Modelling and Control
of Dynamic Platelet Aggregation
under Disturbed Blood Flow

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

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Declaration

I certify that except where due acknowledgement has been made, the work is that of
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which has been carried out since the official commencement date of the approved
research program; any editorial work, paid or unpaid, carried out by a third party
is acknowledged; and, ethics procedures and guidelines have been followed.

Miguel Eduardo Combariza Pacheco.
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Contents

Contents i

List of figures vii

List of tables viii

Nomenclature ix

Publications xii

Abstract 1

1 Introduction 3

1.1 Motivation ................................................. 3

1.2 Thesis Objective ........................................ 4

1.3 Original Scientific Contributions ............................. 5

1.4 Thesis Outline .......................................... 6

2 Literature review 8

2.1 Introduction ............................................. 8

2.2 Overview of Platelet Aggregation ......................... 9

2.2.1 Role of platelets in haemostasis ...................... 9
2.2.2 Factors influencing platelet aggregation ......................... 10
2.2.3 The role of shear rate in hemodynamics ......................... 10
2.2.4 Platelet aggregation mechanisms under laminar and disturbed
    flow ....................................................... 12
2.2.5 Next generation of technologies for blood research ............ 13

2.3 Micro Technologies for Biological Studies ....................... 14
  2.3.1 Flow-based technologies for the study of cell function ...... 15
  2.3.2 Control Systems in microfluidic devices ....................... 22
  2.3.3 Opportunities of microfluidic devices in clinical diagnosis of
    platelet function ........................................ 25

2.4 Modelling of Dynamic Systems ................................. 26
  2.4.1 System identification approach ............................. 27
  2.4.2 Collection of identification data ............................ 28
  2.4.3 Model structure ....................................... 29
  2.4.4 Model estimation ....................................... 32
  2.4.5 Model evaluation ....................................... 33
  2.4.6 Summary ............................................... 34

2.5 Feedback Control Systems .................................... 34
  2.5.1 Feedback control preliminaries ............................. 35
  2.5.2 Nonlinear control methodologies ............................. 38
  2.5.3 Introduction to sliding mode control ......................... 41

2.6 Conclusion ................................................ 43

3 Observation of platelet aggregation dynamics under disturbed flow
    in a microfluidics device .................................. 45
  3.1 Introduction .............................................. 45
3.2 Microfluidics Platform to Study Platelet Aggregation under Disturbed Flow 46
   3.2.1 Experimental methods 47
   3.2.2 Blood perfusion results 49
3.3 Enhanced Feedback Measurements of Platelet Aggregation 52
   3.3.1 Detection of main aggregate in fluorescence images 53
   3.3.2 Representative parameters of dynamic platelet aggregation 55
   3.3.3 Discussion of enhanced feedback measurements of platelet aggregation 60
3.4 Mechanistic Model of Platelet Aggregation under Disturbed Blood Flow 61
   3.4.1 Experimental trials at constant physiological shear rate 61
   3.4.2 Four-stage mechanistic model 62
3.5 Discussion 65
3.6 Conclusions 66

4 Dynamic modelling of platelet aggregation in response to blood flow rate modulation 67
   4.1 Introduction 67
   4.2 Shear Rate Step Response for Identification of Platelet Aggregation 69
   4.3 Identification of Platelet Aggregation in Response to Dynamic Modulation of Shear Rate 72
      4.3.1 The identification process 73
      4.3.2 Formulation of experiment to obtain modelling data 74
      4.3.3 Black-Box model structures 77
   4.4 Nonlinear Grey-Box Dynamic Model of Platelet Aggregation 86
5 Automatic regulation of platelet aggregation dynamics via switching control

5.1 Introduction .............................................................. 96

5.2 Microfluidics Device for Rapid Modulation of Shear Rate ............. 98
  5.2.1 Proposed microfluidics design ................................... 98
  5.2.2 Proof-of-concept experiment .................................... 100

5.3 Control Algorithms for the Regulation of Platelet Aggregation .......... 102
  5.3.1 Relay control ................................................... 103
  5.3.2 Sliding mode control ........................................... 107
  5.3.3 Sliding mode control with pulse width modulation ............... 111

5.4 Simulation of SMC-PWM Algorithm for the Regulation of Platelet
  Aggregation ............................................................. 117
  5.4.1 Case Study I: Regulation of platelet aggregate size ............. 117
  5.4.2 Case Study II: Inter-patient variability .......................... 119

5.5 Conclusions ............................................................ 121

6 Conclusions .................................................................. 122

6.1 Outcomes of This Work .................................................. 123

6.2 Suggestions for Future Work .......................................... 124

References .................................................................... 128
## List of Figures

2.1 Parabolic flow profile in blood vessel. ........................................ 11

2.2 Mechanisms of platelet aggregation under laminar and disturbed flow conditions. ....................................................... 13

2.3 Microfluidic devices for the study of shear-dependent platelet aggregation. ................................................................. 18

2.4 Measures in microfluidic devices for the study of shear-dependent platelet aggregation. ................................................ 20

2.5 Block diagram of open- and closed-loop control systems. ....... 36

2.6 Geometrical interpretation of sliding mode control and equivalent control (in Filippov sense). ........................................... 43

3.1 Microfluidics platform for real-time monitoring of platelet aggregation under disturbed flow. ........................................ 48

3.2 Platelet aggregation results under disturbed flow at physiological constant shear rate ($\gamma = 1800s^{-1}$). ...................... 51

3.3 Image processing algorithm for detection of main platelet aggregate in test microchannel. ................................................. 53

3.4 Sequence of epi-fluorescent images of blood perfusion under physiological conditions ($\gamma = 1800s^{-1}$) and detected main aggregate -white boundary. ..................................................... 55
3.5 *Mean intensity* and *area* measures of platelet aggregate for blood perfusion at physiological shear rate ($\gamma = 1800s^{-1}$). .......................... 57

3.6 *Width* and *height* measures of platelet aggregate of blood perfusion at physiological shear rate ($\gamma = 1800s^{-1}$). ................................. 59

3.7 Aggregation traces and images representing the relative size of the thrombus and four stages of platelet aggregation. ................................. 63

3.8 Four-stage mechanistic model of dynamic platelet aggregation under disturbed flow. ................................. 64

4.1 Platelet aggregation in response to the step change in shear rate $\gamma \in \{1200, 1800, 2400, 3600\} [s^{-1}]$. ................................. 71

4.2 Fluorescence images of platelet aggregation in response to the step change in shear rates $\gamma \in \{1200, 1800, 2400, 3600\} [s^{-1}]$. ................................. 72

4.3 Experimental data employed for model estimation $Z^N$. ................................. 76

4.4 Plots of platelet aggregation in response to shear rate calculated by linear Black-Box models. ................................. 81

4.5 Block diagram of NARX model structure. ................................. 83

4.6 Block diagram of HW model structure. ................................. 83

4.7 Plots of platelet aggregation in response to shear rate calculated by Black-Box nonlinear models. ................................. 86

4.8 Block diagram of conceptual model in which platelet aggregation is regulated by two complementary mechanisms: rheological (biomechanical) and soluble-agonist-dependent. ................................. 88

4.9 Simulation results of grey nonlinear models in comparison to linear model and measured experimental data, and nonlinear function describing time-varying amplification behaviour of platelet aggregation. 91
4.10 Validation of equivalent grey model using the response to a step in shear rate ($\gamma = 1800 \, s^{-1}$). ................................. 92

5.1 Microfluidics device for rapid control of flow rate using negative pressure. ................................................................. 99

5.2 Response times to changes of shear rate in the test microchannel of original and proposed microfluidic devices. .................. 101

5.3 Transfer function diagram of relay control. ................................. 104

5.4 Block diagram of relay control algorithm for regulation of SIPA. . . . . 104

5.5 Simulation of relay control algorithm regulating the dynamics of platelet aggregation. ................................................................. 105

5.6 State trajectories for simulation shown in Figure 5.5 of relay control using two values of switching threshold. ................................. 106

5.7 Block diagram of SMC algorithm for regulation of SIPA. .............. 110

5.8 Simulation of sliding mode control algorithm regulating the dynamics of platelet aggregation. ................................................................. 111

5.9 Waveform of PWM signal and Duty cycle function. ........................ 114

5.10 Block diagram of SMC with PWM Control for the regulation of SIPA. 115

5.11 Simulation results of the SMC and PWM algorithm to regulate platelet aggregation in response to shear rate micro-gradients. ......... 116

5.12 Simulation results of Case Study I: regulation of platelet aggregate size. 118

5.13 Simulation results of Case Study II: inter-patient variability study. . . 120
# List of Tables

2.1 Generation of shear rate conditions in microfluidic devices for the study of platelet adhesion/aggregation. .......................... 16

2.2 Applications of control systems in microfluidics devices. .......... 24

4.1 Performance of standard linear Black-Box models. ................. 81

4.2 Performance of Nonlinear ARX and Hammerstein-Wiener Black-Box models. ................................................................. 85

4.3 Model parameters and performance of linear, and Grey and Equivalent nonlinear models. ................................................. 89

5.1 Parameters of SIPA dynamic model and SMC-PWM algorithm. ... 114

5.2 Model parameters for inter-patient variability study. ............... 120
Nomenclature

**Acronyms / Abbreviations**

ADP  Adenosine diphosphate

AIC  Akaike’s information criterion

$\alpha_{IIb\beta_3}$  Integrin

ALB  Amplification loop blockade

ARMAX  Autoregressive-moving-average model with exogenous inputs model

ARX  Autoregressive model with exogenous input

ATP  Adenosine triphosphate

FIR  Finite impulse response

GPIb  Glycoprotein Ib

HW  Hammerstein-Wiener model

MDL  Rissanen’s minimum description length

$\mu$PIV  Micro-particle image velocimetry

MPC  Model predictive control

NARX  Nonlinear ARX model
PDMS  Polydimethylsiloxane

PEM  Prediction error method

PID  Proportional integral derivative control

PWM  Pulse width modulation

SIPA  Shear-induced platelet aggregation

SMC  Sliding mode control

$TxA_2$  Thromboxane A2

VSCS  Variable structure control system

vWF  von Willebrand factor

Symbols

$c_0$  Initial pro-thrombotic potential

c_1  Enhanced aggregation coefficient

$D$  Duty cycle

e  Error signal

$\gamma$  Shear rate

$\lambda$  Gain parameter of SMC

$Q$  Flow rate

$r$  Input reference

$\tau$  Time constant of SIPA

$\theta$  Model parameters vector
\( T \)  Time period

\( u \)  System input (control signal)

\( u_{eq} \)  Equivalent control signal

\( V \)  Lyapunov function

\( v \)  Fluid velocity

\( x \)  System state vector

\( \dot{x} \)  Time derivative of the state vector

\( y \)  System output

\( \hat{y} \)  Predicted system output

\( y_m \)  Feedback signal

\( Z^N \)  Identification dataset
Publications


Abstract

Diagnosis of platelet function is fundamental for identifying blood disorders of patients, assessing the impact of antiplatelet agents, and enabling the appropriate titration of individual antithrombotic treatments. Following the advancement of new technologies such as microfluidic devices and the use of control engineering methods, new devices have the potential to offer new opportunities in point-of-care diagnosis of platelet function. Such new devices may have significant utility in the development of more tailored antiplatelet therapies.

The aim of this thesis is to investigate modelling and control systems which support the study of the dynamic relationship between newly discovered mechanisms of platelet aggregation and disturbed blood flow, using state-of-the-art micro-engineered technologies.

In order to observe the dynamics of platelet aggregation under disturbed blood flow, blood perfusion experiments carried out on a device mimicking a scenario of severe vessel narrowing are presented. The resulting biological response, that is, aggregation of platelets, is monitored in real-time and synthesised through novel measures developed using image processing techniques. A mechanistic model identifying four distinct stages observed in the formation of the aggregate is formulated, describing the nonlinear relationship between blood flow dynamics and platelet aggregation.

The observed effect of disturbed blood flow on the aggregation of platelets is then modelled mathematically employing System Identification methods. A detailed ac-
count of a novel approach for the generation of experimental data is presented, as well as the formulation of tailored mathematical model structures and the calculation of their parameters using collected data. The proposed models replicate experimental results with low variation, and the reduced number of model parameters is suggested as a novel systematic measure of platelet aggregation dynamics in the presence of blood flow disturbances.

In order to stabilise, optimise, and automate the measurement of platelet function in response to disturbed blood flow, custom-made control algorithms based on principles of Sliding Mode Control and Pulse-Width Modulation are developed. Moreover, the control algorithms are developed to handle the large variability of the aggregation responses from blood types with platelet hyper- and hypo-function. Simulation results illustrate the robustness of the control algorithms in the presence of time-varying nonlinearities and model uncertainty, and indicate the possibility to regulate the extent of aggregation in the device through modulation of the blood flow rate in the microchannel.

The main contribution of this thesis is the development of dynamic models and control systems that allow a systematic measurement of platelet function in response to rapid changes in the blood flow (shear rate micro-gradients), in a microfluidics device containing a scenario of disturbed blood flow. Analysis of the platelet aggregation dynamics revealed that although the aggregate growth appears to be constant at times, measuring its mean fluorescence intensity indicates an increase in the dynamics of platelet density. This densification process appears fundamental for the development of an amplification phase in the aggregation response. The proposed mathematical models and control algorithms facilitate the systematic measurement of platelet function \textit{in vitro} pioneering the development of a novel framework for automated blood disorder diagnosis.
Chapter 1

Introduction

1.1 Motivation

The development of blood clots at sites of atherosclerotic plaque rupture - arterial thrombosis - continues to be a major cause of clinical death worldwide despite decades of research into the underlying thrombotic mechanisms. The inherent platelet functional parameters that put patients at risk of thrombotic disease remain difficult to observe and analyse, and rely on indirect clinical assessment of suboptimal laboratory based tests. Furthermore, current screening and diagnostic tests fail to address the central role of blood flow (hemodynamics) that underlay thrombotic disease. Recently, the International Society on Thrombosis and Haemostasis biorheology subcommittee recommended the development and inclusion of flow-incorporating assays for the screening of platelet function. Essential to this is the development of a robust high-throughput assay to accurately measure flow dependent platelet function.

With the advancement of new technologies such as microfluidic devices, to accurately recreate physiological scenarios of blood clotting, and the use of dynamic modelling and control engineering methods, to extract information and manage these platforms systematically, new diagnostic devices to assess platelet function have the
potential to offer new opportunities in point-of-care diagnosis of platelet function, and may have significant utility in the development of more tailored antiplatelet therapies with the prospect to reduce morbidity significantly.

1.2 Thesis Objective

The aim of this dissertation is to investigate dynamic models and control systems able to assist with the measurement of platelet function in response to newly discovered aggregation mechanisms using a state-of-the-art microfluidics device.

Platelet aggregation is studied \textit{in vitro} in a microfluidics device recreating the conditions of disturbed blood flow present in abnormal narrowing of blood vessels. The growth of the forming aggregate is analysed by tailored image processing techniques which synthesize the experimental measurements in terms of its size and platelet density. A mechanistic model is formulated describing four distinct stages observed in the process of platelet aggregation.

Using this insight and experimental data, dynamic mathematical models are developed using System Identification approaches to describe the effect of rapid changes of blood flow (shear rate micro-gradients) on platelet aggregation. Importantly, the reduced number of model parameters are suggested as a robust diagnostics of platelet function under disturbed blood flow.

Control algorithms are developed with the objective of stabilising, optimising and automating the diagnostics of platelet function, as well as dealing with the large variability of the aggregation responses from blood types with platelet hypo- and hyper function.
1.3 Original Scientific Contributions

The original contributions of this thesis are summarised as follows:

- Observation of the main aggregate size and platelet density in a microfluidics device under disturbed flow, and identification of a required increase in platelet density in stage of plateau of aggregation size as a mechanism to enhance aggregation [1].

- Formulation of a mechanistic model describing four distinct stages of platelet aggregation under disturbed flow observed experimentally, and explanation of nonlinear relationship between blood flow dynamics and aggregate growth [1].

- Demonstration of platelet aggregation in response to multiple shear rates using a single test microchannel and syringe driver using dynamic modulation of shear rate [2].

- Formulation of linear mathematical models that approximate the initial dynamics of platelet aggregation in response to shear rate micro-gradients observed experimentally in a microfluidics device [2].

- Demonstration of a three-parameter nonlinear dynamic model that fits with low variation experimental data of aggregation of platelets in response to a dynamic sequence of shear rates [3].

- Formulation of robust control algorithms for the regulation of dynamic platelet aggregation in response to modulation of shear rate micro-gradients, a novel framework for automated blood disorder diagnosis [4].

- Observation of time-varying amplification effect in the aggregation response of platelets in the presence of major chemical inhibitors of soluble agonists due to nonlinear microrheological events and non targeted soluble agonists [5].
1.4 Thesis Outline

This thesis is structured into six chapters as outlined below:

**Chapter 1** presents an introduction to this thesis by outlining the motivation and objective of this research. Then, a summary of the main contributions and outcomes of this research are highlighted.

**Chapter 2** starts by presenting a brief background on blood clotting, focusing on recently discovered mechanisms of platelet aggregation. Then, a review on microfluidic platforms for the study of shear induced platelet aggregation is presented, and shortcomings of these platforms for clinical diagnostics of platelet function are discussed. A survey on modelling and control systems applied to other microplatforms demonstrates the potential of these methodologies to deal with the issues of current assays for platelet diagnostics. This leads to the formulation of the research question of this investigation on whether these methodologies can be applied to advance the use of microfluidic platforms towards the measurement of platelet function. Finally, a survey on the main dynamic modelling and control methodologies that can be applied to this problem is presented.

**Chapter 3** introduces the experimental platform employed for this research, and evaluates current measures for the monitoring of platelet aggregation. An algorithm is presented to detect the main aggregate and reduce background intensity noise. Measures from the main aggregate are investigated and it is explored whether additional insight into the aggregation of platelets can be gained from observations including the size of the aggregate and its density as indicated by fluorescent intensity. Finally, a mechanistic model of the effect of shear rate micro-gradients on platelet aggregation observed experimentally is proposed.

**Chapter 4** presents a novel method to characterise the aggregation response to a range of shear rates in a single device through dynamic modulation of the
shear rate in the test microchannel. Recorded experimental data is analysed and used to formulate black-box mathematical models which can reveal basic properties of platelet aggregation dynamics. Then, simple nonlinear mathematical models are formulated from the insight drawn in the mechanistic model in Chapter 3 and collected identification data. The reduced number of model parameters obtained when fitting these models to experimental results are suggested as a systematic and novel diagnostics of platelet function.

Chapter 5 investigates feedback control algorithms able to the regulate the growth of the aggregate within the microfluidics device, in order to provide robustness to inter-patient variability in the platelet response, and also optimise the time required for the identification of platelet function. Initially, a modification to the original device is proposed in order to provide a rapid response to changes in shear rate, by controlling a valve that vents an auxiliary chamber to atmosphere. Then, control algorithms based on switching control strategies are investigated to regulate the extent of aggregation in the device by dynamically switching shear rate. Robustness of the control algorithms to the nonlinear platelet aggregation dynamics and inter-patient variability is investigated through simulations.

Chapter 6 concludes this thesis by presenting the main contributions of these investigations. Finally, areas that warrant further investigation are identified and opportunities for future studies are presented.
Chapter 2

Literature review

2.1 Introduction

The aim of this chapter is to identify research questions associated to the objectives of this thesis by reviewing the state of the art of the application of modelling and control methodologies to microfluidic devices in the context of current blood research. This literature review is organised into three main Sections as follows.

First, the context of this research is introduced. Background on platelet function is presented, with an emphasis on recently discovered aggregation mechanisms under disturbed blood flow, shear-rate micro-gradients. Subsequently, the role of micro-engineered technologies in current blood research is highlighted.

Next, opportunities from the application of modelling and control systems to cutting-edge micro-engineered technologies for biological studies (microfluidic devices) are explored. A survey is presented on microfluidic technologies employed for flow-based cell studies. Emphasis is given to the complexity of the devices to generate multiple conditions of shear rate, and the measures employed to representative of the aggregation dynamics. A number of potential issues to be addressed for these type of devices to be successfully translated to the clinical setting are identified. A review of the increasing application of control systems in microfluidic
technologies is then presented to demonstrate the potential of these methodologies to deal with similar issues to the ones encountered above, leading to an elaboration on possible advancements by the application of control systems on the functioning of microfluidics devices dedicated to platelet function measurement.

Finally, a review is presented on modelling and control methodologies that have the potential to address the gaps identified in the use of microfluidic devices for measurement of platelet function. Emphasis is given to the construction of simple dynamic models that can represent relationship between variables observed experimentally through methods of System Identification, and robust control algorithms able to perform in the presence of complexity and nonlinearities of biological systems. A brief introduction to the selected control methodology, Sliding Mode Control, is presented.

2.2 Overview of Platelet Aggregation

Platelets play a key role in the arrest of bleeding (haemostasis) and subsequent repair of vascular injury. This section will present a brief background on platelet function, with an emphasis on technologies able to assist with the recent discovery of mechanisms of aggregation present in scenarios of disturbed flow.

2.2.1 Role of platelets in haemostasis

Platelets are discoid fragments, of approximately 2 µm diameter, produced by megakaryocyte cytoplasm [6]. These circulate in the blood and their main role is to detect any changes in the vascular endothelium. Veins and arteries are covered by a fine line of endothelial cells which protect the blood from becoming in contact with the reactive subendothelium matrix. This way, when an event of endothelial injury occurs, sub-endothelial matrix is exposed to interact with platelets, and these aggregate into a
haemostatic plug to stop haemorrhage.

Platelet’s response to these events require a delicate balance to achieve a successful performance. In the case of an exaggerated physiological platelet response, it can lead to the formation and ejection of blood clots, thrombi, which can obstruct the blood flow inside the circulatory system and eventually lead to ischemia or stroke [7, 8]. These pathological conditions are one of the main causes of clinical death in developed countries [9]. On the other hand, blood with hypo-functional platelets can lead to bleeding disorders.

2.2.2 Factors influencing platelet aggregation

The main factors governing platelet function and vascular thrombosis have been recognised by centuries, and are described by the well-known Virchow’s triad [10]. The first factor is the overall *thrombogenic potential* of blood (hypercoagulability); the second factor is *injury or trauma at the endothelium* (vessel wall injury); the third factor include *changes to the normal blood flow* (hemodynamics). The former two factors have been studied exhaustively and are relatively well understood. On the other hand, the effect of mechanical forces of the blood flow on cellular function and signalling are not very well agreed upon [9].

2.2.3 The role of shear rate in hemodynamics

It has been long recognised that the blood flow properties affect the formation aggregation of platelets in a number of ways [11]. The most common effect of blood flow dynamics is the migration of platelets towards the vessel walls due to interactions with larger particles such as red blood cells, known as "platelet margination" [12, 13]. Blood flow dynamics also determine the localisation of other blood components influencing the function of platelets forces exerted on proteins [14].

Another important parameter in the flow of blood is *shear rate*. Past research
has shown that shear rate has been responsible for several physiological phenomena in blood. For example, it regulates the extent of platelet recruitment and capture at sites of thrombus formation [15, 16]. Since blood is a viscous substance, and owing to drag forces at the vessel walls, blood flows with a parabolic flow profile as illustrated in Figure 2.1. Conceptually, flow laminae travel in parallel alongside at different velocities, with a maximum velocity at the centre of the vessel, and a minimal velocity at the walls. Shear rate is described as

$$\gamma = \frac{\Delta v}{h}$$  \hspace{1cm} (2.1)

where \( h \) is the distance between two laminae in perpendicular direction to the wall, and \( \Delta v \) is difference of their velocities. Shear rate is commonly measured in inverse seconds (s\(^{-1}\)).

![Figure 2.1: Parabolic flow profile in blood vessel.](image)

Physiological values of shear rate in larger veins and arteries are below 1000 s\(^{-1}\), in microvasculature it can range from 1000 to 10000 s\(^{-1}\), and in pathological scenarios such as at the apex of severe vessel narrowing (stenosis) it can reach up to 50000 s\(^{-1}\) [17–19].
2.2.4 Platelet aggregation mechanisms under laminar and disturbed flow

Blood flow can be considered undisturbed and laminar in healthy arteries, away from bifurcation points. In this case, Glycoprotein Ib (GPIb) and Integrin (αIIbβ3) are responsible to promote initial adhesion and aggregation of platelets through tether formation. Then, a cascade of soluble agonists generation occurs (Adenosine Diphosphate, ADP, Thromboxane A2, TxA2, and Thrombin) inducing platelet shape change and degranulation. The result is a very dense and stable platelet aggregate [18]. The process is illustrated in Figure 2.2 a).

Figure 2.2 b) illustrates a scenario of disturbed blood flow produced by a pathological vessel narrowing, as it occurs in atherosclerosis. In this scenario, blood is exposed to high shear rate conditions, and flow disturbances have been shown to be key regulators of platelet function [20]. Nesbitt et al. demonstrated the ineffectiveness of current anti-thrombotic agents in the presence of disturbed flow, and revealed a major role for rapid changes in blood flow (shear rate micro-gradients) in the initiation of platelet aggregation [21]. In this case, platelet aggregation does not require platelet activation of the adhesive function of Integrin αIIbβ3, and instead, aggregation is exclusively mediated by von Willebrand factor vWF – GPIb adhesive bonds [22]. In addition, platelets do not undergo shape change, and interaction occurs between discoid platelets. After some initial formation, flow vortices appear downstream of the aggregate, accumulating soluble agonists, and enhancing the activation and aggregation of platelets. These new insights have brought attention to the importance of hemodynamics in the formation of platelet aggregates.
Figure 2.2: Mechanisms of platelet aggregation under laminar and disturbed flow conditions. a) Thrombosis under laminar flow: soluble agonists are the main drivers of aggregation. b) Thrombosis under disturbed flow: Shear-rate micro-gradients drive initial thrombus formation. Adapted from [18]

2.2.5 Next generation of technologies for blood research

Recent development of high-resolution imaging techniques and micro-engineered technologies, microfluidics, have been essential to cutting-edge blood research [23–27]. The main advantage of microfluidics over traditional assays include remarkable control of the blood flow conditions that mimic physiological and pathological scenarios, and the use of minimal blood volumes. Microfluidic technologies offer several opportunities for biomedical research, cell function under physiological blood flow conditions, and clinical diagnostics.
Several microfluidic devices have been developed for cellular biorheology [28], adhesion dynamics [29], pharmacology [30,31], and coagulation [32]. In particular, a microfluidics device recreating conditions of disturbed flow in arterioles [33] was essential to demonstrate that fluid forces are able to produce platelet aggregation and thrombus formation independently of bio-chemical mechanisms [21], and to suggest a fundamental re-evaluation of the mechanisms of platelet aggregation.

Based on the review presented in this section, microfluidic technologies are selected as a suitable experimental platform to recreate the scenario of disturbed flow present in stenosed vessels, and explore the relationship between recently discovered mechanisms of aggregation (shear rate micro-gradients) and blood clotting. The next section presents a review of microfluidic devices for platelet studies under blood flow.

2.3 Micro Technologies for Biological Studies

The development of micro-engineered technologies has provided a scientific framework to investigate complex biological phenomena with precisely controlled physical and chemical environments, and facilitated the understating of many scientific concepts. On the other hand, modelling and control systems are becoming more crucial for the robust operation and automation of these type of devices.

This section presents a review of microfluidic devices at the frontier of shear-dependent platelet aggregation studies, and evaluates the opportunities of modelling and control systems to advance the usability of these technologies from research towards the clinical setting.
2.3.1 Flow-based technologies for the study of cell function

Microfluidic devices are micro-engineered technologies that are having a large impact in biomedical research [27]. These type of the devices have a vast potential for point-of-care diagnostics by miniaturising large and expensive laboratory equipment into small devices of inexpensive operation [34]. The large capability of parallelism of microfluidic devices, as well as the minute sample handling, make them very well-suited for drug discovery in terms of time and cost efficiency [35]. In general, the use of microfluidics for biomedical research can result in more accurate, rapid, and affordable handling of biological media, and thus enable improved extraction of information.

The effect of shear stress is an important factor for the functioning of many types of cells (mechanotransduction). Previous technologies for the study of shear-dependent platelet aggregation include parallel-plate capillary chambers and cone-and-plate viscometry assays [36]. Parallel-plate capillary chambers comprise a uniform rectangular channel with a characteristic gap of about $100 - 200\ \mu m$, and can be operated with $1 - 10 \ \mu L$ of blood or fluid. These dimensions limit to $2 - 3$ the number of studies that can be carried out with a $20 - 60 \ mL$ blood draw. In cone-and-plate viscometry, the system is formed by the rotation imposed around a cone axis oriented perpendicular to the surface of a flat plate. The cone-and-plate system includes a defined bulk shear rate and linear shear flow which measures platelet aggregation rates in the presence of a chaotic stir-bar-induced flow, poorly representing the hemodynamics found in blood vessels [37].

In contrast to the macroscopic tools identified above, microfluidic devices have been developed to represent physiological shear rate conditions and enable the study of shear rate on several type of cells including endothelial [38–40], fibroblast [41], and epithelial cells [42].

Here, a review is presented on microfluidic devices developed for the study of
CHAPTER 2.

Table 2.1: Generation of shear rate conditions in microfluidic devices for the study of platelet adhesion/aggregation. * Up to 30000 s\(^{-1}\) at the throat of stenosis.

shear-induced platelet aggregation.

**Generation of multiple shear rates in microfluidic devices**

This section presents several approaches employed for the generation of different shear rate conditions in microfluidic devices.

A summary of the approaches in microfluidic devices for the generation of shear rates is presented in Table 2.1. Figure 2.3 a) illustrates an example of an initial approach to implement microfluidic devices recreating the architecture of parallel-plate capillary chambers in microfluidic channels. In order to precisely control the amount of shear rate, microfabrication techniques were employed to achieve nanometer-thick layers of fibrinogen [43, 44] and control the depth of the microchannels.

The next generation of devices increased throughput by having a single outlet and
adding a fluidic resistance to each channel through length-varying flow paths [46, 47, 53], as it is shown in Figure 2.3 b). This configuration allows the creation of multiple shear rate conditions using a single syringe driver. Multi-shear rate devices allowed the demonstration of the shear rate dependency in the process of platelet adhesion to various collagens, such as von Willebrand Factor (vWF) and fibrinogen, and assisted in the identification of thresholds and relationships between chemicals and mechanical variables. Similarly, aggregation in response to multiple shear rates has been accomplished controlling the pressure over well-plates connected to microfluidic channels [54, 55].

Recent studies showed that shear rate micro-gradients, present in scenarios of vessel narrowing such as atherosclerosis can induce the aggregation of platelets without the presence of soluble agonists [21]. These have encouraged the development of platforms that recreate these conditions of disturbed flow. Pioneering the implementation of disturbed flows in microfluidics devices for the study of platelet aggregation, Tovar-Lopez et al. recreated scenarios of severe arterial stenosis through microchannels containing three-dimensional microcontractions [33]. The extent of the shear rate micro-gradients was controlled by the geometry of the microcontractions as illustrated in Figure 2.3 c), which in turn affected the amount of aggregation. Each microchannel was connected to a syringe pump in this device.

Another method for the investigation of platelet aggregation under disturbed flow is the inclusion of spot-injury models. Hansen demonstrated high content evaluation of platelet function in a microfluidic flow assay, depicted in Figure 2.3 d), in which several micro-patterned spots of collagen in parallel channels with different levels of shear rate [51, 52] connected to a single outlet. This work found that a threshold injury size is necessary for stable platelet adhesion.
Figure 2.3: Microfluidic devices for the study of shear-dependent platelet aggregation. 
a) Parallel channels of varying depth with multiple syringe drivers, adapted from [43].
  b) Single outlet and parallel channels with added resistance, adapted from [46].
  c) Realistic microcontractions mimicking arterial stenosis, adapted from [33].
  d) Spot-injury models in parallel channels, adapted from [51].
CHAPTER 2.

Observation of platelet dynamics in microfluidic devices

Measurements for the adhesion/aggregation of platelets has not been standardised, and so, these vary between researches. This section analyses the methods for the interpretation of the results obtained in platforms for shear rate dependent platelet aggregation, in terms of the measurement units, and also how these results are analysed.

Different approaches for the analysis of platelet aggregation are illustrated in Figure 2.4. A common practice in the investigation of platelet research under blood flow is the use of fluorescence microscopy to label platelets in blood. Measurements in planar platforms, such as the ones shown in Figures 2.3 a) and b), primarily study the initial platelet adhesion dynamics. The most common measure is surface coverage, defined as the percentage of pixels above a user-defined threshold in the area of inspection [43,45,51].

Another common method to extract information from the aggregation of platelets is to analyse the fluorescence intensity of the images. For example total florescence, defined as total integrated fluorescence intensity in a.u arbitrary units [46], or similarly, integrated fluorescence intensity, defined as the integral of fluorescence divided by the area of integration in RFU/µm² (where RFU is Relative Fluorescence Units) [51]. Assays that measure bulk aggregates of platelets (or thrombi), as the platforms in Figures 2.3 b) and c), similarly represent the size of the forming aggregate, for example, the area is defined as the number of pixels above certain threshold in the fluorescence images [33,49,50].

Likewise, the analysis of the results from the measurements mentioned above varies between technologies/studies. Studies of platelet adhesion take usually less than 1 – 2 min, and most results are of the and-point type [43,46] (value of the measure at the end of the experiment for each shear rate) as shown in Figures 2.4 a) and b).
Studies of platelet aggregation and thrombus formation usually look at the complete development of a thrombus over a typical duration of $5 - 10$ min, as shown in Figures 2.4 c) and d), including the initial adhesion, growth, and sometimes embolisation. These experiments are usually monitored in real-time [33,51], or close to real time, and analysed in terms of parameters such as lag Time, growth rate, and maximum and plateau size. Lag time is defined as the time lapsed before the aggregates reaches a minimum level of aggregation, e.g. 5% of the maximum. Growth rate can be defined as the slope of the trace between the aggregation points $5 - 95\%$.
of the maximum of aggregation. *Plateau size* is a rather ill-defined parameter, calculated as the average size between after reaching the maximum value and before the aggregate starts goes into embolisation or the end of the experiment.

**Shortcomings of current microfluidic devices for use in clinical setting**

Current platelet tests are specialised into different aspects of platelet aggregation or coagulation, and only provide prognostic and diagnostics of moderate value [56]. Tests such as VerifyNow®, an aggregometry-based platelet function test, and TEGR 5000®, a viscoelasticity-based coagulation test, do not consider the dynamics of physiological platelet aggregation despite demonstrated usefulness [57]. A test including some aspects of blood flow is the PFA-100®, which measures the occlusion time in a perfusion through a microchannel with an annulus coated with ADP or epinephrine. Although the results are correlated to traditional measures of platelet function as platelet count, or anti-platelet therapy, the high levels of shear rate employed by this device make it extremely sensitive to disorders of vWF.

Microfluidic devices possess several attributes that make them well-suited for the measurement of platelet function in the clinical setting. These attributes include the possibility to represent physiological and pathological flow patterns, the handling of small whole blood samples as opposed to requiring platelet-rich plasma, and faster processing times [37]. However, the following issues have been identified in current microfluidic devices for their use in the clinical setting:

- Measurement/analysis of platelet aggregation is not well-defined. Contributing to this, percentages of variation between experiments are very large, usually over 30% [51], and evaluation of multiple parameters (*lag time*, *growth rate*, *maximum* and *plateau size*) require subjective interpretation of specialised personnel. In addition, evaluation of these parameters is required for each of the shear rate conditions tested. While experimental results indicate a clear
dependency of platelet aggregation on shear rate, the relation between these variables, from these large sets of data with large variation, is not straightforward to determine. Experimental results need to be concisely reduced to meaningful and informative measures. Automation in the analysis of the experiments has the potential to reduce the need for expert personnel to carry out tests and interpret the results.

- Effective high-throughput in the evaluation of several flow conditions is required. While microfluidic platforms at the forefront of high content platelet evaluation [46,47,51,53] claim to increase throughput by having parallel test microchannels simultaneously driven by a single actuation mechanism, two main disadvantages outweigh the promised benefits. The first and major problem is that the forming aggregates in the channels generate a build-up of pressure that alters the pre-set conditions of shear rate for the other channels as demonstrated by Colace et al. [48], resulting in a large source of uncertainty in the results. The second problem is that in order to evaluate the multiple test microchannels expensive computerised micro-positioners are required to place each of the channels under the microscope field of view. This invalidates the purpose of portability and simplicity of operation offered by microfluidic devices.

2.3.2 Control Systems in microfluidic devices

The aim of Control Systems is to find the set of required actions for a system to obtain a desired behaviour, in the best possible way. Control System theory has been widely applied in engineering for over a century, having a strong influence in the development of communications and electronics with the introduction of the concept of feedback control [58]. Control systems has been successfully applied to a number of fields such as economics, aeronautics, amongst others. Importantly, it has
been of great utility in biomedical research. This section presents a brief review on applications of control systems to enhance the capabilities of microfluidics platforms, in particular for blood research.

Table 2.2 presents a variety of applications made possible by the incorporation of feedback control algorithms in microfluidic devices. In general, microfluidic devices have mostly applied feedback control systems to actively regulate physical variables in the device with great precision, such as flow pattern [59–64], temperature [65–67], pH [68, 69], electrical fields [70–72], amongst others. Most of the control algorithms were based on simple linear control methodologies. The only application in microfluidics found to use fairly advanced control algorithms was Digital Microfluidics [73–75].

Applications of control systems in microfluidics have enabled unique demonstrations. Some of these applications include single molecule studies for low-cost genome Deoxyribonucleic acid (DNA) sequencing [76], precise time perturbation of conditions (spatial and temporal events) for single-cell analysis [23, 77], bioactuator-powered microdevices [78, 79], nanoliter droplet generation for high throughput drug screening [42, 75, 80–82], and three-dimensional microparticle manipulation [83, 84].

**Modelling and control systems applied to blood research**

A survey of the literature found that only a small number of microfluidic devices for blood research have made use of control systems. A control system was recently applied for regulating the conditions of endothelial cells under hydrostatic pressure and shear stress [88]. Recently, Muthard et al. demonstrated the growth of a thrombus with controllable wall shear rate and transthrombus pressure gradient [24]. A common feature found in the application of control systems to these type of devices is that the control algorithms typically obtain feedback and regulate mechanical variables, and are mainly used to stabilise physical variables at desired points. The
<table>
<thead>
<tr>
<th>Application</th>
<th>Control method</th>
<th>Control variable (actuation)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precise pH control (±0.14 pH)</td>
<td>PD+PWM</td>
<td>pH</td>
<td>[68]</td>
</tr>
<tr>
<td>Control of soft machines</td>
<td>Boolean</td>
<td>Pressure</td>
<td>[85]</td>
</tr>
<tr>
<td>Droplet signal generation</td>
<td>MPC</td>
<td>Pressure</td>
<td>[86]</td>
</tr>
<tr>
<td>Submicron droplet generation</td>
<td>PD</td>
<td>Flow rate</td>
<td>[59]</td>
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<tr>
<td>Transthrombus pressure control</td>
<td>P</td>
<td>Flow rate</td>
<td>[24]</td>
</tr>
<tr>
<td>Multi-droplet actuation enhanced</td>
<td>Fuzzy</td>
<td>EWOD</td>
<td>[73]</td>
</tr>
<tr>
<td>3D manipulation of microparticles</td>
<td>Pseudo-inverse</td>
<td>Flow</td>
<td>[83]</td>
</tr>
<tr>
<td>Colloidal crystal size</td>
<td>Empirical</td>
<td>EPEO and NDEP</td>
<td>[71]</td>
</tr>
<tr>
<td>Microscale segmented flow</td>
<td>I</td>
<td>Pressure</td>
<td>[87]</td>
</tr>
</tbody>
</table>

Table 2.2: Applications of control systems in microfluidics devices. Control algorithms: proportional (P), integral (I), proportional and derivative (PD), model predictive control (MPC), and pulse width modulation (PWM). Microfluidic technologies: electrowetting on dielectric (EWOD), electrophoretic/electroosmotic (EPEO), and negative dielectrophoretic (NDEP).
biological response in the devices is usually not considered in the design of the control algorithms, and instead it is regarded as an external disturbance to the control system.

In terms of modelling platelet aggregation, several models have been reported in the literature developed mainly using Systems Biology approaches [89–91]. Most of these models are based on micro-scale particle-to-particle interactions, describing variables through concentration of chemical species. Only recently, models have incorporated a more detailed relation and emphasis on the interplay of flow variables and thrombus growth [89,90]. Due to their complexity, simulations generally require very high computational time and cost. These aspects mark such models as unsuitable for the replication of experimental results, and to extract from them a reduced number of parameters that represent platelet function. At the same time, these very complex models are not suitable to assist with the design of automatic controllers to regulate the aggregation response in the platform.

2.3.3 Opportunities of microfluidic devices in clinical diagnosis of platelet function

Based on the literature review presented in this section, it was found that microfluidic devices have the potential to play an important role in clinical diagnostics of platelet function. Their advantages include the use of minute volumes of blood, high-throughput analysis, and integration of several aspects such as different shear rate conditions, variety of platelet-adhesive substrates and agonists. However, it was identified that a number of issues have to be addressed for these technologies to be used in clinical diagnosis of platelet function: a) high throughput in testing several conditions of flow, b) evaluation of experimental results with compact and meaningful measures/parameters, and c) incorporation of physiological conditions of blood flow.
It was also found that the application of control systems has enhanced the capabilities of several microfluidics platforms. Nevertheless, the use of these algorithms has been limited to the regulation of physical variables in the platforms without implicitly considering the biological response in the devices. Further, dynamic modelling and control methods have not been applied to the automatic extraction of information from blood research using microfluidic technologies.

In this thesis, it is proposed that dynamic modelling and control systems applied to simple microfluidic devices can lead to a robust and systematic approach to the measurement of platelet function under disturbed flow. The next section presents a review of the main methodologies that can be applied for these purposes.

### 2.4 Modelling of Dynamic Systems

Mathematical modelling is used across various disciplines, such as engineering, natural and social sciences, in order to study the interrelation of components in a system, or to be able to make predictions about future behaviour of a system. In particular, System identification is a term in control systems related to building mathematical models of dynamic systems from observed data. It enables the development of real world applications in the field of control systems [92]. System identification has foundations on classical statistical techniques, and some methods are equivalent to well-known statistical routines of Least Squares and Maximum Likelihood. Other fields with similar endeavours and common foundations include data mining [93], machine learning [94], econometrics [95].

In Section 2.3.3, it was suggested that System Identification can be used to extract compact information from extensive data generated from experimental assays, in order to be able to explain the relation between blood flow dynamics and platelet aggregation. Importantly, the obtained model parameters can then serve as a novel
and systematic diagnostics of platelet function that considers effect of disturbed
flow. This section reviews methods for mathematical modelling that can be applied
to the study of platelet aggregation in response to shear rate micro-gradients in
microfluidic devices.

2.4.1 System identification approach

The process of modelling dynamic systems is also known as system identification,
and its basic components are

1. **Observed Data.** This corresponds to information about the system provided
   by observed data, and prior insight on the behaviour of the system.

2. **Model structure.** Mathematical expression, typically in terms of a set of
   parameters, that represents the relationship between the observed variables.

3. **Model estimation.** Fitting of model to experimental data, i.e. calculation
   of model parameters.

4. **Model Evaluation.** This is usually a scalar measure of the success of a model
   to fit a particular set of observed data.

In brief, the identification process consists of finding a model structure that
best describes observed data according to a fitting criterion. This section first re-
views considerations for the design of experiments to generate experimental data
representative of the system dynamics. Various types of model structures, linear
and nonlinear, are then presented in terms of parameterised differential equations.
Next, methods for calculating the model parameters from empirical data are re-
viewed. Finally, criteria to select the validity and complexity of the models are
evaluated.
2.4.2 Collection of identification data

The identification process starts with the collection of experimental data from the relationship between the underlying variables. In this fashion, experiment design can be defined as the selection of the input $u(t)$, that produces the output $y(t)$, to collect a set of data $Z^N = \{u(1), y(1), \ldots, u(N), y(N)\}$ as informative as possible [96]. In some occasions the system includes a closed feedback loop, in which case recent techniques allow identification using inputs partly formed by output feedback [97, 98].

The input signal must be selected such that all the relevant properties of the system are exposed [99]. An important consideration is the spectral content of $u(t)$. If for example, a sinusoidal signal is selected as input of the system, then only one frequency will be tested. Many systems are characterised by the impulse response, or ideally white noise, however this is not always possible. Sequences of steps contain a broad range of spectral content and are commonly used in identification experiments [100]. For this reason, random binary sequences are commonly selected as the identification input in the case of linear systems, and random noise or multilevel sequences for nonlinear systems.

Another consideration when selecting the input signal is the frequency of the signal, or the duration of the signal at each different step in the sequence. The duration of such steps must be longer than the settling time of the system, so basic prior knowledge on the response time of the system is required. If the input sequence is too fast, the changes in the response will not be observed in the output.

Finally, sampling frequency is an important factor to take into account. A common selection of sampling frequency is ten times the bandwidth of the system, or approximately $5 - 7$ samples along the rise time of a step response [101].
2.4.3 Model structure

The model structure selection is often described as the main challenge in the identification process [102]. There are numerous kinds of model structures, linear and nonlinear, from simple and basic to complex and flexible. Methodologies for model structures have been colour coded as follows. *White-box models* is the case when a model is perfectly known from prior knowledge or physical insight [103]. *Grey-box models* is the case when some physical insight is available, and other parameters need to be extracted from experimental observations [104,105]. Finally, *Black-box models* do not make use of any physical insight, instead standard flexible model structures are employed [102,106]. Here, a review is presented on black-box linear and nonlinear model structures.

**Linear model structures**

First, a brief review on black-box **linear models** is presented. These models are easy to calculate and often reveal basic properties of the system under study.

The simplest dynamical model is the Finite Impulse Response (FIR) model

\[
y(t) = B(q)u(t) + e(t) = b_1 u(t-1) + \ldots + b_n u(t-n) + e(t)
\]  

(2.2)

where \(q\) denotes the delay operator, then the output is calculated as a linear combination of previous values of the input plus noise. The corresponding predictor \(\hat{y}(t|\theta) = B(q)u(t)\) is based on the regression vector

\[
\phi(t) = [u(t-1) \ u(t-2) \ldots u(t-n)]
\]  

(2.3)

Note that the noise term \(e(t)\) is not considered by this model, nor is considered the
effect of previous outputs.

A family of linear models, which considers the effect of noise, can be represented by the general equation

\[ A(q)y(t) = \frac{B(q)}{F(q)}u(t) + \frac{C(q)}{D(q)}e(t) \]  

(2.4)
in which special cases are the Box-Jenkins model \( (A = 1) \) is the most flexible model structure in this family of equations, it assumes no common characteristics between noise and input-output behaviour. On the other hand, the Auto-regressive moving-average with exogenous inputs (ARMAX) model \( (C = F = 1) \) assumes that the system and noise dynamics have common poles, giving a reasonable flexibility of the noise description. The output-error (OE) model \( (A = C = D = 1) \) calculates the prediction based on past inputs, so it concentrates on describing the input-output dynamics. Finally, the simplest model structure of this family is the Autoregressive model with exogenous input (ARX) model \( (F = C = D = 1) \), which gives a simple linear regression with easy calculation of model parameters \( \theta \).

Another slightly more complex linear model structure is the space-state model

\[
\begin{align*}
x(t + 1) &= A(x) + Bu(t) + K[y(t) - Cx(t)], \\
y(t) &= Cx(t) + e(t)
\end{align*}
\]

(2.5)
with the predictor \( \hat{y}(t|\theta) = Cx(t) \), and the states \( x(t) \) being the regressors. In comparison to the input-output regressors, state-space regressors are less restricted in their internal structure.

**Nonlinear model structures**

In reality, most systems are nonlinear, in other words, the output of the system is not directly proportional to the input.
Although the previously presented linear models have been extended to nonlinear black-box models [106, 107], identification of nonlinear systems remains an open question [92, 108]. Black-box nonlinear model structures are reviewed next.

For the case of nonlinear black-box model structures, a general nonlinear mapping function $g$ is introduced

$$\hat{y}(t|\theta) = g(\phi(t), \theta)$$

(2.6)

where $\phi(t) = \phi(Z^{t-1})$.

The nonlinear mapping function can be represented as function expansions

$$g(\phi, \theta) = \sum \alpha_k g_k(\phi)$$

(2.7)

and $g_k$ is referred to as *basis functions*.

A convenient choice of basis functions is to use dilated (scaled) and translated versions of the "mother function" $\kappa(x)$ [92]

$$g_k(\phi) = \kappa(\beta_k(\phi - \gamma_k))$$

(2.8)

then the basis functions are characterised by dilation parameters $\beta_k$, and translation parameters $\gamma_k$. Typical choices of basis functions include sigmoid and Gaussian bell functions. Popular named structures include wavelet networks [109, 110], one-hidden-layer sigmoidal neural networks [111], neuro-fuzzy models [112], and least-squares support vector machines [113].

Following a similar approach to the family of models in equation (2.4) nonlinear model structures can be built as nonlinear FIR (NFIR) (using only $u(t - k)$ as regressor), nonlinear ARX (NARX) (using $u(t - k)$ and $y(t - k)$), nonlinear OE (NOE) (using $u(t - k)$ and $\hat{y}_u(t - k|\theta)$), nonlinear ARMAX (NARMAX) (using
The nonlinear model structures presented above are able to model systems with very complex dynamics at the price of having a complex model structure with a large number of parameters. Another model approach is *Hammerstein-Wiener* structures [116, 117]. These present a hybrid model structure with a dynamic linear structure and input and/or output static nonlinearities. In this way, simple nonlinearities from sensors or actuators, such as saturations or dead zones, can be added to a simple linear model to represent a nonlinear system, whilst having a reduced number of parameters.

### 2.4.4 Model estimation

Once a model structure representing the dynamics of the system has been selected, and input/output data has been collected experimentally, the next step corresponds to fitting the model to the collected data, that is, the calculation of the model parameters. This process is independent of the model structure, and known as the Prediction Error Method (*PEM*) [99].

In *PEM* a model will be a predictor of the next output $y(t)$, given past observations $Z_{t-1}$, parameterised in terms of a parameter vector $\theta$ [101]:

$$\hat{y}(t|\theta) = g(\theta, Z_{t-1})$$  \hspace{1cm} (2.9)

then, a sequence of prediction errors is formed,

$$e(t, \theta) = y(t) - \hat{y}(t, \theta), \hspace{0.5cm} t = 1, 2, \ldots, N$$  \hspace{1cm} (2.10)
and finally, minimisation of the sum of errors in the sequence is formulated as

\[
\hat{\theta}_N = \arg \min_\theta \frac{1}{N} \sum_{t=1}^{N} e_F(t, \theta)
\]

(2.11)

where \(e_F\) is the error signal after being filtered by a stable linear filter.

Search methods for the minimisation of equation (2.11) include the subspace Gauss-Newton direction, or in some ill-conditioned problems the Levenberg-Marquardt search algorithm is recommended [118], as optimum in the search of nonlinear spaces [119,120].

2.4.5 Model evaluation

Several analytical criteria have been established in order to penalise model complexity and quantify the deterioration of fit. The fit is usually measured as the mean square error between the measurements and the predicted signal. To assess the complexity of the model, a criterion evaluates the ability to fit estimation data at the same time that it evaluated the dimensionality of the model parameters.

The most common criteria are the Akaike’s Information Criterion (AIC) [121]

\[
\hat{V}_N(\theta, Z^N) = \left(1 + \frac{2 \dim \theta}{N}\right) F(\hat{y}(t|\theta))
\]

(2.12)

where

\[
F(\hat{y}(t|\theta)) = \frac{1}{N} \sum_{t=1}^{N} \epsilon^2(t, \theta)
\]

(2.13)

and Rissanen’s Minimum Description Length criterion (MDL) [122]

\[
\hat{V}_N(\theta, Z^N) = \left(1 + \frac{2 \log N \dim \theta}{N}\right) F(\hat{y}(t|\theta))
\]

(2.14)
A further criterion is the Generalised Cross-Validation (GCV) \[123\]

\[
\hat{V}_N(\theta, Z^N) = \frac{1}{\left(1 - \frac{\dim \theta}{N}\right)} \sum_{t} F(\hat{y}(t|\theta))
\] (2.15)

These criteria can assist with the formulation of model structures, that can represent the dynamics of a system, with the minimum number of parameters possible.

### 2.4.6 Summary

This Section has presented a review of the main components of System Identification. This methodology is extensively used to describe through mathematical equations the relationship between the variables of a dynamic system using observed data. In particular, the selection of model structure was investigated in detail as this is the most critical component of this methodology. Although the selection of very flexible structures, such as Neural Networks, could fit the dynamics of many complex nonlinear systems, the interest of this research is to obtain a reduced number of parameters which can be interpreted for diagnostics of platelet function.

The approach to finding a suitable model structure adopted by this thesis is to first attempt canonical black-box model structures, and use information criteria to determine basic properties of the system such as the order, time delay, and nonlinear behaviours. Then, more tailored and compact grey models can be formulated to represent knowledge from both experimental data and physical insight on the dynamics of the system to be modelled.

### 2.5 Feedback Control Systems

The inclusion of control systems can provide with a robust and optimum operation of microfluidic devices, as proposed in Section 2.3.3. This section presents a brief
introduction to the concept of feedback control, and reviews the main nonlinear
control methodologies that can be applied for the regulation of platelet aggregation
in a microfluidic device.

2.5.1 Feedback control preliminaries

A control system can be defined as the collection of methods and components added
to a system, established to achieve a desired response in the presence of external
disturbances [124]. In general, there are two applications for control systems: track-
ing and regulation. In the first case, the aim of the control algorithm is to track, or
follow, a specified dynamic trajectory. An example of this type of control system is
a robotic system programmed to grip and transport an object to an assigned loca-
tion. In the second case, the control system is called a regulator, whose function is
to maintain a physical quantity within specified limits. An example of this form of
control system is the thermostat.

Open- and closed-loop operation of control systems

The two basic modes of operation of a control system are open- and closed-loop. A
block diagram presenting the relationships among the variables and processes that
comprise these two types of control system operation is illustrated in Figure 2.5.
In open-loop mode, the control algorithm transforms the input reference signal $r(t)$
into the control action signal $u(t)$, which affects the plant, or controlled system,
hence altering the system output $y(t)$. Concurrently, external disturbances $z(t)$ also
affect the plant behaviour. This is a key limitation of open-loop control systems.
These can perform acceptably provided that the external conditions do not affect
the system considerably.

The closed-loop operation of a control system, as shown in Figure 2.5 b), can
overcome the limitation mentioned above, by reinserting into the system results of
the past performance. In this fashion, the output signal $y(t)$ is measured into the feedback signal $y_m(t)$ and subsequently subtracted from the reference input $r(t)$ to form the error signal $e(t)$. The error signal is then used by the control algorithm to compensate for any changes caused by external disturbances.

![Block diagram of open-loop a) and closed-loop b) control systems.](image)

**Figure 2.5:** Block diagram of open-loop a) and closed-loop b) control systems.

### Stability of dynamical systems

Stability is one of the most important properties to evaluate in any control system. This section presents definitions of stability in nonlinear control systems, and introduces the Lyapunov stability approach [125] to make conclusions about the trajectories of a system.

Consider the system

$$\dot{x}(t) = f(x, t) \quad (2.16)$$

where $x \in \mathbb{R}^n$, $\mathbb{R}_+ = \{x \in \mathbb{R} : x \geq 0\}$, and the function $f : \mathbb{R}^n \times \mathbb{R}_+ \rightarrow \mathbb{R}^n$ is such that a well-defined solution to the differential equation exists. Denote this solution at time $t$ as $x(t, x_0)$ where $x_0$ represents the initial conditions at $t = 0$. 
Suppose that $f(0, t) = 0$ for all $t$.

**Stability** The origin of equation (2.16) is said to be **stable** if given any $\epsilon > 0$ there exists a $\delta > 0$ such that when $\|x_0\| < \delta$ then $\|x(t, x_0)\| < \epsilon$ for all $t > 0$.

In other words, this means that by starting close enough to the equilibrium point, the solution will thereafter always remain arbitrarily close.

**Asymptotic stability** The origin of equation (2.16) is said to be **asymptotically stable** if it is stable and the solution $x(t, x_0) \to 0$ as $t \to \infty$.

An approach for studying the stability of nonlinear systems is the **Lyapunov method**. In simple terms, this method establishes that if a differentiable function $V : \mathbb{R}^n \to \mathbb{R}$ can be found which is positive except at an equilibrium point, and whose total time derivative decreases along the system trajectories, then the equilibrium point is stable. The importance of stability methods is that these methods eliminate the need to solve the nonlinear differential equation when assessing its stability properties.

When dealing with uncertain systems, it may not be possible to guarantee asymptotic stability. Consider the nonlinear system in equation (2.16) and suppose it is subject to an imprecisely known exogenous signal $\xi(\cdot)$ so that

$$\dot{x}(t) = f(x, t, \xi)$$

Let $\epsilon \subset \mathbb{R}^n$ be a bound set, then the following definition can be made.

The solution of $x(\cdot)$ to the uncertain system in equation (2.17) is said to be **ultimately bounded** with respect to the set $\epsilon$ if

- on any finite interval the solution remains bounded, that is if $\|x(t_0)\| < \delta$ then $\|x(t)\| < d(\delta)$ for any $t \in [t_0, t_1)$
• in finite time the solution \( x(t) \) enters the bounded set \( \varepsilon \) and remains there for all subsequent time

The set \( \varepsilon \) is usually an acceptable small neighbourhood of the origin and the concept is often termed practical stability [126].

The Lyapunov theorem states that if the Lyapunov function \( V(x, t) \) is positive definite and decrescent (\( -\dot{V}(x, t) \) is positive definite) then the system is globally uniformly asymptotically stable.

\[
\|x(t_0)\| < \delta \Rightarrow \lim_{t \to \infty} \|x(t)\| = 0
\]  

(2.18)

2.5.2 Nonlinear control methodologies

The use of nonlinear control algorithms is becoming widespread in the recent years, in part due to the vast improvement of computing hardware [127, 128]. Nonlinear control methods have several advantages over traditional linear control algorithms. The main disadvantage of linear control methods is that these rely on the assumption of small range operation. However, when the required operation range is large, a linear controller is unlikely to perform acceptably or present stability. A second major assumption of linear control methods is that the system model can be linearised. Nevertheless, various types of “hard nonlinearities” do not allow linear approximation.

On the other hand, nonlinear controllers can present an easier design and implementation process than linear counterparts. In addition, nonlinear control algorithms are often closely related to the underlying principles of the systems to be controlled. Control systems engineering is a vast field with many strategies developed to date [129–131]. This Section aims to review some of the main methodologies of nonlinear control systems and highlight their applications.
CHAPTER 2.

**Feedback linearisation**

Feedback linearisation is based on the idea of transforming the nonlinear dynamics of a system into a linear form by using state feedback [128,132]. This methodology is limited to certain nonlinear systems. For example, feedback linearisation is not applicable when the relative degree of the system is not defined and lacks systematic global results [127]. In addition, this methodology requires full measurement of the system state, which is not always possible in nonlinear systems. Finally, robustness can not be guaranteed in the presence of parameter uncertainty or unmodelled dynamics. Despite these limitations, this methodology is currently used in practical control applications such as wind power systems, three-phase Uninterruptible Power Supply (UPS) inverter systems, and wheeled mobile robots [133–135].

**Adaptive control**

Adaptive control is a methodology that deals with uncertain dynamic systems, in particular, parameter-uncertain systems [136–138]. Most existing methods require linear parametrisation of the control law or the system dynamics. In Model Reference Adaptive Control the adaptation control law extracts parameter information from tracking errors [139–142]. In self-tuning controllers, the parameter estimator extracts information from prediction errors [143]. Recent hybrid techniques consider an adaptation law which extracts parameter information from both sources, leading to faster adaptation [144,145]. Adaptive control has been applied to various fields including signal processing, power systems, and aircraft systems [146].

**Intelligent control**

Intelligent control is a hybrid approach to control systems based on Fuzzy Logic, Neural Networks and Genetic Algorithms [147,148], as an alternative to traditional mathematical model-based control approaches. These methodologies facilitate the
inclusion of various types of information including numerical empirical (obtained from experimentation with no known cause-effect relation), and qualitative information (e.g. linguistic). Fusion of these methods, plus the optimisation power of evolutionary computation, has enabled the development of flexible systems to perform in several situations with acceptable degrees of efficiency, enhanced adaptive performance, and able to deal with modelling imprecision and uncertainty [112,149–151]. Intelligent control systems have been of great utility to medical equipment [152], robotics and unmanned systems [148], and prognostics and diagnostics [153].

**Sliding control**

Sliding Mode Control (SMC) is methodology that provides a robust systematic approach to the issue of maintaining stability in the presence of modelling imprecisions [154]. The main objectives of these controllers are, firstly, to design a control strategy to account for parameter uncertainty and the presence of unmodelled dynamics, and secondly, to quantify the resulting modelling/performance trade-offs [126,155,156]. In theory, a very high performance is obtained with SMC in the presence of arbitrary parameters inaccuracies given the available bandwidth. In practice, this corresponds to a high frequency switching at the actuator, known as chattering, which can be replaced by a smooth approximation. In some applications in which chattering is acceptable, such as electric and communications systems, pure switching control from SMC can generate extremely high performance. SMC has been applied to numerous systems including electric drives [157], general electromechanical systems [158,159], biomedical systems [160], and chaotic systems [161].

**Summary**

An introduction to the basic concepts of control systems engineering has been presented as background for this thesis. Two of the main nonlinear control strategies
that can provide robustness to model uncertainties are Adaptive Control and Sliding Mode Control. Whilst the former presents excellent performance upon model parameter-related uncertainty, SMC provides robustness to both, unstructured (unmodelled dynamics) and structured (parameters) uncertainties. Thus, SMC would seem best suited to the highly complex dynamics of blood clotting studied in this thesis. However, dealing with the effect of chattering is an important consideration to take into account with the use of SMC. The next Section presents a brief introduction to the concepts of SMC and a method that can overcome the limitation posed by the chattering effect.

2.5.3 Introduction to sliding mode control

Sliding mode control is a robust nonlinear control methodology conceived to deal with the uncertainty of unmodelled dynamics, inherent to the approximation of complex systems by simple mathematical models. SMC is a form of Variable Structure Control Systems (VSCS), characterised by adopting different control actions, based on decision rules (switching functions) and the state of feedback signals.

The main idea of SMC is to drive and then constrain the system state to lie within a neighbourhood of the switching function [126]. This offers the advantage of tailoring the dynamic behaviour of the system by the choice of switching function, and presenting a closed-loop response which is insensitive to a class of uncertainty.

Consider the single-input control system

\[ \dot{x} = f(x, u), \quad x \in \mathbb{R}^n \]  \hspace{1cm} (2.19)

The design of SMC has two main components:

1. Define a switching manifold which prescribe the desirable dynamical properties \( s(x) \)
2. Design a discontinuous control $u(x)$ which will make the switching manifold attractive to the system state, that is

$$
u(x) = \begin{cases} 
  u_{\text{max}} & s(x) > 0 \\
  u_{\text{min}} & s(x) < 0
\end{cases} \quad (2.20)$$

such that

$$\lim_{s \to 0^+} \dot{s} < 0, \quad \text{and} \quad \lim_{s \to 0^-} \dot{s} > 0 \quad (2.21)$$

The concepts of sliding surface and sliding mode control are illustrated in Figure 2.6. The sliding surface (blue) corresponds to the switching manifold $s(x) = 0$ containing the desired state trajectory (red). The sliding mode control guarantees that, starting from any initial condition, the system will reach the sliding surface in a finite time. Then, the state trajectory will slide along the surface towards $x_d$ exponentially.

Since the real implementation of the SMC includes imperfections such as delays, hysteresis, and unmodelled dynamics, the system oscillates in the neighbourhood of sliding surface in a chattering motion. A mathematical construct was proposed by Filippov [162] to find the “average” of the solutions obtained from approaching a point of discontinuity from different directions, known as the equivalent control. If $x_0$ is a point of discontinuity on the surface $s(x)$ and $f^+(x_0, t)$ and $f^-(x_0, t)$ represent the limits of $f^0(x_0, t)$ as the point $x_0$ is approached from opposite sides of the tangent plane to $s(x)$, then the solution

$$f^0(x_0, t) = \alpha f^+(x_0, t) + (1 - \alpha) f^-(x_0, t) \quad (2.22)$$

where the scalar $0 < \alpha < 1$ is such that the vector $f^0$ is tangential to the manifold $s(x)$.

In summary, this Section introduced the main concepts of SMC, and presented
2.6 Conclusion

A review of the state of the art in the application of modelling and control systems to microfluidic technologies in the context of blood research was presented in this chapter in order to refine the objectives of this thesis. It was found that considering the effect of disturbed flow is of great importance to the diagnostics of platelet function. Microfluidic devices have demonstrated the ability to recreate scenarios of physiological blood flow, and allow for precise studies of the effect of flow properties on platelet function. A review on current microfluidic devices for shear rate-dependent platelet aggregation studies found that the prevailing approach is

Figure 2.6: Geometrical interpretation of sliding mode control and equivalent control (in Flippov sense).

the equivalent control as a method to overcome the high-frequency chattering in the ordinary SMC method.
the simultaneous generation of multiple conditions of shear rate in the devices. Two main issues were identified in this approach. To begin with, inspection of multiple microfluidic channels implies the use of additional hardware to position the various inspection areas under the field of view of the imaging systems. In addition, it has been shown that the forming aggregates generate build-ups of pressure capable to alter the pre-set flow conditions on the other microchannels. Furthermore, and most critically, post-processing of experimental results can be time consuming, as results are often sets of data of considerable size and large percentage of variation, and can require the expertise of specialist personnel with somewhat subjective skills.

On the basis of this review, the inclusion of control systems in microfluidic devices that can provide a robust high-throughput assay to accurately measure flow dependent platelet function was chosen as the focus of this thesis. The development of such an assay has the potential to enable a tailored point-of-care diagnostics approach to antithrombotic therapy with the prospect to reduce morbidity significantly.

Finally, a survey was presented on modelling and control methodologies that can be applied to provide a robust and systematic operation of microfluidic devices for the diagnostics of platelet function. System identification was reviewed as a method to obtain a systematic relationship between variables of a dynamic system. These relationships are represented through mathematical models obtained from both, experimental and conceptual information from the system. In addition, robust feedback control algorithms able to regulate complex dynamics with a large degree of uncertainty were introduced and surveyed. Sliding mode control was chosen as a suitable methodology to deal with the regulation of the complex and highly variable platelet function from different blood phenotypes.
Chapter 3

Observation of platelet aggregation dynamics under disturbed flow in a microfluidics device

3.1 Introduction

The ultimate goal of this thesis is to develop a robust, conclusive and potentially automatic means of quantifying the properties of platelet function, as discussed in Chapter 2. To begin pursuing this goal, this chapter investigates methods to obtain feedback information of the dynamics of platelet aggregation response in a microfluidics device recreating a scenario of disturbed blood flow.

From all the microfluidics platforms reviewed in Section 2.3, a platform recreating realistic model of arterial injury was selected for this thesis to observe the dynamics of platelet aggregation under disturbed flow conditions [33]. Advantages of such platform include the representation of physiological and pathological flow profiles (no recirculation), and stable flow conditions. Furthermore, the injury model contained in such platform is more realistic than sphere models and spot-injury models in other platforms [49,51,163].
This chapter is organised as follows. Section 3.2 introduces a microfluidic device employed for the investigation of platelet aggregation under disturbed flow, and evaluates current measures for the monitoring of platelet aggregation. Next, Section 3.3 goes on to present methods which improve the quality of feedback measurements representative of the dynamics of platelet aggregation. Moreover, it is explored whether additional insight into the aggregation of platelets can be gained through the examination of novel measures including the examination of both the size of the thrombus and also its density as indicated by fluorescent intensity. Following, Section 3.4 proposes a mechanistic model of thrombus formation where four distinct stages observed experimentally are identified, and an explanatory physical model is formulated. Finally, discussion and conclusions from the investigations of this chapter are presented in Sections 3.5 and 3.6 respectively.

3.2 Microfluidics Platform to Study Platelet Aggregation under Disturbed Flow

Microfluidic technologies present evident advantages over traditional platelet diagnostics technologies, such as parallel-plate chambers and cone and-plane viscometers [164], including remarkable control of blood flow parameters, the ability to perform multiple experiments with small blood sample volumes, and high throughput [165]. Furthermore, owing to the enhanced precision, complexity and parallelism that is possible with these platforms, there is the potential to evaluate multiple aspects of platelet function and thrombus formation simultaneously [166].

Several microfluidic devices for the study of shear dependent platelet aggregation [33,43,45,46,51,54,55,167–169] were surveyed in Chapter 2, and it was concluded that the platform reported in [33] presents the most realistic representation of the disturbed flow found in scenarios of severe vessel damage. Another advantage in
using this platform is that it only requires a single syringe driver for its operation, as opposed to the required micro-positioners in parallel channel devices. Generation of different shear rate conditions is performed by dynamically regulating the perfusion rate of the syringe driver. This section aims to introduce the operation of the microfluidics device employed for this study, in Section 3.2.1, and evaluate the traditional methods employed for the analysis of the aggregation response, in Section 3.2.2.

3.2.1 Experimental methods

A microfluidics device was designed to provide precisely controlled shear rate micro-gradients and allow real-time monitoring of the resulting aggregation of platelets. Figure 3.1 illustrates a diagram of the components of the device, as well as a photograph of the platform during a blood perfusion experiment.

Blood was perfused through the device using a syringe pump (Harvard PHD-2000), and the forming aggregate of platelets was monitored using fluorescence microscopy as described in [33]. A diagram of the device is presented in Figure 3.1 a), where [A] shows the reservoirs (250 µL each) where anticoagulated whole blood was deposited into. Each reservoir was connected to a microfluidic channel at [C], passing through a filter designed to block undesired inclusions at [B]. The negative pressure exerted in the channel exhaust at [E] induced blood flow from the reservoir into the microchannel. The blood was then funnelled into the main channels and perfused through six replicas of microcontractions, mimicking a scenario of arterial stenosis at 80%. Finally, waste was collected through a bore exhaust connected to the end of the microchannel at [D]. The chip was fabricated using a cast of a biocompatible organic polymer, Polydimethylsiloxane or PDMS, on a mould of negative photoresist (KMPR from Microchem®) using standard photolithographic processes. A photograph of the resulting device is presented in Figure 3.1 b).
Figure 3.1: Microfluidics platform for real-time monitoring of platelet aggregation under disturbed flow. a) Schematics of the microfluidics chip comprising blood reservoirs [A], undesired particles filters [B], interrogation area containing six parallel microcontractiones [C], waste channels [D], and bore exhaust to connect syringe drivers [E]. Close-up of microcontraction geometry [F]. b) Photograph of the microfluidics platform with one of the microchannels operating blood perfusion.

The shear rate near the wall at a point upstream and distant from the microcontraction, where the fluid travels through a uniform rectangular cross section in the $xy$-plane as shown in Figure 3.1 a), was calculated by taking a discrete derivative near the wall of the velocity profile for a Newtonian fluid in rectangular geome-
CHAPTER 3.

tries \cite{170}: 
\begin{equation}
V_x(y, z) = \frac{12Q}{ab\pi^3} \sum_{i=1,3,...} \frac{1}{i^3} \cos \left( \frac{i\pi y}{2a} \right) \left( \frac{1 - \cosh \left( \frac{i\pi z}{2b} \right)}{\cosh \left( \frac{i\pi z}{2b} \right)} \right) \tag{3.1}
\end{equation}

where $Q$ is the flow rate in the channel, $a$ and $b$ are half the channel height and width, respectively; the direction of the flow is $x$. The dimensions of the channels were fixed at $a = b = 100 \, \mu m$, and the flow rate $Q$ was adjusted to obtain the desired shear rate.

In order to isolate the rheological effects of blood flow under disturbances from the major blood-born chemical pathways of platelet activation ($ADP$, $TXA_2$ and $thrombin$), blood was pre-treated with pharmacological inhibitors of the canonical platelet amplification loops (Amplification Loop Blockade ALB) for 10 min prior to perfusion: Indomethacin ($10 \mu M$), Apyrase ($0.02 \, U \, ml^{-1}$), MRS2179 ($100 \mu M$), 2-MeSAMP ($10 \mu M$), and Hirudin ($800 \, U \, ml^{-1}$)) \cite{21}. ALB materials were obtained from Sigma-Aldrich Corporation.

Lipophilic membrane dye DiOC6 ($1 \mu g \, ml^{-1}$), from Molecular Probes, was used to label platelets and an imaging system, comprising of an inverted microscope (Leica DMIRB) and a CCD camera (Hamamatsu Orca ER), monitored the epi-fluorescence illumination of the aggregates of platelets over time, at one frame per second. The size of the forming aggregates was estimated frame-by-frame in Matlab\textsuperscript{®} by thresholding the brightness of video, and calculating the area of pixels above a minimal value. The experiments were carried out in the laboratories of the Australian Centre for Blood Diseases under approval from the Monash University Standing Committee on Ethics in Research Involving Humans.

### 3.2.2 Blood perfusion results

Blood perfusion experiments using the microfluidic device in Section 3.2.1 were carried out at a physiological shear rate $\gamma = 1800 \, s^{-1}$ in order to observe the devel-
 CHAPTER 3.  

opment of shear-driven platelet aggregation, and evaluate these observation using traditional measurements.

Figure 3.2 a) presents images of the shape of the resulting platelet aggregate in the device. The aggregate appeared to grow proportionally in the $x$ and $y$ directions at the start of the perfusion, $t < 3.5\,\text{min}$, commencing the formation at the zone of deceleration near the downstream tip of the stenosis. After the formation of the thrombus there was a disproportionate growth of the aggregate for $3.5 < t < 6.5\,\text{min}$ where the growth was larger in the $y$ direction than in $x$. The size of the aggregate presented small variations for $t > 6\,\text{min}$. When the aggregate reached the bottom wall, it continued to grow in the horizontal direction, $x$, with regular undulations once parts of the aggregate were detached.

The corresponding plot of the total aggregation size, found employing a simple threshold in intensity of the fluorescent images, is illustrated in Figure 3.2 b). The figure shows the presence of an initial delay in the aggregation response, $t < 1.5\,\text{min}$. Following, there is a steep increase in the size of the area for about $2\,\text{min}$. After, the aggregate size oscillates between $1000 < A(t) < 2000\,\mu\text{m}^2$ due to the sudden loss of platelets near the bottom wall, as observed in the last images of Figure 3.2 a).

The aggregation plot in Figure 3.2 b) is typically analysed in terms of parameters such as lag time, and size of the aggregate at the end of the experiment (end point), maximum size, and size of the aggregate during the plateau. While there is large variability in the first three parameters, the size of the aggregate during the plateau is ill-defined and somewhat subjective. Determination of the start and end of the stage of plateau, as well as the calculation of a representative value of this stage, is not straightforward. Furthermore, these parameters do not take into consideration important features from the dynamics of the system such as the time response of the acute growth phase, and the gradual growth observed during and after the plateau.

Observation of the fluorescence images in Figure 3.2 a) indicates an increase in
Figure 3.2: Platelet aggregation results under disturbed flow at physiological constant shear rate (γ = 1800s\(^{-1}\)). a) Image sequence showing the shape of forming thrombus over time for a blood perfusion at physiological shear rate. b) Plots of the size of aggregate over time estimated as the area of pixels above a minimum threshold.

In summary, this section showed that observation platelet aggregation dynamics
using a simple threshold leads to the inclusion of noise from various parts of the platform in the field of view different to the main platelet aggregate, such as pixels corresponding to the microchannel walls, and secondary aggregates growing in the top wall of the device. An algorithm to detect the main developing aggregate in the platform, and reduce noise in the measurement is required. Such endeavour is explored in the next section.

3.3 Enhanced Feedback Measurements of Platelet Aggregation

A survey of the measures used by current microfluidic platforms for the study of platelet adhesion/aggregation was presented in Chapter 2. Such survey found that platforms for platelet adhesion studies primarily use measures such as surface coverage and total fluorescence [43, 45, 46, 169], whereas platforms dedicated to the study of bulk aggregates of platelets are often represented by aggregate size [33, 50, 51]. Section 3.2 presented the observation of the development of platelet aggregation through aggregate size, applying a single threshold on the fluoresce images from blood perfusion. This resulted in the inclusion of noise in the measurement from other areas of the platform different than the main platelet aggregate.

This section aims to develop parameters to represent the dynamics of the main platelet aggregate forming in the microfluidics platform. It is also investigated whether additional information can be obtained from measurements of intensity, area, as well as width and height of the main forming thrombus near the stenosis model. First, an algorithm is developed in order to segment the main aggregate in the images, and reduce the noise caused by the intensity of the microchannel walls. After, several measures from the main aggregate are calculated and their value in providing feedback on the dynamics of the process is evaluated.
3.3.1 Detection of main aggregate in fluorescence images

An image processing algorithm was developed in order to detect the main aggregate and reduce the amount of noise from reflection and gradual increase of intensity in the microchannel walls.

![Image Processing Algorithm](image)

**Figure 3.3:** Image processing algorithm for detection of main platelet aggregate in test microchannel.

The proposed algorithm is summarised in Figure 3.3, and explained as follows. Initially, the epifluorescence image was acquired by a high sensitivity camera using a magnification of 40, and saved using a grey image format with 16 bits. In the next step, the area of interest was cropped from the image, as aggregation is only expected to occur downstream of the stenosis, and the microplatform position is
fixed during the experiment. The image was then binarised (converted to black and white) using an adaptive threshold calculated as

\[
thr(I) = \begin{cases} 
0.2 & \text{if } 0.5\max(I) < 0.2 \\
0.5\max(I) & \text{if } 0.5\max(I) \geq 0.2 
\end{cases} \tag{3.2}
\]

where, \( I \) is the cropped image \((n \times m \text{ pixels})\), with normalised intensity. The lower saturation limit, \( thr(I) = 0.2 \), was determined experimentally as the noise floor found in the experiments. The calculation of the threshold value \( thr(I) = 0.5\max(I) \) was obtained empirically. This relation was found by trial and error by measuring the maximum intensity in the aggregate, usually found in the centre of the aggregate, and comparing this value with the intensity in the edges of the main aggregate. The factor of 0.5 was found to be a good approximation between the two intensities. In the following step, segmentation of the main aggregate was performed by first applying morphological operations of closing, to reconstruct the shape of the aggregate using discs of \( 3\mu m \), and then filling the holes inside the main detected aggregate to create a solid object. Following, the main aggregate was defined as the largest connected object in the vicinity of the stenosis. Finally, the measurements \textit{mean intensity}, \textit{area}, \textit{width} and \textit{height} were extracted from such object.

Figure 3.4 a) illustrates the results of the steps in the algorithm presented in Figure 3.3 to detect the main aggregate in a blood perfusion at a physiological shear rate (\( \gamma = 1800 \text{ s}^{-1} \)). The image in the final step shows the detection of the main aggregate, and the exclusion of other areas with high intensity such as microchannel walls, and secondary aggregates (top wall of the microchannel). Figure 3.4 b) shows the resulting aggregate detection for a perfusion experiment of 10 \textit{min} duration. The adaptive threshold feature of this algorithm enabled a precise detection of the main aggregate at all stages of its formation.

Results from the detection of the main thrombus, shown in Figure 3.4 b), demon-
strate the convenience of this algorithm to detect early dynamics of platelet aggregation, which are hard to detect using static user-defined thresholds. The segmentation of the main thrombus enabled the analysis of not only the total size of the aggregate but also its geometry. The representative parameters of the detected main aggregate will be investigated in the next section.

![Sequence of epi-fluorescent images of blood perfusion under physiological conditions (\(\gamma = 1800 \, s^{-1}\)) and detected main aggregate - white boundary. a) Results from steps in the algorithm presented in Figure 3.3. \(A = \text{area}, \, I = \text{mean intensity}, \, h = \text{height}, \, w = \text{width}\). b) Results of aggregate detection for complete duration of blood perfusion (10 min).](image)

3.3.2 Representative parameters of dynamic platelet aggregation

This section presents the results from the measurements from the main aggregate detected in Section 3.3.1, and evaluates the information these provide on the dynamics of this process. First, evaluation of the information provided by measures of intensity and size of the aggregate is investigated. The geometry of the forming ag-
aggregate is then examined by measuring the growth of the aggregate in the horizontal (direction of flow) and vertical directions.

**Mean intensity and area of the main detected platelet aggregate**

Results from the measurements *mean intensity* and *area* from the algorithm, presented in Section 3.3.1, are illustrated in Figure 3.5.

While Figure 3.5 presents evidence of the correlation between *area* and *mean intensity*, the figure also presents clear differences in the dynamics of each measurement. The first discrepancy occurs during $2 < t < 5 \text{min}$, where the size of the aggregate, represented by the *area*, presents a continuous growth after the initial onset, while the *mean intensity* measure presented two segments; a slow growth for $2 < t < 4 \text{min}$, and a faster growth during $4 < t < 5 \text{min}$. A second difference between the two measures is evidenced between $5 < t < 8 \text{min}$, where the *area* measure appears to remain constant, however the *mean intensity* has a significant rate of growth at this time.

Images from the stage of plateau, $5 < t < 8 \text{min}$, are shown in Figures 3.5 b)-d). These compare aggregates of approximately equal area at different times of the experiment. Figures 3.5 b)-d), show the intensity of the aggregates increases over time, which suggests that for this event to be possible, a higher density of platelets must have been achieved. This finding indicates the possibility of a process of platelet densification during the stage of plateau of aggregate growth, which may be required to further enhance the aggregation response.
Figure 3.5: Mean intensity and area measures of platelet aggregate for blood perfusion at physiological shear rate ($\gamma = 1800\text{s}^{-1}$). a) Plot of Size (represented by area) vs mean intensity. b) - d) Snapshots of platelet aggregate fluorescence with approximately equal size and increasing intensity.
CHAPTER 3.

Width and Height of the main detected platelet aggregate

The width (in the direction of flow) and the height of the aggregate growth appeared to be asymmetrical, as shown in Figure 3.4. This section presents the measure of these parameters in order to establish the dynamics of the morphology of the platelet aggregate formation in the microfluidics device.

Figure 3.6 illustrates the width and height of the main detected aggregates resulting from a blood perfusion at physiological shear rate. It can be seen that for $1 < t < 2 \text{ min}$ the height of the aggregate grew rapidly in comparison to the width, indicating the importance of an initial platelet monolayer formation along the wall of the stenosis.

Then, during $2 < t < 5 \text{ min}$ both the height and the width grew at similar rates and the shape of the platelet aggregate was almost semi-spherical until the aggregate reached the streamlines of high velocity. For $t > 5 \text{ min}$ the growth in the horizontal plane was limited by the high shear of the fast streamlines coming out of the contraction zone, and the only measure to grow slightly was the height.

These results suggest that the measure of height of the aggregate in particular, can provide information on the initial stages of platelet aggregation. This indicates that a particular area of the stenosis wall needs to be covered by a monolayer of platelets prior to a full development of the aggregate. The following stages of the aggregate formation seem to be equally represented by either of these two measures. Further, the area of the aggregate would intrinsically contain the information provided by the width and height measures.
**Figure 3.6:** *Width* and *height* measures of platelet aggregate of blood perfusion at physiological shear rate ($\gamma = 1800 \text{s}^{-1}$). a) Plot of *width* vs *height* of the main aggregate during 10 min of blood perfusion. b) Snapshots of platelet aggregate fluorescence showing the initial growth of the aggregate along the stenosis wall. c) Subsequent proportional growth in vertical and horizontal directions. d) Fully grown thrombus presents sudden episodes of micro-aggregate ejections in the flow direction.
3.3.3 Discussion of enhanced feedback measurements of platelet aggregation

Segmentation of the main platelet aggregate in the fluorescence images resulted in measurements with reduced noise from other objects in the platform, such as intensity from the microchannel walls and secondary aggregates formed in places different to the stenosis. This made possible the detection of the formation of the platelet aggregate throughout its complete development, from its early stages until the aggregate reaches a full size.

By analysing the height of the aggregate it was found that a certain extent of the area in the stenosis wall has to be covered by a monolayer of platelets before the aggregate can start growing in the direction of the flow. In addition, the mean intensity measure provides information on the increase of platelet density of the aggregate. These results anticipate that platelet densification is required for the development of a phase of enhanced aggregation that consolidates the aggregate.

Overall, measurement of the area of the aggregate provides significant information that describes the growth dynamics of platelet aggregation. On the other hand, the mean intensity can complement the previous measure with information on platelet density at the early stages of thrombus formation. However, intensity measurements can suffer saturation effects when the aggregate reaches larger sizes. The next section summarises all these observations, and attempts to explain the physical relation between the variables involved, into a conceptual model of platelet aggregation.
3.4 Mechanistic Model of Platelet Aggregation under Disturbed Blood Flow

This section proposes a mechanistic model of platelet aggregation within the microfluidics platform presented in Section 3.2 emulating a thrombogenic stenosis in an environment independent of chemical pathways and under non-recirculating conditions. This work identifies four distinct stages observed experimentally in the formation of thrombus and formulates an explanatory physical model. In addition, this section investigates whether additional insight into the stages of platelet aggregate formation can be gained from examination of the measures developed in Section 3.3, including the area of the platelet aggregate and its density as indicated by fluorescent mean intensity. Once characterised rigorously, this model will be useful to gain more insight in the mechanistic variables regulating platelet aggregation.

3.4.1 Experimental trials at constant physiological shear rate

Several experimental trials \((n = 6)\) were performed using a constant shear rate \((\gamma = 1800 \, s^{-1})\) and blood from a healthy donor. Results showed a consistent trend in the aggregation, in all experimental trials, in which we identified four distinct stages in the growth of the thrombus as illustrated in Figure 3.7 a). An initial recruitment of platelets occurred after a lag time of approximately 2 min (Stage I). Then, the thrombus presented acute growth, lasting approximately 3 min, (Stage II), followed by a plateau (Stage III) of about the same duration. Finally (Stage IV), the size of the thrombus presented regular undulations accompanied by the ejection of micro-aggregates.

Figure 3.7 b) shows snapshots of the video signal showing the shape that the thrombus takes over time. A correlation between Figures 3.7 a) and b) found that while the size of the thrombus appears to remain almost constant during Stage III, a
measurement of its average intensity, as shown in Figures 3.5 a) and b) respectively, indicated an increase in the platelet density over time. The rate of growth in Stage II appears to be parabolic as the thrombus grows radially (vertically and laterally), and in Stage IV appears to be linear as the thrombus only grows in the lateral dimension.

3.4.2 Four-stage mechanistic model

From the observations in Section 3.4.1, a mechanistic model identifying four stages in the formation of platelet aggregation is proposed, as illustrated in Figure 3.8. The model suggests that the initial recruitment of platelets to form an initial monolayer is driven by kinetic absorption of von Willebrand Factor in the microchannel, influenced by shear, and such monolayer has a low density of platelets. Thereafter, a rapid aggregation starting to form downstream of the apex of the microcontraction is observed, characterised by vertical and lateral growth. This aggregation takes place in the low shear region near the wall and appears to slow down as the aggregate perimeter reaches flow streamlines of higher shear (at the end of Stage II). At this point the thrombus growth seems to plateau, however, platelet recruitment continues with the hydrodynamics forcing the platelets to group together into a denser thrombus.

At the end of Stage III, the thrombus begins to grow again, and this mechanistic model suggests that once a critical density is reached, the advective flow within the thrombus is minimal, and so, the chemical environment of the main thrombus can diffuse and sustain aggregation, allowing the formation and ejection of micro-aggregates - embolisation past the initial thrombus (Stage IV).
Figure 3.7: Aggregation traces and images representing the relative size of the thrombus and four stages of platelet aggregation. a) Plot of the areas from the aggregates ($n = 6$). b) Fluorescence images from the stages of development of platelet aggregation under disturbed flow.
Since the current imaging configuration focuses on a singular plane of the thrombus, it is possible that the observed increase in the average intensity is due to addition in the total number of platelets accrued in other planes. Further experimentation using confocal microscopy could support the conclusion on thrombus densification.

![Figure 3.8: Four-stage mechanistic model of dynamic platelet aggregation under disturbed flow.](image)

Understanding the dynamics of the stages of platelet aggregation is crucial to the development of accurate mathematical models that can predict under which conditions the embolisation of the thrombus can result in vessel occlusion. The interest of this thesis lies in the development of control systems that can stabilise the thrombus at a particular stage through the modulation of the shear rate of the platform. If successful, such control will allow study of dynamic platelet aggregation under stable conditions, providing new insight into aggregation mechanisms. Eventually, these tools will aid the development of safer and more efficient anti-thrombotic treatments that accommodate and exploit the new mechanisms of platelet aggregation being studied.
3.5 Discussion

It is important to note that the main chemical pathways of platelet aggregation were inhibited for the experiments presented in this research, as described in Section 3.2. In this manner, several factors are attributed to the appearance of the nonlinearity observed in the platelet aggregation response. The proposed model hypothesised that the aggregate starts growing linearly in response to transport of platelets through laminar flow [33,171]. However, as the aggregate grows and reaches the fastest flow regions of the stenosis, it reaches a plateau in size, and instead of continuing to grow, the aggregate continues to recruit platelets and becomes denser [90]. As the aggregate grows, it changes the blood flow dynamics and forms a vortex downstream, which starts enhancing the transport of platelets to the thrombus [50]. In addition, other biochemical mechanisms, such as ATP mediated activation, can possibly contribute to the amplification of the aggregation response over time. Methods to measure the calcium flux [166], and other chemical gradients, can be integrated into the micro-platform presented in this thesis to further understand this amplification phenomenon.

The measurement methods developed in this chapter enable the real-time feedback of the stage of development of the platelet aggregate from the start of its formation up to its consolidation. Furthermore, the insight into the dynamics of platelet aggregation in the presence of blood flow disturbances will be of advantage for the formulation of mathematical models able to extract compact and meaningful information from the clotting behaviour of a particular blood type. In addition, the development of such models of platelet aggregation under disturbed flow will facilitate the design of control algorithms able to regulate the extent of aggregation in the microfluidics device. For example, regulation of the extent of platelet aggregation could help analyse the behaviour of platelets at each of the phases of aggregation in response to different anti-thrombotic treatments.
3.6 Conclusions

The enhanced feedback measures developed in this chapter enabled the formulation of a conceptual mechanistic model which describes the interplay between blood flow dynamics and the development of platelet aggregation, observed experimentally under disturbed flow. An important finding of this chapter was that by monitoring both the area of the main aggregate, as well as its density as indicated by the mean intensity of fluorescence in the images, it is possible to gain insight into platelet aggregation development. While the former appears to be constant at times, the latter indicates an increase in the density of platelets. Physiological significance of these findings is still under discussion.

The developed methods for the observation of platelet aggregation dynamics and the insight into the effect of hemodynamics in this process will facilitate the incorporation of shear micro-gradients into mathematical models of platelet aggregation, and eventually provide insight into the mechanistic variables regulating this phenomenon.
Chapter 4

Dynamic modelling of platelet aggregation in response to blood flow rate modulation

4.1 Introduction

The survey of current microfluidic platforms for platelet aggregation studies considering the effects of shear rate, presented in Chapter 2, found that post-processing of experimental results from these devices can be time consuming and can require the expertise of specialist personnel with somewhat subjective skills [172]. Evaluation of platelet function on such devices usually consists of analysing the aggregation response, depicted on time-scale traces for different shear rate conditions. The analysis is commonly represented by parameters such as: lag time, aggregation rate, maximum thrombus size, or total aggregation, for each of the shear rates tested [51]. The result is often a set of data of considerable size and large percentage of variation.

The approach proposed in this thesis is the inclusion of modelling and control systems to microfluidic devices to develop a systematic, robust, and conclusive methodology for the diagnostics of platelet function.
An essential component of control systems is the measurement of the output variable, in other words the obtention of a feedback signal. In Chapter 3, observation of the dynamics of platelet aggregation in a microfluidics device recreating a scenario of disturbed flow was investigated. A measurement of the main aggregate size was shown to provide information of the complete platelet aggregation development, while a measurement of the aggregate intensity can provide additional information on the initial aggregation dynamics. A conceptual mechanistic model describing the nonlinear relationship between blood flow dynamics and platelet aggregation was formulated from experimental results carried out at a physiological shear rate.

The aim of this chapter is to extract a reduced number of parameters that describe the observed platelet aggregation behaviour in blood perfusion experiments at several shear rate conditions. For this, the platelet aggregation in response to a dynamic sequence of shear rates within a microfluidic device [33] is treated as an abstract unknown nonlinear system, and concepts common in Dynamic and Control Systems, namely System Identification [101], are drawn. A simple nonlinear mathematical model is proposed with three parameters representing an abstraction from the conceptual models of platelet aggregation discussed in Section 3.4. The model parameters are calculated using a nonlinear search method [120]. Simulations of the obtained model adequately replicate the dynamic characteristics of platelet aggregation observed experimentally, and the obtained parameters present low variance. These model parameters can possibly be used as an indication of platelet function. This systematic approach to extracting simple parameters from the rather complex blood flow experiments renders the platform a more attractive candidate for point of care diagnostics that can be operated and interpreted by non-specialists.

This chapter is structured as follows. Section 4.2 presents the dynamics of thrombus formation in response to simple step increases in flow velocity from static conditions to sufficient velocity, and hence shear stress, to induce aggregation. Section 4.3
then interrogates the aggregation response to quasi-random fluctuations in flow rate, and hence shear stress, and uses the observed response data to develop black-box mathematical models which can reveal basic properties of the real dynamics of the system. Next, Section 4.4 formulates a simple nonlinear mathematical model of the aggregation response, and uses this model to fit the aggregation response for a given sequence of shear stress rates in a blood perfusion experiment. Finally, the utility of this model is evaluated and discussed in Section 4.5, and in Section 4.6, the key findings of the chapter are presented.

4.2 Shear Rate Step Response for Identification of Platelet Aggregation

A straightforward approach to characterise the dynamics of a system is analysing its transient response [173]. This section investigates the viability of formulating a mathematical model to represent platelet aggregation from the step response of the system.

Transient analysis often uncovers intrinsic characteristics of the system; such as its time constant, value of static gain and, in some cases, an approximate idea of the 'order' of the system (the highest derivative degree of the differential equation that models the system) which is an important characteristic when attempting to construct a model. Transient analysis basically consists of examining the evolution of the output of a system over time, when the input is changed abruptly from one value to another. Let us denote the input and output of the system at time $t$ by $u(t)$ (shear rate) and $y(t)$ (thrombus size), respectively. A step is defined by:

$$
\begin{align*}
    u(t) &= \begin{cases} 
    u_0 & \text{if } t < t_0 \\
    u_1 & \text{if } t \geq t_0 
    \end{cases}
\end{align*}
$$

(4.1)
In order to test the step response of platelet aggregation in the experimental platform, blood flow experiments on single channels ($n = 6$) were carried out at several shear rate conditions $\gamma_n = \{1200, 1800, 2400, 3600\} [s^{-1}]$ for a total time of 10 minutes: $u_0 = 0$, $u_1 = \gamma_n$, $t_0 = 0$, $T = 10$ min.

Traces from the aggregation response of these experiments are shown in Figure 4.1. In all cases aggregation presented a time onset of less than one minute. Following this initial time lag, the platelet aggregate grew rapidly for about three minutes until reaching a plateau, which presented larger variation for the higher shear rates tested. A particular feature of the aggregation response was the presence of a second phase of growth in the aggregate, which contained oscillations in the area of the aggregate, representing the sudden losses of material.

The shear rate condition that produced the largest aggregate was $\gamma \in 2700 \text{s}^{-1}$, which is in close agreement with other studies [43,45]. Larger shear rates, such as $\gamma = 3600 \text{s}^{-1}$, presented a very rapid flow in the platform that caused the aggregate to form and detach unstably from the stenosis region. These effects are shown in fluoresce images from these experiments, in Figure 4.2.

The results of Figures 4.1 and 4.2 indicate the presence of a nonlinearity in the response since the platelet aggregate continues to grow after an apparent plateau, as opposed to approaching a steady state as time goes on. The initial response resembles a typical overdamped response of a physical system of low order, until it reaches the stage of plateau. However, after the plateau is reached the aggregate continues to grow. This second phase of growth is not simply a transient response to the step increase in flow velocity at the syringe pump, as it would be expected that these forced conditions would have reached steady state after the plateau, yet the aggregate continues to grow.

Although approximate linear models can be formulated from these step response experiments, these models are limited in reproducing experimental results with dif-
different shear rates. More importantly, these models do not consider the inherent nonlinearity evident in the experiments, which is of vital importance for the consolidation of the platelet aggregate. A nonlinear model structure, representing the dynamics of platelet aggregation under disturbed flow, in conjunction with a more sophisticated modelling methodology is required. The step responses in Figure 4.1 present sufficient information to characterise the linear transient response of the system, but do not present sufficient information about the nonlinear response as this is only evident towards the end of the experiments. This nonlinear response must be studied further in order to effectively construct a model of platelet aggregation under disturbed flow.

**Figure 4.1:** Platelet aggregation in response to the step change in shear rate $\gamma \in \{1200, 1800, 2400, 3600\} \, [s^{-1}]$ a) to d) respectively. *Area and mean intensities* of the main aggregate showing an initial linear response from the biomechanical response, and a secondary response caused by the nonlinear enhancing effects of aggregation.
CHAPTER 4.

4.3 Identification of Platelet Aggregation in Response to Dynamic Modulation of Shear Rate

System identification was presented in Chapter 2 as a robust methodology to obtain dynamic mathematical models from observed experimental data [99, 101]. As opposed to the step-response experiment presented in Section 4.2, the input is typically varied dynamically over time through different levels of amplitude in order to explore in detail the dynamics of the system. Although this methodology arose from the need to obtain differential equations, space-states, or transfer functions, to design controllers for mechanical systems, it has had increasing application in a number of disciplines, including biomedicine [174–177].

This section investigates whether system identification can be applied to extract

**Figure 4.2:** Fluorescence images of platelet aggregation in response to the step change in shear rates \(\gamma \in \{1200, 1800, 2400, 3600\} \text{ [s}^{-1}]\).
compact and meaningful parameters from platelet aggregation in response to a dynamic modulation of shear rate.

4.3.1 The identification process

This section presents a summary of the methods selected for each of the components of system identification. In brief, the aim of system identification is to find a mathematical model that best describes observed data, according to a fitting criterion, and then evaluate and validate the model properties [101]. In order to solve this, an experiment must be designed to reveal the dynamics of platelet aggregation under different conditions of disturbed flow, and represent this in a dataset $Z^N$. Models will be then constructed to predict the output of the system $y(t)$ based on past observations $Z^{t-1}$, and parameterised in terms of a vector $\theta$

$$y(t|\theta) = g(\theta, Z^{t-1})$$ (4.2)

In general, once a model structure has been selected, the method employed for calculating the parameters in the model ($\hat{\theta}_N$) that best fits the observed data ($\hat{\theta}_N$) is the well-known Predictor Error Method (PEM) [99]. The predicted outputs are calculated:

$$\hat{y}(t|\theta) = g(\theta, Z^{t-1})$$ (4.3)

then, a sequence of prediction errors is formed,

$$e(t, \theta) = y(t) - \hat{y}(t, \theta), \quad t = 1, 2, \ldots, N$$ (4.4)

and finally, minimisation of the sum of errors in the sequence is formulated as

$$\hat{\theta}_N = \arg \min_{\theta} \frac{1}{N} \sum_{t=1}^{N} e_F(t, \theta)$$ (4.5)
where $e_F$ is the error signal after being filtered by a stable linear filter.

Here any minimisation method can be applied, such as subspace Gauss-Newton direction, or Levenberg-Marquardt [118]. We have chosen to work with Nonlinear Least-Squares Method since it is optimum in the search of nonlinear spaces [119,120].

A standard way of evaluating the performance of the models is the percentage of output variation [100]:

$$V_N(\theta) = 100 \left[ 1 - \frac{||\hat{y}(t|\theta) - y(t)||}{||y(t) - \bar{y}(t)||} \right]$$

(4.6)

where $\bar{y}(t)$ is the mean of $y(t)$.

As a means of avoiding model over-fitting, 50% of the experimental data set is used for estimation from the experiments. The remaining samples are employed for validation of the model. Also, the step-response of the model is tested, to verify the ability of the model to extrapolate to data not used in the model estimation. Ultimately, a simple way of inspecting the performance of the models is realised by visual inspection of the model performance in comparison to the measured data.

### 4.3.2 Formulation of experiment to obtain modelling data

The objective of the experiment design presented in this section is to obtain modelling data experimentally which is representative of the dynamics of platelet aggregation in response to shear rate micro-gradients. This data will be used for the evaluation and validation of mathematical models in subsequent sections.

First, an experiment is designed with an input signal $u(t)$ that exposes as many properties of the system as possible, and then record the measured response of the system $y(t)$ to this input over a time interval $1 \leq t \leq N$:

$$Z^N = \{u(1), y(1), \ldots, u(N), y(N)\}$$

(4.7)
where \( u(t) \) represents the shear rate in the device, and \( y(t) \) the resulting aggregate size.

Ideally, it would be desirable to use an infinitely long input signal, containing all possible values of amplitude and frequency in order to fully characterise the system. However, in practice there are several constraints. As we are primarily interested in the effect of the mechanical blood flow factors on platelet aggregation, these are isolated by blocking the chemical pathways of platelet activation with pharmaceutical inhibitors [21], which remain effective for approximately 1 hour and thus the duration of the experiment is constrained to this time frame.

The selected signal for the characterisation of the system \( u(t) \) was a discrete pseudo-random sequence due to its desirable statistical properties [106]. The amplitude values of the levels in the sequence were selected from physiological and pathological values of shear rate reported in the literature [178], and found to be appropriate for the selected microfluidics device in Section 4.2, \( \gamma = \{120s^{-1} - 2400s^{-1}\} \). The duration for which each level was held was 5 min. This was considered sufficiently long for the system to present a complete transient response. Measurements were carried out every second.

A representative experiment from the identification of the aggregation response is shown in Figure 4.3. This figure shows the evolution of the thrombus size, \( y(t) \), as the shear rate experienced by the platelets was altered dynamically, \( u(t) \). Evidently, the physical conditions set by the shear rate, largely dominated the aggregation/disaggregation of platelets.

In general, the amount of aggregation was proportional to the magnitude of the shear rate in the experiment [43, 51, 179]. The lowest value of shear rate set at the input, \( u(t) = 120s^{-1} \), led to complete thrombus disintegration, \( y(t) \approx 0 \). This is in agreement with other studies which have described a minimum threshold in shear rate of 300s\(^{-1}\) to support platelet aggregation [21, 51].
Figure 4.3: Experimental data employed for model estimation $Z^N$. Thrombus size over time $y(t)$ (above) in response to the blood flow conditions $u(t)$ (below). The size of the thrombus is represented by the area measured inside the detected thrombus from the epifluorescent images, and the blood flow conditions are represented by the shear rate in the microchannel upstream of the contraction. The values at the input ranged from low to high physiological shear rates: 120, 600, 1200, 1800, 2400 $s^{-1}$.

At the start of the experiment a time delay of about 1.5 min occurred before the aggregation of platelets was observed, similar to the lag time of 2 min present in Figure 4.1 b). However, once the shear rate was increased again at $t = 10$ min, the aggregation response was almost instantaneous. Aggregation responses to subsequent steps set at the input had an immediate response on the size of the aggregate. Such initial time delay has been explained in the literature as the time required to build an initial monolayer of platelets [18]. Furthermore, Hansen et al. established that the initial delay in the aggregation is insensitive to the value of shear rate set at the input [51]. It is rather, dependent on the absorption kinetics of the glycoprotein vWF to the substrate of the platform (in this case PDMS).
A particularly noteworthy feature of these results is the difference in the aggregation response obtained by apparently identical pulses of the same magnitude and initial conditions at the input, such as the steps at times $t = 0$, and $t = 50\, \text{min}$, indicated with arrows in Figure 4.3. While the former pulse produced a small thrombus size of approximately $1000\, \mu\text{m}^2$, the latter produced a thrombus almost six times larger. This clear increase in the aggregation response verifies the existence of an inherent amplification of the aggregation response over time. In fact, although the measurement of thrombus size was approximately zero at the start of the pulses at $t = 0$ and $t = 50\, \text{min}$, their initial physiological conditions were completely different. Before the latter pulse occurred, $t = 50\, \text{min}$, the stenosis wall included a highly active monolayer of platelets, not registered in the thrombus size measurement due to its small size, expressing an effect that greatly enhanced the amount of aggregation. It is possible that this active monolayer released $\text{ATP}$ (from dense granules) and caused activation of other platelets, through the calcium channel $\text{P2X1}$ [180], producing the observed amplification effect.

The obtained dataset in Figure 4.3 has revealed several properties inherent to the aggregation of platelets under different shear rate conditions. Such information can be used to formulate mathematical models that fit these results.

### 4.3.3 Black-Box model structures

The selection of the model structure is the most important and challenging part in the identification process. Knowledge regarding the order of the system, time delay, and characteristics of the noise in the system dynamics, is not known \textit{a priori} [181]. This section will investigate whether canonical ready-to-use black-box model structures can extract basic properties and fit the experimental data obtained in Section 4.3.2.

As discussed in Section 4.3.2, time delay is only present at the start of the
aggregation response, and the time delay is negligible in subsequent stages of the aggregation response. In particular, this section will investigate on the order of the dynamics of the system. Initially linear model structures are investigated, following by the testing of more flexible and complex nonlinear model structures.

**Black-Box linear models**

The simplest linear model structure corresponds to the Autoregressive model with exogenous input (ARX). This section will first examine the performance of several ARX models with varying number of model parameters, and hence model orders. After, the performance of more flexible models of the output-error type is investigated.

The ARX model represents the output of the system as an equation of differences depending on linear combinations of the previous input and output values:

\[ y(t) = -a_1 y(t-1) - \ldots - a_n y(t-n) + b_1 u(t-1) + \ldots + b_m u(t-m) \]  \hspace{1cm} (4.8)

For compact notation, let us introduce the vectors

\[ \theta = [a_1, \ldots, a_n, b_1, \ldots, b_m]^T \]  \hspace{1cm} (4.9)

\[ \varphi = [-y(t-1), \ldots, -y(t-n), u(t-1), \ldots, u(t-m)]^T \]  \hspace{1cm} (4.10)

Using equations (4.10) and (4.10), equation (4.8) can be rewritten as a parameterised predictor

\[ \hat{y}(t, \theta) = \varphi^T(t)\theta \]  \hspace{1cm} (4.11)

The vector of parameters \( \theta \) can be calculated by minimising the error between the calculated and measured outputs \( \hat{y}(t, \theta) \) and \( y(t) \) respectively. This can be achieved
by using the Least Squares Method:

\[
\min_\theta V_N(\theta, Z^N) \tag{4.12}
\]

where

\[
V_N(\theta, Z^N) = \frac{1}{N} \sum_{t=1}^N (y(t) - \hat{y}(t, \theta))^2 \tag{4.13}
\]

The minimum of this function, \( \hat{\theta}_N \), is calculated by setting the derivative to zero:

\[
\hat{\theta}_N = \left[ \sum_{t=1}^N \varphi(t)\varphi^T(t) \right]^{-1} \sum_{t=1}^N \varphi(t)y(t) \tag{4.14}
\]

As reviewed in Chapter 2, a common method to evaluate the optimum model structure in terms of both, the calculated prediction error, and model complexity, is the Rissanen’s Minimum Description Length (MDL)

\[
\hat{V}_N(\theta, Z^N) = \left( 1 + \frac{2 \log N \dim \theta}{N} \right) \frac{1}{N} \sum_{t=1}^N \epsilon^2(t, \theta) \tag{4.15}
\]

The performance of several ARX models for combinations of different number of poles \( n_a = 1, 2, ..., 5 \) and zeros \( n_b = 1, 2, ..., 5 \) was calculated as a function of the number of parameters in the model \( n_a + n_b \). The MDL criterion found that the optimum model order for the ARX structure that best describes the experimental data presented in Section 4.3.2 is \( n_a = 1 \) and \( n_b = 1 \). Further, calculation of the unexplained output, as the ratio between the prediction error variance and the output variance in percent, resulted in the same value for model structures of higher order. This indicates that the system dynamics of platelet aggregation in response to shear rate can be approximated by a low-order model structure.

In the following, other model structures with greater flexibility in the transfer function than the ARX model are investigated. Models are implemented for low order structures, first, second, and third order, as the MDL criterion above indicates
that there is not significant improvement when increasing model complexity.

The family of black-box Output-Error structures was introduced in Chapter 2. The structure from this group which offers greatest flexibility is the **Box-Jenkins** model structure

\[
y(t, \theta) = \frac{B(q, \theta)}{F(q, \theta)} u(t) + \frac{C(q, \theta)}{D(q, \theta)} e(t) \tag{4.16}
\]

where \( e(t) \) is a source of white noise, and \( q \) is the shift operator; it represents a displacement of a unit in time, as in the \( z \)-transform,

\[
q u(t) = u(t + 1) \tag{4.17}
\]

Another type of structure is the common **state-space** model representation. This model structure has the advantage of having the order of the system as the only information to start the fitting of model parameters

\[
\begin{align*}
\dot{x}(t) &= A(\theta)x(t) + B(\theta)u(t) \\
y(t) &= C(\theta)x(t) + v(t)
\end{align*} \tag{4.18, 4.19}
\]

\( v(t) \) denotes the disturbances and noise.

Finally, models of the type of **continuous-time transfer function** are investigated as these models are easy to estimate. Their structure is simple and parameters relate directly to the transient response observed in the step response. A first order model has the form

\[
y(t) = G_c(p, \theta)u(t) \tag{4.20}
\]

where \( p \) denotes the differentiator operator and \( G_c(p, \theta) \) is

\[
G_c(s, \theta) = \frac{K_p e^{-sT_d}}{(s\tau + 1)}, \quad \theta = [K_p, T_d, \tau]^{T} \tag{4.21}
\]
Table 4.1: Performance of standard linear Black-Box models employing MDL. Model order was defined as the number of poles in the transfer function. Box-Jenkins model included a first order disturbance model.

The performance results from the implemented linear black-box model structures is summarised in Table 4.1 and illustrated in Figure 4.4. These results indicate that other black-box linear model structures with greater flexibility than the ARX model, and hence complexity, do not present significant improvement in the fitting performance.

![Figure 4.4: Plots of platelet aggregation in response to shear rate calculated by linear Black-Box models.](image)

A visual inspection of the performance of the selected models, illustrated in
Figure 4.4, suggests that these linear models capture some parts of the platelet aggregation dynamics, in particular the time constants of the transients. However, the steady-state values from the simulations were in agreement with the experimental results only in a small part of the experiment, from $20 < t < 40 \text{ min}$ approximately. There were major differences at the start and at the end of the experiment due to the inherent nonlinear time-varying behaviour present in the system, where the gain of the system was observed to increase over time. For example, the aggregation output $y(t)$ caused by a stimulus at the input of the same amplitude $u(t) = 1200 \text{ s}^{-1}$ at different times during the experiment produced outputs of $y(t = 2.5 \text{ min}) = 1000 \mu m^2$, $y(t = 27.5 \text{ min}) = 2000 \mu m^2$, and $y(t = 52.5 \text{ min}) = 5000 \mu m^2$, as illustrated in Figure 4.3.

In summary, a simple first order linear model with 2 parameters can capture parts of the aggregation dynamics observed experimentally. Despite increasing the complexity of linear model structures, the performance of these models was limited due to the inherent time-varying nonlinearities of the system.

**Black-Box nonlinear models**

The linear model structures investigated in the previous section suggested that the model structure must contain a nonlinearity to represent the amplification effect observed in Section 4.3.2. In this section, Black-Box nonlinear structures are investigated to determine whether these types of model structures can provide insight into the characteristics of such nonlinearity.

In Chapter 2 several methods for modelling nonlinear systems were presented. These include Nonlinear ARX, Hammerstein Wiener, Neural Networks, and Fuzzy Logic Systems. While Neural Networks and Fuzzy Logic Systems provide great flexibility and fitting performance, the aim of this investigation is to maintain a low number of parameters in the structure in order to be able to extract compact
meaningful information for platelet function diagnosis. Nonlinear ARX (NARX) and Hammerstein-Wiener (HW) model structures are examined in this Section, since these can incorporate the linear models found in the previous section, and add nonlinearities to these models is different ways preserving relatively simple model structures.

The NARX model structure [106, 107] is illustrated in Figure 4.5. In this approach, the output is calculated as

\[ \hat{y}(t, \theta) = F(x)L^T(x - r) + d + g(Q(x - r)) \]  

where \( x \) is the regression vector. The first two terms in equation (4.22) correspond to a transformation of the linear function \( F(x) \), while the third term corresponds to the output of the nonlinear function \( g(x) \). Hence, the model parameters of the NARX
structure are the dilation and offset of the linear function, $L$ and $d$ respectively, the mean of the regression vector $r$, and the projection matrix $Q$.

The nonlinear function $g(x)$ is calculated as follows

$$g(x) = \sum_{k=1}^{n} \alpha_k \kappa(B_k(x - \gamma_k))$$  \hspace{1cm} (4.23)

Two types of basis functions, $\kappa(S)$, were chosen to form the nonlinearity of the NARX models. These were wavelet, and sigmoid networks as these have the advantage of having a reduced number of parameters. In addition, these functions are derivable, and hence continuous optimisation approaches can be applied, resulting in faster computations.

The second nonlinear approach implemented in this section is the Hammerstein-Wiener (HW) model structure, as illustrated in Figure 4.6. The HW structure is formed by two static nonlinear blocks, one at the input and one at the output, and a dynamic linear model in the middle of the structure. The output is calculated in three steps as follows

$$w(t) = f(u(t))$$  \hspace{1cm} (4.24)

$$x(t) = \frac{B(\theta)}{F(\theta)} w(t)$$  \hspace{1cm} (4.25)

$$y(t) = h(x(t))$$  \hspace{1cm} (4.26)

where $f$ and $h$ are the input and output nonlinear functions, and $B/F$ is the linear transfer function. In a similar fashion as for the NARX models, basis functions for the nonlinear functions were selected.

The performance of the implemented NARX and Hammerstein-Wiener models is presented in Table 4.2, and simulations of these models are presented in Figure 4.7. Although the model fit increased up to around 10% in comparison with the estimated linear models in the previous section, this improvement is not significant considering
CHAPTER 4.

Table 4.2: Performance of Nonlinear ARX and Hammerstein-Wiener Black-Box models. Linear components were formed using first order regressors $u(t-1)$, and $y(t-1)$.

<table>
<thead>
<tr>
<th>Model Structure</th>
<th>Nonlinearity</th>
<th>Number of Elements</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>NARX</td>
<td>Wavelet Net</td>
<td>33.67%</td>
<td>33.68%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sigmoid Net</td>
<td>38.85%</td>
<td>42.38%</td>
<td></td>
</tr>
<tr>
<td>HW</td>
<td>Piecewise Linear</td>
<td>38.48%</td>
<td>41.34%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sigmoid Net</td>
<td>34.9%</td>
<td>38.72%</td>
<td></td>
</tr>
</tbody>
</table>

Discussion of black-box model structures

In brief, linear and nonlinear black-box models were implemented in this section using several black-box model structures. Although the performance of the models was limited to fit some sections of the experimental results only, and the time-varying behaviour of the aggregation response was not represented successfully by these models, these low-order models were found to represent reasonably well the transient responses in parts of the obtained experimental data. Tailored model structures incorporating the insight gained on the order of the system and the observed nonlinearity, will have a better chance to describe more accurately the dynamics of platelet aggregation in response to shear rate micro-gradients.
4.4 Nonlinear Grey-Box Dynamic Model of Platelet Aggregation

This section aims to formulate a nonlinear model structure that represents the physiological insight of the mechanisms affecting the dynamics of platelet aggregation under flow disturbances observed in Chapter 3, and the insight on the model structure gained in Section 4.3. The value of the parameters fitted to data from blood perfusion experiments is suggested to be used as an indication of platelet function.

The proposed model structure is conceived to represent the main mechanisms that promote platelet aggregation observed in Section 3.4. A block diagram of this representation is shown in Figure 4.8. In this diagram, the main block represents the biomechanical response of platelet aggregation to shear rate micro-gradients, of which transfer function is approximated by a first order linear system. A second
block represents an enhancement of the aggregation response through a feedback mechanism coupled to the main block. This model suggests that the aggregated platelet density is responsible for generating the release of agonists not targeted by the Amplification Loop Blockade treatment (ALB) presented in Section 3.2.1, which in turn activate more platelets enhancing the aggregation response. This leads to the formulation of the model

\[
\tau \frac{dy}{dt} + y = c(t)u(t) \tag{4.27}
\]

\[
c(t) = c_0e^{c_1 \int I(\eta)d\eta} \tag{4.28}
\]

or, represented in state-space form

\[
x_1'(t) = -\frac{1}{\tau}x_1(t) + \frac{1}{\tau}x_2(t)u_1(t) \tag{4.29}
\]

\[
x_2'(t) = c_1I(t)x_2(t) \tag{4.30}
\]

\[
y(t) = x_1(t) \tag{4.31}
\]

where the state variable \(x_1(t)\) represents the size of the thrombus over time and \(x_2(t)\) represents the magnitude of the enhanced aggregation function. While the former is a variable that can be measured, the latter is a representation of an unmeasurable internal state. The parameter \(\tau\) corresponds to the time constant of the system. In a first-order linear system, a rough calculation of this parameter can be obtained by measuring the time to reach 95% of steady-state when a step is input to the system. This time should correspond to \(t \approx 3\tau\). The parameter \(c_0\) represents the initial proportional gain between output and input. In this specific instance this will be the gain of platelet aggregation in response to shear rate. The parameter \(c_1\) regulates the effect of the enhancing phenomena on the growth of the thrombus. Such enhancing feedback function can be possible due to a number of micro-rheological events including cell-to-cell collisions, and enhanced transport of platelets and minor
 agonists not inhibited by the ALB to the thrombus surface due to the formation of vortices under the aggregate. The function $c(t)$ represents the enhancing nonlinear effect due to expression of agonists not targeted by ALB, and such effect is modelled to be proportional to the amount of blood extracted from the aggregate over time ($\int I(\eta)d\eta$). The function $c(t)$ follows an exponential growth from observations of the time-varying behaviour of the aggregate found in Section 4.3.2.

$\int I(\eta)d\eta$ is the platelet density represented by the measured mean intensity, $c(t)$ is the enhancing aggregation response, $u(t)$ is the input of the system (shear rate), and $y(t)$ is the output of the system (thrombus size measured by its area).

An equivalent model with an ordinary exponential growth function of the enhancing aggregation, $c(t)$ in equation (4.28), is also investigated. This assumption is based on the fact that the input signal $u(t)$ in Section 4.3.2 oscillates around a mean value. Indeed, this exponential behaviour is caused by a positive feedback loop produced when platelet agonists are released near the main aggregate. This causes more platelets to activate, aggregate, and eventually release more soluble agonists into the surroundings of the aggregate. The equivalent model is then formulated as

$$\frac{d}{dt}y + y = c(t)u(t)$$  \hspace{1cm} (4.32)

$$c_{equiv}(t) = c_0e^{c_1t}$$  \hspace{1cm} (4.33)
The next procedure is to realise the estimation of the model parameters $\hat{\theta}$ in equations (4.27) and (4.32). Initial states of equation (4.27), $x_{0,Grey} = [c_0 0]^T$, were required to start the parameter estimation by the Prediction Error Method. An initial computation of the coefficients of the linearised model ($c_1 = 0$) was realised directly using the Least Squares Method (LSM), $\hat{\theta}_{Lin}$ with zero initial conditions $x_{0,Lin} = [0 0]^T$. The calculated coefficients from LSM were employed as a starting point in the nonlinear search of the optimal coefficients for the nonlinear models.

The value of the estimated parameters of the linear and nonlinear models, and their covariance, are shown in Table 4.3. These results show that the time constant ($\tau$) for the three models is consistent, as expected. However, the inclusion of a nonlinearity nearly doubled the fitting performance of the nonlinear models in comparison to its linear counterpart. In addition, the covariance of the parameters from the nonlinear models was significantly lower.

Simulations of the estimated models were carried out using half of the experimental data from Section 4.3.2. Results from these simulations are presented in
Figure 4.9. For these simulations, a saturation block converted to zero any shear rate input below 300s$^{-1}$, in accordance with the minimum threshold for aggregation found in Section 4.3.2. Figure 4.9 b) presents a comparison of the exponential function $c_{equiv}(t)$, the integral of accumulated platelet density $c(t)$, and the shape of the input $u(t)$ after being multiplied by such function, as considered in equation (4.27), $u(t)c(t)$. The equivalent exponential function, in equation (4.33), resulted in a suitable approximation of the accumulated platelet density function in equation (4.28).

Simulation results of the estimated linear and nonlinear models in Table 4.3, in comparison to the experimentally measured thrombus size, are plotted in Figure 4.9 a). These show that, as was discussed in Section 4.3.3, the linear model seems to fit only a section of the experiment, from $20 < t < 40 \text{min}$, presenting a clear underestimation for $t < 20 \text{min}$, and an overestimation for $t > 40 \text{min}$. On the other hand, the nonlinear grey models present a significantly better fit and adequately replicated the time-varying nonlinearity of platelet aggregation observed experimentally. In addition, the calculated parameters of the nonlinear models presented very low variance. Hence, it is proposed to use two of these parameters, $\tau$ and $c_1$, as an indication of platelet function. These simple parameters are believed to present great potential for a point of care diagnostics device that requires minimal training.
Figure 4.9: Simulation results of grey nonlinear models in comparison to linear model and measured experimental data, and nonlinear function describing time-varying amplification behaviour of platelet aggregation. a) Performance of the proposed grey nonlinear model Grey and its equivalent model Equiv, in comparison with its linear model counterpart Lin and measured data $y$. b) Waveform of the input signal $u(t)$, the exponential function representing the enhanced aggregation effect $c(t)$ and its equivalent function $c_{eq}(t)$, and the product $u(t)c(t)$. 
Figure 4.10: Validation of equivalent grey model using the response to a step in shear rate ($\gamma = 1800 \, s^{-1}$). a) Thrombus size $s(t)$, represented by model response $Equiv$ and compared to the average of the step responses obtained experimentally $y$. b) Input $u(t)$ was delayed by 2 min, and had an amplitude of a physiological shear rate.
CHAPTER 4.

A further validation of the equivalent nonlinear model in equation (4.32) obtained is illustrated in Figure 4.10. The step response of the model was compared to the average response of the platform to a constant physiological shear rate input \((\gamma = 1800 \text{ s}^{-1})\), presented in Section 4.2. For this simulation, the input step signal was delayed by 2 min, \(u(t - 2)\) in order to represent the initial time lag not considered by the model and to be able to compare directly the two traces. Despite the initial difference on the rate of aggregation, the model presented a reasonably good approximation of the measured step response. The model response presented a similar trend as the experimental data: the response presented an initial acute growth, followed by a plateau with a gradual positive feedback effect representing the dynamics of enhanced aggregation. The first transient was the result of the linear part of the model, and the second stage corresponded to the effect of the nonlinearity \(c(t)\).

The nonlinear grey models presented in this section, formulated from the insight gained on the relationship between the fluid dynamics and platelet aggregation, approximate the clotting behaviour of blood perfusion experiments in response to dynamic changes in the shear rate micro-gradients. The models, comprising only three parameters, fit experimental data with low variation. Evaluation of the obtained model parameters is suggested as a systematic and robust method for the diagnostics of platelet aggregation under disturbed flow.

4.5 Discussion

In summary, this chapter demonstrated that the dynamics of platelet aggregation can be approximated low order linear models for short periods of time, in particular during the first stages of aggregation. In addition, the long term aggregation response was found to include a time-varying behaviour, in which the aggregate becomes more responsive as the platelet density increases over time. The investigated
models indicate that this amplification response is proportional to the amount of aggregation achieved in the platform, and can be approximated by a uniform exponential function. The amplification effect perhaps can be explained by the expression of platelet agonists not inhibited by the ALB, such as an ATP-mediated response.

The models presented in Section 4.4 will be useful for the development of control systems able to regulate the size of the thrombus in the microplatform through the manipulation of shear rate. This should allow the optimisation of the sequence needed to fully characterise the dynamics of the type of blood being tested, and therefore minimise the time required to perform diagnostics on platelet function. In this way, the random sequence of one hour duration presented in this chapter, may be reduced by a control system to shorter optimal time on the order of minutes. These control systems could also have application in automatic screening of newly developed antithrombotic treatments considering dynamic ranges of shear rate experienced by the platelets.

4.6 Conclusions

A low-order nonlinear dynamic model of platelet aggregation in response to shear rate micro-gradients developed in this chapter was found to fit experimental results of blood perfusion in a microfluidic device mimicking a realistic scenario of disturbed flow produced by severe arterial stenosis. The model is comprised of only three parameters which represent: the time constant response to shear rate $\tau$; an initial prothrombotic coefficient $c_0$; and an exponential coefficient regulating the enhancing effect of nonlinear micro-rheological events and non targeted soluble agonists $c_1$. In particular, the value of the coefficients $\tau$ and $c_1$ can be used as a robust diagnostics of platelet function for a given type of blood. This novel diagnostics methodology has the advantage of requiring only a very small volume of blood and producing an automatic measurement with very low variance. This model will promote further
studies involving robust control systems and advanced microfluidics platforms to perform efficient blood disorder detection tools and eventually gain insight into platelet mechanotransduction.
Chapter 5

Automatic regulation of platelet aggregation dynamics via switching control

5.1 Introduction

Control systems is an established field in engineering that deals with the design of automatic controllers to accomplish a desired behaviour of a system or device [173]. In most cases of design of control algorithms, the system can be represented by a linear approximation of the system dynamics around a desired operating point. However, linear control approaches are inadequate when the operation range of the device is large [127]. This has encouraged the development of nonlinear control systems such as robust control, adaptive control, sliding mode control, and fuzzy logic control. The review presented in Chapter 2 established that Sliding Mode Control (SMC) is a robust methodology for dealing with modelling errors, disturbances, and nonlinear behaviours [155]. In theory, this variable structure strategy makes use of a high frequency switching that offers exceptional results in terms of stability, robustness, and rapid convergence.
Mechanical and electrical systems have limited bandwidth (response time), and instantaneous switching actions may lead to instabilities and excitation of high order unmodelled dynamics. A solution to this problem was proposed by Filippov et al. to smooth out the actuation signal into a continuous signal through the so-called equivalent control design [182]. In practice, some systems have actuators of variable structure such as valves and relays, whose required control action is of discontinuous nature (on/off type). For these cases, Sira-Ramirez demonstrated an equivalence between SMC and Pulse-Width Modulation (PWM) control that allows the transformation from the continuous SMC equivalent control signal back to a two-valued switching signal [183, 184]. This methodology produces a control action of fixed frequency and two states, controlling the duration of one of the states at each cycle. This inclusion of PWM in the control action of SMC algorithms has proved to be effective with a range of classes of nonlinear systems such as robotic manipulators [183], power stabilisation [185] and chaotic systems [186].

In Chapter 4, a simple nonlinear dynamic model that captures the basic dynamics of platelet aggregation and thrombus formation under disturbed flow was proposed [3]. The estimated model parameters were suggested to serve as a systematic diagnostics of the platelet function from the blood tested. However, the signal employed to stimulate the system and obtain experimental data was a pseudo-random sequence of steps with a duration of 1 hour. In order to optimise this sequence, a feedback controller could be employed to automatically regulate the formation and disintegration of the aggregate, and hence, and hence reduce the time required for the test.

This chapter aims to investigate control algorithms able to automatically regulate the growth of the platelet aggregate in a microfluidics device through dynamic manipulation of shear rate. In order to increase the throughput of the original device, a microfluidics device is proposed in Section 5.2 for rapid and dynamic regulation
of shear rate. Next, Section 5.3 aims to identify a control strategy suitable for the regulation of the growth of platelet aggregates in the proposed microfluidics device. Section 5.4 then demonstrates the application of control algorithms for the automated diagnosis of shear rate-dependent platelet function under different simulation scenarios. Finally, the main outcomes of this chapter are drawn in Section 5.5.

5.2 Microfluidics Device for Rapid Modulation of Shear Rate

In Chapter 4, a sequence of shear rates in a microfluidics device was employed in order to test the aggregation response of platelets to several conditions of disturbed flow. Changes in the amount of shear rate were performed by adjusting the withdrawal rate of a syringe driver. As a result, settling times of changes in shear rate were in the order of hundreds of seconds.

A possible solution to improve the time response of the microfluidics system in Chapter 4 could be to reverse the direction of the syringe driver. However, this would contaminate blood upstream of the stenosis causing activation of platelets prior to the inspection area of the device. Similarly, other technologies developed for rapid flow rate control in microfluidic devices, such as positive pressure systems [187,188], and thermally or light actuated on-chip valves [189,190], could also lead to undesired activation of platelets. This section investigates the design of a microfluidics device able to provide rapid shear rate control employing negative pressure.

5.2.1 Proposed microfluidics design

In this section, the inclusion of a pressure relief valve in the proximity of the microcontraction is proposed to improve the response time of the microfluidic system. Such valve can be controlled to open to atmosphere, and reduce the flow rate in the
microchannel, or to close, and allow the original flow between the blood reservoir and the exhaust.

Figure 5.1 presents an illustration of the original and proposed devices, as well as an analogy of their approximation by electrical circuits for a simple analysis of the resulting flow dynamics [191]. An inspection of the fluid network in the original device suggests that the slow changes in shear rate are due to the process of pressure equalisation between the blood reservoir and syringe driver. This is a slow process as the hydraulic resistance of the system is very high, owing to the small dimensions of the microchannel. Moreover, the hydrodynamic resistance is further increased by the presence of the microcontraction.

![Figure 5.1](image-url)

**Figure 5.1:** Microfluidics device for rapid control of flow rate using negative pressure.

In the circuit analogy for the proposed design (Figure 5.1), the flow rate of the syringe driver connected to the exhaust ($P_1$) can be represented by a constant current source $I_s$. Fluidic channels can be represented by electrical resistances, where $R_1$ is the resistance of the original channel containing the microcontraction, and $R_2$ is the resistance of an auxiliary channel connecting the main microchannel with a pressure relief port ($P_2$). The pressure relief port can set the auxiliary microchannel to
atmospheric pressure when it is open, and to the minimal hydrostatic pressure when it is closed. This is represented by a switch $S_w$ in the circuit analogy that connect the auxiliary resistance to ground, or opens the circuit and establishes an infinite resistance in the auxiliary channel. A larger dimension of the auxiliary channel, $R_2$, results in a much smaller electrical resistance than the main test microchannel $R_1$, i.e. $R_2 \ll R_1$. The hydraulic pressure of the reservoirs could be represented by voltage sources. However, these have been neglected for simplification as the hydrostatic pressure generated at the reservoirs is far smaller than the pressure drop across the microchannels.

The principle of operation of the proposed system is as follows:

When the auxiliary reservoir is covered there is no flow through the auxiliary microchannel $R_2$, and the flow rate in the main microchannel $R_1$ is the one set by the syringe driver. In the circuit analogy, this corresponds to the case when the switch $S_w$ is open, and the current through $R_1$ is $I_1 = I_s$.

When the auxiliary reservoir is open to atmosphere most fluid travelling to the syringe driver is drawn from the auxiliary reservoir as it offers less fluidic resistance than the path from the main blood reservoir. This corresponds to having the switch $S_w$ closed, which results in a current divider in the circuit analogy $I_1 = \frac{R_2}{R_1 + R_2}I_s$.

5.2.2 Proof-of-concept experiment

This section aims to verify experimentally the capability of the proposed microfluidics device to rapidly modulate shear rate in the test microchannel.

A prototype device was fabricated by adding a second reservoir in the proximity downstream of the microcontraction as shown in Figure 5.1 b). Micro-particle image velocimetry [192] -\( \mu \text{PIV} \) was employed to measure the average flow rate upstream of the microcontraction, using water as fluid and micro-beads as tracers. The perfusion rate of the syringe driver was set constant at $22 \mu L_{\text{min}}$, which produced a shear rate
near the wall in the test microchannel of 2700 s\(^{-1}\), and a mechanism was applied to the auxiliary reservoir of the proposed device to control the flow of liquid from between the auxiliary reservoir and the microchannel.

![Graph showing response times to changes of shear rate in the test microchannel of original and proposed microfluidic devices.](image)

**Figure 5.2:** Response times to changes of shear rate in the test microchannel of original and proposed microfluidic devices. \(r(t)\) Reference signal, \(u_0(t)\) Time response of original device, \(u_1(t)\) Time response of proposed device.

A pulse lasting one minute was set as a reference signal (auxiliary reservoir covered during this time, switch \(S_w\) was open), and the time response was measured for both the original and the proposed devices. Results from this proof-of-concept experiment are shown in Figure 5.2. The figure shows that the time required for an increase in the flow rate, and hence shear rate, was similar for both cases. However, the time required to decrease the flow rate was significantly shorter for the proposed device, approximately 3 s in comparison to more than 2 min in the original design.

The time response of the proposed system is very fast, however the test microchannel is now limited to presenting two values of flow rate, the one set by the syringe driver, and a minimum flow rate. An approach to achieve intermediate values of flow rate in the test microchannel is to take advantage of the low-pass characteristic of the fluid network, and modulate the flow rate. This requires the
CHAPTER 5.

use of a control system to generate the pulses required to achieve a desired pattern of flow in the channel. The results of this proof-of-concept device showed that rapid control of shear rate in the platform can be achieved by including a relief port near the microcontraction.

5.3 Control Algorithms for the Regulation of Platelet Aggregation

The normal course of platelet aggregation under disturbed flow in a microfluidics device, described in Section 3.4, can lead to complete occlusion of the microchannel depending on the duration of the experiment and the level of reactivity of the type of blood being tested. In addition, the aggregation results for different blood phenotypes can vary significantly. For example, blood types with a platelet hypo-function may not produce a platelet aggregate of significant size when tested at physiological shear rates. The presence of control algorithm in the microfluidics device has the potential to increase the yield of the platform by continuously regulating the shear rate, and therefore the rate of platelets per unit of time exposed to the forming platelet aggregate.

Section 5.2 proposed the use of a relieve valve in the proximity to the microcontraction in order to improve the response time of the device to change of shear rate. This system then takes the form of a variable structure system with two states [193]. The valve can only be controlled between two states, open and closed. When the valve is closed the flow rate (and shear rate) through the microcontraction is maximum, and such flow rate was calculated to maximise the growth of the aggregate as observed in Section 4.2. When the valve is open to atmosphere, the flow through the microcontraction is minimum, which is expected to reduce the size of the aggregate. This Section will investigate switching control algorithms able to regulate
the dynamics of platelet aggregation through modulation of the shear rate in the platform.

5.3.1 Relay control

In this section, a relay control algorithm will be investigated in order to find out a reference of the performance that can be obtained using switching control strategies.

The transfer function of the relay control algorithm proposed to regulate the dynamics of platelet aggregation is illustrated in Figure 5.3, and defined as follows

$$u(t) = \begin{cases} u_{\text{max}} & \text{for } e(t) \geq \rho \quad \text{and} \quad -\rho < e(t) < \rho, \text{ if } \sigma = u_{\text{max}} \\ 0 & \text{for } e(t) \leq -\rho \quad \text{and} \quad -\rho < e(t) < \rho, \text{ if } \sigma = 0 \end{cases} \quad (5.1)$$

where $e(t) = r(t) - y(t)$ is the error signal, and $\rho$ is the trigger that activates the relay control action. The selected values of the control signal correspond to the shear rate values generated by the valve actions. When the valve is closed $u(t) = u_{\text{max}}$, and when the valve is open $u(t) = 0$. The value of $\sigma \in \{0, u_{\text{max}}\}$ corresponds to the value of the control action $u(t)$ after the last relay switching. Figure 5.4 illustrates the block diagram of the feedback relay control and model with the dynamics of platelet aggregation in response to shear rate identified in Chapter 4.

Chapter 2 proposed that a diagnosis of platelet function considering the effects of disturbed flow can be achieved by fitting the aggregation response to a dynamic sequence of shear rate, and evaluating the model parameters. However, Chapter 4 found that the platelet aggregate growth follows a nonlinear amplification behaviour over time. Therefore it would be appropriate to observe the aggregation response at different stages during the experiment. This would allow for a thorough investigation of the phases of platelet aggregation over time, and for a robust measurement able to deal with random disturbances. Consequently, the reference signal $r(t)$ was set
as a square signal with a period of 10 min, which indicates that the desired platelet aggregation size profile is a periodic size of \( r(t) = 2000 \mu m^2 \) for a duration of 5 min, followed by a complete disaggregation \( r(t) = 0 \). The total duration of the simulation was 30 min, so consequently, the reference signal included 3 pulses of thrombus formation and disintegration.

A simulation of the relay control system in Figure 5.4 was realised in Simulink®, for two values of switching trigger \( \rho = \{0, 100\} \). The values of the controller output were selected as \( u_{\text{min}} = 0 \), and \( u_{\text{max}} = 2700 \text{s}^{-1} \). Results of these simulations are presented in Figure 5.5.

The Relay control algorithm with zero threshold, \( \rho = 0 \), tracked the reference sig-
Figure 5.5: Simulation of relay control algorithm regulating the dynamics of platelet aggregation. a) Results for $\rho = 0$. b) Results for $\rho = 100$, (5\% of $r_{\text{max}} = 2000 \mu m^2$).

nal $r(t)$ with precision. On the other hand, the algorithm with a switching threshold of $\rho = 100$ presented a ripple around the reference signal. Both controllers followed the reference signal successfully at all times of the simulation. Although, the choice of a smaller threshold $\rho$ results in smaller error signal, the associated high frequency switching is undesirable. Under certain conditions, high frequency switching can lead to instabilities in the system, as well as the degradation of the actuation de-
Figure 5.6 shows the trajectory of the system states during the simulations from Figure 5.5. Examining Figure 5.6, the time-varying response of the model of platelet aggregation dynamics shows an increase in the state $x_2(t)$. This figure also illustrates the effect of the switching threshold parameter $\rho$ on the system trajectory.

In summary, the relay control algorithms presented in this Section have demonstrated the capability of regulating the dynamics of platelet aggregation dynamics. The main drawback of this algorithm is a high switching activity when the system reaches the reference signal, which can lead to undesirable behaviour. The next sections investigate control algorithms able to provide robustness, stability and performance, suited to this particular system.

![Figure 5.6: State trajectories for simulation shown in Figure 5.5 of relay control using two values of switching threshold: a) $\rho = 0$ and b) $\rho = 100$. For both cases the trend of the state trajectory is equivalent. However, with the increase of $\rho$, the ripple in the state trajectory is increased as well.](image-url)
5.3.2 Sliding mode control

SMC is an advanced nonlinear control technique that has proven to be very effective in controlling nonlinear systems with high degree of uncertainty. A commendable characteristic of SMC is its robustness to model imprecision for both structured (parametric) and unstructured (unmodelled dynamics) uncertainties. This type of control algorithm has the potential to regulate the dynamics of platelet aggregation in response to shear rate, considering the characteristics of the system identified in Chapter 4 and the discontinuous nature of the required control signal for the actuator proposed in Section 5.2. This section investigates whether an SMC algorithm can be formulated to regulate the dynamics of platelet aggregation, and provide stability.

Sliding mode control algorithm for regulation of platelet aggregation

First, let us represent the equivalent model of platelet aggregation in response to shear rate found in Section 4.4, equation (4.27), in state-space form

\[
\dot{x}_1(t) = -\tau^{-1}x_1(t) + \tau^{-1}x_2(t)u(t) \tag{5.2}
\]

\[
\dot{x}_2(t) = c_1x_2(t) \tag{5.3}
\]

\[
y(t) = x_1(t) \tag{5.4}
\]

where the state variable \(x_1(t)\) represents the size of the aggregate over time and \(x_2(t)\) represents the magnitude of the enhanced aggregation function. The output of the system has been assigned to the state \(x_1(t)\), which is the variable to be regulated. Dot notation has been introduced to represent time derivatives \(\dot{x} = \frac{dx}{dt}\).

Consider the variable of interest, \(x_1(t)\)

\[
x_1(t) = f(x) + b(x)u(t) \tag{5.5}
\]
where the scalar signal \( u(t) \) is the control input, shear rate in the microchannel, and \( \mathbf{x} \) the state vector. The control objective is for the system output \( x_1(t) \), platelet aggregate size, to follow the time-varying reference signal \( r(t) \), \( x_1(t) \rightarrow r(t) \), in the presence of model uncertainty on \( f(\mathbf{x}) \) and \( b(\mathbf{x}) \).

Firstly, let us define the tracking error as

\[
e(t) = r(t) - x_1(t) \tag{5.6}
\]

Let us define a time-varying surface \( s(t) \) by the scalar equation \( s(t) = 0 \),

\[
\dot{s}(t) = \dot{e}(t) + \lambda e(t) \tag{5.7}
\]

where \( n \) is the system order, and \( \lambda \) is a strictly positive constant.

The choice of the surface in equation (5.7) implies that once the system states have reached such a surface, the tracking error will decrease exponentially to zero, with a time constant \( (n - 1)/\lambda \) [127]. This is achieved by ensuring that \( \dot{s}(t) = 0 \).

A typical Lyapunov energy function of the system is defined as

\[
V = \frac{1}{2} s^2(t) \tag{5.8}
\]

and the time derivative of the tracking error in equation (5.6) can be expressed as

\[
\dot{e}(t) = \dot{r}(t) - \dot{x}_1(t) \tag{5.9}
\]

Substituting equation (5.2) in equation (5.9)

\[
\dot{e}(t) = \dot{r}(t) - [-\tau^{-1} x_1(t) + \tau^{-1} x_2(t) u(t)] \tag{5.10}
\]
and expressing the equation in terms of the tracking error $e(t)$

$$\dot{e}(t) = \dot{r}(t) + \tau^{-1} [r(t) - e(t) - x_2(t)u(t)]$$  \hspace{1cm} (5.11)

In order to ensure stability, the derivative of the Lyapunov function in equation (5.8) must be negative, $\dot{V} < 0$. The derivative of $V$ is

$$\dot{V} = e(t)\dot{e}(t)$$  \hspace{1cm} (5.12)

Substituting equation (5.11) in equation (5.12)

$$\dot{V} = e(t)\dot{r}(t) + \tau^{-1} [r(t) - e(t) - x_2(t)u(t)]$$  \hspace{1cm} (5.13)

Factorising, and approximating $\dot{r}(t) \approx 0$ (as the input reference is a constant signal at most times)

$$\dot{V} = \tau^{-1} [r(t)e(t) - e^2(t) - x_2(t)e(t)u(t)]$$  \hspace{1cm} (5.14)

Therefore, the critical term of equation (5.14) which can be altered by the control action is $-x_2(t)e(t)u(t)$. In order to minimise the derivative of the energy equation in equation (5.14), $u(t)$ is chosen as follows

$$u(t) = \begin{cases} u_{\text{max}} & \text{for } x_2e > 0 \\ 0 & \text{for } x_2e < 0 \end{cases}$$  \hspace{1cm} (5.15)

A block diagram of the proposed sliding mode controller to regulate the dynamics of platelet aggregation is presented in Figure 5.7. The SMC structure is similar to the relay control algorithm in Section 5.3.1. The only difference is the inclusion of information from state $x_2(t)$, which represents the enhancing effect observed in
platelet aggregation.

\[
\begin{align*}
\dot{x}_1 &= -\tau^{-1}x_1 + \tau^{-1}x_2u \\
\dot{x}_2 &= c_1x_2 \\
y &= x_1
\end{align*}
\]

**Figure 5.7:** Block diagram of SMC algorithm for regulation of SIPA.

**Simulation of sliding mode control algorithm**

Simulation results of the SMC algorithm proposed in the previous section are presented in Figure 5.8. These show the controller achieved tracking of the reference signal, with a performance similar to the relay control algorithm in Section 5.3.1. However, the control signal \(u(t)\) presented high-frequency switching, with slightly less activity than the one presented by the relay control algorithm. In addition, the design of the SMC algorithm guaranteed stability.

Although in theory this SMC algorithm provides excellent performance, direct implementation of this algorithm on the microfluidics device proposed in Section 5.2 is not feasible owing to the high-frequency activity of the control signal. A common solution of this problem is obtaining a continuous equivalent of the control signal through the *equivalent control* [126]. However, as discussed in Section 5.2, the required control signal for the system actuator (valve) must be binary.
5.3.3 Sliding mode control with pulse width modulation

In the previous section, the high performance of the SMC algorithm was achieved at the cost of a high activity of switching in the control action (chattering). This section investigates a control algorithm that aims to eliminate the chattering, while guaranteeing near-optimal response and stability. First, a continuous signal equivalent to the control action from the SMC is derived. This signal is then transformed back to a binary signal of fixed frequency using a Pulse Width Modulator. Finally, simulation results of this algorithm are presented.

Equivalent Control

The equivalent control method aims to replace the discontinuous control of the SMC with a continuous control which directs the velocity vector of the system in the phase plane along the sliding surface [155].

By substituting the derivative of the error signal from equation (5.11) into equa-
the equivalent control \( u_{eq}(t) \) can be found, by solving \( u(t) \) in equation (5.16).

The equivalent control is a continuous control signal that would maintain the condition \( \dot{s}(t) = 0 \) [182]

\[
\dot{r}(t) + \tau^{-1} (r(t) - e(t) - x_2(t)u(t)) = -\lambda e(t)
\]

(5.16)

Assuming that the reference signal will be either constant or a step-wise signal, its time derivative can be approximated by zero \( \dot{r} \approx 0 \), hence equation (5.17) becomes

\[
u_{eq}(t) = x_2(t)^{-1} [e(t)(\lambda \tau - 1) + r(t) + \tau \dot{r}(t)]
\]

(5.17)

In this manner, an equivalent smooth signal of the SMC algorithm has been deducted in this section to eliminate the effect of chattering. Since the required control signal by the device proposed in Section 5.2 is a binary control signal, the equivalent control signal presented here will be transformed in the next section to a pulsating signal of fixed frequency.

**Pulse Width Modulation of the Equivalent Control Signal**

The equivalent control \( u_{eq}(t) \) described in the previous section can be converted to a pulse-width modulated signal provided an appropriate switching frequency is achieved [183]. This assumption is valid as an actuation of the order of fractions of second in the PWM signal is much faster than the time constant of platelet aggregate growth, which is in the order of minutes. The PWM signal avoids the high frequency switching of the conventional SMC, and provides a discontinuous signal of two states suitable for the control of microfluidic valves.
In a pulse-width modulated control, the system actuator signal $u(t)$ is a scalar that takes values in the set $U = \{u_{\text{min}}, u_{\text{max}}\}$. The signal $u(t)$ is allowed to be switched only once during a period of fixed duration $T$. The instant when the switching occurs is determined by the value of the duty cycle $D(x(t))$ at the beginning of each cycle, $t_{\text{on}}$, defined as the fraction of time that the signal should remain at $u_{\text{max}}$ divided by the period of the signal $T$, that is $D(x(t)) \equiv t_{\text{on}}/T$. For example, if $D = 0.2$, the signal $u(t)$ has the value $u_{\text{max}}$ during the time $t_{\text{on}} = 0.2T$, and then $u(t)$ is switched to $u_{\text{min}}$ for the remaining time of the period, $t_{\text{off}} = 0.8T$, as illustrated in Figure 5.9 a). The duty cycle satisfies $0 \leq D(x(t)) \leq 1$. Thus, the control input $u(t)$ is defined as a PWM function of the equivalent control $u_{\text{eq}}(t)$

$$u(t) = \begin{cases} u_{\text{max}} & \text{for } t_k \leq t < t_k + D[u_{\text{eq}}(t_k)]T \\ \text{for } t_k + D[u_{\text{eq}}(t_k)]T \leq t \leq t_k + T \end{cases}\quad (5.19)$$

where the duty cycle function is illustrated in Figure 5.9 b), and defined as

$$D[u_{\text{eq}}(t_k)] = \begin{cases} 0 & \text{for } u_{\text{eq}}(t_k) \leq 0 \\ u_{\text{max}}^{-1}u_{\text{eq}}(t_k) & \text{for } 0 < u_{\text{eq}}(t_k) < u_{\text{max}} \\ 1 & \text{for } u_{\text{eq}}(t_k) \geq u_{\text{max}} \end{cases}\quad (5.20)$$

**Simulation Results of Sliding Mode Control with Pulse Width Modulation Algorithm**

This section aims to evaluate the performance of the SMC with PWM algorithm for the regulation of platelet aggregation in response to shear rate micro-gradients. A block diagram of the implemented controller is illustrated in Figure 5.10. Parameters for the model and controller used in the simulation are summarised in Table 5.1. The model parameters, $\theta = [\tau \quad c_0 \quad c_1]$, were identified in Section 4.4 [3], and are
typical of a healthy patient with normal platelet activity. The gain parameter in the SMC $\lambda$ was chosen to meet the condition $\lambda > \tau^{-1}$ in order to maintain stability of the controller. The period of the PWM signal $T$ was set in the order of seconds, as it is feasible for a microfluidic valve to operate in this range.

As found in Section 4.2, the shear rate that maximised aggregation in the platform was chosen as $u_{\text{max}} = 2700 \text{ s}^{-1}$. Owing to restraints in the operation of the platform, the flow must be positive at all times in order to avoid undesired aggregation upstream of the stenosis. The minimum action control was $u_{\text{min}} = 0$ as a minimal shear rate was found to cause the platelet aggregate to disintegrate.
\[ u_{eq} = x_2^{-1}[e^\tau \lambda + x_1] \]

\[ u = \begin{cases} 
  u_{max} & \text{for } t_{on}(u_{eq}) \\
  0 & \text{for } t_{off}(u_{eq}) 
\end{cases} \]

\[ \dot{x}_1 = -\tau^{-1}x_1 + \tau^{-1}x_2u \]

\[ \dot{x}_2 = c_1x_2 \]

\[ y = x_1 \]

Figure 5.10: Block diagram of SMC with PWM Control for the regulation of SIPA.

Figure 5.11 shows that the SMC with PWM algorithm successfully maintained the tracking performance, \( x_1(t) \rightarrow r(t) \), despite the nonlinear and time-varying dynamics of platelet aggregation. The simulated platelet aggregate size \( y(t) \) regulated by the SMC-PWM controller converged to the reference signal \( r(t) \) each of the steps in a short finite time. In addition, the simulated aggregate remained stable at the desired state as intended, with the design of the sliding surface in equation (5.7).

The adaptation of the control action to the development of the aggregate was reflected on the duty cycle signal \( D(x) \). At the start of the experiment, \( 4 \leq t \leq 5 \text{ min} \), the duty cycle was large, around 80\%, as the initial dynamics of platelet aggregation are known to be slower at this point. Later on in the simulation, \( 24 < t < 25 \text{ min} \), as nonlinear effects stimulated the growth of the aggregate, less control action was required to obtain the same thrombus size in the reference signal where only 40\% duty cycle was set by the controller.

The control signal \( u(t) \) presented smooth operation with a fixed frequency of switching between the states ON and OFF, as expected from the PWM module. A fully saturated action at either the maximum or minimum, \( u(t) \in \{u_{max}, 0\} \), was set at the controller until the state vector reached the sliding surface. After this point, the control action presented a smooth switching with a fixed period forcing
Figure 5.11: Simulation results of the SMC and PWM algorithm to regulate platelet aggregation in response to shear rate micro-gradients. Top, the reference signal $r(t)$ is selected to achieve the periodic formation and dissolution of a platelet aggregate of size $2000 \mu m^2$ in a lapse of $30 \text{ min}$. The output signal $y(t)$ tracks the reference signal during all the course of the simulation in the presence of time-varying nonlinear dynamics of equation (4.27). The switching control action $u(t)$ is a PWM signal with values $u(t) \in \{0, u_{\text{max}}\}$ controlled by the continuous signal of the equivalent control $u_{\text{eq}}(t)$ in equation (5.18). Bottom left, close view of $u(t)$ showing the pulse-width modulated signal at the initial time of experiment $4 < t < 5 \text{ min}$ with a duty cycle of $D \approx 80\%$. Bottom right, close view of $u(t)$ towards the end of the simulation $24 < t < 25 \text{ min}$ with a duty cycle of $D \approx 40\%$.

the system to remain on the sliding surface.

The action of the controller to dissolve the aggregate, when the reference signal transitioned from $r(t) : 2000 \rightarrow 0 \mu m^2$, was limited to setting the control action to zero $u(t) = 0$. Consequently, the response to induce platelet aggregate formation was faster than to cause its dissolution. In fact, this response corresponded to the natural response of the system.

Another noteworthy feature was that error signal $e(t)$ remained bounded throughout all the simulation and the error signal decreased exponentially each time there was a change in the reference signal. This reflected the intention of the sliding surface to force the error to decay exponentially $\dot{e}(t) = -\lambda e(t)$. 
5.4 Simulation of SMC-PWM Algorithm for the Regulation of Platelet Aggregation

This section presents two case studies that demonstrate applications of the SMC-PWM control algorithm proposed in Section 5.3.3 for the study of shear-dependent platelet aggregation.

5.4.1 Case Study I: Regulation of platelet aggregate size

Section 3.4 established that the growth of platelet aggregation under disturbed flow undergoes a series of stages in its development. While some types of blood may have a very slow development of these stages, other blood types can present a rapid growth and reach the stage of embolisation in an extremely short time. This variability in platelet function makes observation of the aggregate difficult at particular stages of its development. The use of a control algorithm to achieve the stabilisation of the aggregate growth at a particular stage would be desirable to study in detail the dynamics of aggregation at that point. This section investigates the application of the SMC-PWM control algorithm proposed in Section 5.3.3 to regulate the growth of the aggregate in the microfluidics device at a particular size by modulating shear rate.

The control objective for this study was to regulate the growth of the platelet aggregate to a size of $1000 \mu m^2$, an intermediate size of the aggregate at the stage of acute growth (as shown in Figure 3.7). Two models representing blood types with hyper- and hypo-platelet function were considered, and the models parameters were estimated as ±50% the value of the parameters found for a healthy individual in Table 5.1. Simulations were carried out in open-loop using a constant physiological shear rate $u(t) = 1800 \text{s}^{-1}$, and in closed-loop with a modulation of the control signal $u(t) = \{0, 2700\} \text{ [s}^{-1}\text{].}$
Figure 5.12: Simulation results of Case Study I: regulation of platelet aggregate size. a) Blood with hyper-reactive platelet function. b) Blood with hypo-reactive platelet function. $r(t)$ reference signal, $y(t)$ platelet aggregate size.

Figure 5.12 shows the simulation results of platelet aggregation for the two models mentioned above. In the case of the hyper-reactive platelet function model, the simulated aggregate size converged to the reference signal in a short time when the SMC-PWM algorithm was used as shown in Figure 5.12 a). In addition, the aggregate size remained at the reference signal until the end of the simulation, despite the time-varying behaviour of the model of platelet aggregation (equation (5.2)). On the contrary, the growth of the aggregate for this type of blood exceeded rapidly the desired size when the system is simulated in open-loop (constant shear rate), as observed experimentally in Section 4.2.
The simulated development of platelet aggregate for a hypo-reactive platelet function type of blood is illustrated in Figure 5.12 b). The inclusion of the SMC-PWM algorithm in the system resulted in the regulation of the aggregation response to the desired reference signal within less than 5 min. However, an inherently slow response of this type of blood was found when a constant nominal shear rate was used (open-loop). The simulated aggregate size did not reach the desired size at the end of the simulation.

This Case Study showed that the inclusion of feedback control (SMC-PWM) in the operation of the proposed microfluidics device can be applied to regulate the size of a platelet aggregate at a desired stage. Such application can be employed to normalise the time required to test blood with different levels of platelet activity. In addition, regulation of the size of the platelet aggregate in the microfluidics device by controlling shear rate has the potential to be used in the evaluation of anti-thrombotic treatments that consider shear-driven platelet aggregation.

5.4.2 Case Study II: Inter-patient variability

The behaviour of blood clotting in aggregation tests can vary significantly between individuals. For instance, the aggregation response of platelets for patient with von Willebrand Disease is significantly slower than the response of a healthy patient. Alternatively, the process of blood clotting for a blood phenotype with a high thrombogenic potential may lead to full occlusion of the test microchannel in seconds. The robustness of flow-based assays of platelet aggregation to inter-patient variability is fundamental for their application in clinical diagnostics. This section aims to investigate the robustness of the controller proposed in Section 5.3.3 to deal with blood types of varying platelet reactivity.

Several blood types with varying degree of platelet activity were represented by \textit{in silico} models as shown in Table 5.2. The models were selected with a random
variation of up to 30% from the model parameters found for a healthy individual in Section 4.4, and simulated with the SMC-PWM controller in equation (5.18).

Simulation results showing a reference signal $r(t)$ and the performance of the controller for each of the models in Table 5.2, are illustrated in Figure 5.13. In all cases, the output of the controller converged to the reference signal. The time of the reaching phase was dependent on the dynamics of each of the models, specially at the start of the simulation. This dissimilarity was reduced after the enhancing effect of the chemical agonists was more evident towards the end of the simulation.

**Table 5.2:** Model parameters for inter-patient variability study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau$</td>
<td>1.8189</td>
<td>1.9025</td>
<td>1.1876</td>
<td>1.9095</td>
<td>1.6515</td>
<td>1.1605</td>
</tr>
<tr>
<td>$c_0$</td>
<td>0.7370</td>
<td>0.8739</td>
<td>1.0833</td>
<td>1.0871</td>
<td>0.6754</td>
<td>1.0900</td>
</tr>
<tr>
<td>$c_1$</td>
<td>0.0218</td>
<td>0.0169</td>
<td>0.0202</td>
<td>0.0134</td>
<td>0.0163</td>
<td>0.0214</td>
</tr>
</tbody>
</table>

**Figure 5.13:** Simulation results of Case Study II: inter-patient variability study. $y_1 \ldots y_6$ correspond to the outputs of control algorithm with the parameters presented in Table 5.2.
These results demonstrate that the SMC-PWM control algorithm is robust to variation in the dynamics of the original system. This increases the utility of the proposed microfluidics device and control system for clinical diagnostics of platelet function for a wide range of blood phenotypes.

The results presented in this chapter constitute an advance towards the automatic diagnosis of platelet function. Other applications of this methodology can be seen in the testing of potential antithrombotic drugs considering the effects of shear rate induced thrombosis.

5.5 Conclusions

This chapter proposed control algorithms to regulate the aggregation of platelets in a microfluidics device, in order to automate, optimise, and provide robustness in the diagnostics of platelet function. A sliding mode control algorithm coupled with pulse-width modulation was found to be suitable the regulation of platelet aggregation in the proposed microfluidics platform. The algorithm proved be robust to the nonlinear dynamics of the system, and its smooth switching control action ensured fast time convergence to the reference signal. Robustness to inter-patient variability of this algorithm suggests an important advantage for automated platelet function diagnostics.
Chapter 6

Conclusions

The aim of this thesis was to investigate the use of modelling and control methodologies that could make more robust a state-of-the-art microfluidics device for the point-of-care diagnostics of platelet function under disturbed flow. Algorithms developed for the measurement of platelet aggregation dynamics allowed the observation of an increase of platelet density, and suggested that this process is fundamental for the development of an enhanced aggregation effect. A mathematical model comprising a first-order linear response coupled with an ordinary exponential function, representing the observed enhancing effect, fitted with low variability experimental data of platelet aggregation in response to a sequence of shear rates. The model parameters were proposed as a systematic method for platelet function diagnostics. Control algorithms based on sliding mode control and pulse width modulation were found to be suitable for the regulation of platelet aggregation in a microfluidics platform. These can optimise the time required for the diagnostics test, and to provide robustness and stability upon different responses of blood phenotypes. Therefore, the objectives laid out at the start of the thesis have been achieved. The next section presents a summary of each of these achievements.
6.1 Outcomes of This Work

In Chapter 3, methods for observing in real-time the dynamics of platelet aggregation under disturbed blood flow were developed. The use of a microcontraction with fixed geometry and a single syringe driver with varying perfusion rate were employed to generate different conditions of shear rate. Experimental results were analysed using novel measures of platelet aggregate size and mean intensity of the main forming aggregate. These results revealed the existence of distinct stages in the formation of the platelet aggregate. The time onset for initiation of aggregation was found to be independent of the response to shear rate, and instead the time onset was found to be dependent on the kinetic absorption of von Willebrand Factor by the substrate of the device, and the formation of an initial monolayer of platelets on the wall of the microcontraction. The forming aggregate presented an initial acute growth, followed by a plateau due to competing forces of growth and fast streamlines of flow. At this stage of plateau, the aggregate was found to undergo an increase in platelet density, as indicated by the mean intensity measure, which promoted further growth of the aggregate. It was hypothesised that the increase in aggregate growth was possible by the release of unidentified chemical agonists from the packed aggregate.

In Chapter 4, system identification methods were applied in order to extract a compact group of parameters from the dynamics of biomechanical platelet aggregation in response to a range of shear rates. Mathematical models were proposed from both, concepts in the relationship of fluid dynamics and platelet aggregation, as well as experimental data. It was found that initial aggregation response may be represented by a first-order linear dynamic model. In addition, a nonlinear model representing the complete aggregation response was proposed. The model considered the observed enhancing effect in the aggregation response which occurs as result of the densification, and release of platelet agonists, and an ordinary exponential function was found to approximate this behaviour well. The model parameters, obtained
from fitting the nonlinear model to experimental data, presented low variation, and were suggested as a systematic and robust methodology for diagnosis of platelet function.

In Chapter 5, control algorithms were formulated to provide robustness to inter-patient variability and to the complex dynamics of blood clotting, and to optimise the operation of proposed microfluidic devices. The addition of a pressure relief point in proximity to the microcontraction of the device improved dramatically the time response of the platform, allowing for rapid control of shear rate in the device. A switching control algorithm, formulated from concepts of sliding mode control and pulse width modulation, demonstrated that the extent of platelet aggregation can be regulated by dynamically modulating the shear rate in the platform. The value of the duty cycle function in the PWM part of the controller gave direct indication of the level of platelet reactivity.

6.2 Suggestions for Future Work

In this thesis, diagnostics of platelet function through dynamic regulation platelet aggregation in response to shear rate micro-gradients was presented for the first time. Experimental data was limited to the main biomechanical response of platelet aggregation as the main soluble agonists were oppressed, and only blood with normal platelet activity was investigated. To eventually assess the potential of modelling and control systems in platelet function diagnosis using microfluidic devices, suggestions for future investigations are outlined as follows.

Experimental validation of SMC-PWM algorithm

In Chapter 5, a switching control algorithm based on SMC and PWM was proposed to regulate the dynamics of platelet aggregation in a microfluidics device with rapid
response to changes of shear rate. Validation of the proposed algorithm was carried out under computer simulation employing a dynamic model of platelet aggregation, formulated in Chapter 4. Prior to experimental validation of the SMC-PWM algorithm with blood, a thorough characterisation of the proposed microfluidics device for rapid control of shear rate is required. Such characterisation can be realised employing \( \mu \text{PIV} \) with water and tracing micro-particles. As discussed in Section 5.2, the switching operation of the device must be such that it does not result in backflow of blood through the microcontraction to avoid activation of platelet before these reach the test point. In addition, the parameters of the switching function of the PWM control signal in equation (5.19) \((T, \text{ and } D)\) can be optimised experimentally to obtain a fast and reliable operation of the platform. Once the microfluidics device has been optimised, experimental trials with different types of blood may be realised to demonstrate the utility of the SMC-PWM control algorithm to regulate robustly the formation of platelet aggregate in the device.

**Real-time measurement of physical and chemical variables**

The approach presented in this thesis only used feedback information from the biological response, that is, the platelet aggregate itself. Inclusion of real-time measurements of variables such as fluid velocity, and chemical gradients of soluble agonists, can lead to the formulation of more precise models. An important phenomenon to measure is the formation of vortices in a post-stenotic region, known to produce enhanced transport of platelets towards the surface of the forming platelet aggregate [50]. Recent optical measurements for thrombus formation at high shear rates in microfluidic devices could detect the formation of these vortices [163]. On the other hand, measurement of thrombin dynamics in the forming aggregate, key enzyme for coagulation cascade, may be investigated using recently developed sensors [194].
Data mining of model parameters for blood disorder diagnosis

The models investigated in this thesis were calculated employing experimental data from a healthy blood donor, and it is expected that the calculated model parameters for blood samples from different phenotypes will vary significantly. A possible framework for automated blood disorder diagnostics can be investigated by collecting a bank of data from experimental results from both, patients with normal platelet activity, and patients with disorders such as von Willebrand Disease or Bernard-Soulier Syndrome. Data Mining methodologies [195] such as Supported Vector Machines or Neural Networks [196] may then be applied to perform classification and diagnostics of blood disorders from blood perfusion experiments.

Microfluidics for rapid flow rate control

In Chapter 5, a microfluidics device including a relieve valve was proposed to improve the time response to changes in the flow rate in device, and characterisation of the average flow rate in the device was performed upstream of the microcontraction for a low switching frequency. A thorough investigation is required in order to ensure that the suggested switching frequencies used by the control algorithms will not result in back flow of blood through the microcontraction. The presence of such back flow would contaminate blood upstream of the microcontraction (inspection area) and most likely would cause undesired activation of platelets prior to inspection tampering the test. In addition, inclusion of embedded deformable features in the fluidic network that respond to time-modulated pressure signals [197] have the potential to add more flexibility and improve the performance of the proposed switching control algorithms.
Inclusion of biochemical aggregation response

This thesis considered the effect of disturbed flow on the aggregation of platelets, represented by the magnitude of shear rate micro-gradients, blocking the main chemical pathways of platelet activation. A key advancement of this work would be the simultaneous investigation of the effect of both soluble agonists as well as disturbed flow on platelet aggregation. Control systems in association with mechanisms for delivering low concentrations of $ADP$ and/or $TxA_2$ into the blood flow as the aggregation is occurring could be investigated for observing of the effects of low dose chemical activation on the shear driven mechanism. Such platform would be very useful clinically to assess the effects of both aspirin and clopidogrel dosing, as differing effects of chemical agonists on the platelet aggregate are expected to be observed.
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142


