Microfluidic disturbances and their impact on blood clotting and platelet aggregation

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

by

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for another academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

Francisco Javier Tovar Lopez.
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Abstract

Multi-disciplinary areas of research like biomechanics or mechanobiology is providing new insights, perspective and scientific frameworks to better understand complex biophysical processes. A particularly interesting bio-physical process is blood clotting. The mechanical aspects of blood flow and its impact on blood cell responses are not well understood, but can be studied from a robust perspective through the implementation of different engineering tools including computational simulations, experiments, and microtechnologies.

The aim of this thesis was to gain insight into the haemodynamics on experiments recently performed in-vivo on mice and in-vitro using capillaries in blood flow. Using this insight a micro-fluidic platform tailored to study the mechanical effects driving blood platelet aggregation independent of biochemical triggers was developed. Detailed account of the haemodynamics of in-vivo and in-vitro experiments are presented as well as the design, fabrication, characterisation of a microfluidic platform that utilizes micro-contractions to test blood dynamics under different scenarios of dynamic strain-rates. The microfluidics platform was characterized using Computational Fluid Dynamics (CFD) and micro-Particle Image Velocimetry. New proof-of-concept blood perfusion experiments that illustrate the versatility of the device are also presented. The newly created platform has been instrumental in elucidating a new mechanism of platelet aggregation that occurs independent of the commonly accepted soluble agonist mediated pathways[1].

The main contribution of this work was building evidence through simulations and the fabrication of a microfluidic platforms to support the claim that with the chemical mechanisms of aggregation blocked, dynamic strain-rates produced by a micro-contraction, are able to produce platelet aggregation and thrombus formation. The microfluidics platform presented facilitates the detailed analysis of the effects of haemodynamics parameters on the rate and extent of platelet aggregation and will be a useful tool to elucidate the haemodynamics and platelet mechano-transduction mechanisms, underlying this mechanical-dependent process.
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Chapter 1

Introduction

1.1 Motivation

The recent development of new multi-disciplinary areas of research like biomechanics or mechanobiology, where physicist and engineers partner with biologist to explore different biological phenomena where mechanics is involved, has provided new insights, perspective and scientific frameworks to better understand complex bio-physical processes important for life sustainability. One of such processes is blood clotting which is a key phenomena for the sustainability of normal blood flow in any human being, but under abnormal conditions can produce diseases such as the acute coronary syndromes and ischemic stroke. The variables regulating thrombosis include hypercoagulability, endothelial injury and blood flow parameters (haemodynamics). The former two variables have been studied extensively and have a well defined role in arterial thrombosis. However, the mechanism by which haemodynamics affects the thrombus formation process are not well understood. With novel scientific frameworks, the role of haemodynamics on thrombus formation can be studied from a robust perspective through the implementation of different engineering tools including computational simulations, experiments, and microtechnologies. A better understanding of how blood clotting occurs will not only have positive impact on basic science by increasing the knowledge on blood biology and its dynamics but also in generating new devices to design better drugs and screen blood cell functional defects.

1.2 Thesis Objective

This dissertation aims to gain deeper insight into the haemodynamics regulating thrombosis at micro-scale, by studying novel experiments recently performed on mice and capillaries. Using this insight a micro-fluidics platform is presented to study the mechanical
effects driving platelet aggregation independent of biochemical triggers (related to hypercoagulability and endothelial injury). This thesis presents a detailed account of the design, numerical and experimental validation of a microfluidic platform that utilizes focally defined changes in strain-rate under non-recirculation conditions to probe platelet dynamics in response to haemodynamics forces. The newly created platforms have been instrumental in elucidating a new mechanism of platelet aggregation that occurs independent of the commonly accepted soluble agonists mediated pathways[1]. Full details of the design and validation of this platform technology as well as blood perfusion experiments that demonstrates the basic haemodynamics parameters driving platelet aggregation are also presented.

1.3 Contributions

The research presented in this work has resulted from a multidisciplinary work. Therefore, my contributions need to be specified for the purposes of intellectual property

Chapter 2: I did not contribute at all to the experiments. This chapter is presented as a background for the thesis. The experiments presented in this chapter were designed and performed by researchers at the Australian Centre for Blood Diseases (ACBD).

Chapter 3: I proposed to study in detail the experiments done on mice described on Chapter 2 by using numerical simulations. After several discussion with biologist from ACBD during the timeframe of this research, I proposed to analyse the flow haemodynamics using streamlines, which was an important step to facilitate the study of strain or shear histories for a single platelet. I wrote the article for submission to the Journal of Biomechanics[3].

Chapter 4: I performed the fluid dynamic simulations as a response to characterizse the second set of experiments described in Chapter 2. The images from these analyses were published in [1]. I proposed to analyse not only the strain-rate distribution at the surface of the bead, but also the fluid streamlines. I compared these simulations with an analytical solution.

Chapter 5: I designed several high resolution masks to perform a versatile micro-fabrication process to test blood under several scenarios of micro-contractions. I fabricated the moulds used for the polymer casting process of the device. I tested and implemented ways to fabricate the reservoirs of the chip. I implemented a process to eliminate debris from the fabrication process that could clog the micro-contraction. I designed and implemented several debris traps. post-processing and analysis of µParticle Image Velocimetry experiments. I performed the blood perfusion experiments and analysis of blood perfusion experiments.
CHAPTER 1.

1.4 Outcomes

During the timeframe of this PhD the following journal articles were produced:


Patent application:
PTC patent application PCT/AU2010/000273

Conference proceedings:


### 1.5 Overview of platelet aggregation

Blood is an essential but complex fluid that transport nutrients to vital organs and waste products away from them. At the same time that regulates body pH and temperature.
CHAPTER 1.

Blood accomplish its important functions by travelling around the body in blood vessels, known as arteries and veins. Blood is composed of liquid (called blood plasma) and various blood cells. Our organism has evolved in such a complex way that blood cells contribute to the self repairing process of blood vessels when they are damaged, a process known as blood clotting.

The key variables regulating thrombosis (the formation of a blood clot inside a blood vessel) have been known for more than 150 years, and are known as Virchow’s triad. These variables include changes in the vessel wall (endothelial injury), alterations in the thrombogenic potential of blood (hypercoagulability) and changes in blood flow parameters (haemodynamics)[5].

Blood clotting is a complex process that controls bleeding in case of an injury. However the same blood clots can, in response to some physiological conditions or disease become life-threatening thrombi, which obstruct the natural blood flow to vital organs. Blood clots can be either thrombocytes–rich clots (formed mainly from platelets), blocking the circulation to the myocardium and brain, leading to acute coronary syndromes and stroke or erythrocyte–rich clots (formed mainly from red cells) which can embolize from the deep veins and threaten the lungs with pulmonary emboli.

This investigation will be focused on the thrombocytes–rich clots formed by platelets. Platelets are highly specialized blood cells that have evolved to develop the complex function of repairing the blood vessel wall (endothelium) when damage occurs. Platelets repair the blood vessel wall by accumulating together driven by biochemical and haemodynamics processes. The methods by which proteins from the injured vessel wall and blood borne chemical factors interact with platelets have been investigated extensively[6, 7, 8, 9, 10] however the critical haemodynamics parameters and their effects on platelet function and aggregation have been less well defined.

1.5.1 Chemical factors on platelet aggregation

From the perspective of biochemical factors affecting platelet aggregation, it is understood that platelets are able to clump together under the influence of flow using two proteins present in blood plasma as a connecting agent. These proteins are fibrinogen (only present in the fluid blood) and von Willebrand factor (vWF, present both in the blood and also in the vessel wall), which becomes exposed when there is an endothelial injury. The process of platelet adhesion to the vessel wall and to each other is performed by the cooperative function of two important platelet adhesion receptors, glycoprotein Ib (GPIb) and integrin $\alpha_{IIb}\beta_3$, with the relative contribution of each receptor dependent on local haemodynamics conditions [9]. These platelet receptors play important roles in cell signaling, as they
transduce information from the vessel wall to the cell as well as reveal the status of the cell to the outside, allowing rapid and flexible responses to changes in the environment.

On the other hand, along with this internal cell signalling, blood borne chemicals (adenosine diphosphate -ADP-, thromboxane A2 TXA2 and thrombin) are able to trigger a series of biochemical and functional platelet responses that coincide with the development of platelet aggregates\cite{11, 9, 10, 12}. Some of these platelet responses include intracellular calcium flux, platelet shape change and granule release. However recent observations demonstrate that platelets can form aggregates in-vivo without a detectable increase in intra-cellular calcium\cite{13, 14}, without undergoing shape change\cite{14, 15}, and without a substantial granule secretion\cite{16}.

1.5.2 Mechanical factors on platelet aggregation (Haemodynamics)

From a fluid mechanics perspective, observations have indicated that platelets in flowing blood tend to aggregate at certain sites in the arterial system such as downstream from bifurcations, sharp bends, constrictions, implanted rings in the vena cava and aneurysms\cite{17, 18, 19}. From fluid mechanical considerations it can be shown that bifurcations, T-joints, sharp bends and constrictions are places where flow is disturbed and where the formation of vortices (eddies) can occur\cite{20}.

How fluid stress from haemodynamics can produce damage in the blood vessel wall (endothelium) has been a matter of several studies (see review of Berguer\cite{21}). However this investigation will be focused on how haemodynamics affects blood cells, specifically to platelets.

Because platelet aggregates form at sites where flow is disturbed, fluid mechanical factors have been cited as a cause of thrombus formation\cite{22}. A number of computational and experimental models of platelet flow through vascular stenoses, consisting of geometries with scales in the order of millimeters and blood flow with Reynolds numbers $\geq 100$, have been carried out\cite{23, 24, 25, 26, 27, 28}. Under these Reynolds numbers, flow may not be laminar and platelets may experience both regions of high shear-rate and, more significantly, zones of recirculating flow, downstream of stenoses. A long standing assumption has been that platelet activation and aggregation within regions of blood flow recirculation is promoted via diffusion and advective transport of blood-borne aggregation agonists \cite{17, 29, 30}, leading to platelet activation and shape change. In some other studies platelet aggregation on stenosis geometries has been reported to be found on the apex of the stenoses, where the highest stress is present \cite{31}.

The other mechanical factor involved is related to the viscous stress and forces related to this stress. According to the Newton’s law of viscosity, any fluid moving into a vessel
experiences a viscous stress that is proportional to the flow velocity. Normal variations of viscous stress regulate changes in vascular diameter and when sustained induce slow, adaptive, structural-wall remodeling[32]. Both processes are endothelium-dependent and are compromised by hypertension, diabetes and inflammatory disorders. This viscous stress produced by the blood flow is in turn experienced by the cells transported and by the endothelium as viscous forces. These forces can enhance the interaction of cells and proteins, stretch cells and proteins, serve as a triggering factor for some mechanotransduction processes, can be involved on mass transport phenomena as advection mechanisms, or deform the cell membrane.

Blood cells and plasma proteins, experience a range of mechanical forces that can have a significant effect on cellular function and signaling. Regions of flow disturbances including arterial branches, bifurcations, curvatures and narrowing (stenosis) result in complex fluid forces and their characteristics can predict atherosclerosis susceptibility [32]. More importantly, in zones of flow disturbance, these forces may not be constant but dynamic. Experimental models comparing the effect of constant versus time varying or pulsatile shear forces on platelet aggregation dynamics have demonstrated that platelets are highly attuned to both temporal and spatial variations in shear forces in contrast to steady state conditions[33, 34, 35, 36]. A distinctive feature of the majority of previous investigations listed is that either they have been conducted primarily on large blood vessels (500µm-2mm), producing large Reynolds numbers or on Couette cells. A long standing but poorly supported assumption in these bio-physical studies has been that platelet activation and aggregation within regions of blood flow recirculation is promoted via diffusion and advective transport of soluble platelet agonists, leading to platelet shape change and activation.

1.5.3 Micro-technology brings new discoveries on platelet aggregation

With the recent development of better imaging systems that allow observation of blood flow through small arteries, a deeper insight into the platelet response under dynamic flow conditions has been possible. Furthermore advances in drug development on platelet biology have enabled experimental designs where platelet chemical receptors are blocked. Recent studies using high quality imaging on mice, demonstrate that even with the chemical mechanisms of aggregation blocked, platelet can aggregate in an inactive state under conditions of perturbed blood flow[1], suggesting that fluid forces can directly drive platelet aggregation independent of biochemical triggers. The former experimental observations served as motivation to investigate the flow conditions of the biological experiments by trying to emulate the phenomena on a synthetic device, using micro-technology.
CHAPTER 1.

By investigating the conditions found in the micro-vasculature, this thesis aims to develop a laminar flow system that emulates such conditions and provides localised disturbances that are of similar size to the platelets themselves. Using this new platform it is hoped that platelet responses can be investigated with unprecedent clarity.

1.6 Thesis Outline

Chapter 2 sets the scene for the present thesis by reviewing two different model experiments where platelet aggregation was observed under the chemical receptors of the platelets blocked. The experiments were done by personnel of the Australian Centre for Blood Diseases. Chapter 3 presents the mechanical modelling of the first experiment presented in Chapter 2, (an artificial stenosis or contraction) by using finite element analysis and computational fluid dynamics to emulate the experiment and gain insight into the haemodynamics governing the conditions of an artificial stenosis in mice. Chapter 4 presents the study of the second experiment presented in Chapter 2, (spherical microprotusion) by using both, an analytical and a numerical simulation of the flow around a spherical protusion. Chapter 5 presents a proof-of-concept microfluidic platform that uses micro-contractions to emulate an artificial stenosis on a synthetic device. This synthetic platform allows independent variation of a number of fluidics characteristics and this finally provides the required experimental evidence to conclude that mechanical forces alone may initiate platelet aggregation. The conclusions and future work are summarized in Chapter 6.
Chapter 2

Review of biological experiments at Australian Centre for Blood Diseases - Monash University

2.1 Contributions

I did not contribute at any part of the experimental work described on this chapter. This chapter is presented as background for the rest of the thesis. The experiments described in this Chapter were designed and performed by researchers at the Australian Centre for Blood Diseases (ACBD) and have been published in:


2.2 Introduction

Platelet activation and associated aggregation is a complex process that occur through the synergistic signaling effects of exposed sub-endothelial matrix proteins (vascular factors), blood borne biochemical activators (thrombin, ADP and TXA2) and blood flow dependent mechanical factors (haemodynamics). Therefore, it is a process hard to study under controlled conditions. This Chapter presents two different model experiments developed at the Australian Centre for Blood Diseases which settled the basis for proposing a new mechanism of platelet aggregation. The experiments are presented here to set the scene for the rest of the thesis.
CHAPTER 2.

Historically in-vitro experimental approaches examining blood flow have been conducted using parallel flow chambers, Couette cells [37, 38, 39] or rectangular capillaries [40, 41, 11, 6, 7], in an attempt to reproduce platelet aggregation phenomena at the laboratory. With these studies, it has been long recognized that platelet aggregation preferentially occurs at regions of flow disturbance after vascular injury where chemical factors play a primary role. These flow disturbances can be understood as stenoses (regions where the blood vessel suddenly contracts and expands), blood vessel bifurcations, protrusions or bumps formed by the accumulation of cells or fatty materials.

As it was presented in Section 1.5.1 some of the platelet chemical responses include intracelullar calcium flux, platelet shape change and granule release. However recent observations demonstrate that platelets can form aggregates in-vivo without a detectable increase in intra-cellular calcium[13, 14], without undergoing shape change[14, 15], and without a substantial granule secretion[16]. These observations suggested that additional mechanisms may be involved in the process of platelet adhesion and aggregation. These observations served as a motivation to investigate platelet aggregation using different approaches by implementing new experimental techniques. Specifically the experiments described in this Chapter include the implementation of advanced imaging techniques to study platelets from a closer and clearer approach.

The first experiment refers to the development of an acute arteriolar stenosis model adapted from the murine mesenteric model of acute thrombosis. This method involves the generation of a stenosis of mesenteric vessels via the progressive compression of the vessel side wall using a blunted micro-injection needle at a site of vessel wall injury ([1]), and its observation using intra-vital imaging techniques. Platelet aggregation was found despite chemical factors being blocked, in the downstream face of the stenosis, rather than in the maximum contraction where the highest shear is expected [31].

On the other hand, the second experiment refers to a modification of a commonly used device in blood research: the rectangular capillary. Rectangular capillaries in particular have become popular in recent years due to their relative ease of use and easy application to transmitted light micro-imaging techniques. A method was developed to locally modify the capillaries to initiate single platelet adhesion at a pre-defined location, modifying the surface of the capillary to create a flow disturbance, similar to Chapter 3, but using a canonical disturbance (sphere of controlled size).

This Chapter presents these two different novel experiments in blood research which set the scene for the coming chapters. Section 2.3 reviews the in-vivo experiments done on mice where an artificial stenosis is produced with a micro-needle on an arteriole. Section 2.4 presents a review of a number of in-vitro experiments where blood is flowed through rectangular channels with micro-beads immobilized on the wall.
The experiments were done by researches at the Australian Centre for Blood Diseases. Because of their importance and intrinsic relationship to the development of this PhD, they are presented in this document as introduction.

2.3 Review of *in vivo* disturbance with an artificial stenosis on mice and blocked chemical receptors

Experiments with artificially induced stenoses provide the most realistic model to study the effects of rheological disturbance on platelet aggregation dynamics. This Section presents a review of experiments done on mice where an artificial stenosis is produced with a micro-needle on an arteriole and platelet aggregation was observed. The experiments were designed and performed at the Australian Centre for Blood Diseases (ACBD).

2.3.1 Description of the experiment done by the ACBD

An experiment was conceived at the ACBD using a blunted needle to compress a capillary vessel in a live mouse to promote a local change in blood flow velocity. The mouse used was from a strain genetically modified and treated with drugs which blocked many of the biochemical and functional platelet responses, effectively turning off the mechanisms for chemical initiation of platelet aggregation and thrombus formation. The aim of this experiment was to discover whether it was possible to still form a thrombus through simple mechanical stresses.

The experiments were performed using intra-vital imaging to visualize the platelet aggregation process in mesenteric arterioles of mice. The mice used was from a genetically modified strain, $P2Y_1^{-/-}$, which lack the platelet receptor $P2Y_1$ an adenosine di-phosphate (ADP) receptor. Furthermore clopidogrel was administrated causing complete inhibition of ADP-induced platelet aggregation, whilst aspirin and hirudin were also introduced to block thromboxane and thrombin generation respectively. The mouse was anesthetized and an injury was performed in their mesenteric arterioles ($40–160\,\mu m$). The degree of stenosis was controllably manipulated using a blunted micro-needle positioned at a site of vascular injury. Platelet interactions was visualized by DIC microscopy using a Leica DMIRB inverted microscope (objectives: 100X PL APO, numerical aperture 1.40–0.7 and 63X HCX PL APO, numerical aperture 1.2) and a DAGE MTI charge–coupled device camera.

Figure 2.1 presents a micro-graph of the experiment with images captured over a 20 second period with the needle being inserted and removed. An aggregate is clearly observed when the needle is inserted and this dissipates when the needle is removed.
CHAPTER 2.

It is observed that initial platelet recruitment occurred specifically at the stenosis apex (Fig. 2.1), with the subsequent formation of platelet aggregates occurring in the downstream expansion zone. Localized stenosis markedly accelerated the rate and extent of platelet aggregation (Fig. 2.1, 16.39 s). Platelet aggregation was observed throughout the expansion zone, but aggregation rapidly ceased at the downstream margin of this zone, suggesting that the stenosis had a spatially restricted effect on thrombus growth.

Figure 2.1: Review of mice experiment at ABCD. Micro-graph of the experiment. After stenosis (11.87 s), a marked increase in platelet aggregation was observed (blue arrow), which specifically developed downstream from the site of vascular injury (red arrow). Aggregate growth ceased at the downstream stenosis margin (yellow arrow; 16.39 s) where the vessel returned to its native geometry. Subsequent removal of the micro-needle led to the rapid embolization of the platelet aggregate (24.13 s). Scale bars, 10 \( \mu \)m.

Figure 2.2 presents an analysis of the thrombus size (% of the maximum size) as a function of compressing the vessel over time at different times, showing that the aggregation is repeatable and there is a distinct threshold for aggregate formation. Thrombus formation was dynamic with cycles of aggregation and disaggregation corresponding to serial increases and decreases in stenosis, with removal of the micro-needle leading to disaggregation of the platelet mass (Fig. 2.1, 24.13 s and Fig. 2.2)

Figure 2.2: Review of mice experiment at ABCD. Aggregation as a function of time. Relative thrombus size a function of inducing the stenosis several times. Gray areas show application of the needle.
From these experiments it can be concluded that a micro scale stenosis can induce the formation of aggregates. Since the strain of the mouse used lacked the ADP receptor and the experiment was conducted under the action of specific drugs to block the aggregation due to thromboxane and thrombin, it is suggested that the aggregation is likely driven by non-chemical factors. One possible mechanism may be relying on the haemodynamics. Since the geometry of the vessel is the most remarkable variable, it is possible that the contribution of haemodynamics is the underlying mechanism of aggregation.

2.4 Review of in vitro disturbance using a spherical protrusion with blocked chemical receptors

Section 2.3 presented an in-vivo experiment where a micro-needle was used to create an artificial stenosis which could initiate platelet aggregation under conditions where the common chemical activating mechanism of platelet aggregation were blocked. Significantly, platelet aggregation was found to occur in the deceleration zone of the stenosis rather than in the high shear zone as has been reported in earlier studies [31, 42, 43].

Using the experimental in-vivo approach described in Section 2.3 it was found that although the peak strain-rate could be accurately controlled using the degree of stenosis, the total strain-rate history including acceleration and deceleration components was highly variable and dependent on parameters such artery wall elasticity which is difficult to control in a systematic manner. Given that platelet aggregation was found exclusively in the deceleration zone of the in-vivo stenosis, it was hypothesised that not just high strain-rates but the total strain-rate history is a significant factor in determining platelet aggregation which may explain why aggregation is only observed under certain specific in-vivo experimental conditions.

Due to the fact that in-vivo parameters are highly complex and difficult to control, with variations existing in geometry, elasticity, platelet function and chemical environment, it was very difficult to conceive of an in-vivo experiment that could prove the importance of total strain-rate history in the initiation of aggregation. It was thus decided to pursue a simplified in-vitro model that could isolate the parameters of interest while eliminating many of the variables encountered in the in-vivo experiment.

Historically in-vitro experimental approaches examining blood flow have been conducted using parallel flow chambers, Couette cells [37, 38, 39] or rectangular capillaries [40, 41, 11, 6, 7]. Rectangular capillaries in particular have become popular in recent years due to their relative ease of use and easy application to transmitted light micro-imaging techniques.
Since the cross sectional area of a rectangular capillary is constant along the direction of flow, the fluid at any point in the cross-section experiences a constant velocity and constant shear-rate as during steady. The rectangular capillary is thus an excellent tool for studying the role of haemodynamics at a constant shear-rate on blood cells. Capillaries can also be easily coated with specific proteins to study the function and interaction of platelets under a wide range of constant shear-rate.

A number of experiments were conducted to study platelet-protein matrix interactions under flow at the Australian Centre for Blood Diseases [33, 44]. During some of these experiments, it was observed that occasionally single platelets adhered to the surface of the capillary and this by itself was apparently sufficient factor to trigger platelet adhesion and subsequent accrual of new platelets from the flow (unpublished observations). However due to the stochastic nature of platelet-surface interactions under flow it was difficult to predict where an platelet aggregation would occur. Based on these limitations a method was developed to locally modify the capillaries to initiate single platelet adhesion at a pre-defined location, modifying the surface of the capillary to create a flow disturbance, a concept similar to Section 2.3, but using a canonical disturbance that could be easily controlled and analysed.

This Section presents a review of a series of experiments of micro-bead collision assays designed and performed at the Australian Centre for Blood Diseases which resulted in the observation of controlled platelet aggregation.

### 2.4.1 Description of the experiment done by the ACBD

An experiment using a micro-spherical protrusion was conceived at the Australian Centre for Blood Diseases by making a modification to a commercially available micro-capillary platform with the aim of observing in real time the blood cells behavior under a localised and size-controlled flow disturbance. The micro-sphere experiments were performed according to a modification of a published method [45]. Since platelets are blood cells of discoid shape (2µm × 0.3µm), fixed polystyrene micro-spheres with similar size diameters 2, 5, 9 and 15µm were used. The micro-spheres were precoated with purified human von Willebrand factor (vWF) and immobilized at the surface of glass micro-capillaries. Sphere concentration near the inlet or outlet of the capillary due to sedimentation effects was neglected since the experiment was performed on beads adhered at the middle of the longitudinal section of the capillary. Blood was treated to inhibit the chemical mechanisms of platelet aggregation (soluble agonists) for 10 minutes with the platelet inhibitors; MRS2179 (100µM) and 2-MeSAMP (10µM) — to block ADP platelet aggregation; Indomethacin (10µM) — to block thromboxane (TXA2) generation; and hirudin (800U/ml) — to block thrombin generation. Blood flow was infused under positive
pressure using a syringe pump. The flow rate was adjusted to produce a shear-rate of \( \dot{\gamma} = 10,000 \text{s}^{-1} \) at the bottom and top wall of the micro-capillary. A separate capillary was used for each bead size. The calculated Reynolds number was 0.01 and 0.64, depending on the bead size. Figure 2.3 presents a diagram of the experiment.

![Diagram of the spherical micro-protrusion experiment in capillary channels.](image)

**Figure 2.3:** Diagram of the spherical micro-protrusion experiment in capillary channels.

Figure 2.4 presents optical Differential Interference Contrast microscope (DIC) images of discoid platelet aggregates, at the downstream face of the sphere. Figure 2.4a), shows that the size of the aggregate becomes larger as function of the bead size for the same flow parameters and channel cross-section. A stable thrombus formation is evident for the 15\( \mu \text{m} \) bead (Re 0.64) and some unstable single platelets are observed at the back of the 2\( \mu \text{m} \) bead (Re 0.01). From a biological perspective it is of interest to note that the single platelets retain a discoid morphology, suggesting that the platelets have not undergone overall shape-change indicative of chemically induced platelet activation [46, 47, 48, 49]). The discoid shape of the platelets can be observed in the Figure 2.4a) and more specifically in the 2\( \mu \text{m} \) bead. Figure 2.4b) presents the aggregate size as a function of bead diameter. This measurement is made as a two dimensional cross-section, since three dimensional aggregate information could not be derived from the DIC imaging approach used. It is interesting to note that if the thrombus area simply scaled in proportion to the bead diameter, then the thrombus area should scale with the square of the bead diameter. It is evident that in fact the thrombus area scales only linearly with bead diameter indicating that the thrombus shape changes significantly as the bead diameter reduces.

This type of experiment is novel [1] and hence there was no previous information on what may have triggered the platelet accumulation at the back face of the sphere. It is evident that the micro-sphere constitutes a very localised fabricated defect where platelets aggregate. The micro-sphere is able to locally change the haemodynamics, therefore it is hypothesised that some factor from the haemodynamics is governing the aggregation.

### 2.5 Conclusion

From these experiments it can be concluded that a micro-scale flow disturbance stenosis can induce the formation of platelets aggregates. Since the strain of the mouse used
Figure 2.4: Review of bead collision assay from [1]. a) DIC image frames showing the nature and extent of discoid platelet aggregation at the downstream face of vWF-coated micro-beads after whole-blood (pretreated with 100µM MRS2179, 10µM 2-MeSAMP, 10µM Indomethacin and 800U/ml hirudin) perfusion at an applied $\dot{\gamma} = 10,000s^{-1}$ (n = 5). Re 0.01-0.64. Aggregation occurs exclusively at the downstream (zone 3) face of the beads, and aggregates can extend up to seven bead diameters (zone 3) downstream of initial platelet-bead adhesion, with overall aggregate width correlating with bead diameter. Top view is shown for all beads. (b) Mean discoid platelet aggregate size (surface area in $\mu m^2$ +/- s.e.m.) as a function of micro-bead diameter (n = 3).

lacked the ADP receptor and the experiment was conducted under the action of specific drugs to block the aggregation due to thromboxane and thrombin, it is suggested that the aggregation is likely driven by non-chemical factors. Since the geometry of the vessel is the most remarkable variable, it is possible that the contribution of hemodynamics is the underlying mechanism of aggregation. The same can be said for the spherical protrusion assay, which was conducted using blood under the action of chemicals to block platelet aggregation and the flow disturbance generated by the sphere produce platelet accumulation at the back face of the sphere. Therefore since the micro-sphere is a very localised fabricated defect where platelets aggregate, which change locally the haemodynamics, it is hypothesised that some factor from the haemodynamics is governing the aggregation.

Thus in both cases it would be of benefit to know precisely the haemodynamics conditions at the micro-scale, particular what flow disturbance they produce, and what strain-rate distribution may be found at the location of the aggregates. Chapter 3 presents the mechanical modelling of the first experiment (an artificial stenosis or contraction) by us-
CHAPTER 2. 

ing finite element analysis and computational fluid dynamics to emulate the experiment and gain insight into the haemodynamics governing the conditions of an artificial stenosis in mice. Chapter 4 presents the study of the second experiment presented in Chapter 2, (spherical micro-protrusion) by using both, an analytical and a numerical simulation of the flow around a spherical protrusion.
Chapter 3

In-vivo disturbance: induced stenosis on mice arterioles

3.1 Contributions

I proposed to study in detail the experiments done on mice described on Chapter 2 by using numerical simulations. After several discussion with biologist from ACBD during the timeframe of this research, I proposed to analyse the flow haemodynamics using streamlines, which was an important step to facilitate the study of strain or shear histories for a single platelet. I wrote the article for submission to the Journal of Biomechanics[3].

The content of this chapter has been published with small modifications in:


3.2 Introduction

Flow disturbance is a principal driver of platelet aggregation and may result from vessel narrowing (stenosis) due to crush injury, vessel wall thickening, stent placement, neointimal thickening and reduction of vessel lumen diameter following angioplasty ([17, 18, 19]). The effects of stenosis on haemodynamics have been studied using both computational and in-vitro modelling approaches on large arteries (Reynolds $\geq$ 100, see [23, 20, 28, 50, 51, 52, 53, 27, 26, 54, 55, 31, 24, 25]).
CHAPTER 3.

Under these conditions, blood cells may experience both regions of high shear-rate and, more significantly, zones of recirculating flow, downstream of stenoses. A long standing assumption has been that platelet activation and aggregation within regions of blood flow recirculation is promoted via diffusion and advective transport of blood-borne aggregation agonists, leading to platelet activation ([17, 29, 30]). A distinctive feature of the majority of previous investigations listed is that they have been conducted primarily on large blood vessels (500µm-2mm).

Advances in intra-vital imaging techniques and the widespread and increasing use of thrombosis models involving rodents have facilitated the study of platelet responses under dynamic and laminar conditions (Reynolds \( \leq 1 \)). As it was shown in Chapter 2, recent biological experiments developed by the Australian Centre for Blood Diseases, Monash University [1] have established that rapid changes in shear-rate in micro-flows can trigger platelet thrombus formation in the absence of the commonly accepted chemical activators (ADP and thromboxane) of platelet activation and aggregation ([1]). A significant outcome of these studies is the recognition that shear gradients at the micro-scale may underlie the lack of efficacy of common anti-platelet drugs such as aspirin ([56]) and clopidogrel ([57]). A key experimental advance underlying this discovery was the development of an acute arteriolar stenosis model adapted from the murine mesenteric model of acute thrombosis. This method involves the generation of a stenosis of mesenteric vessels via the progressive compression of the vessel side wall using a blunted micro-injection needle at a site of vessel wall injury ([1]).

These experiments with artificially induced stenoses have provided the most realistic model reported to date but only allows limited control over the geometric variables, since only the needle width and displacement can be controlled with any precision thus it is hard to know precisely the parameters of the stenoses geometry that has been induced and thus it can be difficult to analyse the fluid dynamics of blood flowing through such a stenosis. Figure 3.1 illustrates possible variables that may be of significance in an in-vivo experiment.

In these experiments, platelet aggregation occurred at the downstream face of the applied stenosis (flow deceleration), rather than within the stenosis throat where shear rates are at their highest. The findings of these experiments arguably suggested that mechanical or haemodynamics variables may play an important role in platelet aggregation when blood is flowing in micron scale capillaries with low Reynolds number.

One important discovery from the biological perspective was the observation that discoid platelet aggregates can form through the development of membrane tethers, independent of integrin and platelet activation. This observation is in contrast to the historically accepted theory that chemical activation is crucial in the initiation and formation of aggre-
gates. One of the frequent observations in the experiments was that platelet aggregation usually tended to occur in regions of rapid changes in blood flow parameters, in particular where significant changes in fluid shear-rates were observed over micron scale distances - so called shear micro-gradients.

To better understand the biology of platelet aggregation and its relationship to the fluid mechanic environment, it is necessary to know precisely the velocity of the fluid at the region of the stenosis. Knowing the fluid velocity subsequent calculations can be performed like the fluid deformation rates (called strain-rates) and the fluid stresses. Thus, there is a persistent challenge to identify the best engineering approach to accurately model the physical form of a stenosis and quantify the haemodynamics of blood flow within this form.

As presented in the Chapter 1, a major goal of this thesis is to investigate the effects of the mechanical and geometrical parameters on fluid flow of stenosed micro-vessel and its effect in the initiation and promotion of platelet aggregation. It is the goal of this chapter to identify the main parameters in the specific in-vivo experiment where a micro-needle is used to create an artificial stenosis in micro-capillaries of mice. Section 3.3 presents a literature review on fluid simulation of stenosis. Section 3.4 presents a qualitative model of the stenosed micro-capillary generated by choosing a specific stenosis geometry and simply subjectively drawing that geometry in three dimensions. Computational fluid dynamics were used to model the stresses that could be experienced by blood flowing through this geometry. A Newtonian and two Non-Newtonian models are implemented for comparison purposes. From this investigation an appreciation for the approximate order of magnitude of the variables in play was obtained. In particular it was possible to determine that blood flow was experiencing a sudden change of strain-rates in a short distance.
A second approach is described in Section 3.5. This second approach aimed to provide a more accurate model of the stenosis geometry by mechanically modelling the capillary as an elastic tube encased in soft tissue which is deformed of stenosis corresponding to different displacements of the needle. In particular it was possible to produce geometric models of extreme stenoses where the cross-sectional area was reduced by up to 90%. Computational Fluid Dynamics was again used to model the strain-rates that would be experienced by blood flow flowing through these geometries and these strain-rates were compared to canonical models reported in the literature. A number of key geometric features were identified and it was found that the elastic properties assumed for the capillary walls are critical in determining the form of the stenosis. Section 3.8 summarizes the findings of this Chapter, and describes how the insight gained can be used to construe new experiments utilizing micro-beads and microfluidic channels that will be discussed in Chapter 5.

### 3.3 Literature Review on numerical models of stenosis

This section presents a brief review of different works on modelling stenosis. Because of observations that atherosclerotic lesions preferentially occurred in blood vessels in regions of flow disturbance, and flow disturbance has been associated with turbulence, stenosis has been a subject of study for several disciplines[21]. Just the combination of words

![Figure 3.2: Scientific articles published with the terms stenosis, stenoses or stenotic, extracted from the database Web of Science, from 1992 to February 2011, including all areas of research.](image)
"stenosis", "stenoses", "stenotic", at the scientific database Web of Science produce more than 85,000 articles, from all areas of knowledge, including medicine, biology, mathematics, engineering, mechanics, and computer science. Figure 3.2 shows the amount of articles published per year containing this combination of words. From this number of articles, around 20% are related with cardiovascular and cardiology studies, that involve mathematics, engineering, computer sciences, mechanics or biophysical Sciences.

From this high number of publications it was of interest to analyse which of them could help to get insight in the experiments done on mice described in Section 2.3. Because the experiments were done on arterioles from a live mice, and low Reynolds number ($< 1$), the presented review was focused to analyse the type of geometry and the Reynolds number used in the investigations. A secondary analysis was focused on the type of fluid they model (Newtonian or Non-Newtonian, Homogeneous or Non-Homogeneous). Table 3.1 presents a summary of this review including the most representative articles with a distinctive feature judged by this candidate.

The first column of Table 3.1 refers to the geometry used for the investigations, grouped as idealized in 2D, 3D, and realistic in 2D and 3D. The second column refers to the type of viscosity used, whether is Newtonian or No-Newtonian. The third column includes the author of the investigations. The fourth column on Table 3.1 presents the Reynolds number range of the investigations selected for this review. In this respect it is worth to note that it has been widely accepted that blood flow recirculation is a key factor in thrombus formation, therefore it is common to find that the models described on mathematics, engineering, computer sciences, mechanics or biophysical sciences, are focused on turbulence or higher Reynolds number than the one used for the present investigation. The fifth column refers to the type of geometry used. In this respect, the fact that the experiments were done on live mice, makes an important difference with most investigations from Mathematics, Engineering, Computer Sciences, Mechanics or Biophysical Sciences, where usually the geometries are idealized or modelled using cosine functions. On the other hand, in medical or cardiology areas, where real vessels are studied, the investigations are either focused to clinical research and no present studies on haemodynamics, or using much larger arteries. Therefore it was of interest to find articles using real vessel geometries to include any three dimensional effects and low Reynolds number.

It has been widely accepted for non-medical sciences, to model the stenosis using cosine functions. This allows researches to formulate questions related to haemodynamics, turbulence or new algorithms to solve the fluid motion equations. There are a high number of works using cosine functions, and two dimensional geometries, some of them include [42, 58, 59, 60, 61]. Investigations including Non-Newtonian viscosity or Non-Homogeneous fluids include [62, 63, 64, 58, 65]. The work of Fogelson[63, 64] is
CHAPTER 3.

particularly interesting since his research deals with modelling the process of platelet aggregation considering advective transport of chemical agonists, in his research they used two dimensional geometries of idealized rectangular shaped stenosis.

Table 3.1: Review on numerical models of stenosis.

<table>
<thead>
<tr>
<th>Geometry</th>
<th>Viscosity</th>
<th>Author</th>
<th>Re</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idealized 2d</td>
<td>Newtonian</td>
<td>Wootton[42]</td>
<td>160</td>
<td>cosine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Varghese[58]</td>
<td>600</td>
<td>cosine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Griffith[59, 60]</td>
<td>50-2500</td>
<td>circular</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Banerjee[61]</td>
<td>50-400</td>
<td>cosine, trapezoidal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fogelson[63, 64]</td>
<td></td>
<td>model of p.a.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sorensen[58]</td>
<td>600</td>
<td>cosine, Casson</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sankar[65]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Idealized 3d</td>
<td>Newtonian</td>
<td>Bark[66]</td>
<td>230</td>
<td>cosine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blackburn[67]</td>
<td>400</td>
<td>cosine, pulsatile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bluestein[26]</td>
<td>300 to 3,600</td>
<td>cosine axisym.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sherwin[68]</td>
<td>400-800</td>
<td>cosine, axisym, exper.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Schoephoerster[52]</td>
<td>300-3600</td>
<td>cosine, PIV, turbulence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vetel[69]</td>
<td>100-1100</td>
<td>cosine, irregularities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anderson[70]</td>
<td>500-2000</td>
<td>cosine, eccentric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raz[25]</td>
<td>200</td>
<td>cosine, Casson square, rect, Carreau</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jung[71]</td>
<td>575-900</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strony[51]</td>
<td>340-472</td>
<td></td>
</tr>
<tr>
<td>Realistic 2d</td>
<td>Newtonian</td>
<td>Kock[72]</td>
<td>0.45</td>
<td>Elastic model of mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Marshall[73]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Realistic 3d</td>
<td>Newtonian</td>
<td>Tovar[3] (model presented in this chapter)</td>
<td>0.45</td>
<td>Elastic model of mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The second row of Table 3.1 presents, three dimensional idealized geometries and Newtonian models investigations [66, 67, 26, 68, 52, 69, 70]. In this respect, it is common
to find that by using a cosine function researches take advantage of the axisymmetry to explore three dimensional haemodynamics effects.

Non-Newtonian viscosity effects on idealized geometries, have been implemented by [25, 71, 51]. Finally on the realistic geometries some works can be found, where the geometry of the vessel was extracted using Magnetic Resonance (MRI) [72, 73]. As far as the author of this thesis is aware, the work presented in this Chapter is the only one so far, considering a realistic geometry and including three dimensional effects[3].

Non-Newtonian investigations on realistic geometries, including two or three dimensional effects have been found so far.

3.4 Fluid mechanics of induced stenoses in live mice using an approximated geometry

This section presents a first approximation to the stenosis geometry observed in Section 2.2. Section 3.4.1 presents the method for obtaining the approximate geometry which is a simple subjective drawing in three dimensions. Section3.4.2 then present computational fluid dynamic simulations of this approximate geometry to find the order of magnitude values of the various fluid stresses that might be expected. A Newtonian and two Non-Newtonian models are implemented for comparison purposes.

3.4.1 Generation of the approximate geometry of the induced stenosis

In order to have a first approximation of the haemodynamics in the described in-vivo experiment, a stenosis case with an approximated geometry from the experiment was modeled. The geometry of the blood vessel was modelled with Solid-Works v. 2004 (Solid-Works Corp.) using the integrated tool “loft“ which enable to construct 3D volumes from different cross sections, see Figure 3.3. Some authors have applied a similar method of drawing in 3D an approximated model, with the aim of get the order of magnitude of the variables[74].

The initial diameter of the cylinder was 42 µm. Based on the in-vivo experiments from video (sec. 2.2) different cross sectional areas were drawn to perform the loft operations into the software, assuming that the diameter of the needle that deformed the vessel was similar in size to the artery (42 µm). Hexahedral elements were used to ensure smooth streamlines in the subsequent computational fluid dynamics simulations. To ensure sufficient resolution in the discretization, the volume of the fluid was split into five regions.
Figure 3.3: Approximated geometry of the stenosis modelled into a 3D software (Solid-Works) using based on different cross-section areas. a) The volume of control under study was divided in 5 sub-volumes to facilitate the construction of a mesh with hexahedral 3D elements. b) Section of the compression or stenosis. c) Lateral view of the vessel modelled.

before building a mesh of hexaedrals. The total volume was composed of a core volume and four volumes around the core (See Fig. 3.3). These five volumes represent the volume of control of the fluid flow inside the blood vessel to analyse. No elastic effects were considered, therefore the perimeter of the transversal section undeformed and deformed was kept constant (131 µm). Care was taken to produce a linear stenosis of bigger than 85%. The stenosis with respect the area resulted in a 60%. We transferred the geometry into a specialized preprocessing grid generation (Fluent, Gambit) to generate a structured mesh of 76,800 elements.

3.4.2 Computational fluid dynamic analysis of the approximate stenosis geometry

To gain insight into the relationship between localised flow changes and platelet aggregation we performed computational fluid dynamic (CFD) modeling of the approximated stenosed vessel geometry. A finite volume scheme using the commercial software (FLUENT 6.0, Fluent USA, Lebanon, NH) was used to solve numerically the Navier-Stokes equations 3.1 and 3.2.

\[ \nabla \cdot \mathbf{u} = 0 \]  

(3.1)
\[
\rho \left[ \frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \nabla \vec{u} \right] = -\nabla P + \mu \nabla^2 \vec{u} 
\] (3.2)

Where \( \vec{u} \) represents the velocity vector, \( \rho \) represents the density, \( P \) the pressure, \( \mu \) the fluid viscosity.

On the other hand it is common to get insight about the regime of the flow whether is laminar or turbulent the Reynolds number can be used and is defined as:

\[
Re = \frac{\rho Ul}{\mu} 
\] (3.3)

where \( l \) is the characteristic length, which in this case is the diameter of the micro-bead, \( U \) the mean fluid velocity. For micro-flows usually the characteristic length is short and the velocities low, this results in a low Reynolds number which means that the viscous forces are dominant.

### Medium of fluid dynamics model

Blood is composed of both a fluid phase and solid cells of different sizes, and can therefore it can be classified as a non-homogeneous fluid. This non-homogeneity produce some non-Newtonian effects. The most common is produced when high shear rates aligns the cells along streamlines of the flow decreasing the viscosity. Therefore blood viscosity decreases with increasing shear rates. For the present simulation, the fluid medium was considered homogeneous with a constant density (\( \rho = 998.2 \frac{kg}{m^3} \)) and since blood flow can be approximated as a Newtonian fluid (constant viscosity) at mid and high strain-rates[75], the viscosity was set as \( \mu = 0.00348 \text{Pas} \). However for comparison purposes, some Non-Newtonian viscosity models will be used in this first section as follows:

Carreau model[75]:

\[
\mu = \mu_\infty + (\mu_0 - \mu_\infty)[1 + (\lambda \dot{\gamma})^n]^{\frac{n-1}{2}} \] (3.4)

where \( \lambda = 3.313 \text{ s} \), \( n = 0.3568 \), \( \mu_0 = 0.056 \text{ Pa s} \), \( \mu_\infty = 0.00345 \text{ Pa s} \).

Power Law model[76, 77, 78]:

\[
\mu = 0.1 \lambda_s \dot{\gamma}^{n_s-1} \] (3.5)

where

\[
\lambda_s = \mu_\infty + \delta \mu \exp[-(1 + \frac{\dot{\gamma}}{a})\exp(-\frac{b}{\gamma})] \] (3.6)

and

\[
n_s = n_\infty + \delta n \exp[-(1 + \frac{\dot{\gamma}}{c})\exp(-\frac{d}{\gamma})] \] (3.7)

where \( \mu_\infty = 0.035 \), \( n_\infty = 1.0 \), \( \delta \mu = 0.25 \), \( \delta n = 0.45 \), \( a = 50 \), \( b = 3 \), \( c = 50 \), \( d = 4 \) according to Walburn[79].
Boundary conditions of fluid dynamics model

In order to solve the Navier Stokes equations numerically, a set of boundary conditions needs to be specified for every surface of the model (walls, inlet and outlet surfaces). Therefore it is needed to estimate the amount of flow that is entering the vessel at the inlet (upstream stenosis), to calculate the shear rate. The blood vessel can be considered as a capillary tube or radius \( R \) and length \( L \). In order to calculate the shear rate in function of the flow rate some assumptions need to be taken as follows:

1. The flow is fully developed, steady, isothermal and laminar.
2. There is no velocity in the radial direction.
3. No slip at the walls.
4. The flow is incompressible with viscosity independent of pressure.

The shear rate for a fluid flowing between two parallel plates, one moving at a constant speed and the other one stationary, is defined by:

\[
\dot{\gamma} = \frac{v}{h} \tag{3.8}
\]

where \( \dot{\gamma} \) is the shear rate, \( v \) is the velocity and \( h \) the distance between two parallel plates, measured in meters. According to [80], the shear rates at the inner wall of a Newtonian fluid flowing within a vessel is

\[
\dot{\gamma} = \frac{8v}{d} \tag{3.9}
\]

The average linear velocity \( v \) is related to the volumetric flow rate \( Q \) by

\[
v = \frac{Q}{A} \tag{3.10}
\]

where \( A \) is the cross-sectional area of the vessel of radius \( r \) given by:

\[
A = \pi r^2 \tag{3.11}
\]

producing:

\[
v = \frac{Q}{\pi r^2} \tag{3.12}
\]

substituting velocity in the equation of shear-rate (Eq.3.9):

\[
\dot{\gamma} = \frac{8v}{d} = \frac{8\left(\frac{Q}{\pi r^2}\right)}{2r} \tag{3.13}
\]

which simplifies to the common expression for Newtonian models of fluids on cylinders 3.14,

\[
\dot{\gamma} = \frac{4Q}{\pi R^3} \tag{3.14}
\]

Since blood flowing in capillary vessels experiences shear-rates (\( \dot{\gamma} \)) between 500 \( s^{-1} \) to 5000 \( s^{-1} \)[81], the flow rate (\( Q \)) was calculated to produce a shear-rate of 1800 \( s^{-1} \).
In equation (3.14), \( \dot{\gamma} \) represents the shear-rate, \( Q \) the flow rate, and \( R \) the radius of the cylinder. Using equation (3.14), we calculate that \( [0.79 \mu \text{L/min}] \) of fluid flowing in a vessel of 42 \( \mu \text{m} \), produce 1800 \( s^{-1} \) of shear-rate, corresponding to a Reynolds number of 0.09 upstream of the stenosis. Once the flow rate was known, the developed flow velocity distribution can be described using the Hagen-Poiseuille equation (3.15),

\[
V_z = \frac{2Q}{A} (1 - \frac{r^2}{R^2})
\]

(3.15)

Where \( A \) represents the area of the cylinder and \( r \) is the radial distance from the center of the cylinder. Equation (3.15) was used as a boundary condition for the inlet velocity. The boundary condition at the walls was set as zero velocity to enforce the no slip condition. The boundary condition at the outlet surface was a relative pressure of zero (Pa).

**Fluid Velocity and Viscous Stress**

If the velocity profile is known it is possible then to calculate the deformation rates that fluid elements and particles transported by the fluid experience. Once the deformation rates are known, these deformation rates along with viscosity value can be used to calculate the viscous stress produced by the fluid flow. Since fluid deformation rates and stress related quantities are commonly used but often confused, this section will present some basic concepts for convenience to the reader as well as some terms and concepts that will be used for the following sections.

The velocity in its vectorial form of fluid elements can be expressed as

\[
\vec{u} = u_1 \vec{i} + u_2 \vec{j} + u_3 \vec{k}
\]

(3.16)

In a general case each component can be a function of \( x, y, \) and \( z \). For fluids in constant motion as is the case of the present investigation, it is preferable to describe the motion and deformation of fluid elements in terms of rates. In order for these deformation rates to be useful they should be expressed in terms of derivatives of velocity. The velocity gradient tensor contains all the derivatives of each velocity component, and it can be expressed in function of a deformation tensor and a rigid body rotation tensor as:

\[
\nabla \vec{u} = \frac{\partial u_i}{\partial x_j} = \dot{\epsilon}_{ij} + \omega_{ij}
\]

(3.17)

The rigid body rotation tensor \( (\omega_{ij}) \) is antisymmetric and relates to the vorticity. On the other hand, the deformation tensor \( (\dot{\epsilon}_{ij}) \) is symmetric and relates to the viscous stress that fluid elements and particles experience due to the fluid motion. This deformation tensor is also known as strain-rate tensor and is of main interest for the present investigation. This tensor can be expressed as:

\[
\dot{\epsilon}_{ij} = \frac{1}{2} (\nabla u + \nabla u^T) = \frac{1}{2} \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right)
\]

(3.18)
Or in its expanded form:

\[ \dot{\epsilon}_{ij} = \begin{bmatrix} \dot{\epsilon}_{11} & \dot{\epsilon}_{12} & \dot{\epsilon}_{13} \\ \dot{\epsilon}_{21} & \dot{\epsilon}_{22} & \dot{\epsilon}_{23} \\ \dot{\epsilon}_{31} & \dot{\epsilon}_{32} & \dot{\epsilon}_{33} \end{bmatrix} = \begin{bmatrix} \dot{\epsilon}_{xx} & \dot{\epsilon}_{xy} & \dot{\epsilon}_{xz} \\ \dot{\epsilon}_{yx} & \dot{\epsilon}_{yy} & \dot{\epsilon}_{yz} \\ \dot{\epsilon}_{zx} & \dot{\epsilon}_{zy} & \dot{\epsilon}_{zz} \end{bmatrix} \]

In cartesian coordinates:

\[ \dot{\epsilon}_{ij} = \begin{bmatrix} \frac{\partial \nu}{\partial x} & \frac{1}{2} \left( \frac{\partial \nu}{\partial y} + \frac{\partial \mu}{\partial x} \right) & \frac{1}{2} \left( \frac{\partial \nu}{\partial z} + \frac{\partial \mu}{\partial y} \right) \\ \frac{1}{2} \left( \frac{\partial \mu}{\partial x} + \frac{\partial \nu}{\partial y} \right) & \frac{\partial \mu}{\partial y} & \frac{\partial \mu}{\partial z} \\ \frac{1}{2} \left( \frac{\partial \mu}{\partial y} + \frac{\partial \nu}{\partial z} \right) & \frac{\partial \mu}{\partial z} & \frac{\partial \mu}{\partial y} \end{bmatrix} \]

Since this is a second order tensor, a scalar quantity obtained from its components represents the magnitude of the deformation rates as a consequence of the fluid movement. The scalar commonly used to represent the magnitude of the total deformation rates of the fluid is known as the second invariant of the strain-rate tensor, which is a mathematical operation that includes any possible contribution of the extensional and shear strain-rates.

The second invariant (J2) of the strain-rate tensor is defined as:

\[ J_2 = \left[ \frac{1}{2} (\dot{\epsilon}_{ij} \dot{\epsilon}_{ij} - \dot{\epsilon}_{ii} \dot{\epsilon}_{jj}) \right]^{\frac{1}{2}} \]  

(3.19)

and it can be written as:

\[ J_2 = \left( \frac{1}{6} [(\dot{\epsilon}_{xx} - \dot{\epsilon}_{yy})^2 + (\dot{\epsilon}_{yy} - \dot{\epsilon}_{zz})^2 + (\dot{\epsilon}_{xx} - \dot{\epsilon}_{zz})^2] + \dot{\epsilon}_{xy}^2 + \dot{\epsilon}_{yz}^2 + \dot{\epsilon}_{xz}^2 \right)^{\frac{1}{2}} \]  

(3.20)

Where \( \dot{\epsilon}_{xy}, \dot{\epsilon}_{yz}, \dot{\epsilon}_{xz} \), are the components of shear strain-rate (s\(^{-1}\)), and \( \dot{\epsilon}_{xx}, \dot{\epsilon}_{yy}, \dot{\epsilon}_{zz} \), the components of extensional strain-rate (s\(^{-1}\)) [82]. For the case of unidirectional flow when there are just shear strain-rate components is common to refer to the quantity obtained from J2 as just shear-rates (like Couette cells and capillary channels), however when the fluid experiences acceleration/deceleration in the flow direction, the extensional strain-rate components are also present together with the shear strain-rate components and the quantity obtained from J2 will be referred just as strain-rates.

Furthermore the viscous stress of a fluid can be calculated using the strain-rate tensor and the fluid viscosity (Pa s). If a Newtonian fluid is considered, then the stress tensor is linearly proportional to the strain-rate tensor. This assumes that the viscosity is constant. It can be show[83] that the viscous stress tensor reduces to

\[ \tau_{ij} = 2\mu \dot{\epsilon}_{ij} \]  

(3.21)

However since most of research done on blood has been refers to the shear or strain rates, in order to facilitate the comparison, all the calculation in this work will be presented as a function of the strain-rates (s\(^{-1}\)) instead of the stress (Pa). Therefore in the following sections any strain-rate quantity referred will mean that is has shear and extensional strain-rate components, and it should be reminded that it is closely related to the viscous stress according to Equation 3.21.
Results of first approximated geometry

CFD modeling of the in vivo vessel geometry predicted a laminar flow regime, no recirculation, and blood strain-rates $\dot{\varepsilon} \leq 20000 \text{s}^{-1}$ at the stenosis apex that rapidly transitioned to $1862 \text{s}^{-1}$ within the post-stenosis expansion zone (Fig. 3.4).

![Figure 3.4: Haemodynamics on approximated model constructed using a 3D modeler (SolidWorks) and a hexahedral mesh. a)Strain-rates on approximated model at 60% stenosis (area) and 85% stenosis (linear) (see Fig. 3.3) b)Streamlines showing laminar flow and no-recirculation conditions.](image)

Fig. 3.5 presents the strain-rate history for a particle travelling at $1 \mu m$ from the wall calculated using a Newtonian, a Power Law and a Carreau model. It can be observed that at this Reynolds number the the strain-rates are high, therefore the assumption of Newtonian viscosity is valid.
In order to explore if our Non-Newtonian model could represent any Non-Newtonian effect, the Reynolds number was changed. Fig. 3.6 presents the dimensionless velocity profiles calculated at different Reynolds numbers, compared with a model of constant viscosity (Newtonian fluid). The figure shows that the model works as expected, as the velocity profile is blunted at low strain-rates (much lower Reynolds number). It can be observed that at this Reynolds number, the strain-rates are high. Therefore the assumption of Newtonian viscosity is valid.

Linking this simulation with the in-vivo experiment result, it can be suggested that a zone of where the strain-rates are increasing followed by a zone where the strain-rates are decreasing (called shear micro-gradient) may be a requirement for the development of discoid platelet aggregates (Fig. 3.4). The name of shear micro-gradient was suggested as a need to differentiate this environment of dynamic deformation from the commonly used deformation in blood research (constant shear) although strictly speaking the history of deformation contains not only shear but also extensional components. On the other hand it is deformation rates occurring in a micron-scale. Using this model it was possible to identify three principal components defining a shear micro-gradient or stenosis, the
Figure 3.6: Comparison of dimensionless velocity profiles for a Newtonian viscosity model and a Power Law at different Reynolds number. Observe that at the Reynolds number of the experiment, the velocity profile using a non-Newtonian viscosity model is similar to the profile using a Newtonian viscosity model, therefore the assumption of Newtonian viscosity is valid.

contraction region which demarcates the acceleration, the throat or apex demarcates the stenotic region where a peak strain-rate is experienced and the expansion that demarcates a decrease in the strain-rates. It can be concluded from this section that micro scale stenosis produce sudden increases/decreases in the flow strain-rate in the micron scale (micro-gradients). Since the accepted chemical factors driving platelet activation were blocked in the mouse experiment, and no recirculation zones were found, it is suggested that platelet formation is likely driven primarily by haemodynamics forces occurring in regions of laminar flow. It was observed that a Newtonian viscosity model behaves in the same way that more complex models of viscosity due the high strain-rate regime of the experiment.

This approximate stenosis geometry was subjectively constructed to experience a fixed stenosis (85% linear and with respect the area 60%). The amounts of vessel contraction impact directly on the maximum strain-rates experienced. The desire to explore slightly different scenarios of contraction with consistency in the geometry shape across each one makes the present method of modelling unreliable, and demands for a more realistic
modelling to obtain the geometry of the deformed shape.

3.5 Fluid mechanics of induced stenoses using a mechanistic model

In the modelling of the first geometry important aspects of the deformation process were neglected, which resulted in a model that likely overlooked subtle, but important aspects determining the platelet aggregation. On the considerations taken we can include the local angles of the geometry, the amount of vessel occlusion, and the shape of the cross sectional area, which was the most subjective parameter, since it is impossible to precisely determine the cross sectional shape from the view that the footage was taken. It was also desired to quantitatively explore different degrees of stenosis (50%, 70% or 90%), with consistency in the deformation history. It was not feasible to use the subjective approach of Section 3.4 to obtain such quantitative data. A more precise method was thus developed where the geometry of the deformed vessel was generated by producing a realistic model of the arteriole-needle interaction with the mechanical properties.

This section presents a more precise approach to analyzing the geometry of the induced stenosis observed in Section 2.3. Section 3.5.1 presents an analysis of video observations of induced stenosis in mice to provide a benchmark for the stenosis models. Section 3.5.2 presents the implementation of a finite element analysis to simulate the mechanical response of the artery. Section 3.5.3 presents the results of the finite element simulation. Section 3.5.4 presents the calculation of the haemodynamics from the geometry generated in Section 3.5.3. Section 3.5.5 presents the relationship between the peak strain-rate, degree of stenosis and arteriole wall elasticity. A similar relationship is presented in Section 3.5.6 but in terms of strain-rate distribution using contour plots. Section 3.5.7 presents the strain-rate history for specific streamlines of interest. Section 3.5.8 presents the role of the needle diameter. Section 3.6 presents a comparison of the stenosis model generated using the mechanistic model geometry and canonical stenosis models used by other researches. Finally the conclusions are summarized in Section 3.8.

3.5.1 Video images of artery wall deformation

To obtain a more detailed description of the artery wall response, a set of experiments were performed on arteriole stenosis by compressing arterioles (blood vessels with diameter $90\mu m-120\mu m$) with blunted needles of different diameter from $(10\mu m-20\mu m)$ to $(90\mu m-120\mu m)$ according to a modified method previously published [1]. Experiments were recorded by DIC microscopy using a Leica DMIRB inverted microscope $(40\times)$. 
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Figure 3.7: Induced stenosis in-vivo using a micro-needle and observed under the microscope at 40×.

Figure 3.7 presents a selection of images recorded during this investigation. Further clear images were obtained of the whole deformation history, which will enable to compare graphically with the simulations of the finite element analysis. These images will be used as a benchmark for comparison in the following sections.

3.5.2 Mechanistic generation of the geometry of the induced stenosis

To obtain a precise model of the geometry of an arteriole deformed using a blunted needle as presented in Section 3.5.1, finite element analysis of the mechanical structure was performed.

Geometry of the finite element analysis

A section of biological tissue (composed by the artery and lipid tissue) was considered mechanically as a cylindrical tube undergoing bending deformation. Blood vessels receive some perivascular constraint from the surrounding tissues [84]. During the development of the simulations, it was observed that lipid tissue, although being less rigid than an arteriole, plays a significant role in defining the local shape of the artery in contact with the needle. Figure 3.8 presents an illustration of the model. The soft lipid tissue was modelled as a cylinder with an external diameter of 500µm and an internal diameter of 110µm. The arteriole was then modelled within this lipid tissue as a hollow cylinder with outer diameter of 110µm and inner diameter 100µm, therefore the thickness of the artery wall was 10µm, which was modelled as a single layer. The model was discretized with hexahedral low order elements of 8 nodes, using Ansys v12.0. (Ansys Corporation, Houston PA).
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Figure 3.8: Schematic of the in-vivo experiment and its discretization in finite elements. A micro needle deforms an arteriole to reduce the cross sectional area. a) Needle tip and biological tissue (artery + soft tissue) modelled. b) Discretization in finite elements of the biological system and the needle head.

Material properties of the structural model

In order to model the mechanical deformation of the biological system a hyper elastic constitutive model was used. Although the arterial wall is known to have heterogeneous and anisotropic structure owing to multiple compositions including collagen, elastin and muscle fibers [85], a classical isotropic Mooney-Rivlin model was employed for the arteriole. Two different set of mechanical properties for the carotid artery wall can be found in the literature [86, 87]. Finite element analysis was conducted using both of these sets to investigate the significance the artery elasticity on the resulting geometry. Table 3.2 presents a summary of the material parameters used in our model.

Boundary conditions of the structural model

From the in-vivo experiments, it was observed that regions of the artery far from the deformation zone experience almost zero or no-movement. Therefore all the degrees of freedom of the nodes representing the extremes of the biological tissue model were set to zero. The movement of the needle was modelled by imposing a boundary condition of displacement to all the nodes of the needle of 2.5 artery diameters in the vertical axis (y axis). According to the in-vivo experimental observations of Section 3.5.1, the needle is able to break just the soft tissue surrounding the artery, however the artery undergoes deformation under contact by the needle tip. This arteriole wall-needle interaction was modelled was modeled using contact elements.
Table 3.2: Mechanical properties used for the biological tissue modelled as solid hyperelastic. \( W \) is the strain energy function, \( \vec{I}_1, \vec{I}_2, \vec{I}_3, \vec{I}_4, \vec{I}_5 \), are the strain invariants, and \( J \) is the local volume ratio. \( C_{10}, C_{01}, C_{20}, C_{11}, C_{02}, \) and \( d \) are material parameters. \( E \) is the Elastic modulus, \( \nu \) is the Poisson ratio, \( \rho \) is the material density. The lipid tissue and needle were modeled as a linear isotropic.

<table>
<thead>
<tr>
<th>Material</th>
<th>Model used</th>
<th>Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriole[86, 88]Elasticity 1</td>
<td>( W = C_{10}(\vec{I}<em>1 - 3) + C</em>{01}(\vec{I}_2 - 3) + \ldots + \frac{1}{d}(J - 1) )</td>
<td>( C_{10} = 2.67 \times 10^3 [Pa] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( C_{01} = 8.365 [Pa] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( d = 4 \times 10^{-6} )</td>
</tr>
<tr>
<td>Arteriole[87]Elasticity 2</td>
<td>( W = C_{10}(\vec{I}<em>1 - 3) + C</em>{01}(\vec{I}<em>2 - 3) + \ldots + C</em>{20}(\vec{I}<em>3 - 3) + C</em>{11}(\vec{I}<em>4 - 3) + C</em>{02}(\vec{I}_5 - 3) + \ldots + \frac{1}{d}(J - 1) )</td>
<td>( C_{10} = 5.04 \times 10^4 [Pa] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( C_{01} = 3.05 \times 10^4 [Pa] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( C_{20} = 4 \times 10^4 [Pa] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( C_{11} = 1.25 \times 10^4 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( C_{02} = 1 \times 10^4 [Pa] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( d = 4 \times 10^{-6} )</td>
</tr>
<tr>
<td>Lipid tissue[87]</td>
<td>Linear isotropic</td>
<td>( E = 1 \times 10^3 [Pa] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( \rho = 998 \frac{kg}{m^3} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( \nu = 0.499 )</td>
</tr>
<tr>
<td>Needle head</td>
<td>Linear isotropic</td>
<td>( E = 1 \times 10^9 [Pa] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( \rho = 7800 \frac{kg}{m^3} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( \nu = 0.333 )</td>
</tr>
</tbody>
</table>

Solution of the structural model

A non linear solution with a sequential algorithm solver was used, with a variable load step in function of the material and mesh size, varying from 50 to 1000 steps. Since the hyper elastic material is assumed incompressible and may lead to numerical errors (volumetric mesh locking, or inaccuracy), a uniform reduced integration with mixed uP element formulation was used. This formulation uses not only displacement (\( u \)) but also hydrostatic pressure (\( P \)) as primary unknown variable. Mesh convergence was obtained after observing no variation in the displacements bigger than 5%, although a finer mesh was used to have a better description of the deformed wall artery.
3.5.3 Results of the mechanistic model of the stenosis geometry and comparison to the experimental observations

The results of the mechanistic model are presented in Figure 3.9. In order to validate the numerical simulation of the biological tissue elastic response a qualitative comparison between the shape of the deformed wall obtained numerically and the experimentally observed in-vivo case at different needle displacements was performed. Figure 3.9a) shows selected images of the in-vivo experiment described in Section 3.5.1. These correspond to approximately 20%, 50%, 70%, 80%, and 90% area reduction from the undeformed case. Figure 3.9b) presents a 2D cross-section of the mechanistic model corresponding to similar degree of stenosis with the two set of different artery wall elasticities defined in Table 3.2. In Figure 3.9c) these two mechanistic simulation results are overlaid on the video images to enable qualitative comparison of the location of the artery walls. In general, good qualitative agreement is evident in each case, and it is evident that the simulation is sensitive enough to show small differences in the deformed shape, produced by different elasticities.

Role of needle displacement and wall elasticity on cross sectional area

Since the algorithm used to solve the structural model was sequential (i.e the total displacement of the needle was imposed progressively, in a similar way that the in-vivo experiment was performed), a quantitative history of the elastic deformation of the artery was obtained. From the structural simulation it was possible to precisely calculate the cross sectional area in the contraction and obtain a precise relation between the needle displacement and the fraction of area reduction, as is shown in Figure 3.10a). Since the whole biological tissue can move relatively freely when is in contact with the needle until some point, the reduction of the area in the artery starts when the needle displacement is around 60\(\mu\)m. This is a result of the low restrained model and occurs in a similar way in the experiments.

In a more elastic tissue more needle displacement is needed to produce an equivalent tissue deformation than is required for a less elastic tissue for the same equivalent cross sectional area. Figure 3.10b), shows the geometric cross section of the vessel for a range of percentage area reductions with respect the original area, for two different artery elasticities. In a more elastic tissue (Elasticity 1) the bottom wall tends to take the shape of the needle tip, contrary to a less elastic tissue where both walls tend to move with a similar displacement. From the results of Figure 3.9 and Figure 3.10 it is evident that the mechanistic approach provide a detailed geometry which qualitatively matches the artery deformation observed for the in-vivo experiment described in Section 3.5.1. Hav-
Figure 3.9: In-vivo experiments and simulations of needle-arteriole interaction. a) In-vivo stenosis at different percentages of area reduction. b) Numerical simulation of in-vivo stenosis at the correspondent stages of stenosis for two different elasticities defined in Table 3.2. c) Qualitative validation of the simulations by overlapping the in-vivo cases and the numerical results.
Figure 3.10: Role of the position of the needle on the cross sectional area and maximum strain-rate. a) Area reduction vs needle displacement. b) Area reduction shapes for two different material properties defined in Table 3.2.

ing confirmed that a precise model of the deformed blood vessel can be achieved using mechanistic modelling, it is now possible to use these precise geometric models to simulate the haemodynamics with in these geometries along the whole deformation history of the artery wall. It should also be noted that Figure 3.9 exhibited significant differences in local geometry, when a different artery wall elasticity is used. It would be informative as well to study the impact of these geometrical differences in the context of haemodynamics.

3.5.4 Computational Fluid Dynamics analysis of the mechanistically modelled stenosis geometry

Having shown in Section 3.5.3 that a mechanistic model of the deformed arteriole can produce qualitatively similar geometries to those observed in the experiments described in Section 3.5.1, the next step was to solve the equations of fluid flow in the geometries.

Geometry of fluid dynamics model for the induced stenosis

To generate the geometry needed to perform computational fluid dynamic analysis inside the artery, it was necessary to translate the deformed arteriole model obtained from Sec-
tion 3.5.2 into a geometry that could be input into the a fluid-solver simulator. Although there are methods that enable fluid dynamic analysis to be performed in conjunction with structural simulations (based on Fluid Structure Interaction algorithms), these rely on automatic mesh generation and this is not a reliable way to produce a good quality discretization especially when the aspect ratio (average feature size of the model/minimum feature size of the model) is changing in a very localised region. This is a problem for the geometries >70% of stenosis. Therefore a method to translate the deformed geometry resulting from the Ansys mechanistic model into a format that could be read by a specialized software for mesh generation (ICEM or Gambit) was developed. Figure 3.11 presents an illustration of the conversion of the structural model to the fluid dynamics geometry. For a given substep of the structural solution, points were defined with the coordinates of the displaced nodes, then lines were constructed connecting these points sequentially, and areas were constructed within these lines. Finally a volume representing the volume of control of the fluid inside the artery was constructed with the areas, and exported as an Initial Graphics Exchange Specification (IGES) file to be read by mesh generation software. All these tasks were written in a APDL (Ansys Parametric Design Language) script. Once the solid volume was constructed the cross-sectional area in the narrowest region of the contraction was measured and the stenosis was determined as a percentage of the occluded area with reference to the original undeformed case. Five cases were selected to conduct the haemodynamics study. These were cases with area reduction of 20%, 50%, 70%, 80%, and 90%. The geometries were discretized with hexahedrals using ICEM (Ansys).

**Medium of fluid dynamics model**

The same fluid medium used in Section 3.5.4 was used. Therefore a Newtonian fluid was considered with a density \( \rho = 998.2 \text{ kg/m}^3 \) and a viscosity of \( \mu = 0.00348 \text{ Pas} \), since blood can be approximated as a Newtonian fluid if the strain-rates are high[75].

**Boundary conditions of fluid dynamics model**

The same methodology described in Section 3.5.4 was applied to calculate the inlet boundary condition. Using Equation (3.14), it was found that a flow rate of \( Q = 10.6[\mu L/min] \) of fluid flowing in a vessel with diameter of \( \phi = 100\mu m \), produces a shear-rate at the wall of 1800s\(^{-1}\), corresponding to a Reynolds number of \( \text{Re}=0.51 \) upstream of the stenosis. The main difference in the boundary conditions compared with the Section 3.5.4, is that in the current model, the flow rate was adjusted according to the degree of stenosis. Observations of flow in coronary arteries[2, 89, 90] suggest that a high degree of stenosis
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b.3) Lines generated from points
b.3) Areas generated from lines
b.4) Volume generated and exported

c) CFD mesh composed by hexaedrals generated from volume

Figure 3.11: Generation of the geometry to perform haemodynamics analysis. a) Structural solution at a given substep of the solution. b) Steps followed in the reconstruction. b.1) Points were defined with the coordinates of the displaced nodes. b.2) Lines were generated from the points. b.3) Areas were generated filling the constructed lines. b.4) A volume of control representing the fluid flow inside the artery. c) A CFD mesh suitable for haemodynamics analyses can be generated from the volume reconstructed.
Figure 3.12: Decrease of flow rate as function of stenosis. Taken from [2]

increases the resistance of the vessel, producing a decrease in the flow rate. According to Figure 3.12, the drop in flow rate was considered as being a 25% of drop of flow rate at 80% of stenosis and a 65% of drop of flow rate at 90% of stenosis[2, 89].

The boundary condition at the walls was set as zero velocity to enforce the no slip condition.

Solution of fluid dynamics model

A finite volume scheme using the commercial software (FLUENT 6.0, Fluent USA, Lebanon, NH) was used to solve numerically the Navier-Stokes Equation 3.1 and Equation 3.2, described in Section 3.4.2. The haemodynamics computation was carried out assuming steady state laminar flow. The vessel wall was assumed to be rigid since minimal deformation due to the pulsatile flow has been observed for these arterioles. A mesh convergence analysis was conducted by increasing the number of nodes and calculating the maximum strain-rate predicted. Figure 3.13 shows the percentage difference between the maximum strain-rate calculated between subsequent refinements as a function of the number of nodes. Plots are shown for area reductions of 80% and 90% and with the arteriole wall elasticity set to Elasticity 1 and Elasticity 2, as defined in Table 3.2. Mesh convergence was assumed after observing variation in the maximum strain-rate values less than 5%.
3.5.5 Relationship between peak strain-rate and degree of stenosis and arteriole wall elasticity.

Having translated the mechanistic model such that it can be used for computational fluid dynamic analysis and having ensured the mesh is of sufficient quality, it is now possible to explore the fluid dynamic conditions that can be expected in the stenosis geometry. The first investigation explored the relationship between the maximum strain-rate experienced by the fluid and the degree of area reduction. Figure 3.14 shows a relationship between maximum strain-rates predicted by computational fluid dynamics as a function of area reduction for the geometries and two different wall elasticities as defined in Table 3.2. A non-linear relationship is evident. The maximum flow strain-rates predicted on both wall elasticities is on the same order of magnitude, being different by less than 15%. Figure 3.14 shows that 80% and 90% stenoses can increase the strain-rate by a factor of 35 and 45 times the strain-rate of the undeformed vessel (from $\dot{\gamma} = 1800\text{s}^{-1}$ to $\dot{\gamma} = 60000\text{s}^{-1}$ or $\dot{\gamma} = 85000\text{s}^{-1}$).

The shear equation for a rectangular section is given by [91] and was used to compare the maximum strain rate at different cases of stenosis, with the numerical model.

$$\dot{\gamma} = \frac{6Q}{wh^2}$$

(3.22)

where $\dot{\gamma}$ represents the shear-rate, $Q$ the flow rate, $w$ the width and $h$ the height ($w << h$).

Although the material properties of the arteriole wall are significantly different between
Figure 3.14: Maximum fluid strain-rates obtained along the artery-needle deformation process for two different artery wall mechanical properties. The strain-rates for a rectangular section calculated using Eq.3.22 are also presented for comparison.

the models, the peak strain-rate obtained by deforming with a needle to a specific degree of stenosis with respect the area, resulted in approximately the same peak strain-rate. For cylinders of regular geometry, the shear-rate is proportional to flow rate but inversely proportional to the cube of the diameter (Eq. 3.14). For a rectangular section when the height is much smaller than the width ($h \ll w$), Eq. 3.22 is used. Figure 3.14 shows the calculated strain-rates approximating the vessel cross-section as rectangular and assuming that the perimeter of the cross-section remains constant. The numerical calculations follow the same trend as Eq. 3.22, but departs from this relationship at between 50-90% area reduction. The higher strain-rates predicted by the numerical model are due mainly to local protrusions in the geometry. For extreme cases of stenosis (90%) both, Eq. 3.22 and the numerical model showed good agreement.

If peak strain-rate were the only important parameter defining the platelet aggregation then it might be predicted that both artificial stenoses would produce the same aggregation response regardless of wall elasticity and local differences of geometry. It is hypothesised, however, that along with the peak strain-rate the strain-rate experienced before and after that peak (strain-rate history) of given platelet at a specific stenosis is also an important factor in determining how a particular stenosis will induce aggregation. In order to explore this hypothesis, a more thorough investigation of the strain-rates predicted by the computational fluid dynamics was conducted, with particular emphasis on studying changes of strain-rate along the direction of flow.
3.5.6 Relationship between strain-rate distribution, degree of stenosis and arteriole wall elasticity.

Having established a quantitative relationship between the maximum strain-rate, the needle displacement and degree of stenoses, we proceed to explore in detail changes in the strain-rate along the direction of the flow that would be experienced by platelets passing through the mechanistically modelled stenoses. To visualize the strain-rate distribution, Figure 3.15 presents a sequence of contour plots of the strain-rates contour plots for Elasticity 1 and Elasticity 2 defined in Table 3.2, and for stenosis area reductions of 50%, 70%, 80% and 90%. Each element of Figure 3.15 presents a longitudinal cross-section (cut along the direction of flow) and a lateral cross-section (cut at the location of peak strain-rate). It should be noted that the location of peak strain-rate is in the center for the cases with Elasticity 2, but is at the circumference of the needle for Elasticity 1.

Figure 3.15: Strain-rates contour maps of the deformed arteriole. Longitudinal and cross sectional view over different degree of stenosis, and two arteriole elasticities defined in Table 3.2.

The observed difference in the location of peak strain-rate is due to the more elastic case (Elasticity 1) being able to adapt to the shape of the needle head, forming significant protrusions corresponding to the circumferences of the needle aperture, which lead to localised changes in the local velocity producing high strain-rates zones (red zones), contrary to the less elastic case (Elasticity 2), where the deformation is more homogeneous therefore velocity does not change as abruptly and the strain-rate distribution presents more gradual changes. As the strain-rate measures the magnitude of the spatial changes in velocity (as explained in Section 3.4.2), the more elastic case (Elasticity 1) presents higher strain-rates for the same amount of area reduction than the less elastic case (Elasticity 2). It can be observed that the interaction between the needle and the artery wall in
contact determines the local shape of the deformation, impacting locally in the haemodynamics strain-rate distribution. This analysis demonstrates that the geometry imposed by the needle can have a significant effect on the distribution of the strain-rates and its rate of change along the trajectory of flow.

Evidently the elasticity of the artery wall has a significant impact on the shape of the stenosis along the direction of flow and consequently significantly impacts the strain-rate history that will be experienced by the platelets. If it is true that the strain-rate history is a major factor in the initiation of aggregation, then these two cases should behave quite differently. To explore the differences between the strain-rate histories that might be experienced by a platelet flowing through each stenosis a more detailed investigation of the strain-rates experienced along specific streamlines was conducted.

### 3.5.7 Strain-rate history predicted along streamlines

The previous section provided quantitative evidence that when arterioles of different wall elasticities are stenosed to the same area reduction they will produce similar peak strain-rate conductions, but may produced significantly different distribution of strain-rates in the regions close to the stenosis. This section aims to quantify the effect that these differences in shape cause in the strain-rates experienced by a platelet passing through the stenosis.

In order to gain a more accurate comparison into the effect of changing wall geometry on the strain-rate experienced by blood cells, the strain-rate histories of streamlines which pass $1\,\mu m$ of the vessel wall at the contraction were studied. Since platelet concentration in blood flow is greatest near the vessel wall [92, 93, 94, 95, 96], streamline trajectories within $1\mu m$ from the wall at the contraction (maximum strain-rate zone) were selected. Figure 3.16a) presents a comparison of the selected streamlines which pass within $1\mu m$ of the wall at the location of the stenosis extracted from the computational fluid dynamics of the geometry with 80% degree of stenosis for both cases arteriole wall Elasticity 1 and Elasticity 2. These streamlines are coloured according to the velocity of the particles travelling along this streamline. It is evident that not only is the path taken by a platelet different in these two cases, but the platelet will also experience a different velocity, acceleration and deceleration as it follows this streamline, therefore it will be subject to different forces along the streamline.

Figure 3.16b) presents the velocity experienced by a fluid particle travelling along the streamlines depicted in Figure 3.16a), as function of distance. The peak velocity achieved is similar in both cases of arteriole wall Elasticity, but the acceleration and deceleration are far more rapid in the case of Elasticity 1 than for Elasticity 2. Figure 3.16c), presents the strain-rate calculated from these velocities. The origins of these streamlines have been
Figure 3.16: Streamline histories of selected stenosis. a) Selected streamlines. b) Velocity experienced by a particle following a streamline 1µm from the wall. c) Strain-rate histories for the selected streamlines aligned to compare the deceleration zone.

offset to the point of maximum strain-rate enable comparison of the deceleration. It can be observed that the overall velocity and strain-rate are in the same order of magnitude however, significative differences exist along the trajectory, in the scale of the platelet size (2µm). Significantly, in the case of Elasticity 1 the strain-rate drops far more rapidly than Elasticity 2 when leaving the stenosis.

If it is true that platelet aggregation response is determined not only by peak strain-rate, but also by strain-rate history outside of the stenosis region, then different aggregation responses might be expected for similar degrees of stenosis applied to arteries of different elasticity.

3.5.8 Role of Artery/Needle diameter ratio on duration of maximum strain-rate

In the previous investigation it was shown that the elasticity of the artery wall could significantly vary the strain-rate experienced by a platelet flowing in close proximity to the wall. It is true that the elasticity of the artery wall may vary from experiment to experiment, but this is a very difficult quantity to control. There are however other variables that could be controlled when inducing an artificial stenosis on a mouse artery. One prominent experimental variable that could be varied is the diameter of the needle used to produce the stenosis. This section explores whether changing the diameter of the needle could produce different strain-rate histories for the same degree of stenosis. The method used to explore the impact of needle diameter on the fluid mechanics of an artificially induced stenosis is similar to that used in Sections 3.5.2, but with a needle diameter of 20µm.
The elasticity of the artery was fixed as Elasticity 2 (Table 3.2) for all investigations. Figure 3.17, shows a compendium of the results obtained for a needle size of 20µm. Figure 3.17a shows the comparison with the in-vivo experiment where an arteriole of 100µm is deformed using a micro needle of 20µm of diameter. Also a comparison of the images recorded from the in-vivo experiment (as described in Section 3.5.1) and different wall geometries obtained from the mechanistically simulated arteriole (as described in Section 3.5.6) are presented. Good qualitative agreement is evident between the wall locations in the mechanistic simulation and the images recorded from the in-vivo experiment. Figure 3.17b), shows the cross sectional area and its calculated strain-rates. It is notice-

**Figure 3.17:** Simulations with a smaller needle (20µm). a) Comparison of intra-vital studies and numerical simulations. b) Strain-rate maps on the cross sectional area c) Streamlines histories. d) Streamlines histories aligned to study the rate of acceleration/deceleration.

able an increase in the magnitude of the strain-rates is noticable compared to the previous case of needle diameter 100µm. This can be explained as following, since smaller spatial changes produce higher changes of velocity, the rate of change of velocity (strain-rate) is larger. Due to the arteriole/needle ratio, the area occluded at 65% represents the maximum occlusion when the lower wall is not in contact yet with the upper wall. For the 100µm a 90% degree of stenosis was possible before the lower and upper walls touched. Figure 3.17c) shows the comparison of the strain-rate history experienced along a streamline located 1µm from the artery wall in the stenosis region, for an equivalent area of 65% reduction and same elasticity for both (Elasticity 2 in Table 3.2) 20µm and 100µm needle diameters. Examining these two strain-rate histories it is evident that a similar maximum
strain-rate is experienced but the length over which this peak strain-rate is distributed is on the order of 5 times less for the $20\mu m$ needle than the $100\mu m$ needle. This is not surprising since the dimensions of the needle tips causing the strain-rate also differ by a factor of 5. Figure 3.17d) shows the comparison of the strain-rate history presented in Figure 3.17c) but with the origin of each plot set to the location of peak strain-rates. This realignment enables comparison of the rate of acceleration/deceleration of the two cases. It can be observed that the rate of change is similar in both cases. This is in contrast to the significantly different deceleration rates that were predicted for differing arteriole wall elasticity as shown in Figure 3.16b). This slope in the velocity therefore depends closely on the mechanical properties of the artery wall. Therefore from this investigation it can be concluded that for a given artery changing the needle diameter will change only the residence time that platelets experience the peak in the strain-rate without impacting the acceleration/deceleration rate. This ability to isolate these two parameters may be of interest for future research into mechanical factors influencing platelet aggregation using live animals.

3.6 Comparison of mechanistically model geometry and canonical stenosis models

Sections 3.5.7 and 3.5.8 have shown using realistic mechanical modelling and computational fluid dynamics that significantly different strain-rate histories can result from various different conditions for artificially induced stenoses. This realistic modelling has revealed how sensitive the strain-rate environment of a stenosis can be to the various experimental parameters. Several previous investigations studying the haemodynamics of a stenosis have assumed that the geometry can be approximated with a cosine function without a rigorous corroboration [97, 98, 99, 100, 101, 102, 69, 103]. It would be instructive to compare some of the geometries utilized in previous investigations to the ones obtained this far in this chapter in order to identify any significant differences. This section presents a comparison of the mechanistically obtained geometries of Section 3.5.4 and their haemodynamics with a selection of the idealized geometries that can be found in the literature. From this analysis, differences in the stenosis parameters are pointed out that may be critical to promote platelet aggregation.
CHAPTER 3.

3.6.1 Canonical stenosis model geometries

To analyse a canonical stenosis model, we utilized a cosine function. The shape of the contraction takes the form according to the Equation 3.23:

\[
\frac{r(z)}{R} = 1 - \delta c [1 + \cos \left( \frac{Z \pi}{L} \right)], \quad -D \leq Z \leq D
\]  

(3.23)

In Equation 3.23, \( R \) is the radius of the unstenosed vessel and \( L \) is the length of the stenosis, \( r \) and \( Z \) are the radial and axial co-ordinates. The parameter \( \delta c \), that controls the percentage of the stenosis of area reduction. For this investigation a value of \( \delta c = 0.2 \) was chosen corresponding to a 80% of stenosis with respect the area. Two different length of the stenosed zone were used \( L = 200\mu m \) and \( L = 300\mu m \) (\( L = 4R \) and \( L = 6R \)), since is the range of variation utilized by the referred investigations [97, 98, 99, 100, 101, 102, 69].

3.6.2 Haemodynamics of the canonical stenosis model

The Navier-Stokes equations were solved numerically using the same method described in Section 3.4.2. Figure 3.18 presents the predicted haemodynamics behaviour for the canonical models with streamlines colored by velocity of the fluid flow. Figure 3.18a) and b) presents the haemodynamics solution for a cosine-shaped geometry where the length of the stenosis is \( L = 200\mu m \) and \( L = 300\mu m \) respectively. It was of interest to compare these geometries to the mechanistic solutions found in Section 3.5.7. To perform this comparison a streamline was selected located at 1\( \mu m \) at the apex of the contraction. Figure 3.19 presents the strain-rate calculated using Equation 3.20 for a streamline which passes 1\( \mu m \) from the wall at the apex of the stenosis. Figure 3.19a) shows the streamlines with the calculated strain-rate, as a function of length for the canonical cosine geometries of Figure 3.18. The strain-rate predicted along similar stream lines in the mechanistic models with the two elasticities from Figure 3.16 are also presented for comparison. Figure 3.19b) shows the strain-rate along the same streamlines as Figure 3.19a) but as a function of time. It can be observed that the peak strain-rate predicted along the streamlines for the cosine shape geometries is significantly lower compared with the mechanistically modelled needle induced stenosis geometries. This can be explained due to the fact that the cosine geometries are smoother and the changes produced in the principal direction of the velocity on these geometries are less abrupt. The strain-rate is related to the gradient of velocity and thus the smoother cosine shaped geometries produce less changes in velocity than the more abrupt needle induced stenoses. Figure 3.19c) presents a comparison of the deceleration regions of the shear stress profiles of Figure 3.19b) aligned such that their origins were set at the value of peak shear stress. Figure 3.19d) shows the
Figure 3.18: Haemodynamics comparison of mechanistic model and canonical stenosis model. a) Streamlines at the middle plane where the maximum velocity is experienced for a cosine shape of $L = 4R$ (Stenosis of 200 $\mu$m). b) Streamlines at the middle plane where the maximum velocity is experienced for a cosine shape of $L = 6R$. (Stenosis of 300 $\mu$m)

same information as Figure 3.19c) but plotted as a function of time. It can be observed that in terms of history over length, using a cosine function with length of stenosis of 200 $\mu$m (red line) is equivalent to the case of an stenosis with lower elasticity (Elasticity 1, blue line). Due to the fact that the changes in velocity over distance may be equivalent. However in terms of history over time, both cosine functions produce a slower deceleration compared with the induced stenosis. This important observation may suggest that the changes in velocity produced by the needle in soft tissue are larger than the changes produced in canonical models. This subtle observation can be supported by the non-linear response of the artery, since it can respond as an hyper-elastic material under load, which constitutes a material that can experience large deformations and easily take the shape of
Figure 3.19: Comparison of strain-rate history of fluid streamlines of mechanistic model and canonical stenosis model. a) Streamlines close 1\(\mu\)m to the wall (B, see Fig 3.18) where aggregation is expected, over length. b) Streamlines close 1\(\mu\)m to the wall (B, see Fig 3.18) where aggregation is expected, over time. c) Streamlines of a) but aligned in the deceleration zone. d) Streamlines of a) but aligned in the deceleration zone.

The presented investigation has shown that the mechanical properties of a stenosed blood vessel can have a significant impact on the behaviour of fluid flowing within the vessel. However, in conducting this study a number of approximations were made to simplify the numerical computation. This section discusses these approximations and whether they
should have significant impact on the conclusions that can be drawn.

In our study we have assumed that blood is a homogeneous, Newtonian fluid with the properties of blood plasma. In fact, blood is a non-homogeneous fluid, in particular about 45-60% of the volume of blood is large red blood cells of around $10^{-30} \mu m$ diameter.

It has been shown that under flow, these large red cells tend to concentrate at the center of the blood vessel, with the fluid at the vessel walls being composed mainly of plasma due to a margination effect ([92, 93, 94, 95, 96]). Conversely platelets tend to concentrate in this plasma at the vessel walls and it is here that shear stress can cause aggregation. Hence our approximation that the fluid properties of blood are the same as for plasma are valid in this region, provided that the stenosis area reduction is significantly larger than the scale of the red cells themselves.

It is worth noting that when the blood vessel is extremely stenosed (80% - 90% area reduction), the channel area will be of similar dimensions to the red cells. At these extreme degrees of stenosis, red cell collision and deformation is likely to occur leading to non-Newtonian fluid dynamics, for example the cells may tend to elongate and line up with streamlines, reducing the viscosity ([104]). However, provided that the stenosis is not blocked by these events, the flow rate through the stenosis should remain constant and the strain-rates close to the vessel wall should remain within the same order of magnitude as the homogeneous plasma case.

An accurate calculation considering the effects of red blood cells including deformation, collision and interaction with the wall, would certainly be valuable but such a rigorous model may be beyond what is tractable currently. Significant work in this field can be found in the literature, ([105]) have considered rigid particles in a simple two dimensional flow while Crowl([96]) has reported a two-dimensional whole blood model where due to interactions with the deformable red blood cells, platelets can be either pushed toward the walls into the cell-free layer or move much more erratically in the center of the vessel. However, to our knowledge numerical models of blood flow in a three dimensional stenosis which include red blood cells have yet to be reported.

In our models we have initiated flow under a uniform inlet condition. In fact, it has been reported ([97]) that in-vivo blood flow can have a helical component and that this may increase the viscous stress. Therefore it is possible that the strain-rates that would be experienced by platelets in-vivo could be higher than predicted by our models. Further investigation of the impact of this helical flow is warranted, however there is no reported data on the magnitude of helical flows within small vessels and at very low Reynolds number.

Along with the approximations made in modelling the fluid dynamics, it is also appropriate to discuss the parameters and validity of the in-vivo experiments on which these models are based. In this study we propose that vessel wall elasticity plays an important
role in the rheology of stenosis. Control of vessel wall elasticity, in the in vivo experimental setting presented, is a technically challenging problem. The elastic properties of the vessels may change locally as a function of vessel wall composition, vessel architecture and the surrounding extravascular tissues. The elasticity of small arteries (capillaries and arterioles) is greatly influenced by the amount of surrounding tissue ([104]). The present computational study is therefore based on carefully chosen (idealized) small bowel mesenteric arterioles. The choice of arterioles was in part limited by the imaging setup utilized, such that only vessels with relatively limited amounts of surrounding fat tissue were assessed.

One possible improvement to the experimental approach would be to directly compare arterioles and adjacent venules that exhibit known differences in vessel wall composition and thickness and therefore elasticity’s. However differences in flow rates and extracellular matrix composition will complicate interpretation of such an experimental approach. A second possibility would be to compare platelet reactivity and modelled haemodynamics and elastic properties in apoE-/- mice fed a high fat diet (which have been demonstrated to exhibit extensive atherosclerotic disease and overt changes in vessel composition), with control (wild type) mice.

Vascular constriction may significantly influence both the extent of the final stenosis and tissue elasticity. The modelling presented was performed on vessels that did not display overt constriction following external application of stenosis. The data presented therefore represents a steady-state snapshot of the vessel at the time of micro-needle compression. Measurements of the inlet and outlet segments along with the region of stenosis were made during this relatively quiescent steady-state phase.

### 3.8 Conclusions

In this chapter it was presented that a stenosis generated by a micro-needle in an arteriole is able to produce sudden increases in the strain-rate on the micron scale (called shear micro-gradients). These increases can be on the order of 30 to 60 times the shear rate of the undeformed arteriole. From the in-vivo experiments of Section 2.3 it was found that aggregates form in the deceleration zone, suggesting that the initiation of platelet aggregation is likely driven primarily by haemodynamics forces occurring in regions of laminar flow, where no flow recirculation or vortex formation is evident. This aggregation occurs when the chemical mechanisms of aggregation (ADP, thrombin and tromboxane) were blocked further supporting the hypothesis that aggregation is initiated by non-chemical means.

In general, the modelling presented in this Chapter has provided the order of magni-
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tude of the haemodynamics parameters that may be expected during platelet aggregation studies in artificially induced stenoses. This will aid understanding of the platelet response observed during in-vivo experiments.

Computational techniques were applied to an approximate geometry to gain insight into the order of magnitude of the haemodynamics variables, particularly blood strain-rates $\leq 20000 \, s^{-1}$ were predicted at the stenosis apex that rapidly transitioned to $1862 \, s^{-1}$ within the post-stenosis expansion zone, suggesting that a zone where the strain-rates are increasing followed by a zone where the strain-rates are decreasing (shear micro-gradients) may be a requirement for the development of discoid platelet aggregates through mechanical means. Using an idealized geometry it was possible to identify three principal components defining a shear micro-gradient or stenosis, the contraction region which causes acceleration of the platelets, the throat or apex of the stenotic region where peak strain-rate is experienced and the expansion region that decelerates the platelets.

A second approach of modelling, which took into account the elastic response of an artery under needle compression and mimicked the in-vivo experiment, enabled detailed haemodynamics analysis. The method presented to generate the geometry deformed artery was based on mechanistic simulation and reliably generated geometries which were very similar to those observed experimentally. Using this model we found that even under the same stenosis conditions, the arterioles elasticity plays a critical role in determining both the cross sectional area in the stenosis region and particularly the rate of acceleration/deceleration that the fluid experiences before and after the stenosis. It is hypothesised that these two variables (cross sectional area and rate of acceleration/deceleration) may impact in thrombus formation for in-vivo studies. Care must be taken in subsequent studies to properly quantify the arterioles elasticity as it is possible that the degree of area reduction is not sufficient to determine the platelet aggregation response. Experimental in-vivo velocity measurements of the fluid are recommended to validate these predictions.

The geometries obtained using the introduced mechanistic modelling approach was compared to canonical geometries based on cosines commonly used in research on stenoses. It was found that the calculation of the strain-rate showed that the more elastic tissue produced larger changes in velocity and this produced larger strain-rates, contrary to the cosine shapes where the changes in velocity are smoother. It was noted that the majority of the investigations using cosine geometries used a stenosis length of twice the vessel diameter. The experiments and simulations presented in this chapter suggest that the length of the stenosis should be shorter than twice the vessel diameter in order to reproduce the aggregation response observed the in-vivo experiment. It can be concluded that under the condition of low Reynolds number the canonical model is not the optimum scenario to study platelet aggregate formation, since more elastic tissue is able to produce a more
abrupt and non-trivial changes in velocity which may be important in determining platelet aggregation response.

It has been shown in this Chapter also that although a certain degree of control of the geometry and haemodynamics conditions can be achieved through displacement of the micro-needle or through choice of micro-needle diameter, these parameters often change more than one variable of the geometry and thus it is difficult to isolate the effects of different geometric components. It has also been shown that the mechanical properties of the blood vessels themselves can be quite significant. These will almost certainly vary from experiment to experiment and thus it is difficult to achieve a strong conclusion about the role that mechanical stresses play in the initiation of platelet aggregates from these studies alone. It is therefore necessary to conceive an in-vitro platform to study platelet aggregation with a more precise control. This is the topic of the subsequent chapters.

The recent adaptation of the mesenteric model of acute thrombosis intra-vital system to the examination of platelet aggregation responses under conditions of controlled vessel stenosis sets the scene for the current study. The causal relationship between vessel elastic response to needle compression and the dependent acceleration and deceleration components of flow through the stenosis may be a significant determinant of the magnitude and rate of the platelet aggregation response, as it has been shown in-vitro on micro-channels ([4]). Differences in the geometry of the applied stenosis from site to site or vessel to vessel could affect the thrombus size. Since different fluid forces experienced as a function of the local geometry affect directly the cell interaction with the wall. This prediction is borne out by experimental findings within our laboratories which suggest that the extent of the platelet aggregation is not a simple function of the degree of stenosis (unpublished observations). This study demonstrates that although a certain degree of control of the geometry and haemodynamics conditions can be achieved through displacement of the micro-needle or through choice of micro-needle diameter, these parameters often change more than one variable of the geometry and thus it is difficult to isolate the effects of different geometric components. The precise way in which stenosis geometry impacts on platelet function is currently under further investigation.

Blood vessel wall composition is complex and can differ markedly across the vasculature. This study highlights the necessity for proper characterisation of the blood vessels under study from a mechanical properties perspective. In contrast to the theoretical cosine shapes, where the changes in velocity are relatively smooth, the simulated geometries experienced higher strain rates.

While the simulation presented has been applied to the specific case of micro-needle stenosis in the small bowel mesenteric thrombosis model it has direct application to and implications for the analysis of platelet function in a number of so called “high shear”
dependent thrombosis models ([106, 107, 51]), where a silk suture is tied around an artery to cause a concentric stenosis.

The simulation presented demonstrates that under generalized externally applied stenosis, the arteriolar non-linear elasticity plays a critical role in determining both the cross sectional area in the stenosis region and significantly, the rate of acceleration/deceleration that the blood experiences before and after the stenosis. Future work will involve more advanced computational models considering non-homogeneous and non-Newtonian fluids under realistic in-vivo stenosis geometries. In conclusion, the simulation presented should be a useful tool in the investigation of the impact of the mechanical blood flow environment on platelet function and thrombosis.
Chapter 4

In-vitro disturbance: spherical micro-protrusions in capillary channels

4.1 Contributions

I performed the fluid dynamic simulations to characterizse the second set of experiments described in Chapter 2. The images from these analyses were published in [1]. I proposed to analyse not only the strain-rate distribution at the surface of the bead, but also the fluid streamlines. I proposed comparing these simulations with an analytical solution. The numerical simulations with minor modifications were published in:


4.2 Introduction

Chapter 3 presented an analysis of an in-vivo experiment where a micro-needle was used to create an artificial stenosis which could initiate platelet aggregation under conditions where the common chemical activating mechanism of platelet aggregation were blocked. The investigation in Chapter 3 attempted to model the haemodynamics conditions that would be experienced by the platelets flowing through this stenosis using a subjective qualitative model and a mechanistically simulated geometry. These investigations demonstrated qualitatively that a micro-scale stenosis produces a sudden increase and subsequent decrease in the flow strain-rates that closely correlate with the regions where platelet
aggregation was found to occur experimentally. Significantly, platelet aggregation was found to occur in the deceleration zone of the stenosis rather than in the high shear zone as has been reported in earlier studies [31, 42, 43]. Using the fluid mechanical insight obtained from the investigation in Chapter 3 in combination with biological models of platelet aggregation it was concluded that the aggregation was most likely due to non-chemical factors. It was thus hypothesised that fluid mechanics play a critical role in the initiation and development of platelet aggregates and thus it was deemed of great interest to study the mechanical conditions and their impact on platelet response. Using the experimental in-vivo approach described in Chapter 3 it was found that although the peak strain-rate could be accurately controlled using the degree of stenosis, the total strain-rate history including acceleration and deceleration components was highly variable and dependent on parameters such artery wall elasticity which is difficult to control in a systematic manner. Given that platelet aggregation was found exclusively in the deceleration zone of the in-vivo stenosis, it was hypothesised that not just high strain-rates but the total strain-rate history is a significant factor in determining platelet aggregation which may explain why aggregation is only observed under certain specific in-vivo experimental conditions.

Due to the fact that in-vivo parameters are highly complex and difficult to control, with variations existing in geometry, elasticity, platelet function and chemical environment, it was very difficult to conceive of an in-vivo experiment that could prove the importance of total strain-rate history in the initiation of aggregation. It was thus decided to pursue a simplified in-vitro model that could isolate the parameters of interest while eliminating many of the variables encountered in the in-vivo experiment. Historically in-vitro experimental approaches examining blood flow have been conducted using parallel flow chambers, Couette cells [37, 38, 39] or rectangular capillaries [40, 41, 11, 6, 7]. Rectangular capillaries in particular have become popular in recent years due to their relative ease of use and easy application to transmitted light micro-imaging techniques. Since the cross sectional area of a rectangular capillary is constant along the direction of flow, the fluid at any point in the cross-section experiences a constant velocity and constant shear-rate as during steady. The rectangular capillary is thus an excellent tool for studying the role of haemodynamics at a constant shear-rate on blood cells. Capillaries can also be easily coated with specific proteins to study the function and interaction of platelets under a wide range of constant shear-rate. A number of experiments were conducted to study platelet-protein matrix interactions under flow at the Australian Centre for Blood Diseases [33, 44]. During some of these experiments, it was observed that occasionally single platelets adhered to the surface of the capillary and this by itself was apparently sufficient factor to trigger platelet adhesion and subsequent accrual of new platelets from the flow (unpublished observations). However, due to the stochastic nature of platelet-surface
interactions under flow it was difficult to predict where an platelet aggregation would occur. Based on these limitations a method was developed to locally modify the capillaries to initiate single platelet adhesion at a pre-defined location, modifying the surface of the capillary to create a flow disturbance, similar to Chapter 3, but using a canonical disturbance that could be easily controlled and analysed.

This chapter presents the application and characterisation of a micro-bead disturbance assay to examine platelet aggregation under defined blood flow conditions and a series of investigations of the fluid dynamics produced by micro-spheres adhered to the wall of a rectangular microfluidic capillary. The Chapter is structured as follows. Section 4.3 presents a review of the theory of fluid mechanics and a solutions for flow around a sphere. Section 4.4 presents a more complete solution for the viscous flow around a sphere fixed on the wall of a rectangular capillary using a numerical approach. It is presented an estimate of the flow disturbance produced by a sphere in a rectangular capillary. Distinct flow parameters are extracted from an estimate of the flow disturbance produced by a sphere in a rectangular capillary, which are correlated to platelet aggregation behaviour. Section 4.5 presents the relative error of using both methods for specific variables of interest of fluid mechanics. Section 4.6 then presents a summary and conclusions from this chapter.

### 4.3 Flow around a sphere: analytic calculation

In this section, an idealized model of the fluid flow around a bead attached to the wall of a rectangular stenosis is presented. A closed-form analytic solution for the strain-rates caused by this bead is developed. Section 4.3.1 presents an overview of the problem definition and the important fluid dynamic variables that are sought. Section 4.3.2 presents the analytic simulation approach used to obtain an approximate model for the strain-rates and also the approximations and limitations associated with this approach. Section 4.3.3 presents the results of this analysis and discusses the validity of these approximate results in comparison with the in-vitro experiments of Section 2.4. Section 4.3.4 discuss some limitations of the analytic approach. Section 4.3.5 then presents a summary and conclusion of the analytic fluid dynamic calculation.

#### 4.3.1 Problem description

It is possible to calculate the haemodynamics of the flow around a spherical object on the wall of a rectangular capillary if a number of important parameters of the flow are known. These include the shear-rate at the walls of the capillary of the unperturbed flow, the
rectangular channel geometry and the sphere diameter. In the experiments of Section 2.4, a constant blood flow rate was maintained to produce a shear-rate of 10,000 s\(^{-1}\) at the bottom and top wall of a rectangular channel. The channel dimensions were 2\(\text{mm}\) \(\times\) 0.2\(\text{mm}\). Fixed spheres with similar size diameters to platelets (2\(\mu\text{m}\)) were used (2, 5, 9 and 15\(\mu\text{m}\)). A diagram of the geometry with the important parameters labeled is presented in Figure 4.1.

![Diagram of spherical obstacle in a constant flow rate field.](image)

**Figure 4.1:** Spherical obstacle in a constant flow rate field.

In order to analyse the impact of the bead on the initiation of platelet aggregation it is necessary to know a number of important haemodynamics parameters. For example, the effect produced by the sphere on the strain-rate at the wall near the bead, the strain-rate distribution on the surface of the sphere itself, the strain-rate distribution within the volume of the channel in the neighborhood of the sphere, and, similar to the investigation of Section 3.5.7 the strain-rate history for particles travelling along streamlines close to the micro-sphere surface, and finally whether recirculation exists.

### 4.3.2 Analytical method of viscous flow around a sphere

In order to solve the problem defined in Section 4.3.1, the analytical solution of Stokes for viscous flow around a sphere can be is presented, which describes the motion of fluid substances. The Navier-Stokes equation for an incompressible Newtonian fluid with uniform viscosity was introduced in Chapter 3 as Equation 3.2. For the convenience of the reader it is repeated in its full form as

\[
\rho \left( \frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \nabla \vec{u} \right) = -\nabla P + \mu \nabla^2 \vec{u} + \vec{f} \tag{4.1}
\]

Where \(\rho\) is the density of the fluid, \(\vec{u}\) is the velocity vector, \(P\) is the pressure, \(\mu\) is the viscosity and \(\vec{f}\) denotes any external body forces. This equation arises from applying the
Newton’s second law to fluid motion, together with the assumption that the fluid stress is the sum of a diffusing viscous term (proportional to the gradient of velocity), plus a pressure term. On the other hand to get more insight about the regime of the flow whether is laminar or turbulent the Reynolds number can be used and is defined as:

$$Re = \frac{\rho Ul}{\mu}$$  \hspace{1cm} (4.2)

where $l$ is the characteristic length, which in this case is the diameter of the micro-bead and, $U$ the mean fluid velocity. For micro-flows, usually the characteristic length is short and the velocities low. This results in a low Reynolds number which means that flow is laminar. For the present case, the Reynolds number is between 0.01 and 0.64 depending on the bead size. In order to present an analytical approach to solve the Equation 4.1, it is assumed that effects of the inertia forces are minimal (i.e. the inertial term $\vec{u} \cdot \nabla \vec{u}$ is disregarded, although for the present case the magnitude of the inertia forces is roughly similar to the viscous forces). Also since the flow is steady the time derivative is also zero, and no external body forces exist, the Navier-Stokes Equation 4.1 can be reduced to:

$$\nabla p = \mu \nabla^2 \vec{u}$$  \hspace{1cm} (4.3)

One method of solution for this equation is by stream functions and assuming that the sphere is fixed in the space. The geometry of the simplified problem with the assumption is depicted in Figure 4.2. For convenience the origin is located in the center of the bead and $r$ is the radial coordinate, $\theta$ is the angle and $R$ is the radius of the sphere. Therefore by applying a stream function, using spherical coordinates and applying the proper boundary conditions, the velocity field can be expressed as Equations 4.4 and 4.5:

$$u_r = U \cos \theta (1 - \frac{3R}{2r} + \frac{R^3}{2r^3})$$  \hspace{1cm} (4.4)

$$u_\theta = U \sin \theta (1 - \frac{3R}{4r} + \frac{R^3}{4r^3})$$  \hspace{1cm} (4.5)
A detailed derivation of the solution that leads to Equations 4.4 and 4.5 can be found in [83]. This result was obtained by Stokes in 1851 considering a flow where the viscous forces are much bigger than the inertial ones ($\text{Re} << 1$), therefore the accelerations of the fluid around the sphere can be ignored, resulting in an equation that can be solved analytically. This analytical solution describes the flow around a sphere, considering that the sphere is fixed in the space, therefore the solution is axisymmetric along the direction of the flow. Equations 4.4 and 4.5 describe the theoretical distribution of velocity as a function of distance $r$ from the center of the sphere and the angle $\theta$ measured around the sphere from $0^\circ$ at the front point to $180^\circ$ at the rear point. It can be noticed from Equations 4.4 and 4.5 that as the radial distance increases ($r \to \infty$) the velocity approaches its free stream magnitude and direction as expected. To calculate the strain-rates, the velocity gradient can be found from Equations 4.5, since the component of drag force per unit area in the direction of the uniform stream is:

$$F = -p\cos\theta + \tau_{rr}\cos\theta - \tau_{r\theta}\sin\theta \quad (4.6)$$

The components of viscous stress are:

$$\tau_{rr} = 2\mu U\cos\theta\left[\frac{3a}{2r^2} - \frac{3a^3}{2r^4}\right] \quad (4.7)$$

$$\tau_{r\theta} = \mu\left[r\frac{\partial}{\partial r}\left(\frac{u_\theta}{r} + \frac{1}{r}\frac{\partial u_r}{\partial \theta}\right)\right] = -\frac{3\mu Ua^3}{2r^4}\sin\theta \quad (4.8)$$

so that Equation 4.6 becomes

$$0 + 3\frac{\mu U}{2a}\cos^2\theta + 3\frac{\mu U}{2a}\sin^2\theta = \frac{3\mu U}{2R} \quad (4.9)$$

From Equation 4.9 it can be observed that the maximum strain-rate around the sphere is located at the apex of the sphere when $\theta = 90^\circ$.

### 4.3.3 Fluid velocity profile in a rectangular capillary

To calculate the strain-rates using Equation 4.9, the undisturbed (upstream) velocity at the bead must be quantified. This can be approximated as the velocity that would be expected at the location of the bead in close proximity to the rectangular channel wall. To calculate this velocity it will be necessary to determine the fluid velocity profile in a rectangular capillary. The velocity profile of a rectangular capillary can be obtained either using computational fluid dynamics or through analytical equations [108]. For the current investigation, computational fluid dynamics using the geometry depicted in
Figure 4.1 (but without the bead) and the numerical technique described in Section 3.4.2 was used. Figure 4.3 presents the velocity profile that would be expected parallel and equidistant from the two vertical walls of the channel at a height ranging from the bottom of the channel to the top. This velocity profile is approximately parabolic as is expected from analytic theory [108]. The inset of Figure 4.3 presents a magnified view of this velocity distribution ranging from the capillary floor to $15\mu m$ above the floor. This is the region where the beads will be located. The values of the tangential velocity and the correspondent shear rate are presented in Table 4.1. Another variable of interest that may be calculated in close form and also extracted from numerical simulation is the drag force experienced by the sphere. This drag force can be calculated by using the Newton’s law of viscosity:

$$\tau = \mu \frac{d\theta}{dr} \quad (4.10)$$

where $\tau$ is the stress. Since stress is force over area, by combining Equation 4.9 and Equation 4.10 the drag force equation can be obtained as:

$$F_x = 6\pi \mu U a \quad F_y = F_z = 0 \quad (4.11)$$

where $D$ is the drag force in Newtons, $U$ the mean velocity and $a$ the radius of the sphere. By applying the use Fourier-Bessel transforms, an exact solution of the Navier-Stokes equations for a sphere touching a plane can be derived [109] and analytical equations for the drag and torque can be obtained. It is deduced for a fixed sphere in a plane, the drag force equation follows the same form but with an additional factor $f = 1.7009$, as follows:

$$F_x = 6\pi \mu U a f \quad F_y = F_z = 0 \quad (4.12)$$

and the torque that the sphere is experiencing can be expressed in Cartesian components as

$$(0, 0, -8\pi \mu U a^2 g) \quad (4.13)$$

where $g = 0.94399$. Using these equations the drag force and torque that the micro-spheres will experience can be calculated and the results are also presented in Table 4.1.

Comparing the mechanical parameters presented in Table 4.1, the first interesting result to observe is that although the flow velocity experienced by the sphere increases in approximate proportion to the sphere diameter, the maximum shear rate predicted at the apex of the sphere falls in the same range, independent of the diameter. This can be explained as follows: Equation 4.9 predicts that the shear-rate will decrease inversely with radius, but the velocity $U$ is increasing approximately in proportion with $r$, due to velocity profile of the rectangular geometry (as shown in Figure 4.3). Therefore a bigger bead experiences a higher velocity $U$ which cancels the increase in shear due to a larger
Figure 4.3: Velocity profile for a rectangular capillary of $2mm \times 200\mu m$ along the $200\mu m$ dimension, with a zoom in in the region 0-15$\mu m$ where the micro-beads are located to obtain the tangential velocity values.

Table 4.1: Fluidic parameters calculated for spherical beads of various diameters. Flow velocity derived from computational fluid dynamics in a rectangular capillary as depicted in Figure 4.3, strain-rates as derived from Equation 4.9, Drag Force calculated using Equation 4.11 and Equation 4.12 and torque calculated using Equation 4.13.

<table>
<thead>
<tr>
<th>Sphere $\phi$ [\mu m]</th>
<th>Vel. [mm/s]</th>
<th>Shear-rate [$s^{-1}$]</th>
<th>Drag Force Stokes [nN]</th>
<th>Drag Force [109] [nN]</th>
<th>Torque [nN\mu m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.2</td>
<td>30000</td>
<td>0.219</td>
<td>0.373</td>
<td>0.276</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>29400</td>
<td>1.344</td>
<td>2.28</td>
<td>4.23</td>
</tr>
<tr>
<td>9</td>
<td>0.87</td>
<td>29333</td>
<td>4.34</td>
<td>7.39</td>
<td>24.60</td>
</tr>
<tr>
<td>15</td>
<td>0.14</td>
<td>28000</td>
<td>11.52</td>
<td>19.58</td>
<td>108.74</td>
</tr>
</tbody>
</table>

bead diameter. Recall that the shear-rate is the rate of change of velocity over the radial distance, then a larger velocity magnitude is compensated by a larger curvature on the sphere size. It can also be noticed that the order of magnitude of the drag and torque is in the order of $nN$ and $nN\mu m$, respectively, and that they are proportional to the sphere diameter.
4.3.4 Limitations of the analytical approach

This analysis assumed that the sphere was fixed in place by some abstract means and was not physically connected to the capillary wall by any means that would disturb the flow. The consideration utilized by Stokes treating the sphere as fixed in space is just an artifact which is useful to understand the viscous flow around a spherical object. A real implementation may exhibit different properties. In the experiment of Section 2.4, the sphere was fixed in place by a small layer of protein (approximated as a disc of dimensions of $0.1\mu m \times 1\mu m$ as shown in Figure 4.1). It has been observed experimentally [94, 95] and corroborated by some recent numerical models [96] that platelets travel close to the wall when flowing in blood, and thus they are likely to experience the perturbation caused by the means of fixing the bead to the wall. The hypothesis of this chapter was that subtle changes in the total shear stress history experienced by the platelet would be significant in determining the initiation of platelet adhesion and aggregation and thus it is important to have a precise model of the shear stress history that would be experienced by a platelet adhering to the surface of the bead. It is thus necessary to accurately model the shear stress history along streamlines of flow travelling close to the wall of the capillary, and this is the zone where the Stokes solution for a sphere (far from a plane) lacks validity. An alternate solution has been proposed by O’Neill [109]. An attempt was not made to implement this model as it was deemed difficult to carry out numerically in order to have a 3D visualization, compared with a finite volume technique, where the author is more familiar. However, O’Neill’s [109] solution has been useful to compare specific values of forces and torques to validate the numerical model. Furthermore, according to the biological experiments platelets seem to adhere to the back of the sphere and adhere to the bottom wall. It would be instructive to know the shear rates associated to these particular zones.

4.3.5 Conclusions of the analytic model of flow around a bead

Although the analytical model of this section provides the interesting insight that the peak shear stress experienced by a platelet interacting with a bead should be independent of the bead diameter, this model is not sufficient to accurately determine the total shear stress history experienced by a platelet flowing around a bead that is physically attached to the wall of a capillary. It is thus necessary to perform more rigorous computational fluid dynamic analysis of the fluid flow in this geometry.
4.4 Flow around a sphere on the wall of a rectangular capillary: numerical calculation

Section 4.3.2 used an analytical approach to estimate the flow field around a sphere. This provided order of magnitude estimates of the mechanical properties of the flow and the interesting insight that maximum strain-rate should be independent of sphere diameter. It was, however, concluded that more rigorous computational fluid dynamics would be required in order to precisely determine the strain-rate history that could be expected along the streamlines close to the capillary wall and the surface of the bead in order to draw any strong conclusions about the role of strain-rate history has in the initiation of platelet aggregates observed in Section 2.4.

Advancements in computational methods allow access to a more complete description of the haemodynamics and visualization in three dimensions of the phenomena which can provide greater insight for both biologists and scientists not necessarily familiar with the theoretical constructs and approximations used to obtain analytic equations such as those presented in Section 4.3.2. Therefore the goal of this section is to achieve accurate estimates of the strain-rate history that would be experienced by platelets aggregating on spherical beads on the walls of rectangular channels, and compare these to the experimental observations of Section 2.4 and the analytic predictions of Section 4.3.2.

4.4.1 Discretisation and Boundary Conditions

The geometry of the beads and capillary was modeled in Ansys by creating a script where the variables were parameterized, the result was exported using an IGES file and imported to GAMBIT (all cases) and ICEM (bead 15 $\mu$m). To create the discretization in GAMBIT, the mesh consisted of two zones, an unstructured region close to the bead and a structured mesh everywhere else.

A symmetrical condition was applied at the center plane of the beads to reduce computational load. The number of cells in the structured mesh generated was directly dependent on bead size ranging from 754,446 ($2 \mu$m beads) to 954,516 ($15 \mu$m beads).

For the geometry in ICEM a structured mesh composed of hexaedrals using the block topology was created for comparison purposes. In order to calculate the velocity for the inlet boundary condition, it is not correct to apply the Equation [3.14] which is useful for cylinders, as the present capillary geometry is rectangular. For geometries where the width is much smaller than the height (at least 5 times) the shear-rate $\dot{\gamma}$ can be written

$$\dot{\gamma} = \frac{6Q}{wh^2}$$

(4.14)
where \( Q \) is the flow rate (defined as \( Q = u \cdot w \cdot h \)) in \([\frac{ml}{sec}]\). The width \( w \) corresponds to 2000 \( \mu m \) and the height \( h \) to 200 \( \mu m \), in \( cm \). Using Equation 4.14 in order to achieve a shear-rate of \( \dot{\gamma} = 10,000[s^{-1}] \) the flow rate must be \( Q = 8[\frac{ml}{min}] \). Therefore it is possible to calculate the velocity that should be imposed at the inlet boundary condition to produce a shear-rate of 10,000\( [s^{-1}] \). The boundary condition at the walls and at the bead surface was set as zero velocity to enforce the no slip condition. The boundary condition at the outlet surface was a relative pressure of zero (Pa).

4.4.2 Computational Fluid Dynamic Solution

Once the discretized volume was built, a finite volume scheme using the commercial software (FLUENT 6.0, Fluent USA, Lebanon, NH) was used in order to solve the Navier-Stokes equations numerically. The same fluid medium used in Section 3.5.4 was used. Therefore a Newtonian fluid was considered with a density of \( \rho = 998.2 [kg/m^3] \) and a viscosity of \( \mu = 0.00348 [Pas] \), since blood can be approximated as a Newtonian fluid if the strain-rates are high\[75\]. The pressure discretization scheme was set as standard and the second order upwind momentum option was enabled.

4.4.3 Strain-rate distribution on sphere and plane

Figure 4.4a) shows the strain-rate calculated at the surface of a spherical bead of diameter 15\( \mu m \) and also the strain-rate at the surface of the channel wall. The streamlines located in a plane 7.5\( \mu m \) above the surface of the channel wall are also depicted (at the center of the sphere). The strain-rate is highest at the top of the sphere. The strain-rate ranges from zero at the base of the bead (where the bead is stuck to the channel), to approximately 30000\( s^{-1} \) on the sides of the bead to approximately 60000\( s^{-1} \) at the top of the bead.

The numerically calculated strain-rate of 30000\( s^{-1} \) observed on the sides of the bead is in good agreement with the analytical predictions presented in Table 4.1. Figure 4.4b) presents a longitudinal cross-section of the bead in flow. Here the streamlines are shown in the plane parallel to the direction of flow and bisecting the bead. Figure 4.4b) illustrates the departure of the computational model from the analytic model of Section 4.3.2. The streamlines divert preferentially over the bead and the peak strain-rate is found on the top of the bead. This peak strain-rate of 60000\( s^{-1} \) is approximately twice that predicted in Table 4.1. This can be explained by the fact that more of the fluid must flow over the top of the bead as the bottom is obstructed by the capillary wall. Figure 4.4b) also shows that the bead produces a zone of low strain-rates on the capillary wall. This can be seen as a dark blue shadow on the upstream face, the downstream face and the underside of the bead and is evident on the capillary wall extending approximately a bead diameter both
before and after the bead.

The streamlines in the plane 7.5\(\mu m\) above the surface of the channel wall flow around the bead in a very symmetric fashion indicating laminar flow with no evidence of flow separation. The symmetric form of these streamlines compares well to the analytic predictions of Section 4.3.2. The streamlines are useful to observe to interpret flow behavior past a stationary sphere. At this low Reynolds number the streamlines are symmetrical before and after passing the sphere. It is interesting to note that the accumulation of platelets downstream of the sphere at low Reynolds numbers is present even although the streamlines are symmetrical. This suggests that a non-physical (biological) mechanism may lead the aggregation.

**Figure 4.4:** Perspective view of the bead of 15\(\mu m\) and streamlines. a) It can be observed that the maximum stress is occurring in the bead surface and following the streamlines, the bead provides a zone of low shear (blue shadow) after the maximum shear. This zone of low shear is located in the bead itself, downstream the flow, but also at the bottom surface of the capillary channel. b) Representation of the streamlines from the bottom wall to 15\(\mu m\). It can be observed that the streamlines are adapted to the geometry of the spherical obstacle.

Figure 4.5 presents the numerically calculated strain-rate distribution for a 2\(\mu m\) and 15\(\mu m\) bead. The 2\(\mu m\) bead produces a very similar strain-rate distribution to the 15\(\mu m\) bead. This is in good agreement with the analytic predictions presented in Table 4.1. It can be observed that the low strain-rate region presents the same distribution proportional to the bead size. It is hypothesised that if platelets experienced the peak strain-rate on the top face of the bead and then drop into this low strain-rate shadow, they will experience the ideal conditions for aggregation where filamentous membrane tethers (cylinders of lipid bilayer) are pulled from the surface of discoid platelets by high strain-rates [44, 1].
but a low strain-rate zone allows them to interact with the bead and channel surface and with each other without being forcibly separated. This bio-physical mechanism may be enhanced by a localised shear-enhanced diffusion of platelets which is a phenomena that allows them to migrate across streamlines to regions of low shear [110].

\[
\phi 2 \mu m \phi 15 \mu m
\]

![Figure 4.5: Perspective view of the strain-rate distribution for a 2 \mu m and 15 \mu m micro-spheres.](image)

### 4.4.4 Strain-rate history along streamlines

In Section 4.4.3 computational fluid dynamics simulations established the strain-rate distribution of the fluid flowing around a spherical bead on the wall of a rectangular profile. It was found that large and small beads should produce approximately the same peak strain-rate. The spherical beads would produce high strain-rates regions at their apex and to a lesser degree on their sides. It was also shown that regions of reduced strain-rates would be created on its upstream and downstream faces.

The next step in this investigation was to compare the history of selected streamlines. Figure 4.6 presents a comparison of the strain-rate experienced by a particle traveling along a streamline that passes at 1 \mu m from the top surface of the micro-bead as a function of distance travelled. The strain-rates for beads of diameter 2 \mu m and 15 \mu m are shown.

Figure 4.6 shows that the flow first decelerates as it approaches the bead which is related to the fluid being diverted by the bead, this results in a flow stagnation region. The flow then changes of direction, accelerates and increases on the strain-rates until a peak at
the apex of the bead. The flow then decelerates on the downstream side of the bead again reaching a stagnation point shortly after the peak strain-rate is experienced. This occurs on a region of 40\(\mu m\).

For the 15\(\mu m\) bead the particle at the capillary wall experiences a strain-rate of approximately 9000\(s^{-1}\) before encountering the bead. The strain-rate then drops below 2800\(s^{-1}\) before rising abruptly to approximately 29000\(s^{-1}\) and then dropping again to approximately 2800\(s^{-1}\) within 10\(\mu m\) of experiencing the peak strain-rate. The streamline then rejoins the flow at 9000\(s^{-1}\) within about 10\(\mu m\) of the center of the bead. For the 2\(\mu m\) bead, the changes are far more rapid with distance.

The minimum strain-rates experienced in the stagnation points before and after the bead are at approximately the same values as the 15\(\mu m\) bead. The peak strain-rates are somewhat lower, which is at first unexpected, considering the predictions of Table 4.1 and Figure 4.4. However through examination of Figure 4.4, the reduced peak strain-rates predicted for the smaller bead are probably limited by the choice of streamlines within 1\(\mu m\) of the surface. For the smaller bead this is an entire bead diameter away from the flow disturbance and the local increase in strain-rates will have reduced significantly. For the larger bead, the 1\(\mu m\) distance from the surface is far less significant. This factor may be important when drawing conclusions about the impact on strain-rates history on platelet aggregation for different sized beads.

Reviewing the in-vitro experiments depicted in Figure 2.4, it is evident that both large and small beads are capable of inducing a dynamic discoid platelet aggregation response at the downstream face of the bead. The location and size of the aggregate corresponds well with the stagnation region shadow on the downstream side of the bead where the strain-rates are lower than for the surrounding regions on the capillary wall. This is less obvious for the smaller beads which may be explained as the aggregate itself is larger than the bead and thus will dominate the local fluid dynamics.

Figure 4.7 presents the strain-rates history of particles flowing along the same streamline trajectories as in Figure 4.6, but with strain-rates experienced plotted as a function of time. The differences between the time scales over which the strain-rates are experienced for the 15\(\mu m\) and 2\(\mu m\) beads is now far less pronounced. This can be explained as the acceleration for a particle 1\(\mu m\) from the surface of the 15\(\mu m\) bead is far greater than for a particle the same distance from a 2\(\mu m\) bead. Thus the particle traverses the larger distance across the surface of the larger bead more quickly.

It is also worth noting that the particles remain in the low strain-rates region for a period on the order of milliseconds. Particles interacting with the 15\(\mu m\) bead remain in the stagnation zone for more than 3 milliseconds while particles interaction with the 2\(\mu m\) bead are exposed to low strain-rates for approximately 0.5 milliseconds. The time...
spent in the low strain-rates shadow of the bead is clearly dependent on bead size but the magnitude of the reduced strain-rates in the stagnation zones is independent of bead size.

It should be noted that once platelets aggregate at the back of the bead they will become important factors that will alter the flow, since they are adhering to the sphere wall and hence changing the sphere geometry. Ongoing platelet recruitment drives the propagation of the thrombus in the downstream of the bead, which may in turn amplify the strain-rate distribution and promote further platelet aggregation. This effect has been ignored in the current analysis, however the nonlinear fluid dynamics of platelet aggregation would provide a very rich avenue for future investigation.

![Image of fluid strain-rates following a streamline over length around a 15μm and 2μm microsphere.](image)

**Figure 4.6:** Fluid strain-rates following a streamline over length around a 15μm and 2μm microsphere.
4.4.5 Recirculation test

Despite the fact that both the analytic theory of Section 4.3 and the computational fluid dynamic results of Section 4.4 predicted no flow separation or recirculation for the Reynolds number of the experiments and simulations, the findings of the biological experiments in Section 2.4 showed platelet aggregation at the downstream face of the bead, where flow recirculation could be observed for much larger Reynolds numbers. Therefore it was deemed important to investigate the conditions under which flow separation could be observed at the back face of the bead and ensure that both the experimental and computational conditions were not in this regime so that recirculation could be ruled out as a cause of the observed aggregation [20]. It is worth mentioning that the analytical Stokes flow solution can not give rise to flow separation. Therefore the recirculation test was performed just on the base of the bead attached to the wall using computational fluid dynamics.

In order to test the mesh capabilities of flow recirculation, different flow rates were imposed. Figure 4.8a) shows the velocity vectors at the downstream face of the bead for the Reynolds number used in the experiment (Re=0.86), Figure 4.8b) and Figure 4.8c) shows the same velocity vectors using flow rates increased by a factor of 10 (Re = 8.6),
and 100 (Re = 86) respectively. It can be observed that flow recirculation start to appear at Re= 86 which is consistent with the theory [111]. This test corroborates that at least for a homogeneous fluid recirculation would only be expected at the downstream face of the bead for Reynolds approximately 100 times that used. It can be concluded that the platelet aggregates at the downstream faces of the beads are due to some other biophysical phenomena other than flow separation.

![Figure 4.8: Recirculation test for a 9µm micro-sphere.](image)

**4.4.6 Comments on blood cell concentration profile**

It has been reported that under physiological conditions, in the presence of red blood cells, platelets concentrate in a region close to capillary walls, for examples of these it is recommended to read the following articles: [92, 93, 94, 95, 96]. This non-homogeneity of the fluid composition suggests experiments were not consistent in comparing the effects of single mechanical variables on platelet aggregation. By changing the bead diameter, the region where the maximum strain-rate is generated is potentially exposed to a different platelet concentration according to the bead diameter. Hence a larger bead is potentially exposed to less platelet concentration than a smaller bead. This effect of non-homogeneous platelet concentration across the channel cross-section has been neglected for the current investigation, but will be the subject of future investigations.

**4.5 Flow around a sphere: Comparison of the analytical and numerical solution**

A parallel step during the numerical solution was to compare the values calculated with the analytical result, for validation purposes. The values of drag force and torque are not relevant to the platelet aggregation phenomena for the present study, but are quantities
of common use in fluid mechanics calculations and useful to compare the methods used in previous sections. Therefore in this Section the values of drag force and torque obtained from CFD are presented and will be compared with the values calculated from the analytical solution presented in Table 4.1.

### 4.5.1 Drag force and Torque value comparison

Table 4.2 presents a summary of the numerical results. The strain-rates were taken from a plane at the middle of the sphere parallel to the surface of the capillary, where the values were obtained from the analytical solution. Due to the condition of axisymmetry, these values should be closer to the numerical values than the numerical values.

Table 4.2: Strain-rates, drag force and torque obtained from the numerical solution for spherical beads of various diameters.

<table>
<thead>
<tr>
<th>Sphere diameter $[\mu m]$</th>
<th>CFD Strain-rates $[s^{-1}]$</th>
<th>Drag Force CFD $[nN]$</th>
<th>Torque $[nN\mu m]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>27500</td>
<td>0.3979</td>
<td>0.271</td>
</tr>
<tr>
<td>5</td>
<td>27000</td>
<td>2.402</td>
<td>4.08</td>
</tr>
<tr>
<td>9</td>
<td>26800</td>
<td>7.658</td>
<td>23.24</td>
</tr>
<tr>
<td>15</td>
<td>26000</td>
<td>20.73</td>
<td>102.6</td>
</tr>
</tbody>
</table>

It can be observed that for the location where the strain-rates were calculated (a plane at the middle of the sphere parallel to the surface of the capillary) falls within the same range of the analytical solution, however as it was presented in Section 4.4.3 the maximum strain-rate is located at the top of the bead and has a different magnitude.

### 4.5.2 Error Drag force and Torque

Table 4.3 presents the calculated error for the analytical solution presented in Table 4.1 and the CFD results presented in Table 4.2 for the drag force and torque calculation. It can be observed that in terms of the drag force, the Stokes force calculated with Equation 4.11 shows a considerable difference with the CFD. Case contrary is the value obtained using O’Neill’s [109] solution (Equation 4.12), where the values obtained using CFD are closer. From this point the next step was to compare the torque obtained using O’Neill’s [109] solution (Equation 4.13) and CFD, it is shown in Table 4.1 that the values are in good agreement.

As a summary it can be concluded from Table 4.1 that the numerical solution applied is in
Table 4.3: Comparison of the analytical solution and CFD for drag force and torque in terms of error.

<table>
<thead>
<tr>
<th>Sphere diameter [μm]</th>
<th>Drag Force Stokes</th>
<th>Drag Force O’Neill</th>
<th>Torque</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>81.35%</td>
<td>6.68%</td>
<td>1.77%</td>
</tr>
<tr>
<td>5</td>
<td>78.73%</td>
<td>5.13%</td>
<td>3.36%</td>
</tr>
<tr>
<td>9</td>
<td>76.29%</td>
<td>3.73%</td>
<td>5.54%</td>
</tr>
<tr>
<td>15</td>
<td>79.92%</td>
<td>5.84%</td>
<td>5.65%</td>
</tr>
</tbody>
</table>

good agreement with the analytical solution even without a grid independent check, that would be of benefit to study the error calculated.

4.6 Conclusion

This chapter focussed on haemodynamics characterisation of an in-vitro experiment where blood flowed through a simple rectangular capillary with spherical micro-beads attached to the walls. Similar to the in-vivo experiments of Chapter 2, the chemical initiators of aggregation were suppressed, yet aggregation was still observed. The simplicity of this experimental platform enabled the application of fundamental fluid mechanics to understand the fluidic behavior and gain insight into the more important environmental conditions that would be experienced by platelets flowing across the surface of the beads. It was shown that the disturbance that the micro-beads produce in the flow close to the surface of the bead is a sudden increase and decrease in the strain-rates. It was demonstrated using theory and simulations that the peak strain-rates produced at the apex of the bead is similar in magnitude regardless of the diameter of the bead, however aggregation was not observed uniformly for all bead diameters. This clearly suggests that it is not only high strain-rate that are required to initiate platelet aggregation. Importantly analysis of the fluid dynamics indicated that the larger beads which exhibited more consistent aggregation produced a prolonged spatial and temporal stagnation zone of low strain-rates downstream of the bead. It is in this region where platelet adhesion and aggregation is observed. It was also shown that the observed aggregation cannot be attributed to flow separation and recirculation as flow rates would need to be nearly 100 times those used in both experiment and theory before recirculation would be expected.

The simplicity of this in-vitro model is also a weakness. Only one variable can be adjusted which is the bead diameter. While changing bead diameter does not fundamentally
change the peak shear observed at the surface of the bead, the streamlines $1 \mu m$ from the surface of the bead do experience significant differences in peak shear and thus it is difficult to examine the effect of acceleration and deceleration in isolation from peak shear for real platelets with dimensions on the order of $1 \mu m$.

By performing analytical and numerical calculations it was demonstrated that although changing bead diameter does not fundamentally change the peak strain-rate observed at the top surface of the bead, by comparing different beads, the location of the peak strain-rate from the bottom surface of the capillary, for different beads is different. Another difference is that the peak strain-rate produced by a larger bead is situated in a higher velocity zone compared with a smaller bead. Therefore platelets may experience the peak strain-rate but different drag forces depending on the bead size.

On the other hand using the spherical geometry of the bead makes it difficult to control the streamlines that platelets $1 \mu m$ may follow in close proximity to the bead meaning significant differences in peak strain-rates may be experienced for equivalent trajectories from the capillary perspective. Thus it is difficult to examine the effect of acceleration and deceleration in isolation from peak strain-rates for platelets with dimensions on the order of $1 \mu m$.

Finally it has been reported that in the presence of red blood cells, platelets concentrate in a region close to capillary walls, this non-homogeneity of the fluid composition produce that the experiment is not consistent in comparing the effects of single mechanical variables on platelet aggregation. By just changing the diameter, the region where the maximum strain-rate is generated is potentially exposed to a different platelet concentration according to the bead diameter. A larger bead is potentially exposed to less platelet concentration than a smaller bead.

Based on the findings presented in this chapter it was identified that a more defined and controllable approach was required to examine the haemodynamics parameters underlying discoid platelet aggregation processes. More precise control of the magnitude, duration, localization of the peak strain-rate and also the rate and duration of the acceleration and deceleration before and after this peak strain-rate is required to accurately map the effects of local haemodynamics to platelet aggregation. Such precise control would enable strong conclusions to be drawn about the relative roles of the peak strain-rate and the local haemodynamics prior to and after this peak strain-rate in initiating and promoting aggregation. Thus a more sophisticated micro-fluidic platform is proposed in Chapter 5.
Chapter 5

In-vitro disturbance: micro-contractions in capillary channels

5.1 Contributions on this chapter

I designed several high resolution masks to perform a versatile micro-fabrication process to test blood under several scenarios of micro-contractions. I fabricated the moulds used for the polymer casting process of the device. I tested and implemented ways to fabricate the reservoirs of the chip. I implemented a process to eliminate debris from the fabrication process that could clog the micro-contraction. I designed and implemented several debris traps. post-processing and analysis of \( \mu \)Particle Image Velocimetry experiments. I performed the blood perfusion experiments and analysis of blood perfusion experiments.

The content of this Chapter has been published in the Journal Lab on a Chip, was selected for the back cover and ranked as a hot article for the Journal during 2010. It is presented here with small modifications.


5.2 Introduction

Chapter 3 and Chapter 4 have identified that a sudden increase followed by a sudden decrease in the strain-rates that the blood is experiencing in the length scale of micrometers
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represent a disturbance where an accumulation of platelets was observed even with the chemical mechanisms of aggregation blocked.

The investigation on Chapter 3 suggested that not only the peak strain-rates experienced by platelets is determinant in platelet response but also the strain-rates history since platelets can also be exposed to different accelerations and decelerations due to the local geometry of the stenosis. As in-vivo parameters are highly complex and difficult to control it was not possible to design an in-vivo experiment to demonstrate the importance of strain-rate history in platelet response. On the other hand in Chapter 4 it was presented that a synthetic device modified with a micro-size protrusion was able to generate a similar strain-rate history that also trigger thrombus formation. The limitation of only being able to change the bead diameter produced that some inconsistencies when comparing beads of different diameters including the inability of choosing an equivalent streamline from the same point reference, therefore it is difficult to examine the effect of acceleration and deceleration in isolation from peak strain-rates.

Such limitations serve as a motivation to develop a platform using micro-technologies to have precise control of the magnitude and duration of the peak strain-rates and also the rate and duration of the acceleration and deceleration before and after this peak strain-rate. In this Chapter, by using current micro-fabrication technologies, a way of combine the previous methods on a mimetic proof of concept platform to emulate and control the haemodynamics conditions that promote platelet aggregation will be presented.

Progress in micro-technologies has attracted the attention of researchers in several areas including haemodynamics and thrombosis. Current applications of micro-devices include lab on a chip applications for clinical diagnostic but also tools for research to better understand biophysical phenomena of blood flow. Although Lab-on-a-chip based devices have been applied to the study of platelet adhesion and aggregation in the past, these methods have either consisted of constant laminar flow systems (constant strain-rates) incorporating immobilized adhesive proteins such as purified von Willebrand factor (VWF), or chemically driven aggregation models involving endpoint biochemical analysis of total platelet-adhesion or aggregate mass [112, 113, 114, 115, 25]. Other microfluidics devices have been applied to study cellular responses to mechanical forces. A device fabricated by You [116] was used to study the mechanical stimulation of osteocyte processes. Schaff[117] utilized a chip that provides a vascular mimetic environment of constant shear stress to study the the leukocyte-endothelial inflammatory response. Rossie[118] fabricated a device that consisted of tapered channels to study the response at sub-cellular level of endothelial cells to shear flow, characterised with CFD and micro-Particle Image Velocimetry (µPIV). Park[119] presented a method to study the generation of chemical concentration and mechanical shear stress gradients in a single microfluidics chip using
an osmotic pump that produces very slow (a few μm/s) and controlled flow, allowing a wide and stable diffusion of specific chemical concentration. Chau[120] fabricated a device that provides an array of different channels that enable the study of cell mechanics under 10 different constant shear stresses ranging over two orders of magnitude (0.07-13 Pa) simultaneously. Wang[121] presented a method capable of generating variable magnitudes, gradients, and different modes of shear flow, to study sensory and force transduction mechanisms in cells. The chip fabricated allowed the study cell culture under spatially resolved shear flow conditions as well as study of cell response to shear flow in real-time. The chip was used to study the effects of shear on cellular functions of Madin-Darby Canine Kidney (MDCK), renal epithelial cells. Shao[122] utilized an integrated micro-pump in a chip to study the response of an endothelial cells culture exposed to a pulsatile and oscillatory shear stress.

The device presented in this Chapter utilizes angular micro-contractions that mimic the haemodynamics conditions of the stenosis found in micro-vessels, producing a platform that offers several advantages over these conventional systems, including; dynamic differential strain-rate control, the ability to achieve discrete periods of highly elevated strain-rate with minimal sample volume, and the ability to precisely control the spatial location and extent of forming aggregates. The latter, in conjunction with the application of state-of-the-art micro-imaging and spectroscopy based techniques, allows for the real-time monitoring of platelet functional responses as opposed to more traditional end-point analyses of aggregate size. This device has been instrumental to replicate under controlled conditions, important in-vivo observations that leaded to the biological discovery of a new mechanism of platelet aggregation that occurs independent of the commonly accepted soluble agonists mediated pathways[1], therefore it has been used as a supporting evidence as well.

This Chapter is organized as follows. Section 5.3 presents the device design and the micro-fabrication methods used. Section 5.4 presents details of the numerical calculation of the haemodynamics in straight sections and in the micro-contractions. Section 5.4 presents the experimental characterisation using μParticle Image Velocimetry. Section 5.5 presents a new proof-of-concept blood perfusion experiments that illustrate the versatility of the device in addressing the basic haemodynamics parameters driving platelet aggregation. A summary of the results and conclusions is presented in Section 5.6.
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5.3 Device Design and Fabrication

5.3.1 Device Design

As it was presented on Chapter 3, the variables of an in-vivo stenosis that impact in the local haemodynamics are in function of the vessel wall elasticity and needle diameter. Figure 5.1 presents again the variables of an in-vivo stenosis. By using micro-fabrication technologies it is possible fabricate micro-channels with accurately and independent control of these variables on the micron-scale. Because of the fabrication technique the micro-channels do not have a cylindrical profile but rectangular.

\[ \text{\% Stenosis \_Area} = \frac{\text{Ar} - \text{Ao}}{\text{Ao}} \times 100 \]

\[ \text{\% Stenosis \_linear} = \frac{\text{lo - lr}}{\text{lo}} \times 100 \]

Figure 5.1: Variables defining a stenosis.

Figure 5.2 illustrates the overall design of the device, comprising a top polydimethylsiloxane (PDMS) chip consisting of: [A] an inlet reservoir of approx. 200\(\mu\)L volume (8 mm diameter) for blood delivery, [B] an upstream trap to prevent fouling of the micro-channels by particulate matter and/or micro-clots that may have formed in the blood sample due to inadequate anticoagulation, [C] a feeder channel connecting the trap zone with the micro-contraction, [D] three replicate high resolution micro-channels with the same defined strain rate micro-gradient geometry, [E & F] a small bore (1.0 mm) exhaust channel and a stainless steel outlet connection [F] attached to 10 cm of medical grade Tygon tubing (0.8 mm I.D/ 1.6 mm O.D) and a Harvard PHD-2000 syringe driver. The PDMS chip was surface adhered to a 65×22 mm borosilicate #1 microscope cover-glass that formed the 0.15 mm thick bottom wall of the micro-channel and allowed for both transmitted light and epi-fluorescence imaging at the micro-contraction geometries. Three symmetric micro-channel design cases were chosen from all possible cases for the proof-of-concept study (Figure 5.2b). These three cases comprised a fixed stenosis of 80%
Figure 5.2: Proof of concept device design. (a) Schematics detailing the main components of the strain rate micro-gradient device (PDMS chip). [A] 200 µL inlet reservoir for blood delivery. [B] Trap to prevent micro-channel fouling by particulate matter and/or micro-clots in the blood sample due to inadequate anticoagulation. [C] Feeder channel connecting the trap zone with the micro-contraction, [D] three replicate high resolution micro-channels with the same defined strain rate micro-gradient geometry, [E] exhaust channel and [F] outlet connection to the syringe pump. (b) Schematic showing three proof of concept micro-geometries selected for this study and an overview of the contraction dimensions within the device; θc= contraction angle at the step wall; θe= expansion angle at the step wall.
(area), with different contraction/expansion angles of 30°, 60° and 90°. In all cases the input and output channel widths were set to 100µm. The contraction angle \( \theta_c \) corresponds to the region of acceleration, the gap distance \( [g] \) corresponds to the region of maximum strain rate and the expansion angle \( \theta_e \) corresponds to the zone of deceleration. For this trial study the length of the contraction was set to 15µm, and the gap distance was set to 20µm (Figure 5.2b). Based on this nomenclature the three cases utilized in the current study were defined as: symmetric 30°: c30g20e30, symmetric 60°: c60g20e60 and symmetric 90°: c90g20e90. Overall the contraction/expansion angles, degree of stenosis and input flow rates were chosen to mimic as closely as possible the in-vivo case under conditions of externally applied stenosis.

5.3.2 Device fabrication

The biomimetic micro-channels were fabricated in (PDMS Sylgard) cast from a patterned mould realized using standard photo-lithography techniques. A high resolution chrome mask was used in order to achieve well defined features and straight side walls. A 4 inch silicon wafer was spin coated with KMPR 1025 (MicroChem Corp.) photo-resist using a spread cycle of 300 rpm and 100 rpm/s for 10 seconds and a development cycle of 1000 rpm and 300rpm/s for 30 seconds, in order to achieve a film of 100µm thickness with good uniformity. A cycle of edge bead removal was conducted for 30 seconds using edge bead removal solvent. The KMPR coated wafer was soft-baked by ramping the temperature at 6°C/min starting from 23°C and holding at 10°C for 4 minutes to dry out the solvents. The KMPR film was exposed with a mask pattern for 2 minutes of UV on a MJB3 contact mask aligner with a wavelength of 360 nm and a power of 8 mW/cm2, using 2 exposures of 1 minute each in order to avoid over heating the substrate. After exposure the patterns were cross linked by baking on a hot-plate for 4 minutes at 10°C, ramping the temperature at 6°C/min starting from 23°C. The exposed and cross linked film was cooled down slowly to room temperature with the sample on the hot-plate to avoid thermal stress on the film and possible cracks due to sudden changes in temperature. The unexposed KMPR was developed for 12 minutes with periodic agitation to remove the unexposed material. After developing the KMPR pattern, the wafer was cleaned with isopropanol and distilled water (DI) and a final hard bake was done by heating the sample to 120°C for 3 hours, in order to improve and strengthen the cross-linked KMPR pattern and extend the service life. The KMPR pattern was then ready for use as a mould for casting PDMS channels. Once the mould was fabricated, PDMS and its curing agent were mixed at a ratio 10:1 and degassed for 30 minutes. The mixture was poured onto the KMPR mould previously made and contained within a polymethyl methacrylate (PMMA) shim. The PDMS was then cured in an oven at 100°C for 20 minutes. The PDMS
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channels were peeled from the KMPR mould and an 8mm inlet reservoir hole was made using a biopsy punch. For the outlet connection to the syringe pump, a 1mm biopsy punch was used. After both holes were punched, the PDMS channel was placed directly onto a 65 × 22 mm glass slide. Adhesion was achieved simply due to the low surface energy of PDMS. Figure 5.3 shows representative images of the fabrication of the device, where it can be observed the rectangular section of the channel.

![Fabrication of the device](image)

**Figure 5.3:** Fabrication of the device. (a) Representative KMPR mould on a 4 inch. silicon wafer. (b) Micro-contractions fabricated. (c) Representative cross-section of the micro-channel. A rectangle marked on dots is shown for comparison.

5.4 Haemodynamics Characterization of the device

5.4.1 Haemodynamics computations of straight section

According to published data, blood flow in capillary vessels produces strain-rates that fall between 500 to 5000 s⁻¹, with around 1800 s⁻¹ being a typical reported mean value
for small arteries/arterioles\[^{[123]}\]. As the micro-channels are designed to mimic haemodynamics in-vivo, the flow rate was adjusted to produce strain-rate close to the mean reported value. A value of 1800 \(s^{-1}\) was choose as an input value upstream of the contraction. Any particle travelling close to the mid-plane given by the thickness of the photo resist dimension will experience a strain-rate with in this range. In order to compute the relationship between the nominal strain-rate (1800 \(s^{-1}\)) with the geometrical variables of the channel (width and height) the analytical solution of the velocity profile for a Newtonian fluid in rectangular geometries\[^{[108]}\] was used:

\[
V_x(y, z) = \frac{12Q}{ab\pi^3} \sum_{i=1,3,\ldots}^{-1} \frac{1-i}{i} \cos\left(\frac{i\pi y}{2a}\right) \left(1 - \frac{\cosh\frac{i\pi z}{2a}}{\cosh\frac{i\pi b}{2a}}\right) \left(1 - \frac{\cosh\frac{i\pi y}{2a}}{\cosh\frac{i\pi b}{2a}}\right) \frac{\tan\frac{\pi b}{2a}}{i^3} (5.1)
\]

In Equation 5.1, \(Q\) is the flow rate, \(a\) is half the channel height which was fixed at \(a = 100\mu m\), and \(b\) is half the channel width which is adjustable during the fabrication process (the width of the channel was given by the thickness of the photo resist); the direction of the flow is in the \(x\) direction and the plane where the micro-geometries are allocated is \(xy\). Once the dimensions were known the flow rate was adjusted to reach the target strain-rate (1800 \(s^{-1}\)). The laminar shear-rate is obtained once the velocity is calculated by taking a discrete derivative near the wall. Equation 5.1 is useful for geometries of constant cross section that produce constant shear-rate. However to compute the haemodynamics of geometries of variable dimension (contraction and expansion zones of the micro-channels), it was necessary to solve over a number of discrete cells that represent the volume occupied by the fluid, the continuity and momentum equations Navier-Stokes Equations 3.1 and 3.2, described in Section 3.4.2.

The geometries of the micro-channels were defined using Gambit 2.2 (Fluent USA, Lebanon, NH) and discretized using a mixed mesh. Once the discretized volume was built, a finite volume scheme using the commercial software (FLUENT 6.0, Fluent USA, Lebanon, NH) was used in order to solve the Navier-Stokes equations numerically. The fluid medium was considered homogeneous with a constant density \(\rho = 998.2 \frac{kg}{m^3}\) and constant viscosity of \(\nu = 0.00348 \text{Pas}\). Blood can be approximated as Newtonian fluid at mid and high strain-rates\[^{[75]}\]. The pressure discretization scheme was set as standard and the second order upwind momentum option was enabled. The boundary condition at the walls was set as zero velocity to enforce the no slip condition. As the micro geometries were symmetric in the \(xy\) plane, a symmetric condition was implemented. The fully developed velocity profile given in Equation 5.1 was used as a boundary condition for the velocity inlet where the channels have a constant cross-section. This significantly improved the time of convergence of the solution and required only a minimal distance to obtain a fully developed flow; hence it was possible to use a high density of elements in the micro-contraction. Under physiological conditions, platelets flowing in blood flow
are transported to the walls due to lateral displacement by high density erythrocytes [93, 94, 95, 96]. CFD simulations were used to predict steady-state models of streamline trajectories within 1µm (equivalent to 1/2 discoid platelet diameter) from the step wall and located at the center of the width of the channel (middle plane of the dimension given by the thickness of the photo-resist mould).

### 5.4.2 Haemodynamics computation of micro-contractions

In order to controllably investigate the effects of the acceleration rate, peak strain-rate and deceleration rate identified as principle geometric parameters from the in-vivo modeling experiments on platelet functional behavior presented in Chapter 3 a proof-of-concept micro-channel device was developed composed of a series of side wall geometries that reflect idealized stages of vessel wall deformation in the in-vivo setting and a series of more synthetic cases. Figure 5.2c) describes the three symmetric micro-channel design cases chosen from all possible cases for the proof-of-concept study. Numerical (CFD) simulations were carried out to predict the velocity field, strain-rate distribution produced, and to study particle behavior within selected streamlines of blood flow within the device. Figures 5.4a) and 5.4b) present comparative strain-rate distributions (upstream of contraction) for the model blood vessel and the c60g20e60 micro-channel case, respectively. Note that in the blood vessel case an axisymetric/homogeneous shear-rate distribution is predicted, however due to the rectangular geometry and low aspect ratio (width/height = 1.3) of the micro-channel case, the shear-rate follows a parabolic distribution along the side walls, with the maximum at the center of the walls and the lowest values at the corners as shown in (Figure 5.4b). However, the strain-rate is fairly uniform for a range of heights from a height of about 30µm above the channel floor (glass cover-slide) to the maximum at half the channel height (at 65µm), as indicated in Figure 5.4b). In this range platelets will experience approximately uniform strain-rates ranging between 1,500-1,960 s\(^{-1}\), falling well within the nominal physiological range reported for mesenteric arteries and arterioles[123]. A more homogeneous distribution of shear-rates across the channel could be achieved by increasing the aspect ratio of the channel (either increasing the width given by the designed mask or increasing the height, given by the thickness of the photo-resist), however, this could affect the hydraulic diameter (affecting the Reynolds number at the contraction and the exposure time of the platelets to the strain-rate gradient). In this investigation it was of interest to keep the lowest possible Reynolds number at the contraction, with a similar residence time, to model a high strain-rate zone with similar inertia effects to the in-vivo case reviewed in Chapter 3 (Reynolds at the in-vivo experiment for a 100µm arteriole Re=0.53 and in the micro-channel Re=0.67).
Figure 5.4: Computed strain-rate distributions in the mesenteric arteriole and the c60g20e60 vascular mimetic micro-channel. (a) Computed strain-rate distribution for blood flow in the mouse mesenteric arteriole (42µm) upstream of stenosis (side-wall compression). Note due to viscous effects and the cylindrical geometry, a uniform strain-rate at the wall is produced by the fluid flow. (b) Computed strain-rate distribution color map for blood flow in the c60g20e60 vascular mimetic upstream of the defined contraction geometry. Note due to the rectangular channel geometry and low aspect ratio the flow inside the micro channel produces a parabolic distribution along the walls, with strain-rate maxima at the center and minima at the corner edges. A plane located at 30µm from the cover-slip was chosen for all imaging experiments such that fluid and particles at this location experience strain-rates in the order of 1700 s⁻¹, with maxima at the 65µm mid-plane exhibiting strain-rates approaching 1960 s⁻¹. (c) Computed strain-rate distribution color map for blood flow in the mouse mesenteric arteriole at a stenosis of 80% area. The geometry of the blood vessel in the contraction zone is imposed by the combination of the shape of the blunted needle and elastic effects of the vessel side-wall. An irregular surface topography is produced which creates a heterogeneous strain-rate distribution in 3-dimensions, with two peaks of 44600⁻¹ that rapidly decrease approaching the expansion zone. (d) Computed strain-rate distribution color map for blood flow in the c60g20e60 vascular mimetic at the defined contraction geometry. Note that in the vascular mimetic a bigger aspect ratio is produced resulting in a more homogeneous strain-rate distribution. The streamlines shown represent the computed trajectories for particles traveling at 1µm (1/2 platelet diameter) from the micro-channel wall; note that at this distance, a maxima of 41200⁻¹ is generated, although higher strain-rates may be experienced at the wall, where flow velocity is zero.
Figures 5.4c) and 5.4d) present the computed results for the strain-rate distributions within the contraction zone for the model arteriole (80% stenosis) and c60g20e60 micro-channel, respectively. Note that in the vessel case, the non-uniform nature of the micro-needle compression results in an uneven side-wall topography producing an irregular distribution of strain-rate, with two local regions of high shear (44,600 s$^{-1}$) positioned at the upstream and downstream edges of the contraction zone as shown in Figure 5.4c). In contrast, a key advantage of the synthetic c60g20e60 micro-channel case is that the geometric shape of the contraction is uniform (with a larger aspect ratio) resulting in a more homogeneous strain-rate distribution as shown in Figure 5.4d). Furthermore, Figure 5.4 demonstrates, that for flow streamlines at 1µm from the c60g20e60 micro-channel wall, platelets will experience a predicted peak strain-rate at the center of the contraction geometry of 41,200 s$^{-1}$ that closely approximates the blood vessel case. Overall the simulations suggest that the c60g20e60 micro channel case represents a good idealized approximation of the haemodynamics conditions generated within the in-vivo model of Chapter 3.

A principle aim of the device design concept was the ability to introduce precisely controlled changes in key haemodynamics variables and explore the subsequent effects on platelet aggregation dynamics. Figure 5.5a) presents comparative strain-rate contour plots for the three micro-channel geometries selected for this study that comprise symmetric changes in contraction and expansion geometry with constant stenosis (80% area). This analysis demonstrates that overall modification of the contraction and expansion angles has a significant effect on the overall strain-rate distribution in the flow. In order to gain more insight into the effect of the changing geometry on the strain-rate history of single platelets travelling through the contraction zone the particle (platelet) flow streamlines within 1µm ($\frac{1}{2}$ platelet diameter) of the step-wall over both time and length were modelled as a function of contraction and expansion angle. Figure 5.5b) and c) present the strain-rate calculation for angles of 30°, 60°, and 90° as a function of time and space respectively. Significantly, all three cases follow temporal and magnitude changes in strain-rate that conform closely to the in-vivo vessel analysis of strain-rate history for the 80% stenosis case. The key characteristics are that platelets flow in from the constant (straight) upstream segment (1500s$^{-1}$ - 1900s$^{-1}$), experience an acceleration through the contraction segments lasting a few milliseconds and rapidly reach a zone of peak strain-rate approaching 41,000s$^{-1}$ (Figure 5.5b).
Figure 5.5: haemodynamics performance of the device. (a) Contour plots of the predicted strain-rate distributions for the c30g20e30, c60g20e60 and c90g20e90 vascular mimetic. Note that the reduction of the hydraulic area produces a progressive increase/decrease of the deformation rates of the fluid, going from zones of dark blue (lowest values) to zones of red (highest values), however, the rate of the progressive increase/decrease is different for each geometry. (b) CFD plots demonstrating the predicted strain-rate history experienced by a model platelet traveling at $1\mu m$ from the step wall for the three designated micro-channel geometries as a function of time. (c) CFD plots demonstrating the predicted strain-rate history experienced by a model platelet traveling at $1\mu m$ from the step wall for the three designated micro-channel geometries as a function distance. The $0\mu m$ reference point is located at the mid-line of the defined contraction geometries. (d) CFD plots showing a comparison of the strain-rate gradient experienced at $10\mu m$ and $30\mu m$ from the mid-line of the defined contraction geometries following a streamline $1\mu m$ from the step wall.

A key haemodynamics variable that can be controlled by changing the expansion angles through in these proof-of-concept geometries was the overall deceleration rate experienced by platelets that initially tether within the contraction zone. Figure 5.5c), shows an analysis of the effect of expansion angle on the strain-rate deceleration experienced by a platelet as it transitions into the expansions for the three micro-channel cases. Examination of the instantaneous values of strain-rate experienced by a platelet $1\mu m$ from the step-wall at $10\mu m$ and $30\mu m$ from the center of the contraction zone (peak phase) demon-
strates that a platelet experiences significant differences in the magnitude of strain-rate
deceleration as a function of the three angles over an equivalent distance, such that; a $\theta_e = 30^\circ$ results in a 35% ($41,000 - 28,000s^{-1}$) reduction, a $\theta_e = 60^\circ$ results in a 46% reduction ($41,000 - 22,000s^{-1}$), and a $\theta_e = 90^\circ$ results in a 65% reduction ($41,000 - 14,400s^{-1}$) in strain-rate over the first $10\mu m$ of the expansion zone (Figure 5.5). Taken together, the
CFD analyses of strain-rate distributions and single platelet trajectories demonstrates the
overall ability of the device design to precisely and independently control and signifi-
cantly modify discrete aspects of the strain-rate micro-gradient imposed on flowing blood
and to generate haemodynamics conditions that represent idealized and more synthetic
versions of the blood flow dynamics generated in the published in-vivo stenosis model of platelet aggregation [1].

5.4.3 Experimental Characterisation of the device

In order to be confident that the predictions from CFD would be an accurate representa-
tion of the real fluid dynamics observed in the practically realized micro-contractions, it
was necessary to characterise the flow within the fabricated device directly using micro
resolution particle image velocimetry ($\mu$PIV). Due to technical limitations of the $\mu$PIV apparatus a low velocity (0.08 $\mu$L/min) approach was used and the findings compared to low velocity CFD predictions and extrapolated to the high velocity scenario utilized in the blood flow studies. This assumption was taken to be valid as at no point in the blood flow experiments were regions of flow separation or recirculation observed. Thus for the CFD simulations, the distribution of the velocity field and strain-rates at low and high flow rates
were assumed to be the same, differing only in magnitude. A constant displacement rate
syringe pump (Harvard Apparatus PHD-2000) was used to impose the flow rate, using a
25 $\mu$L Hamilton Gastight syringe. Before connecting the syringe into the chip, care was
taken to purge all the air in the fluidic line in order to have more control on the small
amount of volume displaced.

$\mu$PIV Set up

Figure 5.6 presents a photograph of the micro-fluidic chip mounted in the $\mu$PIV system.
The low velocity $\mu$PIV system was composed of a back-lit light emitting diode configu-
ration (LED 525 nm) flasher (LaVision GmbH, Gottingen) exciting $1\mu m$ diameter tracer
particles (Duke Scientific Corp) in deionized water as the working fluid. A CCD camera
(LaVision Systems) of $1376 \times 1024$ pixel resolution connected to an inverted microscope
(Nikon TE2000-U) with $60 \times$ (NA = 1.25) was configured to capture 200 image pairs from
the particle flow streamlines. The viscosity of the bead suspension was $\mu = 0.94 \times 10^3 Pas$
and the density was $\rho = 998.2 \text{ kg/m}^3$. The flow rate range was determined by the limitations of the CCD frame rate and available light budget with the maximum measurable range of velocities between $Q = 0.01-1 \text{ µL/min}$. An objective lens of and 60× (NA =1.25 oil immersed) was used to acquire the images in specific xy planes.

Compared to a standard PIV laser system, the LED system has several advantages including small size, adaptability to different fluorescent dyes (due the range of wavelength available), incoherent light (no speckle or interference phenomena), freely adjustable pulse length and repetition rate, low energy consumption and very low cost. The main disadvantages are the lower light intensity, the broad wavelength spectrum and a broad spatial radiation pattern. Because of the back-light configuration, the correlation in the $\mu$PIV was done using the shadows of the particles, hence the fluorescent property of the particles is not needed for the velocimetry measurements, however the fluorescence was useful to find the channel and focus the particles since the pathway of the light of the inverted microscope was blocked by the LED flasher, which should be positioned on the micro-fluidic chip as shown in Figure 5.6.

![Figure 5.6: Experimental set up of the $\mu$PIV. Observe that the led flasher needs to be situated exactly over the sample.](image)

The LED was powered and controlled by a PIV timing system (Lavision Systems). The minimum pulse length of the LED corresponds to the minimum trigger pulse length and an additional fall time, this resulted in a minimal pulse of 8 ms. Image acquisition was performed on a PC with Davis Software (Lavision Systems). A minimum of 200 image pairs were recorded. These images were processed and ensemble averaged using
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Davis software. The final image was produced in Matlab (Mathworks Inc.).

Vector calculation

Taking into account the flow-rate and viewing the optimization of the signal-to-noise ratio, a time delay between LED flasher pulses ranging from 100 to 500 ms and a repetition rate of 4.9 Hz were used to have a maximum particles displacement of approximately 1 to 4 times of the interrogation area length. In order to improve the resolution of the measurements, a multi-pass iteration was employed which comprised the steps described as follows. Interrogation areas of 32x32 pixels with 50% overlap in both stream-wise and crosswise directions were used. This determined the initial grid size of the vector field, resulting in an initial distance between individual vectors of 16 pixels. The multi-pass iteration defined the final grid size and it was performed decreasing the windows down to $8 \times 8$ pixels. The computed vector field information in the first pass (windows of $32 \times 32$ pixels) was used as reference vector field for the next pass. Using the information of the previous pass, the position of the interrogation windows in the new pass was shifted according the determined particle image shift. This helps to correlate the correct particles and improved the signal to noise ratio. In the next pass the window size is half the size of the previous pass and the vector calculated in the first pass was used as a best-choice window shift. In this manner, the window shift is adaptively improved to compute the vectors in the following steps with more accuracy. This ensured the same particles were correlated with each other. This multi-pass with decreasing window procedure resulted in having a much smaller final interrogation window size. This improved the spatial resolution of the vector field since a smaller grid size of the vector field was achieved, and it produces less erroneous vectors.

Scaling

The time between frames was adjusted according to the minimum pulse length of the LED flasher. Even though the minimum pulse length of the LED flasher was $8 \mu s$, previous experience with the particle illumination setup suggested that $30 \mu s$ was a proper time for get a good contrast of the particle shadows with the optical set up used. Using this restriction the minimum time between frames was on the order of $100 \mu s$. Based on this limitation the flow rate was adjusted to observe congruent vectors. The pulse length of the LED needed to be adjusted to at least $200 \mu s$ since a good contrast/illumination was needed since a $60 \times$ objective was used. This resulted in a scaling factor for this setup and magnification of 240 times ($0.06 \mu L/min$ vs $16 \mu L/min$).
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Velocity Profile Prediction vs Experiment: Micro-contraction at 0.06 μL/min with 60× magnification

The next step in experimentally characterizing the platform was to compare the experimentally measured flow in the micro-contraction to that predicted by computational fluid dynamics. At the narrowest point, the micro-contraction is only 20μm wide, this is 5 times narrower than the straight channel examined in the previous section. It was thus necessary to increase the magnification from 10× to 60× and to reduce the flow rate from 4μL/min to 0.06μL/min in order to make accurate measurements with the μPIV system. Using these conditions the μPIV system was used to measure the velocity profile of the c90g20e90 micro-contraction.

Figure 5.7a) presents the measured flow velocity profile for the c90g20e90 micro-contraction with 60× magnification and a flow rate of 0.06μL/min. The velocity vectors measured using μPIV are also presented in Figure 5.7a) as arrows. Figure 5.7b) presents the same velocity profile information presented in Figure 5.7a) but with the μPIV measured velocity profile presented as a three dimensional colored surface plot and the computational fluid dynamics simulations presented as black lines at a sequence of points along the contraction. This plot allows quantitative comparison of the μPIV measured and computational fluid dynamics simulation. It is clear that the magnitude of velocity predicted at all points along the contraction are in good agreement with the μPIV measured velocities. Figure 5.7c) presents the μPIV measured velocity profile from two cross-sections along the length of the contraction. The location of these cross-sections are chosen at 8μm and 25μm from the minimum gap. The velocity profile predicted from computational fluid dynamic simulations is also presented. It is clear from this plot that the agreement between the μPIV measurement and the computational simulation is very good indeed.

The 3D vector velocity field of Figure 5.7a) was used also to calculate streamlines that fluid elements would take when flowing through the geometry. Figure 5.7d) shows the streamlines calculated from the μPIV experiments. The results of Figure 5.7 shows that the measured performance of the realized micro-contraction geometry and the performance predicted by computational fluid dynamics of the nominal geometry profile are in very good agreement. From this it can be concluded that the geometry of the realized micro-channel was very close to the nominal geometry designed and also that the computational fluid dynamics can provide an excellent prediction of the fluid dynamics that would actually be observed in such a channel.
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Figure 5.7: Experimental characterisation of the micro-contraction. a) Velocity contour plot from the measurement at the micro-contraction at the middle plane (yz). b) Velocity profile obtained using µPIV (contour mesh) and velocity profiles across the c90g20e90 micro-channel case at different sections obtained using CFD (black lines) at 0.06µL/min. The fluid flow experiences an acceleration into the contraction reaching its maximum value (red zone) and rapidly experiences a deceleration into the expansion. These rapid changes in velocity occurring in tenths of micrometers produce small regions of high deformation rates that define the strain-rate micro-gradient. c) Velocity profiles comparison in 2D at 8µm (maximum of 900 mm/s) and 25µm (maximum of 300 mm/s) from the contraction. Note the parabolic shape of the velocity profile with some degree of asymmetry due to the differences in velocity imposed by the asymmetric contraction. d) Predicted streamlines calculated from the µPIV experiments that fluid elements follow.
5.5 Micro-device performance as a platform to study platelet aggregation

Having demonstrated in Section 5.4.3 that the micro-channels can be realized precisely and provide a fluid dynamic environment very close to that predicted by computational fluid dynamics, it is now possible to explore the use of these microfluidic channels as a platform for studying platelet aggregation.

To ascertain the effectiveness of the device design to focally promote platelet aggregation and mimic the in-vivo observations a series of whole blood perfusion experiments were carried out. In order to isolate the mechanical effects of blood flow from the well accepted platelet activation effects of the soluble chemical agonists ADP, TXA2 and thrombin, all experiments were performed in the presence of pharmacological inhibitors of the canonical platelet amplification loops (Amplification Loop Blockade [ALB]).

5.5.1 Device Operation

Blood perfusion studies through the device were carried out at the Australian Centre for Blood Diseases, Monash University, using hirudin (800 U/ml) anti-coagulated whole blood, taken from consenting human donors. Approval for these studies was obtained from the Monash University Standing Committee on Ethics in Research Involving Humans. Whole blood samples were incubated at $37^\circ C$ for 10 minutes with the lipophytic membrane dye DiOC6 (1ug/ml) [Molecular Probes]. Flow in the device was induced by a Harvard PHD-2000 syringe driver connected to the outlet channel in the PDMS block using a Becton Dickinson 5ml glass syringe attached to 10cm of Tygon (0.8mm I.D.) tubing. Blood samples were introduced into the micro-channels via the 200$\mu$L reservoir cut into the PDMS block at the channel inlet (Figure 5.2). Prior to sample perfusion, degassed Tyrodes buffer (4.3 mM K2HPO4, 4.3 mM Na2HPO4, 24.3 mM NaH2PO4, 113 mM NaCl, 5.5 mM D-glucose, pH 7.2) at a temperature of $45^\circ C$ was used to prime the channels to remove any air bubbles. Platelet aggregation was monitored via epi-fluorescence illumination (Sutter DG4 Xenon arc lamp [488nm] light source) using an Olympus IX81 invert microscope with a UAPO 40× OI3/340 objective and attached Hamamatsu Orca ER CCD with an exposure time of 80ms. Image acquisition was controlled through Meta-morph 6.0 (multi-dimension acquisition). Blood flow was observed within a focal plane approximately 30 $\mu$m above the cover-slip wall of the channels to avoid side wall effects on platelet behavior (see section 5.4.2). Fluorescently labeled platelet aggregates were segmented via intensity thresholding and the threshold area determined on a frame by
frame basis in Metamorph 6.0. In the case of straight micro-channels, endpoint mean surface areas were determined from 10 random fields-of-view covering the full length of the micro-channels. Figure 5.8 shows a representative chip in operation.

5.5.2 Platelet aggregation as a function of micro-channel design

Figure 5.9 presents real time epi-fluorescence imaging of DiOC6 labeled whole blood perfusion at a constant flow rate of 16µL/min (input strain-rate = 1,800 s⁻¹) through the c60g20e60 geometric case over a 10 min time-frame, following pretreatment for 10 minute with the platelet inhibitors; apyrase (0.02 U/ml), MRS2179 (100 µM) and 2-MeSAMP (10 µM) — to block ADP; Indomethacin (10 µM) — to block thromboxane(TXA2); and hirudin (800 U/ml) — to block thrombin. Perfusion through the c60g20e60 micro-channel resulted in robust platelet aggregation that initiated specifically at the down-stream edge of the peak strain-rate (contraction) zone (Fig 5.10a; yellow arrow). Significantly, as was the case in vivo, platelet aggregation occurred progressively within the downstream strain-rate deceleration (expansion) zone resulting in the formation of a
relatively large and stable platelet aggregate as shown in Figure 5.9 and Figure 5.10a). Comparison across three independent donor samples showed tight agreement in terms of overall aggregation dynamics and time to occlusion (Figure 5.9).

**Figure 5.9:** Proof-of-concept blood flow experiments I. (a) Representative epi-fluorescence image sequence of ALB blood perfusion through the c60g20e60 micro-channel case; the yellow arrow denotes the point of initial aggregation \([t=0\text{ s}]\), the white arrow designates the direction of blood flow (scale bar=15 \(\mu\text{m}\) [representative of \(n=3\) experiments]

To investigate the platelet adhesion receptors mediating the aggregation response in the device, whole blood samples were pretreated with the anti-integrin \(\alpha_{IIb}\beta_3\) Fab c7E3 (20\(\mu\text{g/ml}\)) to block the platelet integrin \(\alpha_{IIb}\beta_3\) or the anti-GPIb IgG Alma12 (50\(\mu\text{g/ml}\)) to block platelet GPIb/V/IX engagement of vWF. Figure 5.10a) demonstrates that in the presence of either integrin or GPIb blockade, platelet aggregation within the c60g20e60 case was completely inhibited, demonstrating a critical requirement for these primary platelet adhesion receptors in the aggregation process.

This proof-of-concept data demonstrates that the device design is capable of eliciting stabilized platelet aggregation mirroring that observed in the in-vivo stenosis experiments and that the introduction of the defined strain-rate micro-gradient imposed by the c60g20e60 geometry can focally induce stabilized integrin dependent platelet aggregation in a highly controlled manner. Furthermore, this data supports the findings of Chapter 3 that imposed strain-rate micro-gradients can induce stabilized platelet aggregation independent of soluble agonists.

### 5.5.3 Platelet aggregation at steady-state strain-rate

A large number of published studies utilizing steady-state laminar shear-rate regimes have identified a direct link between elevated (pathological) shear-rate and increasing levels of platelet activation and resultant aggregation. It could be argued that the aggregation response observed in the prototype device is entirely due to the peak strain-rate generated at the apex of the c60g20e60 geometry rather than as a result of a dynamic strain-rate imposed on the blood flow. To investigate this possibility a series of control steady-state
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Figure 5.10: Proof-of-concept blood flow experiments I. (a) Representative aggregation traces showing the response of whole blood perfusion through the c60g20e60 micro-channel case; c60g20e60-hirudin-anti-coagulated whole blood treated for 10 min with the platelet amplification loop blockers (ALB); apyrase (0.02 U ml$^{-1}$), MRS2179 (100 mM) and 2-MeSAMP (10 mM)— to block ADP; indomethacin (10 mM)— to block TXA2; and hirudin (800 U ml$^{-1}$)— to block thrombin (ALB) whole blood perfusion at an input (pre-stenosis) shear rate of 1800 s$^{-1}$ (n = 3 experiments); $\alpha-\alpha$IIb$\beta_3$ Fab c7E3-ALB whole blood treated for 10 min with 20 mg ml$^{-1}$ c7E3 Fab prior to blood perfusion to block integrin $\alpha$IIb$\beta_3$ engagement (n=3 experiments); $\alpha$GPlb/V/IX IgG Alma12-ALB whole blood treated for 10 min with 50 mg ml$^{-1}$ of the glycoprotein Ib blocking IgG Alma12 (n=3 experiments). (b) Histogram showing mean aggregate size ($\mu$m$^2$) following 10 min of ALB blood perfusion at a pre-contraction strain-rate of 1800 s$^{-1}$ through control c60g20e60 microchannels versus straight microchannels at 1800, 10 000, 20 000 and 40 000 s$^{-1}$ strain-rate (n = 3 experiments).

flow experiments in straight (100 x 20$\mu$m) micro-channels as a function of increasing strain-rate were conducted. Perfusion of ALB whole blood at steady-state strain-rates of 1,800 s$^{-1}$, 10,000 s$^{-1}$, 20,000 s$^{-1}$, and 40,000 s$^{-1}$ demonstrated that at or below a threshold strain-rate of 20,000 s$^{-1}$ no overt platelet aggregation occurred within the device (Fig 5.10c). A constant strain-rate of 40,000 s$^{-1}$, equivalent to that experienced at the peak of the c60g20e60 contraction zone, resulted in the slow formation of small highly unstable rolling mini-aggregates (1-3 platelets) that traversed the micro channel walls. Unlike the c60g20e60 case, these aggregates were randomly distributed and tended to rapidly disaggregate under flow. This data clearly demonstrates that the stabilized aggregation response observed in the c60g20e60 case is not simply a product of the elevated strain-rate achieved at the stenosis apex but is a result of the combined effect of acceleration and the coupled zone of decelerating strain-rate.
5.5.4 Modulation of platelet aggregation as a function of micro channel geometry

As identified in Section 5.4.2 a chief aim of the device design concept was the ability to controllably modulate platelet aggregation by modifying key geometric parameters and therefore the magnitude and extent of the imposed dynamic strain-rate. Figures 5.11 shows a series of test-case experiments in which both the contraction and expansion angles of the micro channel geometry were symmetrically modified as per Section 5.4.2.

Figure 5.11: Proof-of-concept blood flow experiments II. Representative epi-fluorescence image sequences of blood perfusion through the c90g20e90 and c30g20e30 micro channel cases; the yellow arrows denote the points of initial aggregation [t = 0 s], the white arrows designate the direction of blood flow (scale bar = 15 µm) [representative of n = 3 experiments]. Note that in all cases the blood samples were pretreated with amplification loop blockers (ALB); apyrase (0.02 U ml⁻¹), MRS2179 (100 µM) and 2-MeSAMP (10 µM); indomethacin (10 mM) and hirudin (800 U ml⁻¹) for 10 min prior to perfusion (representative of n = 3 experiments for each mimetic).

Figures 5.12 presents a comparison of the aggregation traces for the c60g20e60 test-case and the c90g20e90 test-case and demonstrates no appreciable difference in the overall magnitude of platelet aggregation, where the input pre-stenosis strain-rate was kept constant at 1,800 s⁻¹. However, the c90g20e90 geometry did result in an increased stability of the formed aggregates highlighted by the overall reduction in the variation of aggregate size over time.

In contrast a reduction in contraction and expansion angles from 60° to 30° (c30g20e30 case) significantly reduced both the initial rate and magnitude of platelet aggregation as shown in Figure 5.11. Interestingly Figure 5.11 shows that the site of initial platelet aggregation was shifted in the c30g20e30 case downstream from the stenosis apex, suggesting that an overall strain-rate deceleration must be achieved before significant stabilization of platelet aggregation can occur (Fig 5.11).
Figure 5.12: Proof-of-concept blood flow experiments II. (b) Representative aggregation traces showing the response of ALB treated whole blood perfusion through the c60g20e60, c90g20e90 and c30g20e30 micro channel cases (n = 3 experiments).

Figure 5.13 presents a summary of the strain-rate distribution calculated for each geometry using CFD and its correspondent fluorescence image from the experiments with blood. Based on the current working hypothesis and the detailed CFD simulations, the inability of the c30g20e30 case to support stable platelet aggregation could be explained by the overall higher strain-rates experienced by the developing aggregate (as it is forced to develop within higher velocity regions of the flow therefore platelets can not stand higher drag forces) and the overall reduction in the rate of change in strain-rate within the expansion zone. In contrast, the increase in aggregate stability in the c90g20e90 case could be explained by more rapid strain-rate deceleration and the protection of the formed aggregate from the higher velocity regions of the flow. This proof of concept study clearly demonstrates that modification of the strain-rate geometry and resultant strain-rate distribution (see Section 5.4.2) in the prototype device can be directly used to modulate platelet aggregation dynamics in a controlled way.

5.5.5 Recirculation test

Most research to date on blood associate thrombus formation with the recirculation and eddy formation. To demonstrate that the platelet aggregation produced by this platform is not associated with recirculation, the geometries were simulated with a sequence of increasing flow rates. Figure 5.14 presents the flow behavior in the 90° geometry with flow rates of 16, 19, 29, 48, 64 and 91 µL/min. It can be observed that for the conditions of operation of the device (Q = 16µL/min) no flow recirculation exists at the zone of the contraction. A recirculation zone becomes evident downstream the contraction at Q = 48µL/min, which is around three times faster the experimental conditions. Further-
more, as it will was shown in the biological experiments, this recirculation zone does not correspond with the zone where platelets start to aggregate. Figure 5.15 presents the same study but for the 60° geometry. It can be observed that flow recirculation is not observed until Q = 91µL/min. This evidently shows that the recirculation is not only dependant on the inertial forces but also on the geometry.

Both geometries, the 60° and 90° do not exhibit aggregation even at Q = 16µL/min and hence it can be concluded that the observed aggregation is not due to flow recirculation. The fact that there is no recirculation in the zone of aggregation for the flow rates
used in the experiments, clearly demonstrates an important novel aspect of this platform since this suggest that the aggregation is due to a biophysical phenomena no-related with recirculation.

Comparing the measurement of Figure 5.7d) from Section 5.4.3 and the simulated streamlines of Figure 5.15 it can be observed that good qualitative agreement is again evident.

![Streamline plots](image)

**Figure 5.14:** Assessment of no-recirculation for the 90 degrees geometry at different Reynolds numbers.

Figure 5.16 presents a summary of the recirculation test using numerical simulations (CFD) and the aggregation images from blood experiments. It is evident that under the conditions of the experiment no recirculation was found. For an hypothetical recirculation at high Reynolds number, the zone of recirculation is different to the zone of initial
Figure 5.15: Assessment of no-recirculation for the 90 degrees geometry at different Reynolds numbers.

aggregation (yellow arrow).

5.6 Conclusions

The device described in this Chapter 5 provides a method for controllably manipulating blood strain rates at the micron scale while monitoring platelet aggregation dynamics. Advances in micro-fabrication in combination with detailed numerical analyses of blood flow have enabled the development of a micro-vascular mimetic design that closely reproduces the haemodynamics and platelet aggregation dynamics observed in intra-vital mouse models of acute vascular stenosis. The presented proof-of-concept data demon-
Figure 5.16: Summary of the test of no-recirculation and platelet aggregation experiments. (a) and (d) Representative epi-fluorescence image sequences of blood perfusion through the c60g20e60 and c90g20e90 micro channel cases respectively; the yellow arrows denote the points of initial aggregation \( t = 0 \) s, the white arrows designate the direction of blood flow (scale bar = 15\( \mu \)m). [representative of \( n = 3 \) experiments]. Note that in all cases the blood samples were pretreated with amplification loop blockers (ALB); apyrase, MRS2179 and 2-MeSAMP); indomethacin and hirudin for 10 min prior to perfusion (representative of \( n = 3 \) experiments for each mimetic). (b) and (e) Streamlines of the flow at the Reynolds number of the experiment for c60g20e60 and c90g20e90 micro channel cases respectively. (c) and (f) Streamlines of the flow at a typical Reynolds number where flow recirculation was found numerically for an homogeneous fluid for c60g20e60 and c90g20e90 micro channel cases respectively. Note that the zone of recirculation is different to the zone of initial aggregation (yellow arrow).
strates that the device has particular utility to explore different scenarios of controllable strain rate conditions and should enable a detailed investigation of the role of specific haemodynamics variables regulating platelet aggregation. Lab on chip based devices have been applied to the study of platelet adhesion and aggregation in the past, however these methods have either consisted of constant laminar flow systems incorporating immobilized adhesive proteins such as purified von Willebrand factor (VWF), or chemically driven aggregation models involving endpoint biochemical analysis of total platelet-adhesion or aggregate mass\cite{112, 113, 114, 115, 25}. The application of controlled strain rate micro-gradients inherent in the presented device design offers several advantages over these conventional systems, including; dynamic differential strain-rate control, the ability to achieve discrete periods of highly elevated strain rate with minimal sample volume, and the ability to precisely control the spatial location and extent of forming aggregates. The latter, in conjunction with the application of state-of-the-art micro-imaging and spectroscopy based techniques, allows for the real-time monitoring of platelet functional responses as opposed to more traditional end-point analyses of aggregate size. In the in vivo setting a contributing factor to strain rate micro-gradient induced platelet aggregation is the requirement for prior vessel wall damage and the exposure of adhesive sub-endothelial matrix proteins\cite{1}. PDMS in the absence of oxygen plasma treatment has a highly hydrophobic character that has a demonstrated propensity to adsorb blood proteins such as fibrinogen and albumin\cite{124, 125, 126, 127, 128}. Therefore, an undefined variable inherent in the PDMS micro device presented is the role of adhesive blood proteins and platelet adhesion receptors in mediating platelet aggregation in the presented device. It is conceivable that platelet aggregation under the influence of the imposed strain-rate micro-gradient is the result of a two stage process involving:

i. the advective transport of plasma proteins to the PDMS boundary layer, triggering initial primary platelet adhesion and,

ii. the mechanical effects of the applied strain rate micro-gradient on platelet-platelet cohesive interactions.

Preliminary studies in which the PDMS chip was saturated with bovine serum albumin to prevent plasma protein adsorption during blood perfusion suggest that adsorption of one or more blood plasma proteins is an absolute requirement for platelet aggregation in the presented device. However, observations on constant shear rate PDMS micro-channels (section 5.5.3) demonstrate that blood protein adsorption per se is insufficient to induce platelet aggregation in the absence of an applied strain rate micro-gradient. Investigations examining the adsorption of blood proteins to artificial polymers such as PDMS have identified a hierarchy of preferential protein adsorption (the Vroman effect) characterised by the preferential binding of albumin, followed by IgG followed by fibrinogen, however to date no studies have investigated the place of VWF in this
Further studies need to be carried out to identify the specific plasma protein components and the overall effect of strain rate micro-gradients on the Vroman effect and its impact on aggregation kinetics within the presented micro-fabricated device. Another key variable that may have a significant impact on platelet aggregation dynamics in the device is the secretion of soluble platelet agonists such as ADP and TXA2 under the influence of applied shear stress. Although recently published studies and the new experiments presented in this Chapter 5 suggest that strain rate micro-gradient mediated aggregation can occur in the absence of platelet amplification loops, it is conceivable that different device geometries and therefore different strain rate micro-gradients may display a differential requirement for chemical platelet activators. Future studies involving a broader range of strain rate micro-gradient geometries in combination with pharmacological inhibitors and transgenic animal models will be conducted to explore in detail the possible synergistic interactions of haemodynamics parameters and specific chemical agonists that are known to stabilize platelet aggregate formation. Computational Fluid Dynamic analyses were a fundamental tool utilized in the micro-channel design process.

The application of microfluidics technology has enabled the examination under controlled conditions of the impact of haemodynamics at the micro-scale on platelet function. In the same manner during the development of this platform, the utilization of a high resolution chrome mask as part of the photo-lithographic fabrication process allowed to generate micro-channels of high resolution, however the ability of even micron-scaled deviations to potentially impact on the platelet aggregation process requires a close to perfect fabrication procedure, with little margin for error. To achieve the tolerances needed, a high degree of quality control is required at each stage of the photo-lithographic process. As an example of this, sharply defined corners were part of the preliminary design however due to current limitations in the fabrication process; rounded corners were produced on the order of a $5\mu m$ radius (which was a consideration in the CFD model). Although this limitation did not grossly impact on the platelet response, further refinement of the fabrication method may ultimately result in more precise control of the haemodynamics and resultant platelet aggregation. Although PDMS offers many advantages in terms of cost and ease of fabrication, other materials that allow for more precise geometries may prove to be advantageous.

On the same manner, during the development of the experiments it was found that there exists small changes in the dimensions from chip to chip even fabricated from the same mould. A characterisation on the dimensional changes of PDMS due to moisture absorption is recommended.

Overall the platform technology presented highlights the advantages of microfluidics
and lab on chip devices to study platelet function in response to local changes in strain rate micro-gradients emulating those found in the vasculature. Once fully characterised this platform technology should show considerable utility as a clinical and research tool in the diagnosis of platelet functional defects in patients experiencing bleeding or thrombotic diathesis and also in the screening and development of more effective anti-platelet agents targeting shear-dependent platelet activation mechanisms.
Chapter 6

Conclusions

The main contribution of this work was building the supporting evidence from the engineering perspective through creation of the device concept, through simulations, fabrication of a platform which enabled proof of the claim that fluid forces are by themselves able to produce platelet aggregation and thrombus formation independently of bio-chemical mechanisms. The current research on thrombosis and platelet aggregation, assumes that platelets aggregate by a direct accumulation of soluble agonists (chemical factors) at sites of flow disturbance. It was demonstrated in a synthetic device created as part of this thesis that even with the chemical mechanisms blocked, a localised thrombus as a function of the micro-channel geometry on a controlled fluid strain-rate environment was formed, which was in agreement with the experiments done on mice and micro-spheres. A derived contribution was the demonstration that flow recirculation is not needed in order to produce platelet aggregation, at least for the micro-flows phenomena.

Despite resources spent and improvements in anti-thrombotic therapies, the majority of patients receiving these drugs continue to die. The demonstration that the platelet aggregation process can occur independent of chemical factors, may help explain the limited anti-thrombotic efficacy of the used drugs, particularly aspirin, clopidogrel and thrombin inhibitors.

6.1 Outcomes of this work

Through simulations and micro-fabrication it was possible to improve the understanding in the role that mechanical factors play in platelet aggregation. Particular emphasis was placed on simulations of in-vivo experiments developed in mice (Chapter 3), and micro-sphere disturbance experiments (Chapter 4) where platelet aggregation was found independently of biochemical activators of aggregation. These simulations set the basis of the design of a micro-fluidic platform to study the role on the mechanical variables on
platelet aggregation (Chapter 5). The key areas of investigation were: the development of a model to determine the geometry of a induced stenosis, fluid dynamic simulations to know the role on blood flow fluid stress of small spherical defects as a flow disturbance and its impact on platelet aggregation, and finally the design, fabrication, characterisation and testing of a platform that allows the manipulation of the mechanical variables in a controlled and independent manner. Each of these goals has been realized with a summary of the major achievements of this work following.

A primary outcome of this work was the development of a methodology to perform realistic simulations of the novel experiments of the arteriole geometry deformed by a micro-needle as presented in Chapter 3. By using this methodology a direct relationship on how the needle displacement affects the local fluid strain-rate, therefore it was possible to estimate accurately the order of the strain-rates experienced by the platelets aggregating at the downstream zone of the stenosis. In the same investigation it was found that the elastic properties of the arteriole wall affect the local vessel geometry which in turn affect the strain-rate and mechanical forces experienced by platelets. It was found that the hyper-elastic nature of the living tissue is determinant in creating local velocity gradients closely related with platelet aggregates.

Through fluid mechanics theory and simulations conducted in Chapter 4, it was shown that the flow disturbance produced by a micro-spherical bead is a sudden increase and decrease in the strain-rate magnitude by a factor of 6. It was demonstrated that the strain-rate produced by the bead is similar in magnitude across each bead irrespective of bead diameter. The fact that platelets would experience the same peak strain-rate for each bead but in fact exhibited different aggregation responses depending on bead diameter, clearly suggested that it is not only the value of high strain-rates that was promote the aggregation of platelets but also the deceleration zone that provides a low shear region where the platelets are allow to recruit.

In Chapter 5 using micro-fabrication technologies in combination with detailed numerical analyses of blood flow, a micro-vascular mimetic platform was fabricated that closely reproduces the haemodynamics and platelet aggregation dynamics observed in intra-vital mouse models of acute vascular stenosis. The proof-of-concept data presented demonstrates that the device has particular utility in enabling the exploration of different scenarios of controllable fluid strain-rate conditions and should enable a detailed investigation of the role of specific haemodynamics variables regulating platelet aggregation.

Overall the platform technology presented highlights the advantages of microfluidics and lab on chip devices to study platelet function in response to local changes in strain rate micro-gradients emulating those found in the vasculature. Once fully characterised this platform technology should show considerable utility as a clinical and research tool in the
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The work presented in this investigation open the door for interesting projects in microfluidics and blood research. A first area to work on is the computational fluid mechanics. An underlying assumption presented in this thesis was the overall Newtonian and homogeneous composition of the blood. However better approaches are needed to represent more closely the haemodynamics experienced by blood flow. In micro-flows the most distinctive Non-Newtonian features, and particularly in blood, come from the non-homogeneity, which is given by the deformable blood cells. Therefore, any effort considering the non-homogeneity of the blood will be worth pursuing further.

A second area quite interesting to investigate further is the experimental validation of the fluid mechanics. In the current investigation, the experimental validation using μPIV correlated well with the numerical simulations because in both cases the fluid was considered homogeneous with constant viscosity and low velocity. However, further studies and more comprehensive models would be advantageous that take into consideration the heterogeneity of blood composition (composed of deformable elements), the interaction between these elements and changes in viscosity as a function of elevated and/or time varying strain rate. More extensive modeling and experimental investigations need to be carried out to further define the way in which blood elements such as platelets and erythrocytes impact on local haemodynamics conditions and mass transport phenomena to the micro-channel walls. Blood cells can be stained with fluorescence dyes, therefore explorations towards the velocimetry of blood flow would be desirable using the blood platelets themselves as tracer elements. In the same trend, measurements of instantaneous velocimetry during thrombus growth will definitely give further insight in the haemodynamics regulating thrombosis.

A third area also important is the proper characterisation of the material of the device. An undefined variable inherent in the micro PDMS micro-channel device presented is the role of adhesive blood proteins and platelet adhesion receptors in mediating platelet aggregation in the device. Therefore further studies need to be carried out to identify the specific plasma protein components and the overall effect of strain rate micro-gradients on the PDMS protein absorption and its impact on aggregation kinetics.

Another area to further investigate, which is probably the most obvious to the look, is to study the role of the different variations in the geometry of the stenosis. This is an
area currently under investigation. Figure 6.1 presents one example of suggested micro-contractions to study in detail the variables of micro-stenosis using micro-fabrication.

Figure 6.1: Suggested micro device for future work, in order to study in detail the variables of micro-stenosis using micro-fabrication. (a) Schematics detailing the main components of the strain rate micro-gradient device (PDMS chip). [A] 200 μL inlet reservoir for blood delivery. [B] Trap to prevent micro-channel fouling by particulate matter and/or micro-clots in the blood sample due to inadequate anticoagulation. [C] Feeder channel connecting the trap zone with the micro-contraction, [D] six high resolution micro-contractions, [E] exhaust channel and [F] outlet connection to the syringe pump. (b) Schematic of the micro-contractions allocated in [D].

Overall the platform technology presented highlights the advantages of microfluidics and lab on chip devices to study platelet function in response to local changes in strain rate micro-gradients emulating those found in the vasculature. Once fully characterised this technology platform should show considerable utility as a clinical and research tool in the diagnosis of platelet functional defects in patients experiencing bleeding or thrombotic diathesis and also in the screening and development of more effective anti-platelet agents targeting shear-dependent platelet activation mechanisms.
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References


BIBLIOGRAPHY.


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BIBLIOGRAPHY.


BIBLIOGRAPHY.


BIBLIOGRAPHY.


BIBLIOGRAPHY.


[132] H. Elwing, A. Askendal, and I. Lundstr
"om, “Competition between adsorbed fibrinogen and high-molecular-weight