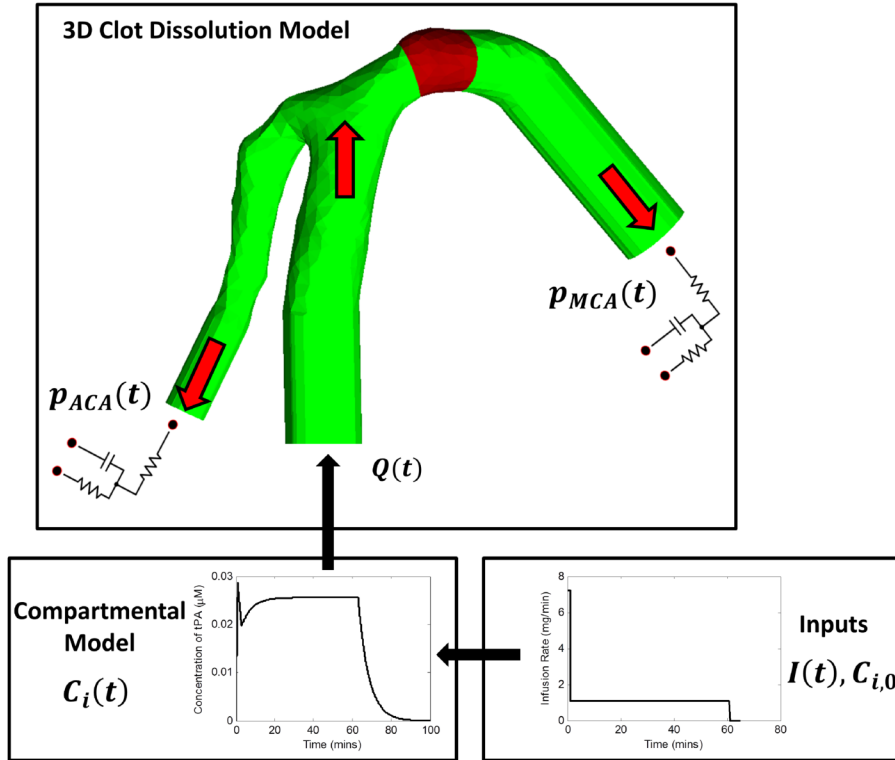


Figure 6.1: Overview of the computational model. Compartmental model solves transient plasma concentrations of free phase proteins for a given therapeutic regimens which are then used as inputs for the 3D clot dissolution model.

6.3 Results

6.3.1 Validation of the Compartmental Model

The compartmental model was validated against experimental data in the literature. [Noe and Bell \(1987\)](#) collected blood samples from patients suffering from myocardial infarction who were treated with a continuous rt-PA infusion of 0.5 mg/kg over a 60 minute period. The plasma concentrations of PLG and fibrinogen for 13 patients were evaluated before drug infusion and at 1.5, 2, 4 and 24 hours after treatment. The average change in the lysis proteins over time for these patients be found in Figures ?? (a and b) where solid lines show the corresponding predictions by the computational model for an initial concentration of $1.21 \mu M$, $8 \mu M$ and $1 \mu M$ for PLG, fibrinogen and AP respectively. The activation and generation of PLG show a good agreement with the experimental data using the constants defined in Table 6.1. The reduction in the fibrinogen concentration on the other hand had to be fitted with a k_{cat} value of $23 \mu M^{-1} s^{-1}$ to ensure a good agreement.

[Tanswell et al. \(1989\)](#) evaluated the plasma concentrations of tPA for 12 healthy patients after 30 minute infusions of either 0.25 mg/kg or 0.5 mg/kg tPA over a 3 hour period. Figure 6.2 (c) shows a comparison between the measured and predicted tPA concentration. During infusion, a hepatic clearance coefficient of 0.183 min^{-1} (corresponding to a tPA half-life of just under 4 minutes) was found to have a good agreement with the experimental data at both tPA concentrations.

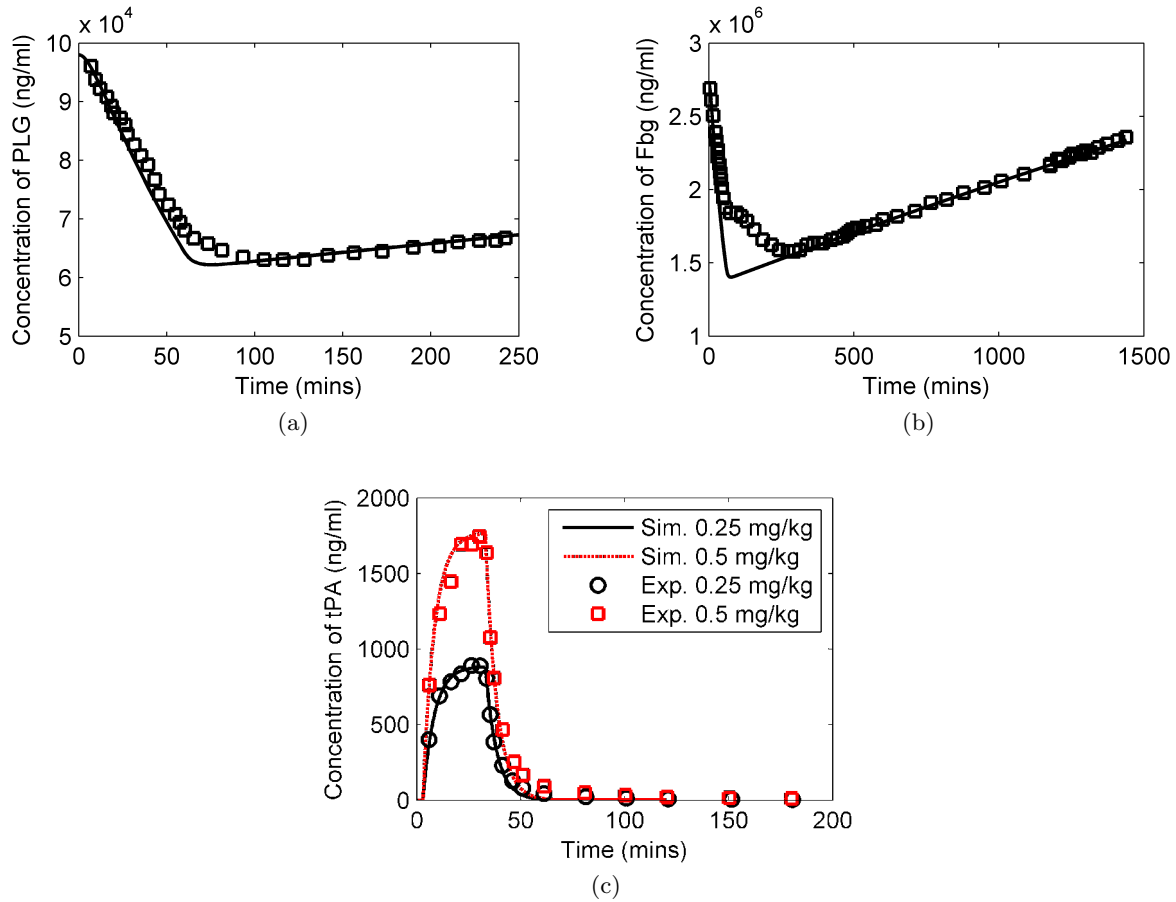


Figure 6.2: Comparison of simulation results of the compartmental model and experimental data for plasma concentrations of (a) PLG ([Noe and Bell; 1987](#)), (b) fibrinogen ([Noe and Bell; 1987](#)) and (c) tPA ([Tanswell et al.; 1989](#)).

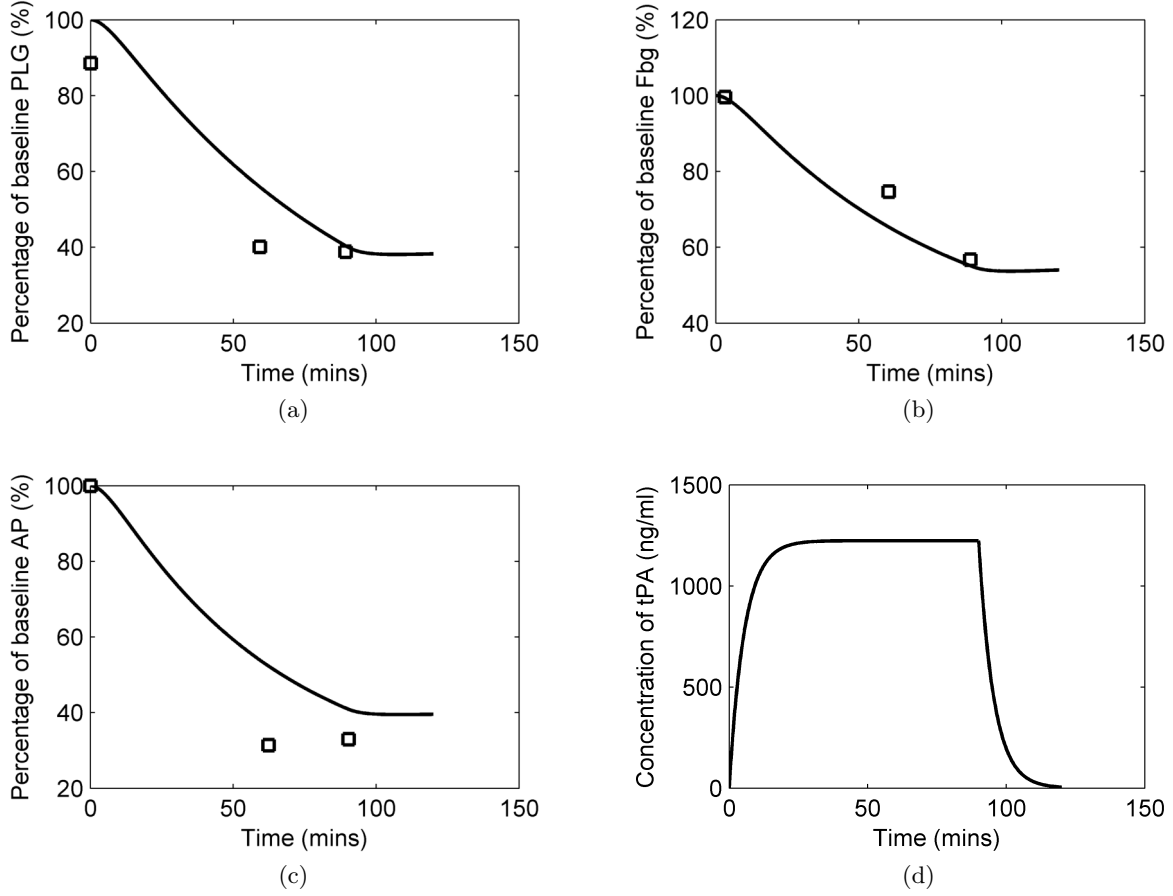


Figure 6.3: Comparison of simulation results with experimental results from [Collen et al. \(1986\)](#) for plasma concentrations of (a) PLG, (b) fibrinogen, (c) AP and (d) tPA.

[Collen et al. \(1986\)](#) measured the concentrations of PLG, fibrinogen and AP of 101 patients who received a 0.75 mg/kg rt-PA infusion dose over a 90 minute period. The concentrations were evaluated with respect to changes in the baseline value at 0, 60 and 90 minutes after infusion as shown for PLG, fibrinogen and AP in Figures 6.3 (a, b and c), respectively. The plasma tPA concentration predicted by the model is shown in Figure 6.3 (d) and is consistent with [Collen et al. \(1986\)](#)'s observation of a steady rt-PA concentration of 1200 ng/ml following infusion. The PLG concentration shows a reasonably good agreement with the experimental data using the same Michaelis-Menten reaction coefficients given in Table 6.1 and an initial PLG concentration of $2 \mu M$. Using a $k_{cat,f}$ value of $23 \mu M^{-1}s^{-1}$ was found to lead to a significantly more rapid degradation of fibrinogen than those measured by [Collen et al. \(1986\)](#). As such, a value of $6 \mu M^{-1}s^{-1}$ for $k_{cat,f}$ was used as it gave a better prediction for the degradation of the fibrinogen over time for an initial concentration of $8 \mu M$ as shown in Figure 6.2 (b). The concentration of the primary lysis inhibitor AP was initialised with an assumed value of $1 \mu M$ with the same reaction coefficients as those given in Table 6.1. The results show an over prediction of the percentage of remaining AP following tPA infusion.

6.3.2 Infusion of Alteplase during Ischemic Stroke

Using the values defined in Table 6.1, changes in plasma concentrations of PLG, tPA, AP, PLS and fibrinogen can be determined for different tPA infusion regimens. As mentioned before, the recommended dose of tPA can differ by country (0.9 mg/kg in the USA vs. 0.6 mg/kg in Japan) and

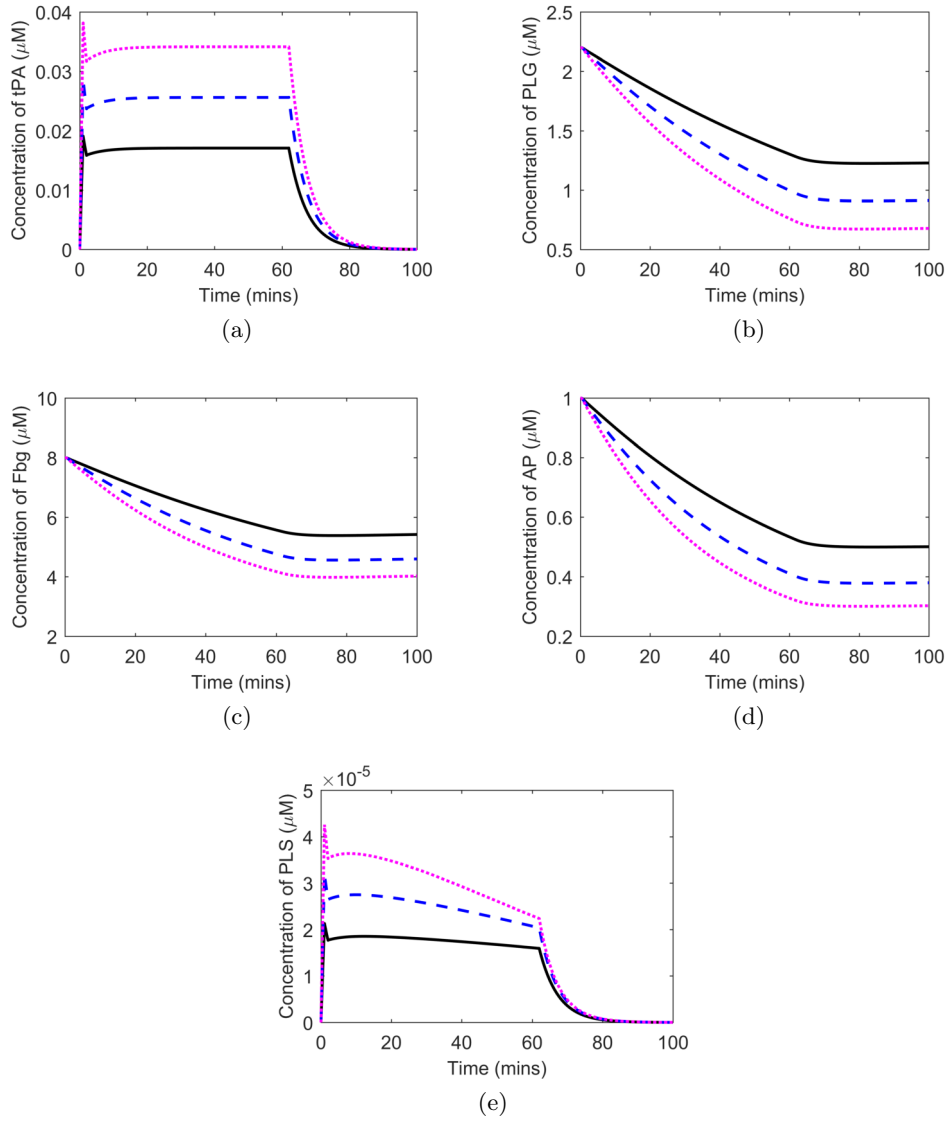


Figure 6.4: Simulation results for different tPA regimens with a dose of 0.6 mg/kg (solid black line), 0.9 (mg/kg) (dotted red line) and 1.2 mg/kg (dashed blue line). Free phase concentrations are given for (a) tPA, (b) PLG, (c) fibrinogen, (d) AP and (e) PLS.

clinical trial (1.1 mg/kg used in the ECASS trial). As such, 3 different tPA regimens were simulated using the compartmental model (0.6 mg/kg, 0.9 mg/kg and 1.2 mg/kg) with 10% initially given as a 1 minute bolus infusion, followed by the remaining 90% provided as a 1 hour continuous infusion. A delay of 1 minute was assigned between the bolus and continuous infusions. The results for tPA, PLG, fibrinogen, AP and PLS are given in Figures 6.4 (a, b, c, d and e).

6.3.3 3D Patient Specific Model

Results obtained from the compartmental model can be used to determine the inlet conditions of free phase proteins for the 3D clot dissolution model. Using the patient-specific model described in chapter 5, 3D clot dissolution simulations were performed for the three different therapeutic regimens with an initial concentration of $2.2 \mu\text{M}$ PLG and $1 \mu\text{M}$ AP. Figures 6.5 (a and b) show the predicted changes in the ACA and MCA flowrate for the 3 different regimens under steady flow. The ACA flowrate starts at an initially high value of around 4.3 ml/s that then drops to around 1.6 ml/s as clot breakthrough

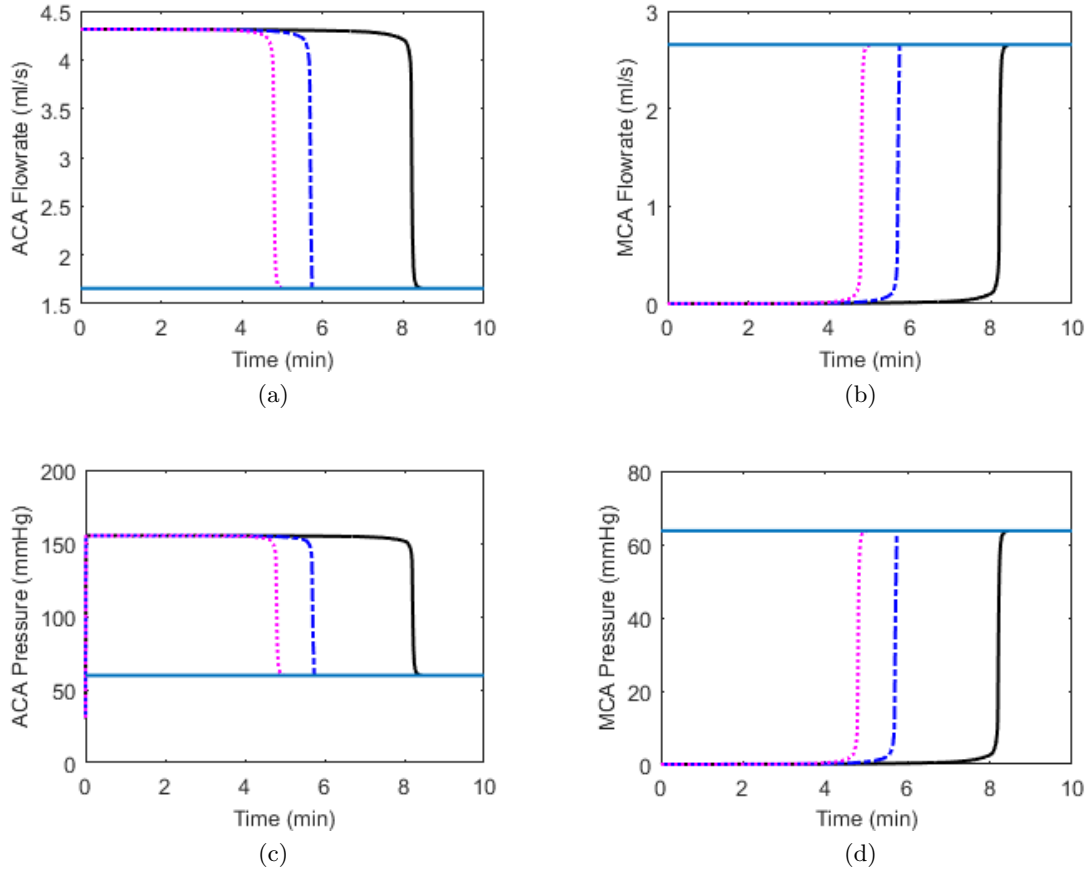


Figure 6.5: Hydrodynamic variables over times for a tPA dose of 0.6 mg/kg (solid black), 0.9 mg/kg (dashed blue) and 1.2 mg/kg (dotted magenta). (a) ACA flowrate, (b) MCA flowrate, (c) ACA pressure and (d) MCA pressure over time. Blue line represents the limits of the ACA and MCA pressures and flowrates.

is achieved. In a similar manner the MCA flowrate rises from an initially low value of around 0 ml/s to around 2.7 ml/s following re-introduction of flow into the MCA branch. The changes in pressure at each outlet mirror the changes in the flowrate (shown in Figures 6.5 b and c for ACA and MCA correspondingly). The initial pressure difference between the MCA and ACA outlets was around 150 mmHg and drops to 0 following clot dissolution. These results show the influence of tPA regimen on the breakthrough time for a coarse 3 mm fibrin clot with an average fibre radius of around 85 nm. The predicted breakthrough time times are around 5, 6 and 8 mins for a tPA dose of 1.2, 0.9 and 0.6 mg/kg, respectively.

The free phase concentrations of lysis proteins (averaged over the thrombus domain) over time is shown in Figure 6.6. The tPA concentration increases in a linear fashion with tPA dose (a) with the initial bolus dose causing a rapid increase in the circulating concentration of tPA and the continuous dose maintaining a uniform concentration throughout the dissolution process. The free phase concentration of PLG in the clot region is influenced by the tPA regimen (b), with a high dose leading to a lower concentration of PLG. The concentration of free phase PLS can be seen in Figure 6.6 (c). The free phase PLG is activated faster for higher tPA doses due to the higher concentration of tPA and decreases more quickly as the fibrin binding sites are rapidly degraded.

Changes in the resistance of the clot (Figure 6.6 d) show that after breakthrough has been achieved, there are still clot fragments in the artery that are being dissolved for all the therapeutic regimens.

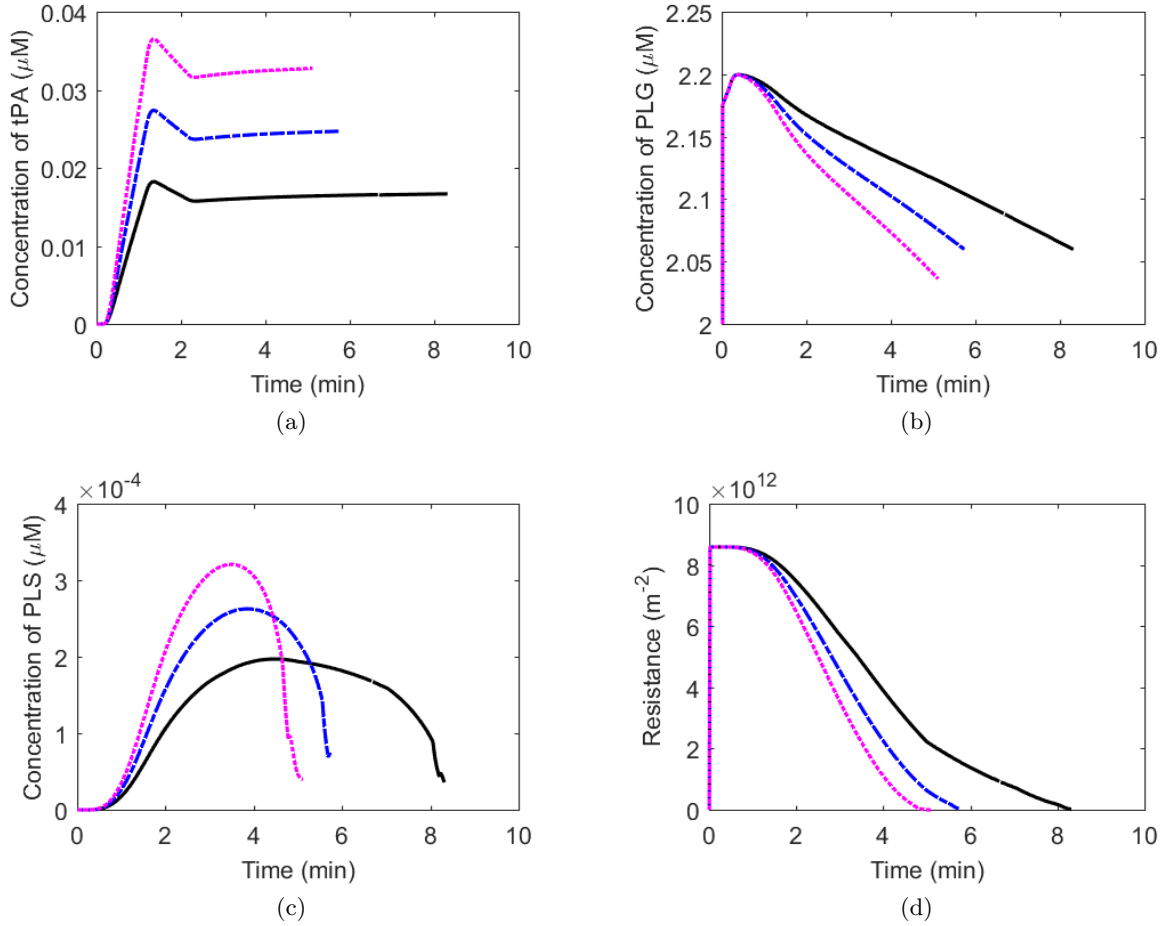


Figure 6.6: Average free phase concentrations of lysis proteins in the clot region for 0.6 mg/kg (solid black), 0.9 mg/kg (dashed blue) and 1.2 mg/kg (dotted magenta) of tPA. Free phase concentrations of (a) tPA, (b) PLG and (c) PLS, and (d) resistance to blood flow during thrombus dissolution.

The lowest tPA dose shows the slowest decrease in the resistance of the clot during thrombolysis. No visible difference was observed in the clot lysis patterns indicating that the concentration of tPA does not affect the shape of dissolution. Since the patterns are similar to those observed in the previous chapter, they have not been included here.

6.3.4 Clot Lysis Time and Risk of ICH

The compartmental model can be used to determine the maximum and minimum concentrations of key lysis proteins during IV infusion for different tPA doses. Figure 6.7 (a) shows that a linear change in the tPA dose causes a corresponding change in the maximum concentration of tPA, which has a consequent effect on the time till clot lysis in well-mixed fibrinolysis as shown in Figure 6.7 (b). An exponential relationship shows that lowering the tPA dose from 1.2 mg/ml to 0.6 mg/ml increases the time till lysis from around 5 mins to 8 mins. The use of a high tPA dose is restricted by the risk of ICH (as estimated by the minimum fibrinogen concentrations shown in Figure 6.7 c) while low doses of tPA have physiological levels of circulating fibrinogen ($>6 \mu\text{M}$). Low plasminogen levels (shown in Figure 6.7 d), as caused by high tPA doses, may also retard clot lysis by decreasing PLS generation in the clot.

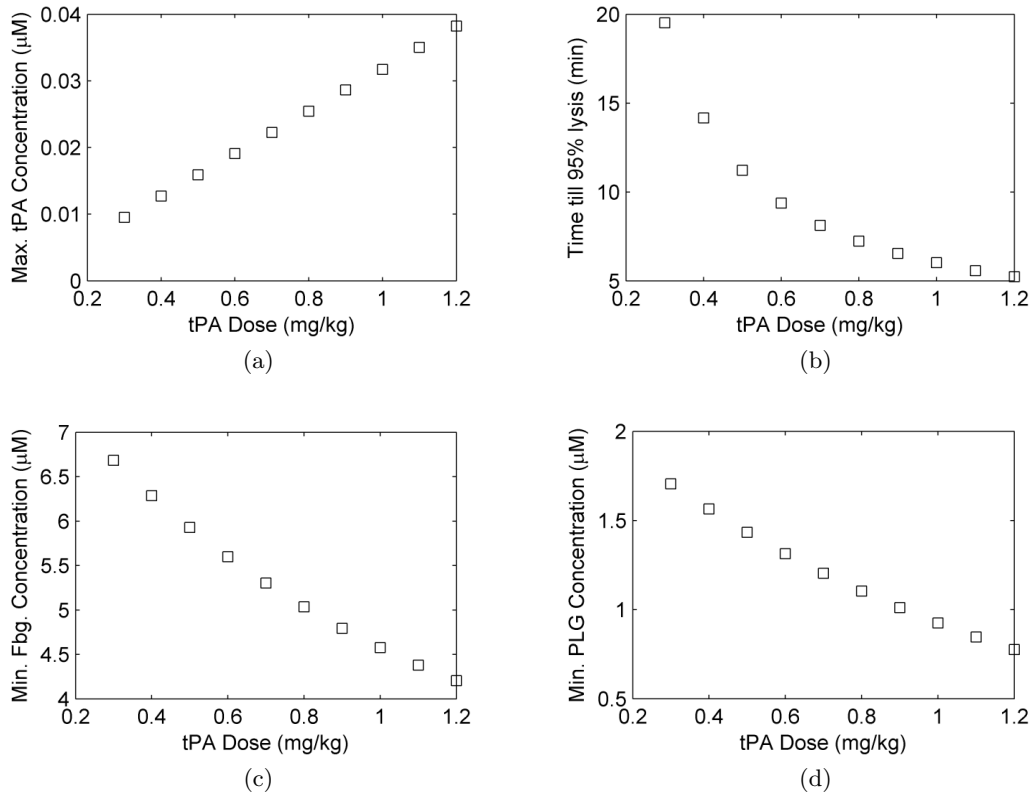


Figure 6.7: Effect of tPA dose on the (a) maximum circulating tPA concentration, (b) the time till 95 % lysis as predicted by a well-mixed model, (c) minimum circulating fibrinogen concentration and (d) minimum circulating PLG concentration.

6.4 Discussion

In this chapter, the effect of drug dose on the efficacy of thrombolytic therapy was examined through the use of a computational model. Changes in the lysis proteins over time during an IV infusion were simulated via the aid of a compartmental model. In order to ensure its accuracy, the model results were compared against experimental data, and the simulated plasma concentration of tPA agreed well with the experimental measurements of [Tanswell et al. \(1989\)](#) for a 0.25 mg/kg and a 0.5 mg/kg continuous infusion (Figure 6.2 c). The half-life of tPA that best fitted the data was found to be at around 4 minutes which is within the range of values reported in the literature ([Noe and Bell; 1987](#); [Verstraete et al.; 1985](#)).

Computational predictions were also compared with the clinical results of [Noe and Bell \(1987\)](#) who evaluated plasma PLG and fibrinogen concentrations following a continuous infusion of 0.5 mg/kg tPA over a 60 minute period. Using similar values for PLG activation as documented in [Tiefenbrunn et al. \(1986\)](#) for a Michaelis-Menten reaction, the simulation results compare well with the experimental data (Figure 6.2 a). The increase in the PLG concentration after the cessation of the tPA infusion was well reproduced with a constant generation term G_{PLG} . However, an interesting anomaly of the experimental data of [Noe and Bell \(1987\)](#) is the initially low concentration of free phase PLG. Most studies have found an initial circulating PLG concentration of 2.0-2.5 μM ([Anand et al.; 1996](#), [Collen et al.; 1972a](#)) while this experiment found a concentration of 1.14 μM . The initial level of PLG in the blood stream is important in determining the production of PLS and the corresponding reduction in fibrinogen. Using an initial PLG concentration of 1.14 μM , a $k_{cat,f}$ value of 23 $\mu M^{-1}s^{-1}$ was needed

to adequately describe the degradation of fibrinogen. Furthermore, the experimental results indicate a relatively quick recovery of fibrinogen levels as shown in Figure 6.2 (b).

Due to the slight anomalies in the data of [Noe and Bell \(1987\)](#), the computational model was compared to the experimental data of [Collen et al. \(1986\)](#) who collected blood samples from over 100 patients after they received a large dose of 0.75 mg/kg tPA over a 90 minute period. Using the same half-life for tPA and the same PLG activation kinetics, the simulation results are able to match the experimental data for an initial PLG concentration of 2 μM (Figure 6.3 a). The change in AP concentration is slightly under predicted (Figure 6.3 c) while the steady concentration of tPA is the same 1200 ng/ml as given in [Collen et al. \(1986\)](#) (Figure 6.3 d). The change in the fibrinogen concentration, however, was found to best fit with a $k_{cat,f}$ value of 6 $\mu M^{-1}s^{-1}$ (Figure 6.3 d). This radical reduction in the free phase lysis constant is thought to be due to the higher initial concentration of PLG that allows for a greater production of PLS, resulting in the degradation of more fibrinogen. Furthermore, the simulation shows a rapid inhibition of PLS by AP (shown by the declining concentration of AP in Figure 6.3 c) as has been seen in previous experiments ([Leebeek et al.; 1990](#)).

Although the experimental studies mentioned above involved continuous infusions, thrombolytic therapy for ischemic stroke typically involves 2 modes of tPA administration: a bolus infusion of 10% of the entire dose over a minute, followed by a continuous infusion of the remaining 90% provided over 60 minutes ([NINDS; 1995](#)). The dose can range from 0.6 mg/kg ([Nakagawara et al.; 2010](#)) to 1.1 mg/kg ([Hacke et al.; 1995](#)). Furthermore, a delay of around 8 minutes can occur between the administration of the bolus and continuous infusions. [Acheampong et al. \(2014\)](#) found that although there was no difference in 3 month outcomes for delays greater than 8 mins, there was a slight decrease in the likelihood of functional independence. In the simulation presented in this chapter, a modest 1 minute delay is applied between the two infusion modes. This can be seen in Figure 6.4 (a) where the bolus infusion allows for a rapid increase in the free phase concentration and the continuous infusion allows for a steady tPA concentration to be sustained. The delay between the infusion of the bolus and continuous tPA doses can be seen by the sharp drop in the free phase tPA concentration. This drop becomes more noticeable at higher tPA doses due to the low-half-life of tPA that can cause a rapid reduction of circulating tPA following the bolus infusion. The pattern of free phase tPA release is similar to that presented by [Smith et al. \(2014\)](#).

A higher tPA dose allows for rapid reductions in the concentration of PLG (Figure 6.4 b) and fibrinogen (Figure 6.4 c) during infusion, and a higher rate of PLS production (Figure 6.4 d) that is rapidly inhibited by AP (Figure 6.4 d). A key observation is the slow recovery of fibrinogen and PLG after the infusion of tPA. Low fibrinogen levels have been implicated in the development of ICH, with concentrations around 150 mg/dL or lower being linked to an increased likelihood of bleeding ([Skeik et al.; 2013](#)). In addition to this, a low concentration of PLG can be associated with suppressed lytic efficacy ([Andrew et al.; 1992](#)). Thus, although a high tPA dose is expected to enhance the rate of thrombolysis, the increased risk of ICH (due to low fibrinogen levels) and a drop in lytic efficacy (caused by low PLG concentrations) limit its use. For instance, a tPA dose of 1.2 mg/kg brings the level of fibrinogen to 50% of its original level. This may be even lower for certain patients as the initial concentration of fibrinogen can vary between 5 and 13 μM ([Noe and Bell; 1987](#)). As such, using high levels of tPA may be favourable for some patients while for others it would bring about an unacceptable risk of ICH. For all 3 tPA doses, the level of PLS in the circulation is very low due to

its rapid inhibition by AP.

When the compartment model was used in conjunction with the 3D clot dissolution model, changes in the flowrate at the MCA and ACA outlets were obtained (Figures 6.5 a and b). The drastic change in the ACA and MCA flowrates marks the moment when clot breakthrough occurs at around 5, 5.8 and 8.2 minutes for tPA doses of 1.2, 0.9 and 0.6 mg/kg, respectively. The changes in pressure for the MCA and ACA outlets (Figures 6.5 c and d) mirror the changes in flowrate in that the pressure across the clot, (which can be roughly estimated as $P_{ACA} - P_{MCA}$), drastically drops to 0 as clot breakthrough is achieved. It can be seen that the choice of tPA dose can have a substantial effect on the clot lysis time. Since the clot under consideration is a short fibrin clot through which the dissolution front passes in a matter of seconds, the dissolution time is limited by the onset of clot lysis. As such the time till clot dissolution is inversely proportional to the change in tPA concentration (Zidansek and Blinc; 1991). This, however, may not be the case for long and fine fibrin clots which have a substantially slower lysis front. In this scenario, the onset of clot lysis would be the same but the slow movement of the lysis front could severely impede the dissolution time. Under this circumstance, the tPA dose would have less bearing on the efficacy of thrombolysis, but more on the risk of ICH.

The average concentration of tPA in the clot for different tPA doses mirrors the inlet conditions set by the compartmental model (Figure 6.6 a). The concentration of PLG however changes in a more unusual fashion (Figure 6.6 b) whereby the rate of decrease of PLG over time is much slower for the lowest tPA dose. This is mirrored by the rapid production of PLS for the highest tPA dose (Figure 6.6 c) that rises quickly and drops as the number of binding sites available for binding are dramatically reduced. The average resistance of the clot (Figure 6.6 d) is also substantially affected by the tPA concentration. The clot lysis patterns were not affected by the tPA concentration and are similar to the ones presented in the previous chapter.

The effects of tPA dose on clot lysis are summarised in Figure 6.7, where increases in the tPA dose lead to linear increases in the maximum free phase tPA concentrations (Figure 6.7 a). For instance, an increase in the tPA dose from 0.3 to 1.2 mg/kg increases the maximum free phase tPA concentration from $0.01 \mu M$ to around $0.04 \mu M$. The clot lysis time as determined in a well-mixed scenario increases exponentially with a decrease in the tPA dose (Figure 6.7 b). This is consistent with results in the literature where the dissolution time was reported to be inversely proportional to the initial concentration of the plasminogen activator (Zidansek and Blinc; 1991). It should be noted that for a 3 mg/ml fibrin clot, the decrease in dissolution time between a tPA dose of 0.9 mg/kg and 1.2 mg/kg is only around 1 minute. The increase in dissolution time between 0.9 and 0.6 mg/kg is around several minutes, while the change between 0.6 mg/kg and 0.3 mg/kg is around 10 minutes. The minimum fibrinogen concentration decreases in a more or less linear fashion according to the tPA dose (as shown in Figure 6.7 c). The relatively large tPA dose halves the circulating fibrinogen concentration following thrombolytic therapy to around $4 \mu M$ while a 0.6 mg/kg dose has around $5.5 \mu M$ of fibrinogen remaining.

The minimum PLG concentration shown in Figure 6.7 (d) also displays a sharp fall from $1.7 \mu M$ for 0.3 mg/kg tPA to $0.7 \mu M$ for 1.2 mg/kg tPA. From this analysis it can be seen that the choice of a tPA dose can have important ramifications on the clot lysis time. At low tPA doses, the time till lysis can increase dramatically. On the other hand, at very high tPA doses, the effect on clot lysis time is negligible compared to the effect of causing dangerously low fibrinogen concentrations. The influence on clot lysis time is expected to be even more pronounced on retracted clots, where certain

binding sites are obscured from PLG activation. Therefore, it seems that the choice of therapy should ideally be kept between 0.6 mg/kg and 0.9 mg/kg. The tPA dose should be chosen according to the composition of the clot as well as the patient's own fibrinogen levels. However, it should be noted that the entire dissolution of a clot is also dependent on the movement of the lysis front within the clot. Thus we would expect that a fully occlusive clot with a low permeability and a long length where the lysis front movement occurs over minutes rather than seconds is less sensitive to the tPA dose.

6.5 Conclusion

This chapter presents the development of a compartmental model and its coupling to a 3D clot dissolution model to investigate the influence of drug dosage on thrombolysis. The compartmental model has been verified with experimental results from the literature. The results show that in the interest of attaining a reasonable dissolution time, a minimum of 0.6 mg/kg is recommended as tPA doses lower than this level can radically increase the dissolution time. On the other hand, the dose effect on dissolution time is reduced at high dose levels (e.g. reducing the dose from 1.2 mg/kg to 1.1 mg/kg would cause a 5% reduction in the dissolution time), but its effect on fibrinogen concentrations becomes more important. At doses greater than 0.9 mg/kg, the fibrinogen concentration in plasma becomes so low that they may make the patient more susceptible to ICH. tPA doses can cause a noticeable difference in the dissolution time for short and coarse clots, but will have less inference on long and dense clots. The results shown no difference in the clot lysis pattern for different tPA doses.

Chapter 7

Development of a Hybrid Stochastic-Continuous Model of Thrombolysis

7.1 Introduction

The previous chapters were focused on modelling of clot dissolution in 3D macroscale geometries, where clot properties changed as a function of time according to a microscopic model of fibrinolysis that described the dissolution of the fibrin network as the cleavage of binding sites. This continuum based model is suitable for simulating the lysis of fibrin clots and can be easily scaled to evaluate the dissolution of occlusive thrombi in large vessels. However, blood clots extracted from patients are heterogeneous mixtures of many different components such as platelets, erythrocytes and leukocytes in addition to fibrin (Ahn et al.; 2016). The presence of these cellular elements can alter the fibrin architecture (Collet et al.; 2002) and cause clot retraction (Kunitada et al.; 1992) thereby affecting the rate of fibrinolysis. As such, it is important to develop a thrombolysis model that can account for the effects of different cellular components on lysis time and can also be easily extended to model the dissolution of large clots.

Experimental studies attempting to evaluate the effect of fibrin architecture on fibrinolysis have shown contradictory results. Some studies have shown that coarse fibrin clots (composed of thick fibres) lyse faster than fine fibrin clots (composed of thin fibres). Carr Jr and Alving (1995) for instance, found that the lysis time increased with a decrease in the fibre mass-length ratio; a 30% reduction led to a 7.5 fold increase in the lysis time for a 29000 IU dose of tPA. Collet et al. (2000) discovered that while the lysis front of a fine fibrin clot was slower than that of a coarse fibrin clot, the lysis speed of a single thin fibre was faster than that of a thick fibre. Wu et al. (1994), on the other hand, found that coarse fibrin clots and fine fibrin clots were lysed at a similar rate when exposed to any concentration of PLS and high concentrations of urokinase ($>1 \mu M$). At lower concentrations of urokinase, the lysis front of a fine fibrin clot advanced at a slower rate than that of a coarse fibrin clot.

The presence of cellular components in a clot can affect the rate of fibrinolysis in many different ways. Platelets, for instance, can release inhibitors such as PAI from their α granules thereby slowing down the lytic rate (Fay et al.; 1994). However, one of the main mechanisms whereby cellular compo-

nents increase the clot lysis time is by reducing the volume of free space in a clot in a process known as clot retraction (Kunitada et al.; 1992). This can impede the movement of lysis front by creating clots of high fibrin density (>3 mg/ml) (Collen et al.; 1972b) and low permeability (Sabovic et al.; 1989). Furthermore, the presence of phospholipid surfaces during clot formation can cause locally high concentrations of thrombin (van der Spuy and Pretorius; 2013) that lead to the generation of fine fibrin clusters. These regions of thin fibres can hinder the movement of the lysis front causing heterogeneity in the clot dissolution pattern (Collet et al.; 2002) and a marked deterioration in the lytic rate (Wohner et al.; 2011). In addition to this, the presence of cellular components alone can cause a notable reduction in clot permeability thereby increasing dissolution time (Wufsus et al.; 2013). As such, it is important to have a fibrinolysis model that can incorporate the influence of cellular components and be easily implemented in the 3D model of clot dissolution used in the previous chapters.

A number of numerical studies have attempted to model the process of fibrinolysis. One of the earliest works was by Diamond and Anand (1993) who described fibrinolysis as a homogeneous shrinking of the fibres radius. The key macroscopic properties of the clot such as the voidage (volume fraction of empty space) and permeability were determined as a function of fibre radius which gradually decreased over time. Although computationally efficient, the model fails to predict the process by which fine clots dissolve slower than coarse fibrin clots. In fact, for the same initial number of binding sites, the model would predict a coarse fibrin clot to dissolve at a slower rate due to its large diameter. However, in reality this is not expected to be the case as thin fibres have a larger fibre pore size due to a greater intra-protofibril distance (Yeromonahos et al.; 2010). Despite these drawbacks, the model has been shown to be good at describing the dissolution of fibrin clots, for a calibrated value of k_{cat} (Wootton et al.; 2002; Anand and Diamond; 1996).

In reality, fibrinolysis has been experimentally shown to occur via transverse cutting of fibrin fibres rather than a homogeneous dissolution of the fibrin radius (Collet et al.; 2000). Bannish et al. (2014b) attempted to account for this in a 1D continuous model where an initial heterogeneous fibrin distribution would recreate fibres of different sizes. Binding locations are assumed to be composed of binding doublets where only tPA and PLG in proximity to one other can generate PLS. The initial binding doublets that are accessible are modelled as specific whereby a binding site in a doublet was reserved exclusively for tPA. As lysis progresses over time, the model predicts the exposure of cryptic non-specific binding doublets that accelerate the rate of fibrinolysis. Bannish et al. (2014b)'s model predicts that if all binding sites are initially accessible for both a fine and coarse clot, then the rate of fibrinolysis would be identical. However, if the initial amount of specific and non-specific binding sites is different according to the accessibility of the outer protofibrils, then the dissolution time would be different. The authors concluded that in order to properly account for the transverse cutting of fibres, a stochastic model is needed.

The stochastic model of Bannish et al. (2014a) determined the time required to dissolve a single fibrin cross-section on the microscale and then used this to determine the dissolution of fibres on the macroscale. Their study found that the influence of fibrin architecture on clot lysis depends on the fibre density (number of fibres in a given control volume) and the number of tPA molecules per fibre cross-section. The authors speculate that the larger number of cross-sections in a fine fibrin clot for a relatively low tPA concentration can cause an increase in clot dissolution time. The study argues that a stochastic model is required to model fibrinolysis at hemostatic concentrations of tPA (~ 80 pM).

To the author's knowledge, there are no models of fibrinolysis in the literature that consider the

presence of cellular components. The influence of cellular components on clot lysis can be expressed through modifications in the fibrin architecture, clot retraction and reduction in clot permeability. Although the stochastic model of [Bannish et al. \(2014a\)](#) can account for differences in clot architecture, their formulation may be difficult to implement in the 3D model of clot dissolution described in the previous chapters. Furthermore, none of the previous models have attempted to account for the influence of clot retraction and cellular components on fibrinolysis. To this end, an initial attempt was made to develop a hybrid stochastic-continuous model that can describe the dissolution of cellular clots. This chapter presents details of the hybrid model that describes fibrinolysis as the collapse of the fibrin network and determines the extent of lysis according to the distribution of fibrin degradation products (FDPs). Furthermore, the model can be readily scaled up to the macroscale to study the lysis of blood clots of different compositions and structures in realistic arteries.

7.2 Methods

7.2.1 Fibrin Microstructure

Fibrinolysis can be described on 3 different scales as shown in Figure 7.1. The macroscale involves analysing blood flow and evaluating the clot resistance. The thrombus can be divided into small control volumes where the mixture of cellular components and fibrin fibres is used to describe the clot properties on the mesoscopic scale. Information on the key macroscopic variables such as the voidage, extent of lysis and the total number of binding sites can be found by examining the fibrin microstructure. At the microscale, the fibrin fibres and the arrangement of protofibrils are described. Fibrin monomers bind together in a half staggered manner to form strands of protofibrils, which then laterally aggregate to form fibrin fibres (Figure 7.2).

In the present model, binding sites are grouped together in fibrin fibre cross-sections (shown by the dotted line) that are repeated twice for every fibrin monomer length. The extent of lysis is determined via the cutting of these cross-sections in the fibrin network ([Collet et al.; 2000](#)) into small remnants termed fibrin degradation products (FDPs). The number of cross-sections and the total length of a fibrin network in a control volume is evaluated with a fibrin fibre mass balance (shown in equation 7.1).

$$\sum_{i=1}^{N_{CS}} \rho_{f,i}(R_{f,i})R_{f,i}^2 = \rho_{f,1}R_{f,1}^2 + \rho_{f,2}R_{f,2}^2 + \dots + \rho_{f,N_{CS}}R_{f,N_{CS}}^2 = \frac{\rho_0 U_0}{\pi L_M/2} \quad (7.1)$$

where $R_{f,i}$ is the radius of a fibrin fibre segment i , N_{CS} is the number of cleavable cross-sections in a fibrin fibre, U_0 is the volume of the control envelope, $\rho_{f,i}$ is the density of fibrin in a fibre of radius $R_{f,i}$, L_M is the length of a fibrin monomer and ρ_0 is the initial density of fibrin. The radius of each fibrin segment is assumed to follow a normal distribution such that the probability P of attaining a radius of R_f is :

$$P(R_f) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(R_f - \mu_f)^2}{2\sigma^2}} \quad (7.2)$$

where σ is the standard deviation and μ_f is the mean of the fibrin fibre distribution. It has been

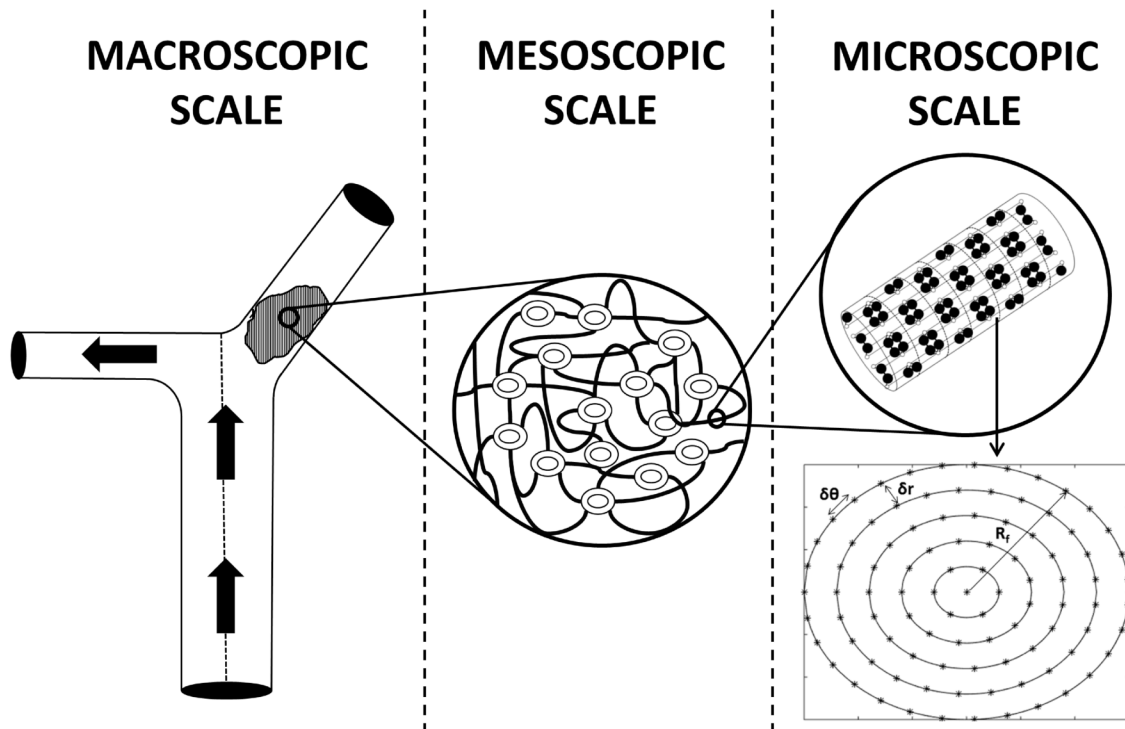


Figure 7.1: Schematic of the different scales of fibrinolysis. The macroscopic description include flow features and average properties of the clot (~ 1 mm). The mesoscopic scale takes into account the local effects of cellular components (~ 10 μ m). The microscopic scale is concerned with fibrin fibres and fibrinolysis (~ 10 nm).

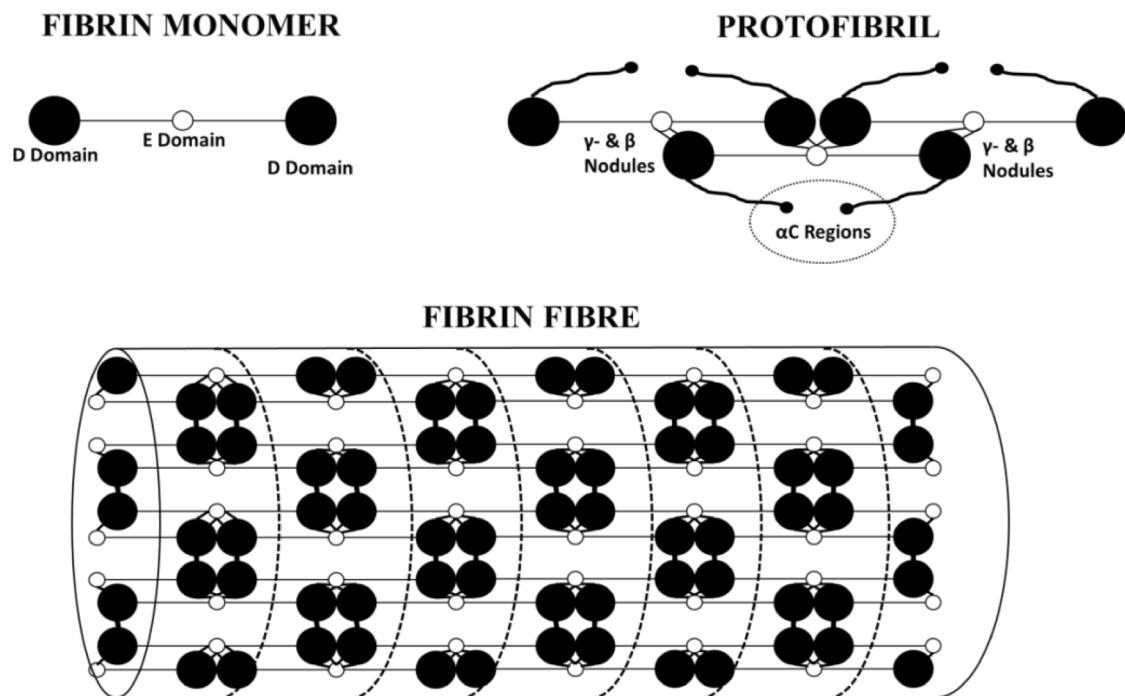


Figure 7.2: Schematic of the microscopic structure of a fibrin fibre. Fibrin monomers join together in a half-staggered structure to create protofibrils that then laterally aggregate to form fibrin fibres. The binding locations occur at the intersections between the D and E domains which repeat every half monomer distance.

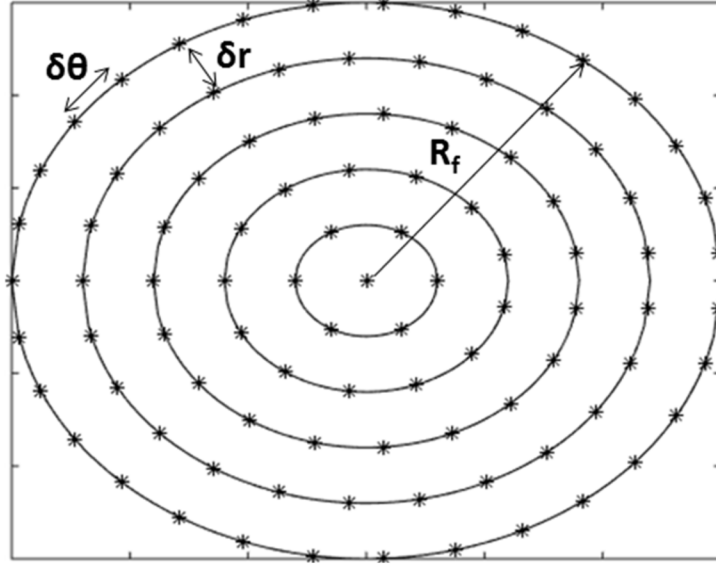


Figure 7.3: Schematic of a fibre cross-section where binding locations are denoted by asterisks.

seen experimentally that the protofibril packing of a given fibre cross-section changes depending upon the radius (Yeromonahos et al.; 2010). Assuming that the density of fibrin in a fibre is 280 mg/ml, the amount of cleavable cross-sections in a fibrin fibre for an arbitrary control volume (U_0) can be determined by satisfying the fibrin mass balance (equation 7.1) for a given σ , μ_f and ρ_0 .

The total number of binding sites can be found by considering the binding site distribution for each fibre cross-section. Binding site groupings in each cross-section are spaced out between each protofibril layer radially by an interprotofibril distance (δr) and angularly by a distance ($\delta \theta$) as shown in Figure 7.3. The total number of binding locations can thus be found by tallying the total number of binding sites for each cross-section as given in equation 7.3.

$$N_{BS} = \sum_{z=1}^{N_{CS}} \sum_{n=1}^{N_{layer}=R_f/\delta r} \frac{\pi}{\arcsin(\delta \theta / 2n\delta r(R_f))} \quad (7.3)$$

where N_{layer} is the number of protofibril layers in a particular cross-section, δr is the radial distance between two protofibril layers (as a function of fibre radius) and $\delta \theta$ is the closest distance between 2 binding site locations on the same layer (assumed to be constant).

7.2.2 Stochastic Simulation of Bound Species

Blood clots are composed of different cellular components (platelets, erythrocytes, lymphocytes, etc.) strung together by a complex mesh of fibrin fibres. Clot lysis can be described as the dynamic interplay of 3 lytic proteins: tPA, PLG and PLS. tPA and PLG adsorb onto binding sites located on the fibrin fibre and react together to form bound PLS. This protease enzyme then goes on to cleave the binding sites in the fibrin fibre, gradually dissolving the clot over time. Clot dissolution occurs via the lateral transection of these fibres at specific binding sites (a process known as fibrinolysis) that collapses the fibrin mesh and releases the cellular components back into the blood stream (Collet et al.; 2000).

The kinetics of clot lysis can be expressed in terms of probabilities as shown in Table 7.1. Each lysis protein (tPA, PLG and PLS) can adsorb onto a free binding site. The reaction between tPA and

Table 7.1: Microscopic kinetics of clot lysis

Reaction (i)	Probability of reaction i (a_i)
Protein α adsorption	$K_{a,\alpha}M_\alpha N_{free}$
Protein α desorption	$K_{r,\alpha}N_{tPA}$
PLG consumption/PLS generation	$\frac{k_2 N_{tPA} N_{PLG}}{K_{M,n} + N_{PLG}}$
Binding site cleavage/ L_{PLS} generation	$k_{cat}N_{PLS}$
Desorption of PLS from L_{PLS}	$K_{r,PLS}L_{PLS}$
Liberation of bound cellular component β	$K_{E,\beta}(E_L)O_\beta$

PLG to form PLS is assumed to follow a Michaelis-Menten reaction rate (Tiefenbrunn et al.; 1986). The cleavage of a binding site by PLS results in the generation of a lysed fibrin fragment through which the absorbed protease enzyme enters the free phase. The cellular components are assumed to be restricted in their movement by the fibrin network and have a greater chance of being liberated as the extent of fibrinolysis (E_L) approaches unity.

M_α and N_α represent the number of free phase and bound phase molecules for species α (tPA, PLG or PLS) respectively, N_{free} is the number of available binding sites, $K_{a,\alpha}$ and $K_{r,\alpha}$ are the coefficients of adsorption and desorption for species α , K_{cat} is the coefficient of binding site cleavage, ϵ is the voidage, K_M and k_2 are the Michaelis-Menten rate constants for plasminogen activation, $K_{E,\beta}$ is the coefficient for the liberation of cellular component β (erythrocytes, platelets or leukocytes) and O_β is the number of cellular components of type β . It is assumed that the binding sites are not protein specific and the number of free binding sites can be defined as the total number of binding sites (N_{tot}) subtracted by the ones occupied by lytic proteins. Any kinetic contributions to the lysis pathway by the cellular components and the effect of lysis inhibitors such as PAI and AP are not considered in the present model. Equations for the bound phase can be solved using the master equation (a set of equations that determine the continuous temporal evolution of the probability of every state occurring in the system) as given in equation 7.5.

$$N_{free} = N_{tot} - (N_{tPA} + N_{PLG} + N_{PLS}) \quad (7.4)$$

$$\frac{\partial}{\partial t}P(S, t) = \sum_{I=1}^{N_{rxns}} (a_I(S - v_I)P(S - v_I, t) - a_I(S)P(S, t)) \quad (7.5)$$

where S is the state of the system, I is the index of a particular reaction, v is the stoichiometry matrix of the system, a_i is the propensity function (probability rate that reaction I occurs given state S) and $P(S, t)$ is the probability that state S is reached at time t . A possible stochastic trajectory based on these equations can be solved using Gillespie's stochastic method (Gillespie; 1977). As the fibrin network is degraded over time, the values of the voidage and the extent of lysis change.

7.2.3 Modelling the Transverse Cutting of Fibres

In order to model clot lysis as the translateral cleavage of fibrin fibres, it is necessary to model the stochastic trajectory of each fibrin fibre cross-section. This can be done by explicitly modelling the change in the population of binding sites for each fibre cross-section and determining how the fibrin fibre matrix is changed when a cross-section is cleaved. A cross-section is considered cleaved when 80% of its initial binding sites has been dissolved. Initially, the fibrin fibre is assumed to be composed of a single long fibre with a total number of N_{CS} cross-sections. As more and more cross-sections are cleaved, the long fibrin strand is cut up into various smaller fibres until all the remaining fibres are only $L_M/2$ long. The extent of lysis is determined according to the total fraction of these fibres as shown in equation 7.6:

$$E_L = 1 - \sum F_1/N_{CS} \quad (7.6)$$

where $\sum F_1$ is the total number of FDPs that are $L_M/2$ long (i.e. a cross-section that is not connected to any other) and E_L is the extent of lysis. This is done under the assumption that FDPs of this length are soluble in plasma, and as such no longer provide any resistance to fluid flow. This value is then used to determine the change in the fibrin volume fraction as done in chapter 4. The extent of lysis is also used to predict the chances for a cellular element to be liberated from a fibrin mesh. The parameter that defines this likelihood can be calculated using equation 7.7 where α_β is the release coefficient.

$$K_{E,\beta} = \frac{\alpha_\beta E_L}{1 - E_L} \quad (7.7)$$

The release coefficient is the probability of a cellular element to leave the fibrin matrix at 50% lysis ($E_L = 0.5$) and is determined by the connectivity of the cellular component to the fibrin matrix. This parameter affects the release of a cellular constituent and causes a change in the cellular volume fraction, (θ_c), given in equation 7.8:

$$\phi_c = \frac{\sum O_\beta V_\beta}{U_0} \quad (7.8)$$

where V_β is the average volume of a cellular component β and O_β is the number of cellular components of type β . As the fibrin fibre matrix is dissolved, there is a greater probability that the cellular components are no longer restricted in their movement by the fibrin fibres. Therefore, the value of the liberation coefficient, $K_{E,\beta}$, is expected to tend towards infinity as the extent of lysis tends to unity.

7.2.4 Reaction of Free Phase Proteins

The reactions in the free phase can be modelled continuously for each species using equation 3.9 where the source terms are defined according to 7.9.

$$R_{\alpha,1} = -(k_{a,\alpha} n_{s,free} C_\alpha - k_{r,\alpha} n_{s,\alpha}) \quad (7.9)$$

where k_a and k_r are the adsorption and desorption coefficients of component α respectively, $n_{s,\alpha}$ is the bound phase concentration of component α and n_{free} is the concentration of binding sites that are free for adsorption. The kinetic contribution of the free phase PLS has an additional term due to the resolubilisation of its bound counterpart following binding site cleavage (shown in equation 7.10). The equations describing the kinetics of free phase proteins are solved using a 4th order Runge-Kutta method.

$$R_{PLS,2} = k_{r,PLS} L_{PLS} \quad (7.10)$$

7.2.5 Solution Procedure for Hybrid Model

The following procedure was devised to solve for clot lysis in a control domain with a given volume U_0 .

Step 1

Specify the following initial parameters: ρ_0 , μ_f , σ , C_{tPA} , C_{PLG} and O_β .

Step 2

Calculate the initial properties of the clot, namely N_{CS} , N_{BS} , $n_{tot,0}$, ϕ_{f0} and ϕ_{e0} . Further to this, allocate memory space to store the stoichiometric matrix (v_{ij}), the state array (X) and the propensity function (a) needed for the stochastic calculation.

Step 3

Create a for loop iteration between the initial time t_0 and the simulation end time t_{end} . Choose a value of the time step size dt sufficiently small to solve accurately for species transport.

Step 4

Calculate the free phase kinetic source terms using a 4th order Runge Kutta discretisation method. These can then be used to calculate the new free phase protein concentrations for the next time step.

Step 5

Since the number of free phase PLG molecules is very high and would require excessive computational time to calculate using a discrete method, the number of bound PLG molecules has been calculated using a quasi-steady state method.

$$N_{PLG} = \frac{N_{tot} - N_{PLS} - N_{tPA}}{1 + \frac{K_r}{K_a M_{PLG}}} \quad (7.11)$$

Step 6

The other bound phase species have been resolved using the stochastic Gillespie Method. Each cross-section is considered as an independent species with its own number of bound and free phase molecules. It is assumed that the free phase molecules are uniformly distributed at each cross-section.

Step 7

Generate a while loop (a continuous if loop) that allows the simulation time to change

within a given timestep (dt). This essentially considers any microscale reactions that are likely to occur within the time period set by the solution of the continuous algorithm.

$$t_n = t \quad (7.12)$$

$$\text{while}(t_n < t + dt) \quad (7.13)$$

Step 8

Based on the values for free phase concentrations and those defined in the state array, the probability of each reaction (defined in Table 7.1) for every cross-section is calculated. This is known as the propensity function and its values are temporally stored. The sum of these values is calculated according to equation 7.14 where N_{rxns} is the total number of reactions that can possible occur and a_0 is the sum of the propensity function.

$$a_0 = \sum_{i=1}^{N_{rxns}} a_i(X) \quad (7.14)$$

Step 9

A Monte Carlo step provides the generation of 2 random numbers (r_1 and r_2) that will determine which reaction is likely to occur next and at what subsequent time it will occur (τ refers to the stochastic timestep).

$$I = \min\left(\sum_{i=1}^I a_i(X) > a_0 r_2\right) \quad (7.15)$$

$$\tau = \frac{1}{a_0} \ln\left(\frac{1}{r_1}\right) \quad (7.16)$$

Step 10

The time and the state vector can be updated using the stoichiometric matrix.

$$t_n = t_n + \tau \quad (7.17)$$

$$X = X + v(I) \quad (7.18)$$

Step 11

If the new simulation time is within the constraint demanded by the while loop, then iterate from step 8. Otherwise break the loop.

Step 12

Cycle through the state array and evaluate if any fibre cross-sections have a binding site population that has fallen under 20% of its initial value. These cross-sections are now considered to be cleaved.

Step 13

Update the fibrin fibre network to take into account the cleavage of any binding sites

Table 7.2: Parameters for the hybrid model

Symbol	Meaning	Value	Units	Source
dr	Radial distance between each protofibril	8	nm	Yang et al. (2000); Yeromonahos et al. (2010)
$d\theta$	Angular distance between each protofibril	8	nm	Yang et al. (2000); Yeromonahos et al. (2010)
$E_{L,crit}$	Extent of lysis level when the clot is considered cleaved	95	%	-
L_M	Length of a single fibrin monomer	45	nm	Ferri et al. (2000)
N_{AV}	Avogadro's number	6.02×10^{23}	1/mol	Fox et al. (2007)
nt	number of timesteps for the larger loop	100	-	-
U_0	Volume of control volume	100	μm^3	-
σ	Standard deviation of radii	0-50	nm	Wohner et al. (2011)
μ	Average fibrin fibre radius	50-200	nm	Yeromonahos et al. (2010); Diamond (1999)
η_{crit}	Percentage of remaining binding sites that cause a cross-section to be cleaved	20	%	Bannish et al. (2014b)
ρ_0	Initial Fibrin Density	3	mg/ml	Collen et al. (1972a)
ρ_{fibre}	Density of fibrin in fibre	210-280	mg/ml	Diamond (1999)

and calculate the length of any new threads. If the thread reaches the smallest subunit ($L_M/2$), then update ΣF_1 appropriately.

Step 14

Determine the new extent of clot lysis and update all parameters accordingly. Iterate from step 4 onwards until $t = t_{end}$.

7.2.6 Model Parameters

The same constants and parameters as given in Table 4.3 have been applied. All kinetic constants with units of concentration need to be converted into molecules as shown for the adsorption coefficient and the Michaelis-Menten coefficient (equations 7.19 and 7.20) where N_{AV} is Avogadro's number. Additional parameters used in the hybrid model are given in Table 7.2.

$$K_{a,\alpha} = \frac{k_{a,\alpha} N_{CS}}{N_{AV} U_0} \quad (7.19)$$

$$K_{M,n} = \frac{K_M N_{AV} U_0}{N_{CS}} \quad (7.20)$$

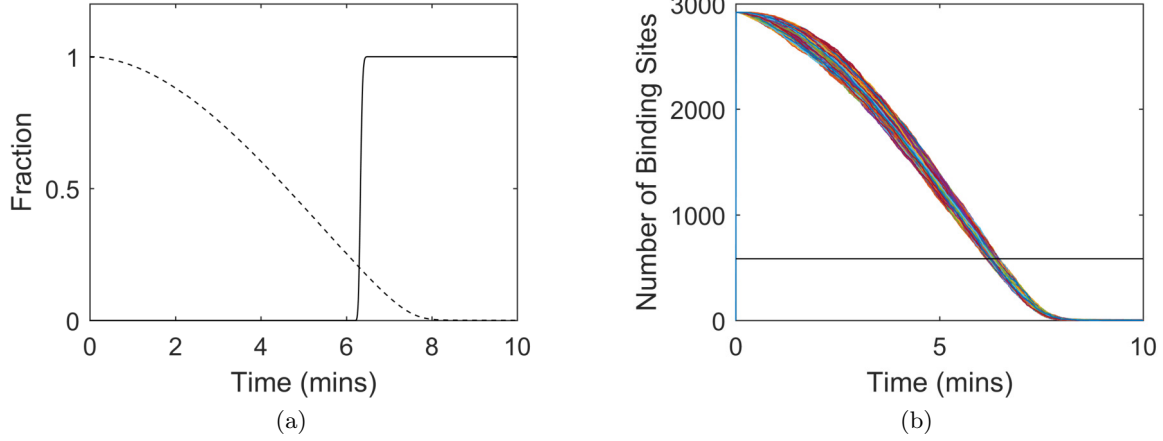


Figure 7.4: Fibrinolysis modelled as the transverse cutting of fibres. (a) Extent of lysis and (b) binding site concentration over time. Solid line represents the extent of lysis while dotted line represents the normalised total binding site concentration. Number of binding sites over time for each cross-section. Horizontal black line represents the minimum amount of binding sites needed for a cross-section to be considered cleaved.

7.3 Results

7.3.1 Fibrinolysis as Transverse Cutting of Fibres

The computational model presented in this chapter is capable of modelling fibrinolysis as the transverse cutting of fibres. The stochastic module evaluates changes in the number of binding sites for each cross-section and is considered cleaved when over 80% of its original sites have been dissolved. Figure 7.4 shows how the variation of lysis extent and the number of binding sites over time for a $100 \mu m^3$ system with 0.04 nM tPA and $2.2 \mu M$ PLG for an initial fibrin density of 3 mg/ml and an initial fibre radius of 100 nm. The number of binding sites gradually decreases over time due to the activity of PLS (seen in Figure 7.4 a). The extent of lysis on the other hand as defined by the connectivity of the fibrin fibre is evaluated according to the cleavage of cross-sections. The decrease in the binding sites for each cross-section can be seen in Figure 7.4 (b) where each line represents the stochastic trajectory of a cross-section. Once the number of binding sites for a given cross-section falls below 20% of its original value (shown by the black horizontal line), the cross-section is considered cleaved.

The extent of lysis is determined by the population distribution of FDPs calculated at each timestep. Figure 7.5 shows the distribution of FDPs for different extents of lysis. Initially the diversity of fibre lengths is very small as it is assumed that all the fibrin monomers are attached to the fibrin fibre. However, as more and more cross-sections are cut when lysis takes place, the large single fibres are cut up into smaller segments that eventually accumulate to a large group of indivisible fibrin monomers. It is these components that are considered to be so small that they can be considered dissolved in the system.

7.3.2 Effects of Fibre Radius

The purpose of describing fibrinolysis as the transverse cutting of fibres is to be able to distinguish between the lysis of fine and coarse fibrin clots as shown in Figure 7.6. The extent of lysis over time

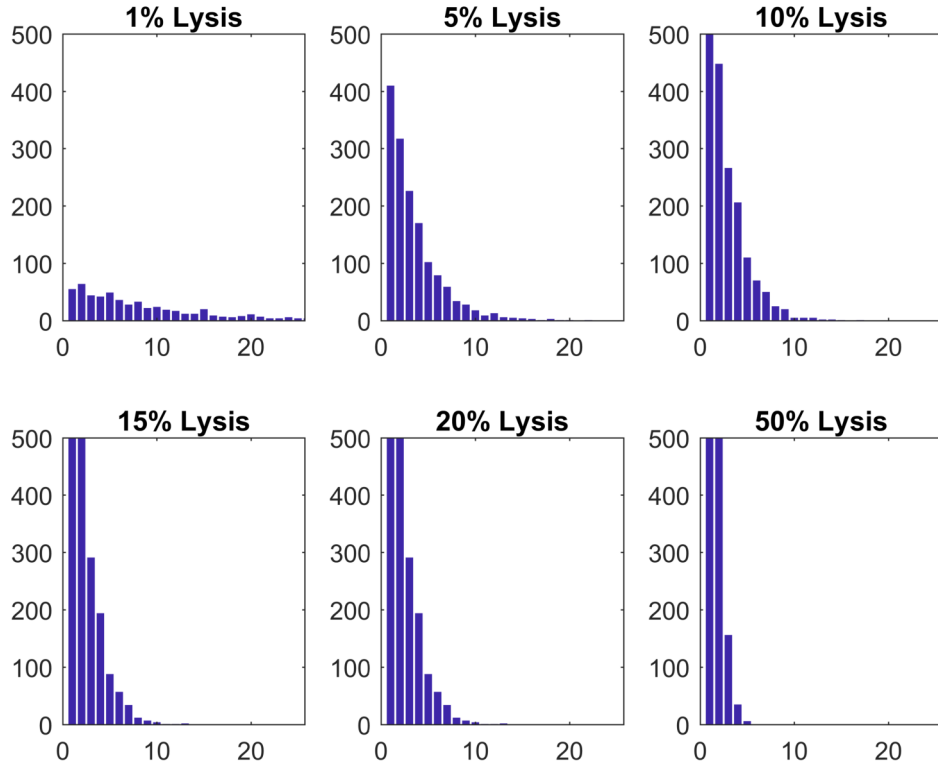


Figure 7.5: Fibrin degradation product distribution for different extents of lysis. The horizontal axis refers to the length of each FDP while the vertical axis reflects the number of FDPs of a particular length. Length is measured in terms of fibrin monomer units.

for different initial fibrin radii is given in Figure 7.6 (a) where the extent of lysis gradually shifts to the left for larger fibrin radii. This gives different lysis times as shown in Figure 7.6 (b) where the lysis time is determined when the extent of lysis reaches 0.95. The binding site fraction for different radii is shown in Figure 7.6 (c) where it gradually decreases to 0 at different rates. Clearly, the model is able to predict the lysis time and lysis rate in response to a change in fibrin architecture.

The hybrid model, since it treats the dissolution of each cross-section separately, takes into account how different distributions of radii in the fibrin network can affect the lysis time. For a normal distribution, the dependence of the lysis curve and the final lysis time on the standard deviation of the fibre radius can be evaluated. Figure 7.7 shows the lysis of a fibrin network whose cross-section size is normally distributed with a mean (μ_f) of 100 nm and a standard deviation (σ) of 50 nm. Each line in Figure 7.7 (a) shows the lysis of a single cross-section. Since, the fibre radius is non-uniform, it can be seen how each cross-section has a different initial number of bindings sites that gradually tends to zero over time. Figure 7.7 (b) shows how changes in the standard deviation of fibrin fibre radius can influence fibrinolysis. This illustrates that the clot lysis time increases sharply with σ .

7.3.3 Release of Cellular Components

The presence of cellular components in a blood clot can be measured by the volume fraction they occupy. Over the course of clot lysis, the fibrin network is degraded and the cellular components that are trapped can be released. This causes a change in the cellular volume fraction as the clot is

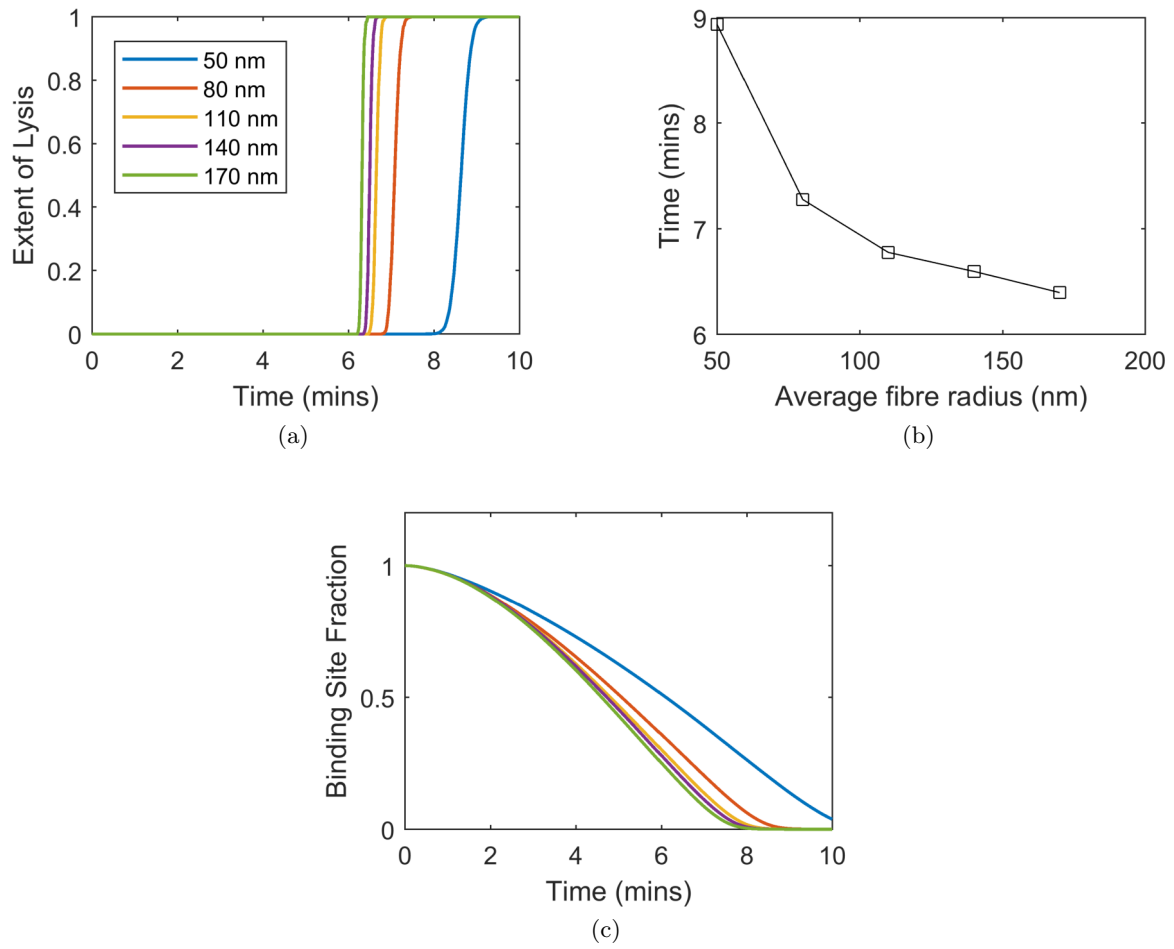


Figure 7.6: (a) Extent of lysis curves, (b) lysis time and (c) binding sites concentration as a function of initial fibrin fibre radius. Each colour represents the lysis simulation results for a different initial fibrin radius starting from 50 nm to 170 nm in 30 nm increments.

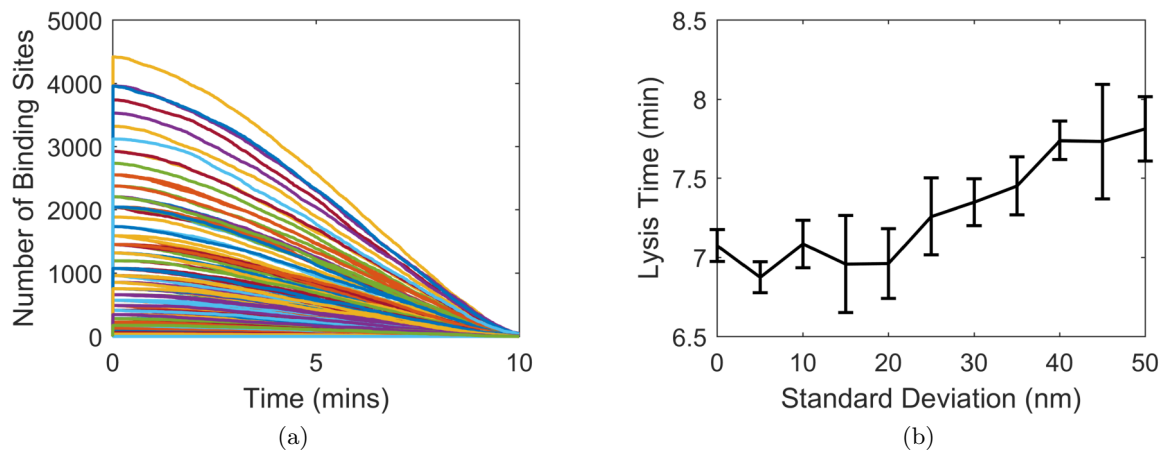


Figure 7.7: Sensitivity of fibrinolysis to the standard deviation of fibrin fibre radius; (a) Binding sites for each cross-section and (b) final clot lysis time against σ_{radius} .

dissolved as can be seen in Figure 7.8 (a). The release coefficient α is a parameter that measures the tendency that a cellular component has to leave the fibrin fibre network. The value of this parameter indicates the relative ease at which a cellular component can ‘escape’ the fibrin matrix. Since the value for α is unknown, it has been tested for different values at 1 s^{-1} , 0.1 s^{-1} , 0.01 s^{-1} and 0.001 s^{-1} . It can be seen that the higher the value of α , the quicker the release of the cellular components. A notable difference can be seen for the lowest value where there is a more gradual change in the movement of cellular components from the fibrin matrix. This is important as the presence of cellular components will alter the permeability of the clot thereby changing the overall dissolution time as seen in the previous chapter.

One of the most important effects of cellular components on fibrinolysis is manifest through clot retraction. A retracted clot causes a reduction in the volume of the clot and an increase in its fibrin density. Therefore, for the same volume, more binding sites will need to be dissolved. Figure 7.8 (b) shows the degradation of a clot that has 40% of its volume occupied by cellular components at various levels of retraction (from 0% to 50% of total volume). As it can be seen, a larger concentration of the fibrin binding sites results in slower dissolution. The extent of lysis for each level of clot retraction is shown in Figure 7.8 (c) where increasing retraction causes the lysis curve to shift to the right. The lysis time as a function of clot retraction is given in Figure 7.8 (d) where there is an increase in the lysis time at high levels of retraction. Thus clot retraction has a strong effect on the rate of dissolution of cellular clots.

7.4 Discussion

Fibrinolysis refers to the dissolution of fibrin matrix and has been thought to occur either as a homogeneous shrinking of the fibre radius (Gabriel et al.; 1992; Blinc et al.; 2000) or transverse cutting of fibrin fibres (Collet et al.; 2000). Accurately describing this process is important in order to take into account the effect of fibrin architecture on fibrinolysis which some previous models have failed to address (Diamond and Anand; 1993; Wootton et al.; 2002). Although stochastic models can simulate a greater amount of detail (Bannish et al.; 2014b), their scalability remains to be demonstrated for the dissolution of large macroscale clots (Bannish et al.; 2014a). To this end, a hybrid computational model has been developed which evaluates fibrinolysis as the transverse cutting of fibres but describes free phase proteins as a continuous system.

The fibrin network is assumed to be composed of a single long fibre which is discretised into a number of cross-sections that are mapped onto a network graph. The binding sites in each of these sections can be cleaved by bound PLS and their outcome is determined stochastically using the Gillespie algorithm (Gillespie; 1977). When the number of binding sites in a particular cross-section reaches 20% of its initial value, it is considered cleaved and is used to update the distribution of fibre lengths using the network graph. The lowest indivisible fibre length is the distance between 2 adjacent cross-sections ($L_M/2$) which is assumed to readily dissolve in the plasma. The structural integrity of the fibrin network and the extent of fibrinolysis are determined by the distribution of these fibre lengths.

The results of the model can be visualised in Figure 7.4 for a $100 \mu\text{m}^3$ system with a fibrin density of 3 mg/ml and a uniform fibre radius of 100 nm that was injected with a tPA and PLG concentration of $0.04 \mu\text{M}$ and $2.2 \mu\text{M}$, correspondingly. The binding site concentration (Figure 7.4 (a)) decreases in

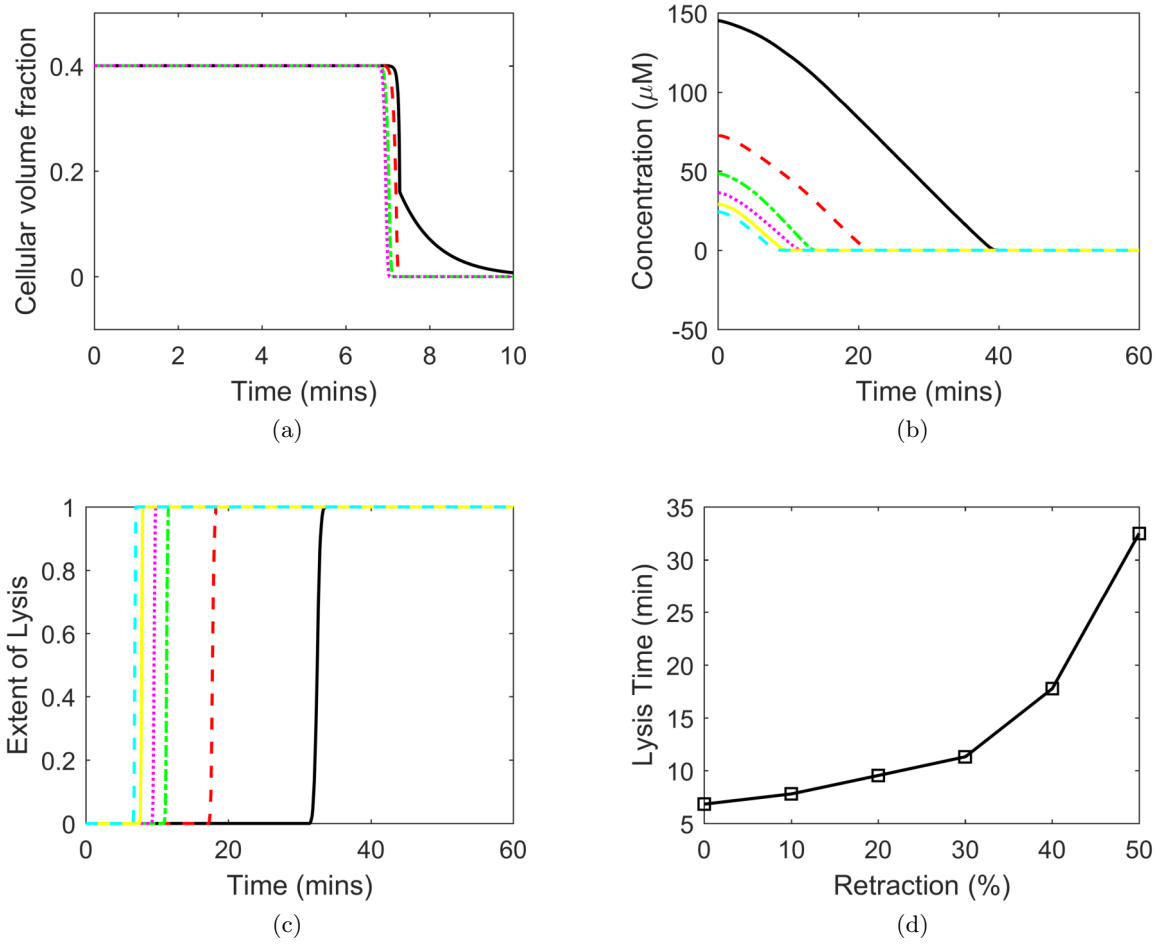


Figure 7.8: (a) Cellular volume fraction against time for $\alpha = 0.001 s^{-1}$ (solid black), $\alpha = 0.01 s^{-1}$ (dashed red), $\alpha = 0.1 s^{-1}$ (dash-dot green) and $\alpha = 1 s^{-1}$ (dotted magenta). Clot lysis for a clot composed of 40% by volume of cellular components with a total cell volume retracted by 0% (dashed cyan), 10% (solid yellow), 20% (dotted magenta), 30% (dash-dot green), 40% (dashed red) and 50% (solid black). (b) Concentration of binding sites over time, (c) extent of lysis over time and (d) lysis time as a function of clot retraction for $\alpha = 0.001 s^{-1}$.

a smooth and continuous manner as typically seen in turbidity studies of fibrinolysis (Kunitada et al.; 1992). The extent of lysis, on the other hand, as defined by the transverse cutting of fibres shows a sharp increase from 0 to 1 at around 6 minutes. This rapid decrease in the integrity of fibrin matrix is characteristic of experimental studies of fibrinolysis using ball sedimentation assays (Wohner et al.; 2011). In these studies, the clot lysis time is determined by how long it takes for the ball to suddenly drop as the fibrin network is dissolved. It appears that the hybrid model is capable of capturing the features observed in both experiments. The continuous decrease in the turbidity of the fibrin clot is captured by the binding site concentration while the clot dissolution time is accounted for by the collapse of the fibrin matrix due to the cleavage of fibre cross-sections.

The fibrinolysis model is also capable of tracking the stochastic trajectory of each cross-section as shown in Figure 7.4 (b). The number of binding sites decreases throughout fibrinolysis and determines the point when a cross-section is considered cleaved (when the value reaches about 20 % of the initial number of binding sites). This threshold can be altered depending on the strain that the fibrin fibre is under. Bucay et al. (2015) found that thin fibres are likely to lyse faster due to the strain that they encounter in the fibrin network. Thick fibres on the other hand elongate during fibrinolysis thereby losing some of their pre-strain and slowing down the lytic rate. This is consistent with the results of Collet et al. (2000) who found that thick fibres lyse at a slower rate than thin fibres. Although our model of fibrinolysis predicts this to be the case (by virtue of coarse fibres having a greater number of binding sites), the findings of Bucay et al. (2015) can be accommodated by altering the limit at which a cross-section is considered to be cleaved. For instance, a thin fibre needs only to have 80% of its binding sites cleaved to be considered lysed while a coarse fibre would require 90%. This could potentially have an effect on the difference in lysis rate of fine and coarse clots.

The cleavage of fibre cross-sections causes a change in the distribution of fibre lengths as seen in Figure 7.5. Initially, the long fibrin fibre is cut into several relatively long fibre strands. As more cross-sections are gradually cleaved, the length of these fibres decreases (1% lysis) until a substantial portion reaches $L_M/2$ long (50% lysis). At this point, the fibres are considered soluble and the extent of lysis rapidly increases to 1. The ability to predict the distribution of fibre lengths during fibrinolysis can provide insights into how fibrin network properties such as branching can affect the dissolution time. Although in this study, the fibre architecture is assumed to be composed of a single long fibre, many different configurations can be considered in the future. Properties such as these may be important as they can affect clot rigidity and the stiffness of the fibre network (Weisel; 2007).

One of the main motivations of developing a hybrid model is to understand the effect of fibrin architecture on fibrinolysis. The effect of average fibre radius on the time as predicted by the model is shown in Figure 7.6 (a). It can be seen that a smaller average fibre radius gives rise to a longer dissolution time (Figure 7.6 b). In fact, a decrease in the fibre radius from 180 nm to 50 nm causes an increase in the clot lysis time by around 40%. Since the initial binding site concentration is similar regardless of fibre radius, the main reason for the increase in the clot lysis time is due to the sharp increase in the number of cross-sections at lower fibre radii. This is consistent with the model of Diamond and Anand (1993) whereby fibre length was found to be inversely proportional to the square of the fibre radius. Bannish et al. (2014a) also found that fibre density was important in determining the difference in lysis time between coarse and fine fibrin clots. The binding site concentration for each radius (Figure 7.6 c) decreases at different rates due to the lower availability of tPA for each cross-section for progressively finer clots.

Experimental data in the literature show that fine fibrin clots made up of thin fibres dissolve faster than coarse clots at the same initial fibrin density (>3 nM) (Carr Jr and Alving; 1995). Collet et al. (2000) found this to be the case at higher tPA concentrations (>3 nM) for a fine clot with an average fibre diameter around half of that for a coarse clot (~ 400 nm) in a 0.12 mL system. Wu et al. (1994), on the other hand, found that at high concentrations of urokinase (>10 μM) and at all concentrations of PLS, there is no considerable difference in the lysis times between fine and coarse clots. The same has been found with the hybrid model for clots with an average fibre radius of 100 nm and 200 nm where the predicted lysis times only differ slightly. Any differences can be further reduced if only the outer protofibrils are accessible for binding as finer clots have a larger surface area and therefore a greater number of exposed binding sites (Diamond and Anand; 1993).

Another capability of the hybrid model is its ability to determine the influence of the distribution of fibrin radii on the clot lysis time. This is important as the fibrin network is composed of several different fibre radii rather than a single homogeneous fibre dimension. For instance, Wohner et al. (2011) found that the presence of erythrocytes can narrow the standard deviation and promote the formation of thin fibres which reduce the clot lysis time. Figure 7.7 shows the effect that any deviation in the fibre radius can have on clot lysis for a system with a mean fibrin radius of 100 nm. The stochastic trajectory for each cross-section can be seen in Figure 7.7 (b) where the initial number of binding sites depends on the fibre radius. It can be seen that thin fibres lyse at a slower rate than thick fibres as observed by experimental studies (Collet et al.; 2000; Bucay et al.; 2015). The lysis time for a fibrin network with different standard deviations is given in Figure 7.7 (b) where increasing standard deviations leads to a notable increase in the lysis time. This is due to the considerable increase and modest decrease in the lysis time for lower and higher fibre radii, respectively.

The presence of cellular components in clots can affect the dissolution time in several different manners. Firstly, the presence of phospholipid surfaces during clot formation can cause locally high thrombin concentrations that favour the formation of thin fibres (Varin et al.; 2013). Secondly, α granules in platelets can release lysis inhibitors. This however, is suspected to be in too low a quantity to affect dissolution rates at pharmacological levels of tPA (Fay et al.; 1994). Thirdly, the presence of cellular components can affect the total permeability of the clot which, as seen in the previous chapters can have a profound affect on the dissolution time. This may become especially important when attempting to describe the heterogeneous nature of blood clots. As such, in order to determine the total permeability of a clot, the volume fraction of each component should be determined (Diamond; 1999; Wufsus et al.; 2013).

The change in the volume fraction as calculated by our mathematical model is shown in Figure 7.8 (a). The sudden drop in the volume occupied by the cellular components is due to the collapse of fibrin matrix as determined by the distribution of fibre lengths. The rate of release of these cellular components can be controlled by the value of α which describes the ‘willingness’ of the cellular component to leave the fibrin matrix. For instance, erythrocytes can bind to each other and the fibrin matrix through inter-digitation (van der Spuy and Pretorius; 2013). At high values of α ($0.1s^{-1}$), the cellular components readily re-enter into the plasma phase while lower values allow for a much slower re-entry ($\alpha = 0.001s^{-1}$). Although cellular components can take up a substantial portion of the clot volume, they contribute to a modest reduction in clot permeability (Wufsus et al.; 2013).

One of the most important effects to consider in the lysis of cellular clots is the mechanism of retraction (Kunitada et al.; 1992; Sabovic et al.; 1989). Figure 7.8 shows the lysis of a cellular clot

composed of 40% erythrocytes for different retraction rates. The retraction of the clot causes a decrease in the total volume of the clot thereby increasing the fibrin density. This means that for the same volume, there is a greater number of binding sites that need to be cleaved as shown in Figure 7.8 (b). For instance, the number of binding sites for a clot retracted by 50% is around $150 \mu M$ which is around 5 times that of the unretracted clot. This radical increase in the binding site concentration causes a slow down in the extent of lysis that can be seen in Figure 7.8 (c). Consequently, the lysis time of the retracted clot increases as a function of the retraction rate (Figure 7.8 d). This sharp increase is very much dependant on the total composition of the clot. As the clot becomes gradually more compressed such that the total volume begins to approach the volume occupied by the cellular components, there will be a radical increase in the fibrin density. This increase in the fibrin density causes a significant increase in the lysis time.

The fibrinolysis model can be further improved by including some modifications. For instance, retracted clots may delay clot lysis due to increased steric hinderance thereby decreasing access to binding sites. Furthermore, we have neglected the influence that other components such as myosin (Mutch et al.; 2010) and fibre strain play in fibrinolysis (Varjú et al.; 2011). In order to further consolidate the use of this model, the model should be tested directly against experimental data and incorporated into our 3D clot dissolution model to evaluate the lysis of heterogeneous and cellular clots.

7.5 Conclusion

In this chapter, a new way of modelling fibrinolysis has been presented that can be readily applied to the dissolution of clots with different fibrin fibre networks and compositions. Fibrinolysis is modelled as the transverse cutting of fibrin fibres and lysis is defined according to the distribution of fibre lengths that are considered dissolved in the fluid once they reach a certain length. The strength of the model lies in its ability to address the key features observed in experiments of clot lysis, to take into account the complexity of the fibrin fibre network and to solve for the change in cellular volume fraction throughout clot lysis. The model results show that fine fibrin clots dissolve slower than coarse clots but the difference in lysis time is highly dependant on the fibrin fibre radius. Clot retraction has been found to severely delay clot lysis by increasing the density of fibrin in the clot. Future work will attempt to test the fibrinolysis model against experimental data and implement it into the computational model of 3D clot dissolution described in the previous chapters.

Chapter 8

Conclusions and Recommendations

8.1 Summary of Main Contributions

8.1.1 Development of a CFD Model for Clot Dissolution

Early work in the modelling of thrombolysis looked at the 1D and 2D dissolution of clots using analytical and numerical approaches ([Anand et al.; 1997](#); [Zidansek and Blinc; 1991](#)). These were used to determine the importance of permeation enhanced thrombolysis ([Diamond and Anand; 1993](#)) and the occurrence of finger lysis patterns in fully occluding clots ([Zidansek et al.; 1995](#)). Subsequent studies looked at the dissolution of mural thrombi and the parameters that affect their rate of lysis ([Wootton et al.; 2002](#); [Sersa et al.; 2007a](#)). Recently, stochastic models have been used in order to better quantify the effect of certain parameters on thrombolysis ([Bannish et al.; 2014a](#); [Bajd and Sersa; 2013](#)). Building upon this existing body of work, a multiscale CFD model has been developed to investigate 3D clot dissolution in patient-specific geometries.

Blood flow is solved with modified Navier-Stokes equations where a Darcian momentum source term accounts for the hydrodynamic resistance of the clot. The transport of lysis proteins such as tPA, PLG, PLS and AP is determined through the use of diffusion-convection-reaction equations while fibrinolysis is modelled on the microscale as the degradation of binding sites on fibrin fibres by bound PLS. Changes in binding site concentration are then used to determine variations in the macroscopic properties of the clot such as the fibrin volume fraction and clot resistance. To model physiological conditions, a 3-element Windkessel model is implemented as an outlet boundary condition. A compartmental model has also been developed to calculate the circulating concentration of each lysis protein for a given tPA dose.

In chapter 3, some of the early work in modelling thrombolysis was replicated and a modified computational model was used to analyse the lysis transition of fully occluding clot at different pressure drops. The breakthrough in the central region marks the transition of a fully occluding clot to mural thrombi that cause a local acceleration in the velocity and bring about disturbed flow at higher pressure drops. Chapter 4 focused on the analysis of an idealised 3D geometry of the ICA bifurcation with a fully occluding clot in the MCA segment. Physiological flow conditions were incorporated and the effect of flow pulsatility was examined. The fibrinolysis model was modified by simulating clot lysis as the cleavage of binding sites rather than homogeneous shrinking of the fibre radius. The model predicted a change in ACA pressure from 150 mmHg before thrombolysis to 60 mmHg after

breakthrough was achieved. The lysis front was found to proceed in a uniform manner that took from several seconds to minutes to pass through the clot depending on the initial clot length. A longer clot led to a significantly longer dissolution time due to reduced perfusion velocity through the clot.

8.1.2 Parameter Investigation for the Dissolution of an M1 Occlusion

The computational model was used to determine the effect of certain parameters on clot lysis in a patient-specific geometry of the ICA bifurcation. In chapter 5, clot length was found to be an important determinant of the breakthrough time as a longer length would result in a lower perfusion velocity thereby slowing down clot lysis. The predicted substantial increase in the dissolution time for clots longer than 9 mm is consistent with clinical observations that longer clots are associated with a low probability of recanalisation. This effect is expected to be more prominent for fine fibrin clots which have permeabilities of around one order of magnitude lower than that of coarse clots (Diamond; 1999). The clot position along the MCA was found to have a minor effect on the dissolution time but a strong influence on clot lysis patterns. This was primarily controlled by the recirculation region proximal to the clot face that grew in length and decreased in strength the further away the clot was from the ICA bifurcation. The presence of a stagnant flow region for clots more than 12 mm away from the ICA bifurcation may help to explain why thrombus can form at the proximal surface of a clot (Qazi et al.; 2015).

Results in chapter 6 showed that different drug regimens could affect the onset of thrombolysis but not the lysis front velocity. As such, using higher tPA doses would have little effect on improving the dissolution of long and highly resistant clots. This is expected to be the case for fine fibrin clots that have a similar rate of dissolution to coarse fibrin clots (Wu et al.; 1994) but have a significantly lower permeability. Retracted clots on the other hand, in addition to having a higher hydrodynamic resistance, also dissolve at a slower rate than fibrin clots (Sabovic et al.; 1989). In this scenario, increasing the tPA dose may have a significant impact on the dissolution time.

It was found that both the lysis time and fibrinogen concentration in plasma were sensitive to tPA dose. Drug doses greater than 0.9 mg/kg led to dangerously low plasma levels of fibrinogen which may increase the risk of ICH, while tPA doses lower than 0.6 mg/kg would dramatically increase the clot dissolution time. tPA doses of between 0.6-0.9 mg/kg may be a suitable therapeutic choice for assuring treatment effectiveness while minimising the risk of ICH. Thus, the simulation results seem to indicate that an optimal tPA dose could be found depending on the clot length, its location and hydrodynamic permeability.

8.1.3 New Fibrinolysis Model to Account for Fibrin Architecture

The fibrin composition of a clot has a strong influence on clot lysis time. For the same fibrin density, experimental studies have produced conflicting results with fine clots composed of thin fibres being dissolved at a similar (Wu et al.; 1994), slower (Collet et al.; 2000) or faster rate (Kolev et al.; 1994) than coarse clots with thick fibres. The fibrinolysis model used in chapters 4-6 treated clot lysis as the degradation of fibrin binding sites, which could not differentiate lysis between fine and coarse clots provided that all protofibrils are accessible. If only the outer protofibrils are available for tPA and PLG binding (Diamond and Anand; 1993), then fine clots can dissolve at a faster rate than coarse clots due to increased binding site exposure. However, in order to effectively model the delay in clot lysis for

thin fibres, it may be necessary to model clot lysis as the lateral transection of fibres (Bannish et al.; 2014a,b). To this end, a stochastic-continuous hybrid mathematical model was developed, which has an advantage of being readily usable for the modelling of thrombolysis in patient-specific geometries.

The hybrid model presented in chapter 7 can account for the difference in lysis time between coarse and fine fibrin clots. The fibrin fibre is discretised into a number of cross-sections and each cross-section has a certain number of binding sites. Since PLG is present in large quantities, the binding of this protein is approximated using a quasi steady-state condition. The binding of free phase tPA and PLS is determined using a continuous Runge-Kutta approach while the production of bound PLS and binding site cleavage are calculated with the stochastic Gillespie method. This is calculated for each cross-section which is considered cleaved once a certain percentage of binding sites has been removed. The cross-sections are projected onto a plane that shows the connections of the fibrin fibre, which can then be used to calculate the length of FDPs. The smallest indivisible unit of fibrin is half a monomer which is assumed to readily dissolve in the plasma and as such is used as a determinant of the extent of lysis. The model predicts that fine fibre clots are dissolved at a slower rate than coarse clots due to the exponential increase in the number of cross-sections that need to be cleaved.

The newly developed model also accounts for the effect of cellular components on the rate of fibrinolysis. Although cellular components are expected to make a modest contribution to the overall clot resistance (Wufsus et al.; 2013), they have a strong effect on the fibrin network. The results show that clot retraction can significantly retard thrombus dissolution due to its effect on increasing the fibrin density of the clot.

8.2 Model Limitations and Recommendations

The fibrinolysis models presented in this thesis are subject to a number of assumptions and limitations. These are provided in the following section, together with discussions of their significance and suggestions on how they can be accounted for.

8.2.1 Model Limitations and Validation

Blood is modelled as a Newtonian fluid

In all computational simulations of clot dissolution, blood has been assumed to be a Newtonian fluid with a constant viscosity which is independent of shear rate. In reality, blood is a non-Newtonian fluid, exhibiting shear-thinning behaviour as it is a mixture of plasma, red blood cells, leukocytes and platelets, amongst other components.

The non-Newtonian behaviour of blood becomes more prominent at low-shear rates ($<100 \text{ s}^{-1}$) and in small vessels ($<500 \mu\text{m}$). Studies have found that a Newtonian model can accurately approximate the behaviour of blood in vessel diameters $>0.5 \text{ mm}$ (Nichols et al.; 2011). The smallest vessel in the patient-specific model is the ACA which has a diameter $>2 \text{ mm}$. Furthermore, blood flow in the ICA is always positive, even under pulsatile conditions. As such, previous simulations of blood flow in a healthy circle of Willis have found that the assumption of a Newtonian fluid is sufficient for realistic results (Razavi and Sahebjam; 2014). A potential concern may be the stagnant flow region

proximal to the occluding clot in the MCA, which can arise if the clot is sufficiently far from the ICA bifurcation. For a shear-thinning fluid, this is expected to increase the viscosity in this region. A number of non-Newtonian models of blood are available such as, for example the Quemada model (Quemada; 1983).

Arterial walls are rigid

The vessel walls have been assumed to be rigid whereas in reality the arterial wall is compliant. Modelling arterial wall compliance requires the use of a FSI (Fluid Structure Interaction) model that solves for the deformation of the vessel wall which is coupled with the CFD solution of blood flow. This requires the computational domain to be remeshed at every time-step to account for the new vessel volume. Brown et al. (2012) found that including FSI is a computationally expensive process that can increase the computational time by over a multiple of 20. Furthermore, another difficulty in accommodating vessel wall motion is concerned with determining the mechanic properties of the wall, which can be difficult to obtain for patient-specific models (Morris et al.; 2016).

The effect of vessel wall compliance on blood flow can be important for certain vessels such as the coronary arteries and the thoracic aorta. However, it becomes noticeably less important for the cerebral arteries as shown by Grinberg et al. (2011) who found that although there can be slight discrepancies in the instantaneous flow rate and pressure values between a rigid and compliant model, the time-averaged flow rate and pressure drop are very similar. Furthermore, the arterial wall becomes stiffer with age due to calcification of the vessel wall (Jani and Rajkumar; 2006). Since the present study is concerned with flow in the cerebral arteries of patients suffering from ischemic stroke (whose average age is around 60), the effect of vessel wall compliance is expected to be negligible. If the wall compliance was accounted for, a slight reduction in the pulse pressure and systolic and diastolic flowrates would be expected.

Laminar blood flow

Blood flow has been solved by the modified Navier-Stokes equations assuming that the flow is laminar. This assumption can be justified because the peak Reynolds number (Re) is well below 2000, which is considered a suitable threshold for laminar flow in biological systems (Nichols et al.; 2011). However, as the clot is lysed, thrombus fragments stuck to the wall can cause a constriction that may increase the local Reynolds number temporarily to higher values. Furthermore, under conditions of hypertension, the inlet velocity could potentially increase, leading to disturbed flow.

For turbulent or transitional flows in the aorta, the shear stress transitional model (SST-Tran) has been shown to be able to capture the important flow features (Kouser et al.; 2013). The same model has also been tested for flow in stenosed carotid arteries (Tan et al.; 2011), making it a suitable choice for the application concerned in this study. Flow constriction by the presence of mural thrombi is expected to lead to higher shear stress which may enhance the erosion of the clot (Bajd and Sersa; 2013). However, modelling turbulence requires the solution of additional equations that is expected to increase the computational time.

Model validation

The computational model has been used to predict clot lysis patterns and dissolution times in idealised and patient-specific geometries. As is the case for many computational models, direct validation is difficult to achieve for complex systems (for example [Kandail et al. \(2016\)](#)). Although certain parameters such as pressures, flow rates, dissolution times and velocities have been shown to be similar to literature values, a direct comparison is important to assess the accuracy and reliability of the computational model.

Although the kinetics model has been validated against the corresponding experimental data in the literature and the clot lysis model has been tested on simple systems as shown in chapters 3 and 4, there has been a lack of validation of the lysis front patterns obtained in the patient-specific geometries presented in chapters 5 and 6. An experimental study similar to that of [Kefayati et al. \(2014\)](#) should be undertaken. The fluid flow patterns can be measured using PIV (particle image velocimetry) and the lysis patterns of the clot can be tracked using confocal microscopy or MRI. This should ideally be done for clots of different fibrin densities and radii to ensure that clot composition is properly accounted for.

8.2.2 Enhancing Clinical Relevance

Influence of the collateral circulation

The status of the collateral circulation has been shown to be an important predictor of the success of thrombolytic therapy ([Bang et al.; 2011](#)). This is because good collateral circulation helps to sustain the penumbra following ischemic insult, allowing for increased flow near the site of occlusion thereby aiding drug transport and preventing the formation of thrombus by eliminating stagnant flow regions ([Qazi et al.; 2015](#)). The effect of collateral circulation on thrombolysis has been neglected in the present model. Good quality MRI images are needed to extract the collateral circulation from clinical scans as CT scans cannot distinguish these vessels from brain matter. A new set of boundary conditions would also be required to predict the overall influence of the collateral circulation on fluid flow proximal to the clot.

Influence of the upstream and downstream vasculature

In the present model of clot dissolution, a pre-defined inlet profile was used and the pressure at the outlets was approximated using a 3-element Windkessel model which represents the influence of downstream vasculature through the use of lumped parameter models. In reality, the inlet flow rate is not constant but varies before and after the occluding clot is lysed. Clinical studies have found changes in the peak velocity in the ACA as the occluded clot in the MCA is dissolved ([Alexandrov et al.; 1994](#)).

Blood vessels distal to the MCA form a system of arteries and veins that gradually feeds back into the heart. The heart determines the distribution of blood according to the resistance of the arteries that branch out from the aorta. This entire linked system can be approximated through the use of a closed-loop model that describes the system as a series of resistors, transistors, capacitors and inductors ([Marsden and Esmaily-Moghadam; 2015](#)). The influence of the heart is determined by a

parameter known as the elastance which is cyclical for pulsatile motion and constant for steady flow. In addition to taking into account the shape of the circle of Willis, the model will be able to simulate the influence of diseases such as carotid stenosis on patient outcome.

Determining clot properties

Clot composition and length have a very strong effect on clot dissolution time (Santos et al.; 2016; Riedel et al.; 2011). For instance, retracted clots can have a permeability several orders of magnitude lower than that of coarse fibrin clots thereby lowering the perfusion velocity and the lysis front velocity. In order to improve the predictive power of the computational model, the properties of the clot should closely represent those found in ischemic stroke.

It may be possible to determine the clot permeability based on CT images by making use of thrombus attenuation increase as determined by Santos et al. (2016) in Hounsfield units. An important step forward would be to determine how the thrombus attenuation increase relates to clot permeability in SI units. Furthermore, the clot length and position can be determined using clinical scans (Friedrich et al.; 2015). This valuable information can then be fed into the model to determine the likelihood of recanalisation for a given thrombolytic regimen and compared to the clinical outcome of the patient. Any parameters that affect the difficulties in recanalisation can then be determined and different therapeutic regimens can be tested to investigate any improvements in treatment that can be achieved.

Predicting Clot Heterogeneity

Clot heterogeneity has been implicated in the generation of finger-like lysing patterns observed experimentally (Anand et al.; 1997; Zidansek et al.; 1995). This departure from a uniform lysis front can be achieved by varying fibrin densities in the clot with thin fibres forming around cellular components (Collet et al.; 2002). The clot properties in chapters 3, 4, 5 and 6 were assumed to be homogeneous while in reality it is likely to be heterogeneous. In the future, blood clots extracted from thrombectomy can be preserved and scanned using a micro CT. The images can be reconstructed in 3D and analysed to determine the void space in the clot. This can then be converted to variations in clot permeability that can be used in the computational model. The inclusion of realistic estimates of clot heterogeneity will help determine the true clot lysis patterns that may occur during thrombolytic therapy.

8.2.3 Extending the Kinetics of Fibrinolysis

Influence of clot composition

The composition of the clot can have a complex effect on the rate of thrombus dissolution. A retracted clot has been shown to impair fibrinolysis by amongst other things inhibited binding of lysis proteins and expulsion of free phase PLG (Sabovic et al.; 1989). Chapter 7 presents an initial attempt towards developing a fibrinolysis model to incorporate this effect based on the the binding sites that each fibrin clot exposes and the number of cleavable cross-sections. A detailed experimental and computational study should be performed to ensure that the fibrinolysis model can determine the correct clot dissolution for a variety of different clot compositions.

Influence of other fibrinolysis components

Aside from PLG, PLS, tPA and AP, fibrinolysis can be affected by a myriad of different components such as myosin, DNA, histones, etc. Furthermore, cellular components such as leukocytes can release substances such as neutrophil elastase that act as lytic agents and enhance PLG activation. These substances can all modify fibrinolysis and cause changes in clot dissolution time. In order to simplify the computational model, a number of components were neglected. For instance, the effect of PAI was considered negligible due to its low plasma levels and the correspondingly high pharmacological concentration of tPA ([Anand and Diamond; 1996](#)). Myosin has also been shown to be negligible at relatively high tPA concentrations (>1 nM) ([Mutch et al.; 2010](#)).

Influence of thrombosis

Some studies have speculated that the presence of stagnant flow regions proximal to the face of a clot may encourage the development of thrombosis ([Qazi et al.; 2015](#)). The growth of the clot in these regions may account for the reason why patients with poor collateral circulation have a lower likelihood of success during thrombolytic therapy. Thrombus forms on a much longer timescale than thrombolysis, as the former is expected to take several months. However, the likelihood of thrombus development can be predicted by evaluating the residence time distribution in the early stages of clot lysis as residence time is associated with the likelihood of thrombus formation. If there is a high probability of clot formation, then thrombosis may be modelled during the treatment time (1 hour to 4.5 hours) using the residence time distribution as a guide for clot growth ([Menichini et al.; 2016](#)).

Influence of drag on fibrinolysis

Various studies have shown that drag may contribute to an increase in the thrombolytic rate. [Wootton et al. \(2002\)](#) found that a biochemical model was unable to explain the degradation of mural clots at larger shear rates while [Sersa et al. \(2007a\)](#) found that turbulence can lead to a substantial increase in the rate of clot lysis. While we expect that this will have a limited effect on the dissolution of fully occlusive clots in the early stages of lysis, the influence of shear stress on the dissolution of a residual mural clot may be substantial. The influence of fluid shear on clot dissolution can be addressed by adding an extra degradation term to the lysis of binding sites in the biochemical model. The rate of degradation will be a function of shear rate and will follow similar parameters to those developed by [Sersa et al. \(2007a\)](#). However, it should be noted that shear rate is very sensitive to mesh density, and as such will require a highly resolved mesh to model accurately.

8.3 Further Recommendations

Investigating other types of occlusions

In chapters 5 and 6, clot lysis was modelled in a patient-specific geometry with a complete occlusion in the M1 branch of the MCA. However, stroke can occur in many other regions in the cerebral vasculature such as the M1/M2 segment of the MCA, the terminal segment of the ICA and the vertebral artery. Some of these locations have a low level of recanalisation and a high rate of mortality at 3 months.

As such, investigating clot lysis in these geometries may offer insights as to why certain areas may be more difficult to treat with thrombolysis. Furthermore, certain arteries such as the M1/M2 MCA segment is subject to high variability from patient to patient and could potentially affect the success of recanalisation.

Movement of the thrombus in the system

In evaluating the clot lysis patterns, it was found that micro-emboli could form during clot dissolution. This could lead to secondary complications such as micro-occlusions. Furthermore, the breaking off of clot chunks during thrombolysis could potentially allow for rapid recanalisation. This is expected to happen towards the latter stages of dissolution where the pressure may force the clot to dislodge. To account for this, an additional term for the thrombus will be required when solving for the voidage, and it can be activated when a critical value for the residual force is reached.

Using the computational model to investigate novel technologies

A recent exciting development in the field of thrombolysis is the creation of nano-particles that can encapsulate tPA and release the drug at the site of the clot. This can be achieved via shear stress sensitivity ([Korin et al.; 2012](#)) or ultrasound induced cavitation ([Shaw et al.; 2007](#)). The release of tPA from these nano-carriers can be incorporated into the computational model by using an additional transport equation and solving for the acoustic pressure field generated by ultrasound transducers ([O'Neil; 1949](#)). Predicting the thrombolytic efficacy of these novel technologies may help optimise the technology and aid their translation into clinical application.

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Appendix A

Additional Mathematical Information

A.1 Mathematical Operators

Two types of mathematical shorthand are used to describe transport equations in this work: Einstein summation notation and operators represented with Greek symbols. The divergence operator can be expressed as the dot product of 2 vectors:

$$\nabla \cdot \eta = \frac{\partial \eta_i}{\partial x_i} = \frac{\partial \eta_x}{\partial x} + \frac{\partial \eta_y}{\partial y} + \frac{\partial \eta_z}{\partial z} \quad (\text{A.1})$$

where η is a vector quantity defined as

$$\eta = \begin{bmatrix} \eta_x \\ \eta_y \\ \eta_z \end{bmatrix} \quad (\text{A.2})$$

The gradient operator of a vector field on the other hand creates a second-order tensor:

$$\nabla \eta = \frac{\partial \eta_i}{\partial x_j} = \begin{bmatrix} \frac{\partial \eta_x}{\partial x} & \frac{\partial \eta_x}{\partial y} & \frac{\partial \eta_x}{\partial z} \\ \frac{\partial \eta_y}{\partial x} & \frac{\partial \eta_y}{\partial y} & \frac{\partial \eta_y}{\partial z} \\ \frac{\partial \eta_z}{\partial x} & \frac{\partial \eta_z}{\partial y} & \frac{\partial \eta_z}{\partial z} \end{bmatrix} \quad (\text{A.3})$$

The convection term in the momentum equation involves the dot product between a vector and the gradient of a vector. This can be expanded as:

$$\eta \cdot \nabla \eta = \eta_j \frac{\partial \eta_i}{\partial x_j} = \begin{bmatrix} \eta_x \frac{\partial \eta_x}{\partial x} + \eta_y \frac{\partial \eta_x}{\partial y} + \eta_z \frac{\partial \eta_x}{\partial z} \\ \eta_x \frac{\partial \eta_y}{\partial x} + \eta_y \frac{\partial \eta_y}{\partial y} + \eta_z \frac{\partial \eta_y}{\partial z} \\ \eta_x \frac{\partial \eta_z}{\partial x} + \eta_y \frac{\partial \eta_z}{\partial y} + \eta_z \frac{\partial \eta_z}{\partial z} \end{bmatrix} \quad (\text{A.4})$$

A.2 Derivation of Transport Equations

In order to derive the transport equations of a fluid flowing through a porous medium, microscopic balance equations are used and then averaged over a representative control volume.

Microscopic Equations

The movement of a fluid can be expressed with a mass and momentum conservation balance as showing in equations A.5 and A.6, respectively.

$$\frac{\partial \rho}{\partial t} = -\nabla \cdot u\rho \quad (\text{A.5})$$

$$\frac{\partial \rho u}{\partial t} + \nabla \cdot (\rho u u) = -\nabla p + \nabla \tau + \rho g \quad (\text{A.6})$$

$$\tau = \mu(\nabla u + (\nabla u)^T) \quad (\text{A.7})$$

where g is a vector that defines the gravitational acceleration for each component and τ is the stress tensor. The latter can be elaborated as a Newtonian fluid by using equation A.7. Fluid flow is subject to a no-slip condition as it encounters the surface of the porous medium.

The transport of a component in the fluid phase can be defined on the microscopic scale as:

$$\frac{\partial C_\alpha}{\partial t} = -\nabla \cdot (uC_\alpha + j_\alpha) + \Sigma R_\alpha \quad (\text{A.8})$$

where j_α is the diffusive mass flux that can be determined using Fick's law, defined as:

$$j_\alpha \equiv C_\alpha(u_\alpha - u) = -D_\alpha \nabla C_\alpha \quad (\text{A.9})$$

where u_α is the velocity of the α component.

Volume Averaging Identities

The microscopic equations in the previous section govern the transport of a fluid in the porous medium. In order to determine the fluid properties as an average over a control volume that contains a porous medium, the method of volume averaging can be applied (Bear and Bachmat; 1991; Whitaker; 1986; Wang et al.; 2015). The microscopic components can be averaged in 2 fashions: using phase average (equation A.10) and intrinsic phase average (equation A.11) variables.

$$\bar{\eta} = \frac{1}{U_0} \int_{U_f} \eta \, dU_0 \quad (\text{A.10})$$

$$\bar{\eta}^f = \frac{1}{U_f} \int_{U_f} \eta \, dU_0 \quad (\text{A.11})$$

where U_0 is the volume of the entire control volume and U_f is the volume of the fluid phase within it.

The two expressions can be related by the voidage as

$$\bar{\eta} = \epsilon \bar{\eta}^f \quad (\text{A.12})$$

These averaging procedures can be applied to temporal and spatial derivatives as shown in the equations below.

$$\frac{\partial \bar{\eta}}{\partial t} = \frac{\partial \bar{\eta}}{\partial t} - \frac{1}{U_0} \int_S \eta w \cdot n_c dS \quad (\text{A.13})$$

$$\bar{\nabla} \eta = \nabla \bar{\eta} + \frac{1}{U_0} \int_S \eta \cdot n_c dS \quad (\text{A.14})$$

$$\bar{\nabla} \cdot \eta = \nabla \cdot \bar{\eta} + \frac{1}{U_0} \int_S \eta \cdot n_c dS \quad (\text{A.15})$$

where w refers to the velocity of the interface, S is the interfacial area of the clot with the fluid and n_c is a vector that is normal to its surface.

Mass Conservation

Using the averaging rules applied in the previous section, the microscopic balance of the fluid phase can be expressed in terms of phase averaged variables. We begin with the mass conservation as represented by equation A.5. The last term corresponds to a mass exchange between the 2 phases and can therefore be set to 0 if the 2 phases don't react with one another.

$$\frac{\partial \epsilon \rho}{\partial t} + \nabla \cdot (\rho \bar{u}) = \frac{\partial \epsilon \rho}{\partial t} + \nabla \cdot (\rho \bar{u}^f \epsilon) = -\frac{1}{U_0} \int_S \rho(u - w) \cdot n dS = 0 \quad (\text{A.16})$$

Momentum Conservation

General Formulation

The microscopic momentum equation can be expressed in terms of the intrinsic phase average as:

$$\frac{\partial \rho \epsilon \bar{u}^f}{\partial t} + \nabla \cdot (\rho \epsilon \bar{u}^f \bar{u}^f) = \nabla \cdot (\epsilon \bar{\sigma}^f) + \frac{1}{V} \int_S (\sigma \cdot n + \rho u(u - w) \cdot n dS) + \epsilon \rho g \quad (\text{A.17})$$

$$\sigma = -pI + \tau \quad (\text{A.18})$$

where σ is the total stress tensor. The integral term represents the momentum transfer across phases (which is neglected due to the no slip condition) and the total drag force in the control envelope. The stress terms can thus be simplified as:

$$\nabla \cdot (\epsilon \bar{\sigma}^f) + \frac{1}{U_0} \int_S \sigma \cdot n dS = \epsilon \nabla \cdot \bar{\sigma}^f + \frac{1}{U_0} \int_S (\sigma - \bar{\sigma}^f) \cdot dS \quad (\text{A.19})$$

This then gives rise to the following expression for the momentum equation:

$$\frac{\partial \rho \epsilon \bar{u}^f}{\partial t} + \nabla \cdot (\rho \epsilon \bar{u}^f \bar{u}^f) = \epsilon \nabla \cdot (\bar{\sigma}^f) + \frac{1}{U_0} \int_S (\sigma - \bar{\sigma}^f) \cdot dS + \epsilon \rho g \quad (\text{A.20})$$

$$= -\epsilon \nabla \bar{p}^f + \epsilon \nabla \bar{\tau}^f + \frac{1}{U_0} \int_S (\sigma - \bar{\sigma}^f) \cdot dS + \epsilon \rho g \quad (\text{A.21})$$

The integral term in this equation can be expressed in macroscopic variables as a resistive force between the porous domain and the fluid (with aid of the Ergun equation).

$$\int_{A_f} (\sigma - \bar{\sigma}^f) \cdot dS = -\epsilon \left(\frac{\mu \epsilon}{k} (\bar{u}^f - w) \right) \quad (\text{A.22})$$

Bear and Bachmat (1991) derived the Darcian source term with a slightly different method and attained the following expression:

$$F = -\mu \alpha_{ij} \frac{C_f}{\Delta_f^2} q_j^m = -\epsilon q_j^m T \frac{\mu}{k} \quad (\text{A.23})$$

$$\Delta_c = \Delta_f / C_f \quad (\text{A.24})$$

$$q_j^m = \epsilon (\bar{u}^f - w) \quad (\text{A.25})$$

where α_{ij} refers to a tensorial property of the porous-fluid interface, C_f is a dimensionless shape factor, Δ_c is a measure of the pore size, Δ_f is the hydraulic radius, T is the tortuosity and q_j^m is the mass weighted specific discharge. Thus the most generalised equation for momentum conservation can thus be expressed as:

$$\frac{\partial \epsilon \bar{u}^f \rho}{\partial t} + \nabla \cdot (\rho \epsilon \bar{u}^f \bar{u}^f) = -\epsilon \nabla \bar{p}^f + \epsilon \nabla \bar{\tau}^f + \epsilon \rho g - \epsilon \left(\frac{\mu \epsilon}{k} (\bar{u}^f - w) \right) \quad (\text{A.26})$$

Further to this, the left hand side of the equation can be notably simplified by using the mass conservation balance in equation A.16. For instance, the LHS of equation A.26 can be elaborated as:

$$\frac{\partial \epsilon \bar{u}^f \rho}{\partial t} + \nabla \cdot (\rho \epsilon \bar{u}^f \bar{u}^f) = \epsilon \rho \frac{\partial \bar{u}^f}{\partial t} + \bar{u}^f \frac{\partial \rho \epsilon}{\partial t} + \rho \epsilon \bar{u}^f \nabla \cdot (\bar{u}^f) + \bar{u}^f \nabla \cdot (\rho \epsilon \bar{u}^f) \quad (\text{A.27})$$

$$= \epsilon \rho \left(\frac{\partial \bar{u}^f}{\partial t} + \bar{u}^f \nabla \cdot (\bar{u}^f) \right) + \bar{u}^f \left(\nabla \cdot (\rho \epsilon \bar{u}^f) + \frac{\partial \rho \epsilon}{\partial t} \right) \quad (\text{A.28})$$

$$= \epsilon \rho \left(\frac{\partial \bar{u}^f}{\partial t} + \bar{u}^f \nabla \cdot (\bar{u}^f) \right) \quad (\text{A.29})$$

If the fluid's behaviour can be described as Newtonian, then the stress tensor can be expressed as a function of the velocity. This takes the form as

$$\epsilon \bar{\tau}^f = \mu(\overline{\nabla u \epsilon}^f + (\overline{(\nabla u \epsilon)^f})^T) \quad (\text{A.30})$$

$$= \mu(\nabla \epsilon \bar{u}^f + (\nabla \epsilon \bar{u}^f)^T) + \frac{1}{U_0} \int_S u \cdot n + n \cdot u dS \quad (\text{A.31})$$

where the last term takes into account the movement of the porous interface. If the movement of this interface can be neglected and gravity is not included then the momentum equation can be written in terms of both the intrinsic phase average (equation A.32) and the phase average velocity (equation A.33) as

$$\rho \epsilon \left(\frac{\partial \bar{u}^f}{\partial t} + \bar{u}^f \nabla \cdot (\rho \bar{u}^f) \right) = -\epsilon \nabla \bar{p}^f + \mu \nabla^2 \bar{u}^f \epsilon - \frac{\mu \epsilon^2}{k} (\bar{u}^f) \quad (\text{A.32})$$

$$\rho \left(\frac{\partial \bar{u}}{\partial t} + \bar{u} \nabla (\bar{u}/\epsilon) \right) = -\epsilon \nabla (\bar{p}^f) + \mu \nabla^2 \bar{u} - \frac{\mu \epsilon}{k} (\bar{u}) \quad (\text{A.33})$$

Component Conservation

The microscopic balance equation presented in equation A.8 can undergo volume averaging to take the following form:

$$\frac{\partial \epsilon \bar{C}_\alpha^f}{\partial t} = -\nabla \cdot (\bar{u}^f \bar{C}_\alpha^f + \bar{C}_\alpha \dot{u}^f + \bar{j}^{\alpha f}) - \frac{1}{U_0} \int_S (C_\alpha (v - u) + j_\alpha) \cdot n dS + \epsilon \Sigma R_\alpha \quad (\text{A.34})$$

where $\bar{C}_\alpha \dot{u}^f$ refers to convective dispersion, the integral term refers to the exchange of the α species between the porous and fluid domains and $\epsilon \Sigma R_\alpha$ refers to the generation of α in the fluid phase. The diffusive and dispersive fluxes are combined into a single term (as defined by Fick's law) and the the integral term is replaced with the net rate of adsorption onto the surface. This gives the following equation:

$$\frac{\partial \epsilon \bar{C}_\alpha^f}{\partial t} + \nabla \cdot (\epsilon \bar{u}^f \bar{C}_\alpha^f) = \nabla \cdot (\epsilon (D^* + D) \nabla \bar{C}_\alpha^f) - \frac{\partial (1 - \epsilon) A_\alpha}{\partial t} + \epsilon \Sigma R_\alpha \quad (\text{A.35})$$

where A represents the concentration of the component on the porous surface. If the porous medium is highly porous such that there is no considerable spatial difference in the porosity, then the equation can be rearranged to solve for the superficial concentration $C_\alpha^s = \epsilon C_\alpha$. The equation for the bound phase component on the porous media (where there is no convection or diffusion) can be expressed as

$$\frac{\partial (1 - \epsilon) n_\alpha}{\partial t} = \frac{\partial (1 - \epsilon) A_\alpha}{\partial t} + (1 - \epsilon) \Sigma G_\alpha \quad (\text{A.36})$$

where n_α is the concentration and ΣG_α refers to the rate of generation of species α in the bound phase.

Appendix B

Additional information for Chapter 3

B.1 Finite difference discretisation

Parameters related to numerical discretisation for the finite difference methodology used to solve for clot lysis under diffusion controlled transport are given in Table B.1.

Table B.1: Values used for FD discretisation

Symbol	Value	Units	Meaning
D	5×10^{-11}	$m^2 s^{-1}$	Dispersion Coefficient
dt	0.1	s	Time-step Value
dx	0.01	mm	Spatial-step Value
L	3	mm	Clot Length
nt	2.4×10^4	—	Number of Discrete Time Points
nx	300	—	Number of Discrete Spatial Points

B.2 Changes in the property of the clot

This section provides a description of the clot lysis fibrin microstructure model as outlined by [Diamond and Anand \(1993\)](#). The blood clot is assumed to be a fibrous porous medium composed only of strands of fibrin fibres. The fibrin mesh is assumed to be compromised of fibrin monomers that combine in a half-staggered manner to form protofibrils that go on to laterally aggregate and form fibrin fibres. The microstructure model assumes that the fibrin radius is uniform across the entire fibre and degrades homogeneously as lysis goes on. The amount of fibrin lysed can be used to evaluate the change in fibrin fibre radius

$$R_f = \sqrt{R_{f0}^2 - \frac{LN_{AV}}{\pi p_0 L_{pf} a_0 / U_0}} \quad (B.1)$$

where R_f represents the fibre radius, p_0 represents the amount of protofibrils per fibre cross-sectional area, L_{pf} is the length of each protofibril, a_0 is the amount of fibrin monomers per length of protofibril, N_{AV} is Avogadro's constant and U_0 is the volume of the control surface (computational cell). For a fibrous porous medium, the radius of the fibrin fibre can be used to calculate the voidage

and permeability. The voidage is expressed as

$$\epsilon = 1 - \pi R_f^2 L_{pf} / U_0 = 1 - \rho_{f,0} / \rho_{fibre} \quad (\text{B.2})$$

where ρ_β is the fibrin density in the control volume and ρ_{fibre} is density of fibrin in a fibre. The concentration of the total amount of binding sites that are sterically accessible to the proteins can be found by calculating the amount of binding sites per fibrin monomer and the total amount of these monomers in a protofibril.

$$\theta_\alpha = a_0 L_{pf} \xi q_\alpha \frac{1}{N_{AV} U_0} \quad (\text{B.3})$$

Where ξ is the number of protofibrils that are sterically accessible and q_α is the number of binding sites in a fibrin monomer for species α . In this study, we assume that all the protofibrils in the fibrin fibre are sterically accessible.

$$\xi = \pi p_0 (R_f + r_0)^2 \quad (\text{B.4})$$

where r_0 is the average radius of a protofibril. Finally, the permeability of a fibrous porous medium can be expressed as a function of the fibre radius and porosity:

$$k = \beta \frac{R_f^2}{(1 - \epsilon)^n} \quad (\text{B.5})$$

where k is the permeability, n is the permeability coefficient and λ is the permeability constant (chosen within the range specified by [Wufsus et al. \(2013\)](#)).

B.3 Comparing with experimental data

We matched our numerical predictions with the experimental results given by [Blinic et al. \(2000\)](#) and [Rijken et al. \(1982\)](#). Here we outline the experimental set up of each and describe any modifications that have been made in the kinetic constants or the modelling equations. [Blinic et al. \(2000\)](#) created different types of clots by varying NaCl concentrations and analysed their fibrin microstructure upon lysis with PLS using atomic force microscopy. The clots were created using 1.8 mg/ml fibrinogen and had a range of average fibrin diameters between 150 to 600 nm. A plasmin concentration of 0.17 U/ml was applied to 2 clots with a fibrin diameter of around 100 nm and 300 nm. Because the plasmin was in the system for a very long time (>20 mins), we added an extra degradation term to account for the denaturing of the plasmin. This is assumed to occur only in the free phase and can be represented by the following equation.

$$R_{PLS} = -k_{decay} C_{PLS} \epsilon \quad (\text{B.6})$$

The value of k_{decay} was obtained from [Blinic et al. \(2000\)](#) where it can be clearly seen that the majority of initial plasmin (>99%) was denatured by 80 minutes. A k_{cat} value of 1 s^{-1} was found to best fit the experimental data. All other kinetic parameters are the same as those in Table B.2. [Rijken](#)

et al. (1982) evaluated the lytic efficacy of extrinsic tPA in its one-chain and two-chain forms. They created clots using 2 mg/ml fibrinogen in a 0.14 M NaCl solution and then evaluated the clot lysis time for varying concentrations of one-chain tissue plasminogen activator. Using the results obtained by Blinc et al. (2000), we assumed that the average initial diameter of fibrin fibres in the clot created in these conditions was 300 nm. The initial plasminogen concentration was 0.15 mg/mL and the kinetic parameters for plasminogen activation were $K_M = 2.42\mu M$ and $k_2 = 0.22s^{-1}$. The kinetic value of $k_{cat} = 5s^{-1}$ used by Wootton et al. (2002) and Anand and Diamond (1996) was chosen. All other kinetic parameters are the same as those in Table B.2.

B.4 Kinetic Constants

The well mixed kinetics model defined in the paper was used to generate Figures 3.3 and 3.4. The values for the constants used are given in Table B.2. The well mixed kinetics model was extended to analyse clot lysis in 1D diffusion limited transport. This was done to generate Figures 3.5 and 3.6 in the research article. The same kinetic parameters as those defined in Table B.2 were used.

Table B.2: Values for model parameters

Symbol	Value	Units	Reference
a_0	0.0444	nm^{-1}	Diamond and Anand (1993)
k_2	15	s^{-1}	Diamond and Anand (1993)
$k_{a,PLG}$	1.087×10^{-4}	$\mu M^{-1} s^{-1}$	Anand et al. (1995)
$k_{a,PLS}$	0.500×10^{-3}	$\mu M^{-1} s^{-1}$	Anand et al. (1995)
$k_{a,tPA}$	1.148×10^{-4}	$\mu M^{-1} s^{-1}$	Anand et al. (1995)
k_{AP}	10	$\mu M^{-1} s^{-1}$	Tiefenbrunn et al. (1986)
k_{cat}	10	s^{-1}	Diamond and Anand (1993)
k_{decay}	8.45×10^{-4}	s^{-1}	Blinc et al. (2000)
K_M	0.13	μM	Diamond and Anand (1993)
$k_{r,PLG}$	4.131×10^{-3}	s^{-1}	Anand et al. (1995)
$k_{r,PLS}$	5.435×10^{-5}	s^{-1}	Anand et al. (1995)
$k_{r,tPA}$	6.658×10^{-5}	s^{-1}	Anand et al. (1995)
MW_{fbrn}	3.4×10^5	$g\ mol^{-1}$	—
n	1.8	—	—
N_{AV}	6.02×10^{23}	mol^{-1}	—
p_0	0.01116	nm^{-2}	Diamond and Anand (1993)
r_0	2.39	nm	Diamond and Anand (1993)
R_{f0}	250	nm	—
γ	0.1	—	Diamond and Anand (1993)
β	0.025	—	—
ρ_{fibre}	0.28	$g\ ml^{-1}$	Diamond and Anand (1993)
$\rho_{f,0}$	0.003	$g\ ml^{-1}$	—

Appendix C

Additional information for Chapter 4

C.1 Inlet conditions

The equation used to specify a pulsatile inlet flowrate is given by:

$$Q = a_0 + \sum_{m=1}^8 a_m \cos(mwt) + \sum_{m=1}^8 b_m \sin(mwt) \quad (\text{C.1})$$

where the values of each coefficient are provided in Table C.1.

C.2 Distribution of binding sites on a fibre cross-section

Below we present a derivation for expressing the number of binding sites as a function of the cross-section radius with the aid of Figure C.1. The angle α between two binding sites on the same protofibril layer of distance R_f can be expressed as follows:

$$\alpha = 2 \arcsin\left(\frac{\delta\theta}{2R_f}\right) \quad (\text{C.2})$$

Therefore the total number of binding site locations per layer can be expressed as

$$N_{BS,L} = 2\pi/\alpha = \zeta \frac{\pi}{\arcsin(\delta\theta/(2R_{f,L}))} \quad (\text{C.3})$$

Where $N_{BS,L}$ is the number of binding sites per layer, $R_{f,L}$ is the radius of the layer from the origin and ζ is the number of binding sites in each location (assumed to be 1). Thus the total number of binding site locations for that cross-section is related to the quantity of layers it has. Therefore the total number of binding sites for a cross-section can be expressed as

$$N_{BS,1} = \sum_{n=1}^{N_{layer}=R_f/\delta r} \frac{\pi}{\arcsin(\delta\theta/(2n\delta r(R_f)))} \quad (\text{C.4})$$

Table C.1: Values of the Fourier coefficients for the expression of blood flowrate at the inlet

Coefficient	Value (ml/s)
a_0	4.307
a_1	-0.2427
b_1	1.125
a_2	-0.9454
b_2	0.5766
a_3	-0.4395
b_3	-0.3121
a_4	-0.2677
b_4	-0.4183
a_5	0.3666
b_5	-0.2777
a_6	0.07413
b_6	0.05799
a_7	0.111
b_7	-0.01202
a_8	0.0431
b_8	0.1057
w	6.283185

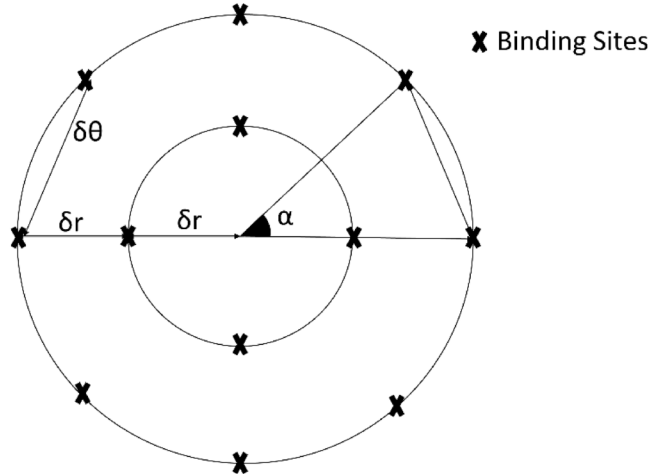


Figure C.1: Binding site distribution for a cross-section. Each cross represents a location where a number of binding sites (ζ) can be found and each circle is a protofibril layer. δr represents the distance between each protofibril layer and $\delta \theta$ is the distance between 2 locations on that same layer

Appendix D

Additional information for Chapter 7

The following chapter provides any extra information needed for the model of fibrinolysis defined in chapter 7. The stoichiometry matrix that specifies the change in the state array after each timestep is presented in Table D.1. Each row corresponds to a reaction that can take place and each column within that row defines the corresponding change in the number of species. For instance, the row tPA ads refers to the adsorption of tPA onto a fibrin binding site and a causes a change in the number of bound phase tPA molecules at a certain cross-section. The species represented in each column refer to the number of bound phase molecules of tPA, PLS, binding sites and degraded binding sites with PLS attached. The reaction in each row represents tPA adsorption, tPA desorption, PLS adsorption, PLS desorption, generation of PLS, degradation of fibrin binding sites and the release of PLS from the degraded binding site.

The state array which refers to total number bound phase molecules at each cross-section is given in equation D.1. For instance, $N_{tPA,10}$ refers to the number of bound tPA molecules at the 10th cross-section. The propensity function, which specifies the probability of a certain reaction occurring, is given in equation D.2. This array specifies the probability of all the reactions occurring in each cross-section according to the number of molecules that are present in that cross-section.

$$X = [N_{tPA,1}, \dots, N_{tPA,N_{CS}}, N_{PLG,1}, \dots, N_{PLG,N_{CS}}, N_{PLS,1}, \dots, N_{PLS,N_{CS}}, \\ N_{tot,1}, \dots, N_{tot,N_{CS}}, L_{PLS,1}, \dots, L_{PLS,N_{CS}}] \quad (D.1)$$

Table D.1: Stoichiometry matrix for hybrid stochastic continuous model of fibrinolysis

RXNS/SPCS	N_{tPA}	N_{PLG}	N_{PLS}	N_{tot}	L_{PLS}
tPA ads	1	0	0	0	0
tPA dis	-1	0	0	0	0
PLS ads	0	0	1	0	0
PLS dis	0	0	-1	0	0
PLS gen	0	-1	1	0	0
N_{tot} deg	0	0	0	-1	1
L_{PLS} dis	0	0	0	0	-1

$$a = \begin{pmatrix} k_{r,PLS}N_{PLS,1} \\ \vdots \\ k_{r,PLS}N_{PLS,N_{CS}} \\ k_{a,PLS}M_{PLS,1}N_{free,1} \\ \vdots \\ k_{a,PLS}M_{PLS,N_{CS}}N_{free,N_{CS}} \\ \frac{k_2N_{PLG,1}N_{tPA,1}}{K_M + N_{PLG,1}} \\ \vdots \\ \frac{k_2N_{PLG,N_{CS}}N_{tPA,N_{CS}}}{K_M + N_{PLG,N_{CS}}} \\ k_{cat}N_{PLS,1} \\ \vdots \\ k_{cat}N_{PLS,N_{CS}} \\ k_{r,PLS}L_{PLS,1} \\ \vdots \\ k_{r,PLS}L_{PLS,N_{CS}} \\ k_{a,tPA}M_{tPA,1}N_{free,1} \\ \vdots \\ k_{a,tPA}M_{tPA,N_{CS}}N_{free,N_{CS}} \\ k_{r,tPA}N_{tPA,1} \\ \vdots \\ k_{r,tPA}N_{tPA,N_{CS}} \end{pmatrix} \quad (D.2)$$

Appendix E

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Appendix F

Presentations and Awards

F.1 Academic Presentations

- **A 3D multiscale model of thrombolysis for patient-specific applications**, International Conference on Biomedical Engineering, Singapore, 2016
- **Towards a 3D patient-specific computational model of thrombolysis**, International Society of Fibrinolysis and Proteolysis, Shizuoka, 2016
- **Modelling thrombolytic therapy in ischemic stroke: a 3D multiscale model of clot dissolution**, European Society of Biomechanics, Lyon, 2016
- **Towards a multi-physics framework for thrombolysis in middle cerebral artery during ischemic stroke**, Chemical Engineering PhD Symposium, Imperial College London, London, 2016
- **Mathematical modelling of oxygen transport in the retina**, Summer Biomechanics, Bioengineering and Biotransport Conference, National Harbor, Maryland, USA, 2016
- **Development of a computational model to investigate clot dissolution in stroke**, Chemical Engineering PhD Symposium, Imperial College London, London, 2015
- **Computational multiphysics model of clot lysis in a completely occluded stenotic artery**, Summer Biomechanics, Bioengineering and Biotransport Conference, Snowbird, Utah, USA, 2015

F.2 Scholarships and Awards

Second Place, ICBME Young Investigator Award	2016
Second Place, Imperial College Chemical Engineering Symposium Best Presentation	2016
Finalist, Imperial College London Best Graduate Teaching Assistant	2016
ESB Travel Award	2016
Winner, Departmental Best Graduate Teaching Assistant Award	2015
Finalist, Student Academic Choice Awards Best Graduate Teaching Assistant	2015
Doctoral Training Scholarship for PhD Studies (Full Scholarship)	2013–2017