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EVALUATION OF A NUMERICAL THROMBOSIS MODEL FOR A HIGH SHEAR ROTATING FLOW

By

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A thesis submitted in partial fulfillment of the Requirement for

Master of Science

in

Mechanical Engineering

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Abstract

Blood clotting, or thrombosis, is an interesting biological application for computational fluid dynamics. Existing numerical thrombosis models have previously been shown to be effective for low shear rates and simple geometries. For these models to be used in biomedical applications such as the design of rotary blood pumps, however, they must first be experimentally validated for high shear rates and complex geometries. In this study, we test the ability of a numerical thrombosis model to predict thrombosis related phenomena in a high shear flow by creating a geometry similar to that of a rotary blood pump. We have applied an existing numerical thrombosis model to an annular gap between rotating concentric cylinders, a geometry that is closely related to rotary blood pumps. Additionally, we created a physical model of the same geometry and exposed blood to a range of shear rates in both the empirical and numerical model. The empirical and numerical results are compared in order to evaluate the ability of the numerical model to predict thrombosis in similar geometries, such as high shear blood handling pumps.

Fluent was used to solve the coupled convective-diffusion equations along with user defined equations that include production and consumption of 7 species critical to thrombosis. These equations, along with equations of fluid motion, were solved iteratively within the Fluent solver. All reaction constants were from previously published work. At each of the shear rates and exposure times tested, the numerical model calculated platelet deposition, platelet-platelet aggregation and the two-dimensional distribution of three primary agonists (ADP, thromboxane and thrombin) in addition to the standard fluid variables (velocity, pressure, shear rate, etc.)

A physical model was designed and constructed to control the shear rate to which blood is exposed. An annular gap of 360 μm was chosen in order to induce a shear rate of up to 10,000 s^{-1}

while maintaining laminar flow. In a series of experiments, fresh, heparinized, bovine blood was exposed to a constant shear rate ranging from 1,000 to 10,000 s^{-1} for 120 seconds. Prothrombin time (PT) and activated partial thromboplastin time (APTT) of the blood was then measured for each stress level.

While the observed changes in thromboembolic potential (as measured by PT and APTT) of the whole blood test samples qualitatively correspond to platelet activation and agonist concentration predicted by the numerical model, further work is needed to quantitatively verify the numerical model. Thrombosis models based on coupled convective-diffusion equations show promise, but need further refinement and validation before they can be trusted to authoritatively predict thromboembolic potential.

Table of Contents

Abstract.....	v
Table of Contents	vii
List of Figures.....	ix
List of Tables	xi
1 Introduction.....	1
2 Background	3
2.1 Biological Contributors to Thrombosis	3
2.1.1 Platelet.....	3
2.1.2 Coagulation Cascade	5
2.1.3 Haemostasis.....	8
2.2 Experimental Thrombosis Research	9
2.2.1 Effect of Shear on Platelet Deposition	9
2.2.2 Effects of Shear on Platelet Activation and Aggregation	10
2.2.3 Effects of Shear on Platelet Diffusivity.....	11
2.2.4 Platelet Embolization	12
2.3 Analytical and Computational Models of Thrombosis.....	13
2.3.1 Haemodynamic Models.....	13
2.3.2 Cascade Models.....	14
2.3.3 Platelet Adhesion Models.....	14
3 Methods.....	18
3.1 Geometry	18
3.2 Test Rig Design and Construction.....	19
3.3 Blood Collection.....	19
3.4 Exposing Blood to Shear	20
3.5 Coagulation Measurement.....	21
4 Computational Methods.....	22
4.1 Numerical Methods	22
4.2 Thrombosis Model.....	26
4.2.1 Included Species.....	26
4.2.2 Platelet Deposition	29
4.2.3 Platelet Activation and Agonist Release	30
4.2.4 Thrombus Growth	34
4.2.5 Enhanced Platelet Diffusion.....	34
4.3 Validation of Model by Comparing with Previously Published Results.....	35

4.4 Numerical Model Applied to Annular Gap	35
4.4.1 Boundary Conditions	35
4.4.2 Modifications to the previous model.....	36
4.4.3 Model convergence	37
5 Results	38
5.1 Experimental.....	38
5.2 Comparison of Numerical Model with Previous Results	39
5.3 Annular Gap	40
5.3.2 Grid Study	40
5.3.2 Velocity Field.....	43
5.3.3 Platelet Concentrations.....	44
6 Conclusion and Discussion	49
6.1 Experimental Insight and Discussion	49
6.2 Numerical Insight and Discussion	49
6.3 Comparison of Numerical and Experimental Results	50
6.4 Future Improvements to the Experiment	50
6.5 Potential Improvements to the Current Numerical Model	51
6.6 Potential Clinical Applications of the Numerical Model	52
References	54
Appendix A Implementation into Fluent.....	I
Appendix B Some notes on Compiling the UDF	VII
Appendix C Source Code	VIII
Appendix D Standard Operating Procedure for Blood Collection	XXX
Appendix E Standard Operating Procedure (SOP) for Concentric Cylinders Test Stand	XXXII
Appendix F Drawing Package for Concentric Cylinder Test Stand.....	XXXIV
Appendix G Standard Operating Procedure for PT and APTT tests from Stiene-Martin, Clinical Hematology, pg. 638-639	L

List of Figures

Figure 1: Image of titanium surface with attached unactivated platelets (top) and attached activated platelets (bottom). Note the change in morphology of the activated platelets. From Jones, 2000 pg 416.....	4
Figure 2: Attached platelets on a polycarbonate surface at 10x magnification. The platelets are a purple / pink color from the Romanowsky stain.....	5
Figure 3: Coagulation cascade from Stiene-Martin, Clinical Hematology pg. 614.....	7
Figure 4: Illustration of the hemostatic process showing platelet attachment, activation and fibrin clot formation. From McKenzie, Textbook of Hematology, pg 490.	8
Figure 5: The difference in the enhanced platelet diffusion of Goodman and Wootton. Goodman used a diffusion enhancement coefficient of $6E-10$ and Wootton used $7E-9$	12
Figure 6: Flow geometry.....	18
Figure 7: Test rig assembly, 1: DC motor; 2: Inner cylinder held static; 3: Outer cylinder mounted in bearings.....	19
Figure 8: Flow chart of solution process in Fluent. Items in circles are input or output values to or from the solver. Items in solid squares are functions performed by the solver. The dashed line encompasses the loop of functions that Fluent performs each iteration.	24
Figure 9: Schematic of platelet activation and thrombus formation. Solid lines are pathways to the various platelet states and dashed lines represent the feedback loop due to agonist release. For clarity, either the equation or the table are listed for each reaction.	33
Figure 10: Schematic of complete flow geometry compared to computational domain with labeled boundary conditions 1: Rotational periodic; 2: Stationary wall; 3: Rotating wall.....	36
Figure 11: Measured PT and APTT.....	38
Figure 12: Top Left: Schematic of Goodman et al. geometry showing line of symmetry in red. Bottom Left: Resulting simulation done as a part of this work, showing attached thrombus. The colors identify the grid cells as fluid (blue and light blue), and thrombus (red and yellow). Right: Experimental (A and B) and computational (C) results from Goodman 2005, pg 788 showing attached thrombus. Note similarity in both size and location to Goodman et al. results.....	40
Figure 13: Shear rate at the stationary (inner) wall for three different grid sizes. Less than 1% change was observed from the 10 micron grid to the 5 micron grid.	41
Figure 14: The shear rate at moving (outer) wall for three different grid sizes. Less than 1% change was observed from the 10 micron grid to the 5 micron grid.	42
Figure 15: Normalized platelet deposition on the stationary (inner) wall for grid elements of 10 and 5 microns. Platelet Deposition is normalized to the minimum value for each simulation for comparison purposes.....	43
Figure 16: CFD velocity profile development.....	44

Figure 17: Total number of platelets in the simulation at 0.0005, 0.5, 1, 10, 30, and 100 seconds. The number of platelets deposited on the moving and stationary wall is less than 3% different than the number of plates lost from the bulk flow at any time.	45
Figure 18: The mass fraction of activated and resting platelets decrease over time in the CFD simulations.	46
Figure 19: Activated platelet concentration for the entire computational domain at 100 sec of flow time. Note the high concentration in the center and low concentration near the walls.	47
Figure 20: Results from computational simulations demonstrating increasing thrombin mass fraction with increasing shear rate. Each point represents an individual CFD simulation of 120 seconds of solution time.	48

List of Tables

Table 1: Summary of platelet deposition experiments	10
Table 2: Flow geometry parameters at max shear Rate of 10,000s ⁻¹	18
Table 3: List of included species and their source terms	27
Table 4: List of symbols in Table 3	28
Table 5: Parameters for all the species in the coagulation model	29
Table 6: Critical concentrations for activation and weights for agonist induced activation	31

1 Introduction

Thrombosis, the formation of blood clots, remains one of the most significant challenges in the design of blood contacting medical devices such as implantable blood pumps, hemodialysis, and blood oxygenators [Gartner, 2000]. An attached clot can grow until it obstructs the natural flow and a detached or suspended embolus may travel through the blood stream until it eventually obstructs a vessel. The design methodology of blood handling devices has progressed remarkably in the past years, largely because of the use of finite element analysis and computational fluid dynamics [Burgreen, 1996]. Nonetheless, the prediction of thrombosis in devices is unsatisfactory because there is not a validated model that can be used for the predictions.

Thrombosis is principally due to three factors: platelet transport, coagulation cascade, and haemodynamics. While considerable research has gone into studying the underlying mechanisms of thrombosis [Basmadjian, 1997; Chang, 1988; Fogelson. 1984] these models are generally not quantitative enough to decidedly predict the occurrence of thrombosis. While many studies of thrombosis focus on one of the three mechanisms in order to gain insight into specific area of the larger problem, the design process needs a single comprehensive model that includes effects of platelets, surface chemistry and haemodynamics. One of the more promising models was developed by Sorensen [1999] and included platelets and 5 agonists, chemical species that are part of the biochemical cascade of thrombogenesis. The model accounts for the agonist induced activation of platelets, enhanced species diffusion, attachment of platelets to the solid surfaces, and convective and diffusive transport of platelets by the flow. Goodman [2005] implemented the Sorensen model using Fluent, a commercially available CFD package and determined reaction rate constants of platelet deposition and embolization from his own experiments.

To date, this coagulation model has been applied to relatively simple geometries and flow regimes such as an abrupt cylindrical contraction or expansion [Goodman, 2005], or physiological conditions of shear rate and material [Sorensen, 1999]. The model was compared to empirical data and was shown to be effective for these cases [Goodman, 2005; Sorensen, 1999]. However, the experimental data have been case dependent and more research is needed to determine how robust this numerical model is. Unlike physiological flows, the flow within blood prostheses is often characterized by high shear rates, bio-reactive materials, and turbulence. While the implementation of the model in Fluent make it feasible to apply the model to complex geometries such as mechanical blood pumps, the validity of the model in these applications is questionable because of both high shear rates and non-biological materials.

The objective of this study is to test the validity of applying these numerical models to a geometry that more closely resembles rotary blood pumps. Specifically, we use the annular flow between rotating cylinders to create a shear flow with controllable shear rate. We have implemented the thrombosis model on this geometry and conducted numerical experiments by varying the shear rate. Additionally, we have designed and constructed a mechanical apparatus to expose whole blood to the same conditions. We compare the numerical and empirical results in an effort to determine under what, if any, conditions the model is valid.

2 Background

2.1 Biological Contributors to Thrombosis

2.1.1 *Platelet*

The platelet is a non-nucleated blood fragment of irregular shape. It will typically have a diameter of 2 to 4 microns. In healthy, whole blood they number 250,000 platelets/mm³, but make up less than 1% of the total blood volume. The main function of platelets is to maintain hemostasis by forming a thrombus or clot. Platelets exist in two states: activated and unactivated. When visually inspected, unactivated platelets have a variable disc shape. When a platelet activates, it forms pseudopods. The pseudopods stretch out like tentacles and give the platelet an irregular star shape as seen in Figure 1. When a platelet activates, it also releases chemicals such as thromboxane A2 and adenosine diphosphate which further encourage clot formation. Platelet activation can occur due to many factors or a combination of factors including contact with sub-endothelial cells, high concentrations of agonists or exposure to high shear rates. Platelets appear purple when viewed under a microscope when a Romanowsky stain is used as seen in Figure 2. [Stiene-Martin, 1998; McKenzie, 1996]

Figure 1: Image of titanium surface with attached unactivated platelets (top) and attached activated platelets (bottom). Note the change in morphology of the activated platelets. From

Jones, 2000 pg 416.

Figure 2: Attached platelets on a polycarbonate surface at 10x magnification. The platelets are a purple / pink color from the Romanowsky stain.

2.1.2 Coagulation Cascade

The coagulation cascade is the enzymatic process that ends with fibrin formation and ultimately leads to clot formation. The cascade can be activated by contact with either tissue factor (extrinsic) or a negatively charged surface (intrinsic). The extrinsic pathway begins with tissue thromboplastin (FVII) and the intrinsic pathway begins with Hageman factor (FXII). Both pathways lead to the common pathway. In the common pathway, prothrombin (FII) in the

presence of calcium is converted to thrombin (FIIa). Thrombin with calcium will convert fibrinogen that is present in whole blood into a stable fibrin clot.

2.1.3 Haemostasis

Haemostasis is the control of bleeding when a blood vessel is damaged. When blood flows past damaged, exposed sub-endothelial tissue, platelets immediately begin to adhere to the exposed collagen. In the presence of ADP, platelets will quickly clump to one another as well as

Figure 4: Illustration of the hemostatic process showing platelet attachment, activation and fibrin clot formation. From McKenzie, Textbook of Hematology, pg 490.

to the damaged tissue. Platelets can also adhere to the damaged tissue with the electrostatic force between the positively charged calcium ions and the negatively charged platelets and damaged tissue. The platelets then release proteins that, in the presence of ATP and glucose, pull together

the fibrin clot. This process is called clot retraction. [Platt, 1979] **Error! Reference source not found.** illustrates the clot formation process.

2.2 Experimental Thrombosis Research

There are many different areas of thrombosis research. Many of them are clinical or diseased state studies, and have little direct impact on the current model. The current research is focused on platelet transport, platelet attachment to biomaterial surfaces and platelet activation. More specifically, the the study focuses on the role that a shear fluid plays in these areas. The following sections outline experiments studying the effects of shear on platelets and platelet functions.

2.2.1 *Effect of Shear on Platelet Deposition*

Platelet deposition on a biomaterial surface has been the focus of much research; however, the influence of shear rate on platelet deposition is still not fully characterized. A comparison between Neumann et al. [1980] static exposure and Leonard et al. [1967] flowing exposure of fluorinated ethylene propylene (FEP) shows that flowing blood will deposit more platelets in the same amount of time. Grabowski et al. [1972] exposed glass to whole canine blood at shear rates of 20, 40, and 80 sec^{-1} . Grabowski et al. showed that a material has a saturation point where that material has the maximum number of platelets deposited on the surface. The authors also demonstrated that increasing shear rate increased the rate that a material reached its saturation point. There have been several experiments demonstrating increasing platelet deposition with increasing shear rate; [Grabowski, 1972; Strong, 1987; Turitto, 1975; Hubbell, 1986; Alevriadou, 1993; Schaub, 1999; Jones, 2000] however, the

highest shear rate studied was 3000s^{-1} . [Alevriadou, 1993] These experiments are summarized in Table 1.

Table 1: Summary of platelet deposition experiments

Reference	Fluid	Material	Shear Rates (s^{-1})	Exposure Time (sec)	Highest Recorded Deposition	
Schaub, 1999	Heparinized human whole blood	Ti, LTIC, DLC, PCD, Dacron, HEM, ePTFE, dePTFE	50, 250	300	$1.70\text{E}+10$	plt m^{-2}
Alevriadou, 1993	Heparinized human whole blood	Collagen coated glass	100, 800, 1500, 5000	60	$1.90\text{E}+11$	plt m^{-2}
Turitto, 1975	Sodium citrated whole rabbit blood	Mounted subendothelium	6.6, 52, 208, 247, 832	180	55	% surface coverage
Grabowski, 1972	Heparinized canine whole blood	Glass	20, 40, 80	2700	$6.60\text{E}+10$	plt m^{-2}
Hubbell, 1986	Hepranized whole human blood	Collagen coated glass	100, 500, 1500	120	$6.00\text{E}+11$	plt m^{-2}

2.2.2 Effects of Shear on Platelet Activation and Aggregation

Platelet activation plays a large role in thrombosis. There have been several studies investigating how exposure to a shear field influences platelet activation. Anderson et al. [Anderson, 1978] used a concentric cylinder viscometer to expose platelet rich plasma (PRP) to a controlled shear field. The cylinders were designed to be biologically inert to minimize the platelet-surface interaction. The authors then measured platelet aggregation and lysis. At a shear of 5000 s^{-1} , there was a 60% decrease in platelet count due to platelet aggregation in the bulk flow. The threshold for platelet lysis for a 5 minute exposure was 5000 s^{-1} and, for a 30 second

exposure, it was $20,000 \text{ s}^{-1}$. Hellums has a vast body of research in the field of thrombosis and provides a review in Hellums, 1993. Equation 12 governing shear induced platelet activation was developed from the data provided in this review.

2.2.3 Effects of Shear on Platelet Diffusivity

Many experiments have been conducted to quantify the enhanced diffusion of platelets in whole blood. The earliest experiments used platelet rich and platelet poor blood to create a washout curve. Platelets were counted using an automatic platelet counter. The highest shear rate tested was 440 s^{-1} . These experiments generated a power-law relationship between shear rate and effective diffusivity; however the experiments were not sensitive enough to accurately determine the relationship between diffusivity and shear rate [Leonard, 1972; Turitto, 1975; Grabowski, 1972i; Strong, 1987]. Currently, there are two popular methods of characterizing the enhanced diffusion of platelets: Keller [1971] or Zydney and Colton [1982]. Both methods use a linear relationship between shear rate and enhanced diffusivity, however each uses a different method of determining the diffusion enhancement coefficient (the constant that is multiplied by shear rate). To determine the diffusion enhancement coefficient, Keller derives a relationship to the diameter of a red blood cell, while Zydney and Colton determine it empirically. Sorensen [1999] used the method proposed by Keller, while Goodman [2005] and Wootton [2001] used the method proposed by Zydney and Colton. Equation 13 is the equation for the enhanced platelet diffusion coefficient used by Goodman and the current research. 5000 s^{-1} is the highest shear rate that the enhanced diffusion of platelets has been experimentally validated for [Keller 1986].

Wootton and Goodman both used the method of describing the enhanced diffusion of platelets proposed by Zydney and Colton, but each used a different diffusion enhancement coefficient. Wootton only considered the experimental results of Zydney and Colton, while

Goodman calculated the diffusion enhancement coefficient using experimental results from 4 separate researchers [Hubbel 1986; Turitto 1972; Aarts 1986; Zydney 1982]. Wootton used a diffusion enhancement coefficient of $7\text{E-}9$, while Goodman calculated it to be $6\text{E-}10$. The change in the enhanced diffusion of platelets due to these values is shown in Figure 5.

Figure 5: The difference in the enhanced platelet diffusion of Goodman and Wootton.

Goodman used a diffusion enhancement coefficient of $6\text{E-}10$ and Wootton used $7\text{E-}9$.

2.2.4 Platelet Embolization

Goodman et al. provide the only quantitative measure of platelet embolization due to shear. Goodman conducted a series of experiments in which he exposed polyethylene (PE) tubing to whole blood at a steady flow rate for 3 min. The authors then rinsed the tubing with a saline

solution at varying shear rates from 0 to 6000 s⁻¹. Goodman et al. then took a measure of percent platelets remaining. The authors fit the experimental data with a curve shown in Equation 1

$$F_{emb} = e^{-0.0095\gamma}$$

Equation 1

where F_{emb} is the percent platelets remaining and γ is the shear rate. Equation 1 is used to calculate Boundary Source Term #1 in Table 3.

2.3 Analytical and Computational Models of Thrombosis

2.3.1 Haemodynamic Models

Haemodynamic models use information from the flow field such as shear rate and reattachment points to estimate how and where thrombosis or other particle mediated deposits will form [Nazemi, 1989; Kleinstreuer, 1991; Lee, 1995]. Fry reported findings of atherosclerotic plaque formation in vascular bifurcations and proposed a critical shear stress at which plaque begins to form. [Fry 1969, 1973] Fry defined the critical shear stress to be 0.5 of the shear stress for Poiseuille flow in a pipe of the same diameter. Nazemi et al. applied Fry's method to an aortic bifurcation. [Nazemi, 1989] They solved the conservation of mass and momentum equations using the Galerkin finite element method. The wall shear stress was then calculated. The locations with wall shear stress less than the critical shear stress were identified and a thin sinusoidal mass layer was placed onto the wall. The mesh was redefined to incorporate the new sinusoidal wall and the governing fluid equations were resolved. This iterative process developed a solution for plaque formation and growth on the vascular wall. The size and location of the plaque formation coincided with clinical observations of the aortic bifurcation. This model has

only been applied to bifurcating geometries. While this model was shown to be effective at predicting plaque formation in a bifurcation, this model is highly influenced by the previous knowledge of plaque formation in this particular geometry. The applicability of this model to more complex geometries is questionable mainly do to the difficulty of determining a critical shear stress where Poiseuille flow is not applicable (i.e. blood pump).

2.3.2 Cascade Models

The coagulation cascade has total of 13 inactive factors and another 6 active factors. Hockin et al. [2002] developed a strict stoichiometric model that contained all the coagulation factors. This model contained 27 chemical expressions to stoichiometrically describe the coagulation cascade. This model provided insight into the limiting factors of the cascade. However, the rate constants and results only apply when phospholipid concentrations are saturated. The work of Basmadjian et al. [1997] provided results that are much more applicable to biomedical applications. First, they took into account mass transport, addressing the fact that the blood is flowing over a surface. Second, they developed solutions for low, intermediate and high flow. Next, the authors investigated the intrinsic and extrinsic paths separately. Finally, a time dependent solution was generated for thrombin concentration. This solution was sensitive enough to capture the positive feedback loop created by the coagulation factors. This model, while powerful, was still relatively simple and could potentially be implemented into CFD in its entirety.

2.3.3 Platelet Adhesion Models

One of the first mathematical models of platelet mediated thrombosis came from a group of researchers out of Columbia University [Grabowski, 1972; Leonard, 1972; Turitto, 1975]. This research group used the coupled convection-diffusion equation (Equation 5) to model platelet transport in whole blood flowing through a cylinder. They non-dimensionalized the equation and developed an asymptotic solution. This research group did extensive work developing the enhanced diffusivity of platelets in whole blood, the adhesion rate constant for platelets on biomaterials, and began to look at how the biomaterial surface affects platelet adhesion. These researchers performed a strict and thorough analytical solution to the governing differential equations; however, some limiting assumptions had to be made to render these equations analytically solvable and these assumptions limit the efficacy of the solution. The largest of these assumptions include a time independent solution and diffusion-limited platelet adhesion. It was later shown that a time dependent solution is necessary to capture the positive feedback loop of platelet adhesion [Sorensen, 1999], and that platelet adhesion in a non-creeping flow cannot be considered diffusion limited [Strong, 1987]. This model also did not include any other species or factors of the coagulation cascade. Given the assumptions made and the relative simplicity of the model, this model determined the diffusion enhancement coefficient with 95% confidence and compared well with experimental results of platelet surface coverage on glass.

Research from the University of Utah lead by Aaron Fogelson took advantage of new technology to develop a finite element scheme to describe the phenomena of thrombosis. In Fogelson's early work [Fogelson, 1984], he did rigorous development of the numerical methods required to solve the governing equations. This publication was the foundation for numerical solutions to not only thrombosis models, but any simulation using the coupled convection-

diffusion equation. Fogelson continued with a similar model to Grabowski et. al. but made many improvements. This improved model could differentiate between activated and unactivated platelets where activated platelets were more likely to attach to the biomaterial surface and other platelets. Fogelson also added the species ADP to his model. ADP was released when a platelet was activated and platelets would activate when a threshold concentration of ADP was reached. Platelets were also modeled with a radius and not as discrete points, so when platelets deposit on the surface or aggregate together, the flow field is re-solved around them. Fogelson also developed a complex scheme to describe the connection between aggregated platelets. In this scheme, the link between platelets could stretch and flex from the forces of the flow field. While this model contained many novel improvements, it also contained some shortcomings. Fogelson applies this model to a narrow blood vessel that has a Reynolds number less than 0.1, and he therefore neglects inertial effects. Due to this simplifying assumption and low Reynolds number, he now only has to solve the Stoke's equation of fluid motion. This flow field also has a low, physiological shear rate, so he can neglect the effects of shear (activation, aggregation and enhanced diffusion) on platelets. While this makes his solution simpler, it renders his model useless for anything besides a creeping flow. Later, Fogelson went on to develop different versions of thrombosis models including an in-depth study of the platelet-platelet link [Fogelson, 1992] and a model purely of the coagulation cascade [Fogelson, 2003]. While Fogelson has made tremendous contributions to the field of thrombosis modeling, currently there is no experimental validation for any of Fogelson's models.

The research group from Rice University developed a model for thrombosis that included more of the platelet released agonists [Hubbell, 1986; Folie, 1989]. This model specifically looked at the effects of an attached thrombus on the flow field. They used the convection-

diffusion equation to calculate the concentrations of ADP, TxA₂, and thrombin in the flow field around the attached thrombus. This group also used the commercial computational fluid dynamics (CFD) program FIDAP to solve the conservation of mass and momentum equations as well as the convection-diffusion equations. [Folie, 1989]

Sorensen *et al.* [1999] developed a fairly comprehensive model of thrombosis. This computational simulation was able to generate a time dependent solution that was able to model the synergistic effect of platelet attachment and agonist release. This model also included a surface availability function to simulate the tendency for platelets to initially adhere as a monolayer [Grabowski, 1972]. The Sorensen *et al.* model compared well to experimental results of parallel plate flow for shear rates of 100 and 500 s⁻¹ but did not compare well for a shear rate of 1500 s⁻¹. Goodman *et al.* [2005] added to this model an embolization term that allows adhered platelets to embolize and return to the bulk flow after sufficient exposure to shear. The Goodman *et al.* model is used in the current research and is discussed further in the *Methods* section.

The highest shear rate used in the prior computational models was 5800 s⁻¹. This shear rate was produced in the small diameter of Goodman's *et al.* abrupt expansion and contraction geometry. However, in Goodman's single pass flow cell, exposure to this shear rate was brief (~.01 sec) compared with the overall length of the experiment (~120 sec).

3 Methods

3.1 Geometry

An annular gap between rotating concentric-cylinders was chosen for its machinability and similarity to an axial flow blood pump (shown in Figure 6). The shear rate within the annular gap is varied by controlling the rotational speed of the outer cylinder. The maximum shear rate is 9500 s^{-1} , which is typical of an axial flow blood pump and higher than previously tested with this numerical model. The radial dimensions, angular velocity, and Reynolds number at this shear rate are listed in Table 2. The annular gap is 0.014 in (0.36 mm) in order to maintain laminar flow ($Re < 1,500$) over the range of rotational speeds. The outer cylinder is rotated in order to avoid any Taylor instabilities [Tritton, 2003].

Figure 6: Flow geometry

Table 2: Flow geometry parameters at max shear Rate of $10,000 \text{ s}^{-1}$

Parameter	Value
Inner radius (mm)	16.3
Gap (mm)	0.36
Angular velocity (rad/s)	445
Reynolds number	459

Based on blood viscosity of 3 cp and density of 1.06 g cm^{-3}

3.2 Test Rig Design and Construction

A device (Figure 6) was designed and constructed for the purpose of exposing the blood to a known, constant and controlled shear rate. Natural polycarbonate was chosen for the blood contacting test section because of its optical clarity and its common usage in blood handling devices. Appendix F gives a full parts list and drawing package for this test rig.

Figure 7: Test rig assembly, 1: DC motor; 2: Inner cylinder held static; 3: Outer cylinder mounted in bearings

3.3 Blood Collection

Fresh, whole bovine blood was collected by relieving the hide and puncturing the carotid artery. The freely flowing blood was then collected in polyvinyl chloride (PVC) container containing enough heparin (porcine intestinal mucosa, EMD Chemicals INC (Biosciences), Darmstadt, Germany) to reach a final concentration of 1.5 U/ml. The blood was then transferred to a sterile Polyethylene Terephthalate Glycol (PETG) container (VWR International) for storage. The process was done at a local slaughterhouse in accordance with all USDA regulations. Appendix D gives a full standard operating procedure for the blood collection.

3.4 Exposing Blood to Shear

Because of the instability of blood, great care was taken to replicate the method for each individual trial. 2 mL of blood was pipetted from the PETG storage container using a variable volume pipettor (VWR International). The blood was then placed in the annular gap test section. The test rig was then turned on and the speed adjusted to the desired rpm. The time from when the blood is placed in the test section until the desired rpm was reached was 30 seconds for all trials. The blood was exposed to the desired shear rate for an additional 120 seconds. The blood was pipetted from the test section and placed in a sterile, borosilicate glass test tube.

Three different shear rates, 2200, 6600, and 9500s^{-1} , were tested in this experiment and compared to a control of 0 shear (simply exposing the blood to the section). This control was chosen in order to isolate the effects of shear stress on the whole blood, while including any effect of exposure to the material. 9500s^{-1} was chosen as the maximum shear rate. Two or three trials were run at each shear rate. One outlying data point was omitted from the results because the BBL Fibro System malfunctioned.

3.5 Coagulation Measurement

Each whole blood sample was centrifuged for 10 minutes and approximately 1mL of plasma was collected for each trial. The BBL Fibro System (Becton Dickinson Microbiology Systems, NJ) was used to measure coagulation by conducting the prothrombin time (PT) and activated partial thromboplastin time (APTT) tests. PT and APTT are medical standards for measuring coagulation and detailed methods have been well documented [Stiene-Martin, 1998]. PT measures the extrinsic pathway, which is activated by exposure to damaged endothelial cells, and APTT measures the intrinsic pathway, which is activated by exposure to any negatively charged surface. Both the PT and APTT are indirect measures of prothrombin and thrombin which are the final and most important factors for fibrin clot formation, and both of these coagulation factors are included in my computational model. A shorter PT or APTT indicates that more coagulation factors are present and clot formation is occurring more rapidly. The vise versa is also true. PT and APTT of the blood were then measured for each stress level. The variance of the test data “R” was found by linear regression and solved in Microsoft Excel.

After the trials at the highest shear rate, the polycarbonate inner cylinder was inspected for platelet attachment with microscopy. Directly after each trial, the inner cylinder was rinsed with a 0.9% NaCl solution. The surface was then stained with Wright stain in order to visualize the platelets. A Keyence VHX-500 (Keyence Corporation of America, NJ) digital microscope was used to inspect the surface. An area representing approximately 10% of the total surface area was inspected.

4 Computational Methods

4.1 Numerical Methods

The governing fluid equations were solved using a commercially available CFD package, Fluent 6.3 (Fluent Inc., Lebanon, NH). A control-volume approach was used and the geometry was discretized into quadrilateral elements. Blood was modeled as a Newtonian fluid with a viscosity of 3 cp [Goodman, 2005]. The conservation of mass or continuity equation can be written as Equation 2

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{u}) = S_m \quad \text{Equation 2}$$

where $\vec{u}$ is the velocity vector, ρ is the density of the fluid and S_m is the source term to account for any mass added to the system. This is the general form of the equation and is applicable to both compressible and incompressible fluids. The general form of the conservation of momentum equation can be written as Equation 3

$$\frac{\partial}{\partial t}(\rho \vec{u}) + \nabla \cdot (\rho \vec{u} \vec{u}) = -\nabla p + \nabla \cdot \left(\begin{smallmatrix} \tau \\ \tau \end{smallmatrix} \right) + \rho \vec{g} + \vec{F} \quad \text{Equation 3}$$

where p is static pressure, $\rho \vec{g}$ and $\vec{F}$ are the gravitational and body forces, and the stress

tensor τ is described in Equation 4

$$\vec{\tau} = \mu \left[\left(\nabla \vec{u} + \nabla \vec{u}^T \right) - \frac{2}{3} \nabla \bullet \vec{u} I \right] \quad \text{Equation 4}$$

where μ is the molecular viscosity and I is the unit tensor. The coupled convection-diffusion equation can be written as Equation 5

$$\frac{\partial}{\partial t} (\rho C_i) + \nabla \bullet (\rho \vec{u} C_i) = -\nabla \bullet \vec{J}_i + S_i \quad \text{Equation 5}$$

where C_i is the mass fraction of the i th species, S_i is the source term for the i th species and J_i is given as Equation 6

$$\vec{J}_i = -\rho D_{i,E} \nabla C_i \quad \text{Equation 6}$$

where $D_{i,E}$ is the enhanced diffusivity for the i th species. Fluent solves each of these equations iteratively with time to generate the flow field and species concentrations for each grid cell.

The species transport solver in Fluent uses the convection-diffusion equation (Equation 5) to model individual species transport in a flowing medium. This equation is valid for species with a low (<0.001) mass fraction and particles small enough that they do not disrupt the flow field. The entire solution process is illustrated in Figure 8. The simulation is started by initializing the problem by inputting values of initial velocity and species concentrations. Fluent then performs its first iteration by iterating the momentum, continuity, energy, and species transport equations. This generates values of the velocity field, shear rate and species concentration for each grid cell. These values are inputted into the UDF. The UDF then calculates species concentration based on the source terms given in Table 3. Fluent then checks

for convergence by comparing the residual of each equation to the convergence criteria. If the residual is larger than the convergence criteria, Fluent performs another iteration beginning by iterating the equations of fluid motion. If the residual is smaller than the convergence criteria, Fluent moves to the next physical time step. A listing of the steps taken to implement this model in Fluent are given in Appendix A.

Figure 8: Flow chart of solution process in Fluent. Items in circles are input or output values to or from the solver. Items in solid squares are functions performed by the solver. The dashed line encompasses the loop of functions that Fluent performs each iteration.

The CFD simulation is not an exact representation of the actual physical geometry, so some assumptions must be made. A CFD is 2-D and therefore assumes that there is a negligible gradient longitudinally along the cylinder and also neglects the end effects of the bottom of the

cylinder. Periodic boundary conditions were used to reduce the computational domain and assume a negligible circumferential gradient due to the symmetric geometry. The time for the cylinder to reach full speed is different between the experiment and the CFD and the effects of these beginning seconds is assumed negligible when compared to the 120 second duration of the experiment. The flow regime is assumed to be laminar due to the calculated Reynolds number of 450 for the highest shear rate. And finally, while blood is known to be a non-Newtonian fluid it is assumed to have a constant viscosity because these flow regimes are well above the critical shear rate 1000 s^{-1} [Hellums, 1993].

The nondimensionalization of Equation 5 leads to the dimensionless group of the Peclet number

$$Pe = \frac{Ua}{D} \quad \text{Equation 7}$$

The Peclet number is a measure of relative importance of convective terms to diffusive terms where U is a characteristic velocity, a is characteristic length and D is normally the Brownian diffusivity of a species, but for this research the effective diffusivity will be used. A large Peclet number compared to unity indicates a dominant convective term. For this geometry, the Peclet number is only dependent on the characteristic length a . For the bulk flow $a=3.5\text{e-}3 \text{ cm}$ and Pe is on the order of 10^4 in the circumferential direction. In the radial direction, velocity is close to 0 and Pe is small compared to unity. Therefore, convection is the dominant term for platelet transport in the bulk flow, but diffusion is dominant for platelet transport in the radial direction. Also, the velocity near the inner wall will be close to zero while the shear will remain constant, so a boundary layer exists near the inner wall where diffusion is the dominant term for platelet transport.

4.2 Thrombosis Model

4.2.1 *Included Species*

The species included in the numerical thrombosis model consist of unactivated platelets, activated platelets and 5 agonists of thrombogenesis (ADP, TxA2, prothrombin, thrombin and antithrombin). The abbreviation and source terms for each species are given in Table 3. These species were chosen due to their major role in platelet activation, deposition, and aggregation. The use of these species was first proposed by the model developed by Hubbell and McIntire (Hubbell, 1986). While von Willebrand factor has been included in previous models, it is not included in the current model. The given parameters for each of the species including initial concentration and Brownian diffusivity D_b are given in Table 5.

Table 3: List of included species and their source terms

#	Species	Abbreviation	Bulk Source Term	Boundary SourceTerm
1	Unactivated Platelets	PI	$-k_{pa} [PI]$	$-S(x,t)k_{ps} [PI]+F_{emb} M(x,t)$
2	Activated Platelets	AP	$+k_{pa} [PI]$	$-S(x,t)k_{as} [AP]-M_a(x,t)/M_{\infty} k_t [AP]$
3	Adenosine diphosphate	ADP	$+\lambda k_{pa} [PI]$	----
4	Thromboxane A ₂	TxA2	$+s_{tl}[AP]+k_j [TxA2]$	$+M_a(x,t) s_t$
5	Prothrombin	pT	$-[pT](\Phi_{atl}[AP]+\Phi_{rt} [PI])$	$-[pT](\Phi_{pt} M_u(x,t) + \Phi_{at} M_a(x,t))$
6	Thrombin	T	$+ [pT](\Phi_{atl}[AP]+\Phi_{rt} [PI])-\Gamma [T]$	$[pT](\Phi_{pt} M_u(x,t) + \Phi_{at} M_a(x,t))$
7	Antithrombin III	aT	$-\Gamma [T]$	---

Table 4: List of symbols in Table 3

Symbol	Description	Value
M_u	Concentration of unactivated platelets on the surface (plt m ⁻²)	-
M_a	Concentration of activated platelets on the surface (plt m ⁻²)	-
M_∞	Total capacity of the surface for platelets (plt m ⁻²)	-
k_{ps}	rate constant for unactivated platelets adhering to surface (m s ⁻¹)	2.5×10^{-6}
k_{as}	rate constant for activated platelets adhering to surface (m s ⁻¹)	3.5×10^{-3}
k_t	rate constant for platelets adhering to other platelets (m s ⁻¹)	3.5×10^{-3}
F_{emb}	fraction of surface-adherent platelets that embolize during each time step	Eq. 1
λ	Amount of ADP released from platelet granules (nmol / plt)	2.4×10^{-8}
S_t	rate of synthesis of TxA2 (mol [AP] ⁻¹)	9.5×10^{-12}
k_j	rate of TxA2 inactivation (s ⁻¹)	0.0161
Φ_{at}	thrombin generation rate on the surface of activated platelets (U plt ⁻¹ s ⁻¹ [pT] ⁻¹)	6.5×10^{-10}
Φ_{rt}	thrombin generation rate on the surface of unactivated platelets (U plt ⁻¹ s ⁻¹ [pT] ⁻¹)	3.69×10^{-9}
Γ	calculated rate for heparin-catalyzed inactivation of T by aT (mole [T] ⁻¹ s ⁻¹)	-

Table 5: Parameters for all the species in the coagulation model.

Species	Initial Concentration	Initial Mass Fraction	MW (g/mol)	$D_b(\text{cm}^2 \text{ s}^{-1})$
PI	$3 \times 10^8 \text{ plt ml}^{-1}$	0.0006	1.28×10^{12}	1.58×10^{-9}
AP	1% [PI]	0.000006	1.28×10^{12}	1.58×10^{-9}
ADP	0 μM	0	424.4	2.57×10^{-6}
TxA2	0 μM	0	352.5	2.14×10^{-6}
pT	1.1 μM	0.000075	72000	3.32×10^{-7}
T	0 μM	0	36600	4.16×10^{-7}
aT	2.84 μM	0.000167	62000	3.49×10^{-7}

4.2.2 Platelet Deposition

Platelet deposition and attached platelet agonist release are modeled using a set of boundary source terms shown in Table 3 in the Boundary Source Term column. These boundary source terms are written by the user as a User Defined Function (UDF) and each is implemented in separate convection-diffusion equation (Equation 5). Because these are boundary source terms, they are only applied to nodes designated as wall nodes.

The kinetic constant of platelet adhesion (k_{ps} and k_{as} in Table 3 Boundary Source Terms #1 and #2) is the constant that determines the rate at which platelets adhere to the surface. Unfortunately, this constant is not widely used and there is little available empirical data. Previous researchers have used scanning electron microscopy (SEM) to measure platelet adhesion to polyethylene and fit the numerical data to the experimental data by changing the constant [Goodman, 2005]. In addition, previous experiments with polyethylene and silicon rubber lead to the conclusion that the difference in platelet adhesion on polymer surfaces is not significant [Goodman, 2005] Therefore, this research used the most recently published value for the kinetic constant of platelet adhesion of $2.5\text{e-}6 \text{ m s}^{-1}$ for unactivated platelets and $3.5\text{e-}3 \text{ m s}^{-1}$ for activated platelets.

Platelets have been observed to initially adhere in monolayer on the biomaterial surface [Grabowski, 1967] and also are more likely to adhere to a surface that is not already populated by adhered platelets. These phenomena are taken into account by the surface availability function $S(x,t)$ (Table 3 Boundary Source Term #1 and #2) defined as Equation 8

$$S(x,t) = 1 - \frac{M(x,t)}{M_{\infty}} \quad \text{Equation 8}$$

where M_{∞} is the total available surface area and $M(x,t)$ is current surface area occupied by platelets and shown as Equation 9.

$$M(x,t) = \int_0^t S(x,t) (k_{ps}[Pl] + k_{as}[AP]) (1 - F_{emb}) dt \quad \text{Equation 9}$$

When a platelet attaches to the surface, it releases agonists (txA2, pt, th) that further promote platelet deposition and activation. Attached platelet agonist release is included in this model as Boundary Source Terms #4, #5, and #6 in Table 3.

4.2.3 Platelet Activation and Agonist Release

Platelet activation and agonist release are governed by the set of source terms shown in Table 3. These source terms are written by the user in a UDF. Each source term is implemented as the source term in Equation 5. Each species has a separate convection-diffusion equation (Equation 5) with the appropriate source term.

There are two modes of platelet activation included in this model: agonist induced activation and shear induced activation. Agonist induced activation is dependent upon a weighted summation of the concentrations of ADP, TxA2, and thrombin. When this summation reaches a

critical threshold, k_{pa} (Bulk Source Terms #1, #2, and #3 in Table 3) changes from 0 to >0 and platelets begin to activate as shown in Equation 10

$$k_{pa} = \begin{cases} 0, & \Omega < 1.0 \\ \frac{\Omega}{t_{act}}, & \Omega \geq 1.0 \end{cases} \quad \text{Equation 10}$$

where Ω is the agonist summation described as Equation 11

$$\Omega = \sum_{i=1}^n w_i \frac{[a_i]}{[a_{i,crit}]} \quad \text{Equation 11}$$

where a_j is the concentration of the jth agonist and $a_{j,crit}$ and w_j are the critical concentration for activation and the weight of the jth agonist as shown in Table 6

Table 6: Critical concentrations for activation and weights for agonist induced activation

Species	Concentration needed for activation	Weight
ADP	2 μM	1
TxA2	0.6 μM	3.3
T	0.01 U ml ⁻¹	220

The rate of platelet activation is proportional to the concentration of the agonists.

Shear induced activation is based on the work of Hellums [Hellums, 1993] and is described in Equation 12

$$t = 4 \times 10^6 \tau^{-2.3} \quad \text{Equation 12}$$

t is the time to activation and τ is the shear the platelet is exposed to (dynes cm^{-2}). The time to activation and the shear stress are tracked in the UDF.

Upon activation, platelets release ADP, TxA₂, prothrombin, and thrombin (Bulk Source Terms #3, #4, #5, #6 in Table 3) which creates a positive feedback loop because these species further encourage platelet activation. Figure 9 is a flow chart illustrating the feedback loop as well as the pathways for platelet activation and thrombus formation.

Figure 9: Schematic of platelet activation and thrombus formation. Solid lines are pathways to the various platelet states and dashed lines represent the feedback loop due to agonist release. For clarity, either the equation or the table are listed for each reaction.

4.2.4 Thrombus Growth

The current model allows for grid cells that contains a higher volume of platelets than the volume of the grid cell to be converted to a solid. This solid represents a thrombus and does not allow fluid to flow through the grid element. In this way, a clot can “grow” by converting adjacent numerical elements to thrombus. Additionally, attached thrombus can also embolize and attached platelets may return to the bulk flow as unactivated platelets.

4.2.5 Enhanced Platelet Diffusion

The motion of red blood cells (RBC) in flowing whole blood has been observed to enhance the diffusivity of other particles in the blood [Strong, 1987; Wootton, 2001]. The enhanced diffusivities for unactivated platelets, activated platelets, prothrombin, thrombin and antithrombin III were accounted for. The other species (ADP and TxA2) are too small to be affected by the RBC motion; therefore, the Brownian diffusivity was used for these species. The diffusion coefficient for each species (enhanced and Brownian) was taken from the literature. [Sorensen 1999] Following Wootton et al. [2001] the enhanced diffusivity is given as Equation

13

$$D_e = D_b + \beta\gamma \quad \text{Equation 13}$$

where D_e is the effective diffusivity, D_b is the Brownian diffusivity, β is the diffusion-enhancement coefficient ($=6.0e-10 \text{ cm}^2$) and γ is the maximum local shear rate. The effective diffusivity can be several orders of magnitude greater than the Brownian diffusivity for high shear flows. The Brownian diffusivity for each species is listed in Table 5.

4.3 Validation of Model by Comparing with Previously Published Results

The previously published results of flow through an axisymmetric stenosis from Goodman [2005] were reproduced to ensure that our numerical model was working and implemented correctly. All of the simulation parameters were exactly replicated with the exception of mesh size, due to computational expense. We used a mesh size of 2 microns whereas Goodman used 0.5 micron. The geometry of an abrupt contraction and expansion was modeled with a two-dimensional, axi-symmetric mesh.

4.4 Numerical Model Applied to Annular Gap

4.4.1 Boundary Conditions

The cylindrical annular gap was solved using a two-dimensional, unsteady, laminar solver in Fluent. Rotational periodic boundary conditions were used to simulate a 0.048 radian portion of the geometry (Figure 10). A structured mesh was used to represent this characteristic volume. The outer wall was set as a rotating boundary condition and the inner wall is a fixed wall. The bulk fluid is initially at rest, but develops as it is driven by the outer wall rotating at a constant angular velocity. For a shear rate of $10,000 \text{ s}^{-1}$, the outer wall was set to rotate at 4000 rpm. Each simulation was run for 120 seconds for a total of 8000 revolutions.

Figure 10: Schematic of complete flow geometry compared to computational domain with labeled boundary conditions 1: Rotational periodic; 2: Stationary wall; 3: Rotating wall

4.4.2 Modifications to the previous model

The following modifications of the implementation of Goodman were made in order to produce a well posed problem. First, periodic boundary conditions were used. Periodic boundaries must come in pairs and the paired boundaries are treated as neighbors, so whatever is the output of one boundary is the input to the other. Secondly, as there was no flow rate or pressure difference specified for this simulation, the rotating outer boundary is the only cause for convection. Because there is no inlet boundary condition, a method of inputting initial species concentration was determined. A user defined function was written that added a second source term to the diffusion equation. This source term was turned on only for the first time step of the

simulation and the appropriate initial species concentration was reached. The source was then turned off and only the original source terms remain.

4.4.3 Model convergence

The rotating boundary problem is a fairly common problem in CFD, especially in the field of turbomachinery. To produce a converged solution, the rotation boundary condition was set to an RPM of about 0.5 to 0.25 of the final RPM. A converged solution is reached using a small numerical time step ($1e-5$ sec) when compared to the total solution time (120 sec). The RPM is then raised incrementally and a converged solution is reached for each RPM until the desired RPM is reached. This “ramp up” method produces a better converged solution with less computational expense. For this research, the desired RPM was reached in about $1e-3$ sec of flow time.

The simulation was run to convergence criteria of $1e-4$ for the weighted residuals of continuity, velocity and convection-diffusion of each species. The simulation was started with a time step of $1e-5$ sec for stability, and was later incrementally increased to 0.01 sec. The PISO pressure-velocity coupling proved to be more stable than other methods. The under-relaxation factors were not changed from the default settings. A grid study comparing shear rate with element sizes of 10, 5, and 2 microns and a separate grid study comparing platelet deposition with element sizes of 10 and 5 microns were conducted. 120 sec of flow time solution required about 48 computer hours on a single 2.8 GHz AMD Opteron processor with 32GB of RAM for a grid size of 5 microns (5600 grid elements). A solution for a 2 micron grid was attempted. The rate of solution for one week was interpolated out to determine that 120 sec of flow time would require ~9000 computer hours or about 1 year.

5 Results

5.1 Experimental

Figure 11 shows the results from the PT and APTT tests described in chapter 3.5 as a function of shear rate. Although regression showed the data to be statistically insignificant, a trend of decreasing time is seen from the linear fit lines. The decreasing PT and APTT indicate that coagulation is occurring more rapidly and higher levels of coagulation factors such as thrombin and prothrombin are in the blood. The control PT and APTT (0 shear) of these experiments were approximately 75 and 200 seconds

Figure 11: Measured PT and APTT

respectively. The normal PT and APTT are about 12 and 25 respectively. The PT and APTT were elevated in these experiments by the addition of heparin in an effort to make any changes in PT or APTT more pronounced. Microscopy showed that no platelets were attached to the surface.

5.2 Comparison of Numerical Model with Previous Results

The result of the simulation of flow through the stenosis compared very well with the published results [Goodman, 2005]. Figure 12 illustrates the thrombus growth that is similar in size, shape and location to that published by Goodman [2005, Fig #13]. The time when the thrombus reaches this size is about 30% faster in the current model than calculated in the prior study due to the larger mesh size.

Figure 12: Top Left: Schematic of Goodman et al. geometry showing line of symmetry in red. Bottom Left: Resulting simulation done as a part of this work, showing attached thrombus. The colors identify the grid cells as fluid (blue and light blue), and thrombus (red and yellow). Right: Experimental (A and B) and computational (C) results from Goodman 2005, pg 788 showing attached thrombus. Note similarity in both size and location to Goodman et al. results.

5.3 Annular Gap

5.3.2 Grid Study

A grid study of only the fluid motion was conducted. Less than a 1% change in the wall shear rate was observed when the grid elements were reduced in size from 10 microns to 2 microns. Figure 13 and Figure 14 are plots of the wall strain rates for the 3 grid sizes. Note the

Figure 13: Shear rate at the stationary (inner) wall for three different grid sizes. Less than 1% change was observed from the 10 micron grid to the 5 micron grid.

increase in deviation when the grid is reduced from 5 microns to 2 microns. There are two potential causes for this increase in deviation. First, it is possible that when a grid is smaller than a critical size, the numerical error increases as the grid size decreases. Future work could consist of a grid study to determine the critical grid size. A more likely cause of this increase in scatter is inability for the 2 micron simulations to converge to the same residual as the 10 and 5 micron grids. The 10 and 5 micron grids converged to a residual of 1e-5 for continuity while the 2 micron grid could only converge to a residual of 1e-4 for continuity.

Figure 14: The shear rate at moving (outer) wall for three different grid sizes. Less than 1% change was observed from the 10 micron grid to the 5 micron grid.

Another grid study using the entire thrombosis model was then conducted with grid elements of 10, and 5 microns. Platelet deposition on the wall at 100 sec of flow time was measured for each grid. Platelet deposition for the 10 micron grid was over 3 times higher near the center of the domain than at the boundaries. The asymmetry observed in the 10 micron grid was eliminated when the grid size was reduced to 5 microns as shown in Figure 15. For this reason, a grid size of 5 microns was used for all simulations.

Figure 15: Normalized platelet deposition on the stationary (inner) wall for grid elements of 10 and 5 microns. Platelet Deposition is normalized to the minimum value for each simulation for comparison purposes.

5.3.2 Velocity Field

The annular gap geometry generated expected flow properties. The steady-state velocity profile was linear, which resulted in a near uniform shear across the entire domain. Because the fluid is initially at rest, there is a transitional period in the fluid before it reaches a steady state velocity profile (Figure 16). The development time (~0.025 seconds) is short compared to the total solution time (>60 seconds) and any shear stresses imparted by this transitional period have minimal impact on the final solution.

Figure 16: CFD velocity profile development

5.3.3 Platelet Concentrations

Platelet mass was conserved to within 3% over the 120 sec simulation. This change is likely due to round-off error of the solver.

Figure 17: Total number of platelets in the simulation at 0.0005, 0.5, 1, 10, 30, and 100 seconds. The number of platelets deposited on the moving and stationary wall is less than 3% different than the number of plates lost from the bulk flow at any time.

The mass fraction of resting and activated platelets in the bulk flow decreases over time due to platelet deposition. Figure 18 illustrates the higher rate of deposition for activated platelets compared to resting platelets.

Over the three shear rates tested, the mass fraction of thrombin was observed to increase with increasing shear (Figure 20). This increase in thrombin is due higher levels of platelet activation and deposition.

Most of the species including thrombin maintained a nearly uniform concentration throughout the solution. However, after several seconds of flow time, a concentration gradient developed for activated and unactivated platelets across the domain. The center of the domain

contained a high concentration of platelets while platelet concentration was low near the walls as shown in Figure 19. This is due to the fact that the walls act as a sink for the platelets. As platelets deposit on the wall, platelet concentration in the bulk flow near the wall decreases. As this process continues in time, a gradient develops with the highest concentration of platelets in the center of the flow.

Figure 18: The mass fraction of activated and resting platelets decrease over time in the CFD simulations.

Figure 19: Activated platelet concentration for the entire computational domain at 100 sec of flow time. Note the high concentration in the center and low concentration near the walls.

Figure 20: Results from computational simulations demonstrating increasing thrombin mass fraction with increasing shear rate. Each point represents an individual CFD simulation of 120 seconds of solution time.

6 Conclusion and Discussion

6.1 Experimental Insight and Discussion

After exposing whole blood to a specific shear for 120 seconds, PT and APTT were measured as described in chapter 3.5. These tests provide a measure of time until clot formation and relative amounts of coagulation factors. A decrease in PT and APTT would indicate more rapid clot formation and higher levels of coagulation factors. Therefore, the results shown in Figure 11 indicate that blood exposed to high shear rate contains more coagulation factors than blood exposed to a lower shear rate.

6.2 Numerical Insight and Discussion

Three separate numerical simulations were solved with three different shear rates. As the simulation progressed in time, the flow field and the biomaterial surface interacted with the species to simulate coagulation as described in chapter 4. In the numerical model, thrombin is generated due to platelet activation and platelet deposition as governed by the source terms in Table 3. Figure 20 demonstrates that thrombin concentration in the numerical model increases with increasing shear rate. Therefore, increasing shear is causing more platelet activation and more platelet deposition. However, increasing the shear rate also increased the rate of embolization, so no significant difference in thrombus growth was observed. Another, interesting insight is the non-zero thrombin concentration for the 0 shear simulation. This indicates that the numerical model is capable of modeling the effect of a biomaterial surface without a flow field.

6.3 Comparison of Numerical and Experimental Results

The trend seen in the experimental results of PT and APTT decreasing with increasing shear indicate that higher levels of coagulation factors are present. This is in agreement with the numerical models prediction of increasing thrombin and other coagulation factors with increasing shear rate. This agreement indicates that the numerical model can qualitatively predict the effects of shear on whole blood. A qualitative evaluation of the numerical model will require further research with more sophisticated experiments. This initial evaluation of the numerical coagulation model is necessary to guide future research with the ultimate goal of applying this model to a mechanical blood pump.

6.4 Future Improvements to the Experiment

The experimental method and test rig described in this thesis is a baseline for developing an experimental apparatus that can conduct controlled experiments studying whole blood in a high shear flow field. Ultimately, this apparatus will be similar to a commercial ventricular assist device (VAD). Immediate improvements would include: the direct measure of coagulation factors, a geometry that induced thrombus formation, and a controller for the DC motor. The validation of the numerical model would benefit from experimentation that directly measured values of the individual coagulation factors that are included in the numerical model. A geometry that included areas of high and low shear would be a better representation of the blood pathway through a poorly designed assist device that is more likely to produce a clot. This geometry would provide more information on platelet deposition and thrombus growth that is more readily seen in clinical applications. A computer controlled DC motor could provide some interesting

experiments such as pulsing the motor on and off. Experiments such as this can demonstrate the effect repeated exposure to high (implanted VAD device) and low (venous return) shear on whole blood.

6.5 Potential Improvements to the Current Numerical Model

One area of possible improvement to this computational model is the threshold nature of platelet activation. It has been shown that weak agonists (ADP, epinephrine, etc.) and strong agonists (thrombin, etc.) act synergistically, and that different concentration combinations of each have varying effects [Huang, 1981]. The synergist effect of agonists is current not included in the model at all.

In the current model, agonist induced activation and shear induced activation are two completely separate modes of platelet activation. It has been shown that a sub-critical concentration of agonists combined with a sub-critical shear rate can produce significant amounts of platelet activation. [Anderson, 1978] This synergistic effect of shear and agonists could be large source of error for this numerical model. While incorporating the synergistic effects would be the best solution, perhaps a more efficient use of resources would be to determine the regimes that these synergistic effects would be most influential and evaluate if the current numerical model can be applied in these regimes.

The experiment and the numerical model both showed no signs of clot formation. This is likely due to the high shear washing the surface. This agrees with Goodman et al. experimental data on embolization (Equation 1). According to Goodman et al., at the shear rate and exposure time tested, less than 5% of platelets would remain attached. This type of flow, while uniform

and controllable, does not promote thrombogenesis. This numerical model is best suited for comparing with experiments that encourage platelet deposition and clot formation.

In the future, a similar comparison of numerical and physical models should be made for a high shear rotating flow that also includes regions of stagnation or recirculation.

The current numerical model only contains two factors of the coagulation cascade (prothrombin and thrombin). These two factors are part of the common pathway (coagulation factors that are in the extrinsic and intrinsic pathways). While these two factors are precursors to thrombus formation, the model would benefit from the inclusion of other coagulation factors from the individual pathways.

The current research is an effort to characterize the applicable domain of this numerical model. Further investigation into potential applications would greatly benefit the progress of this model. Future work could include a parametric analysis of the current research's results to determine the relative importance of individual species to a particular application or flow regime.

6.6 Potential Clinical Applications of the Numerical Model

As CFD becomes more robust and computational power becomes cheaper, geometries are becoming more physiologically realistic. The applicability of this model to physiological applications has currently not been tested. However, there are several clinical applications that possess a large potential for this model and should be investigated. Some well studied clinical cases that thrombosis plays a large role include: vascular stents [Czemak, 2004], aneurysms [Nagai, 2008], and the internal carotid artery bifurcation [Kanazawa, 2004]. Each of these cases has several publications that provided *in vivo* computed tomography (CT) measurements of an attached thrombus that can readily be compared with CFD simulations of a similar geometry.

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References

- Alevriadou, B. R., J. L. Moake, N. A. Turner, Z. M. Ruggeri, B. J. Folie, M. D. Phillips, A. B. Schreiber, M. E. Hrinda, and L. V. McIntire. "Real-Time Analysis of Shear-Dependent Thrombus Formation and Its Blockade by Inhibitors of Vonwillebrand-Factor Binding to Platelets." *Blood* 81, no. 5 (1993): 1263-76.
- Anderson, G. H., J. D. Hellums, J. L. Moake, and C. P. Alfrey. "Platelet Lysis and Aggregation in Shear Fields." *Blood Cells* 4, no. 3 (1978): 499-507.
- Basmadjian, D. "Coagulation on Biomaterials in Flowing Blood: Some Theoretical Considerations." In *Biomaterials*, 1511. [Guildford, Eng.]: IPC Science and Technology Press, 1997.
- Burgreen, Greg W. "Design Improvement Strategy for Axial Blood Pumps Using Computational Fluid Dynamics." In *ASAIO journal*, M354. Hagerstown, MD: Published for the Society by J.B. Lippincott Co., 1996.
- Chang, G., D. R. Absolom, A. B. Strong, G. D. Stubley, and W. Zingg. "Physical and Hydrodynamic Factors Affecting Erythrocyte Adhesion to Polymer Surfaces." *Journal of Biomedical Materials Research* 22, no. 1 (1988): 13-29.
- Czermak, B. V., A. Mallouhi, R. Perkmann, I. E. Steingruber, P. Waldenberger, B. Neuhauser, G. Fraedrich, T. Jung, and W. R. Jaschke. "Serial Ct Volume and Thrombus Length Measurements after Endovascular Repair of Stanford Type B Aortic Dissection." *Journal of Endovascular Therapy* 11, no. 1 (2004): 1-12.
- Day, S. W., McDaniel, J. C., "Piv Measurements of Flow in a Centrifugal Blood Pump: Time-Varying Flow." In *Journal of biomechanical engineering*, 254. [New York, N.Y.]: American Society of Mechanical Engineers, 2005.
- Feuerstein, I. A., S. M. Buchan, T. A. Horbett, and K. D. Hauch. "Platelet Adherence and Detachment - a Flow Study with a Series of Hydroxyethyl Methacrylate-Ethyl Methacrylate Copolymers Using Video Microscopy." *Journal of Biomedical Materials Research* 25, no. 2 (1991): 185-98.
- Fogelson, A. L. "A Mathematical-Model and Numerical-Method for Studying Platelet-Adhesion and Aggregation During Blood-Clotting." *Journal of Computational Physics* 56, no. 1 (1984): 111-34.
- Fogelson, A. L. "Continuum Models of Platelet-Aggregation - Formulation and Mechanical-Properties." *Siam Journal on Applied Mathematics* 52, no. 4 (1992): 1089-110.
- Fogelson, A. L., H. Y. Yu, and A. Kuharsky. "Computational Modeling of Blood Clotting: Coagulation and Three-Dimensional Platelet Aggregation." *Polymer and Cell Dynamics* (2003): 145-54.

- Folie, B. J., and L. V. McIntire. "Mathematical-Analysis of Mural Thrombogenesis - Concentration Profiles of Platelet-Activating Agents and Effects of Viscous Shear-Flow." *Biophysical Journal* 56, no. 6 (1989): 1121-41.
- Gartner, M. J., C. R. Wilhelm, K. L. Gage, M. C. Fabrizio, and W. R. Wagner. "Modeling Flow Effects on Thrombotic Deposition in a Membrane Oxygenator." In *Artificial organs*, 29. Malden, MA: Blackwell Science, 2000.
- Goodman, Paul D, E. T. Barlow, P. M. Crapo, S. F. Mohammad, and K. A. Solen. "Computational Model of Device-Induced Thrombosis and Thromboembolism." In *Annals of biomedical engineering*, 780. Cambridge, Eng.: Blackwell Scientific Publications, 2005.
- Grabowsk.Ef, E. F. Leonard, and L. I. Friedman. "Effects of Shear Rate on Diffusion and Adhesion of Blood-Platelets to a Foreign Surface." *Industrial & Engineering Chemistry Fundamentals* 11, no. 2 (1972): 224-&.
- Hellums, J. D. "1993 Whitaker Lecture - Biorheology in Thrombosis Research." *Annals of Biomedical Engineering* 22, no. 5 (1994): 445-55.
- Hockin, M. F., K. C. Jones, S. J. Everse, and K. G. Mann. "A Model for the Stoichiometric Regulation of Blood Coagulation." *Journal of Biological Chemistry* 277, no. 21 (2002): 18322-33.
- Hopkins, L. M., J. T. Kelly, A. S. Wexler, A. K. Prasad. "Particle Image Velocimetry Measurements in Complex Geometries." In *Experiments in fluids*, 91. Berlin: Springer International, 2000.
- Huang, E. M., and T. C. Detwiler. "Characteristics of the Synergistic Actions of Platelet Agonists." *Blood* 57, no. 4 (1981): 685-91.
- Hubbell, J. A., and L. V. McIntire. "Platelet Active Concentration Profiles near Growing Thrombi - a Mathematical Consideration." *Biophysical Journal* 50, no. 5 (1986): 937-45.
- Hubbell, J. A., and L. V. McIntire. "Visualization and Analysis of Mural Thrombogenesis on Collagen, Polyurethane and Nylon." *Biomaterials* 7, no. 5 (1986): 354-&.
- Jacobs, H. A., T. Okano, and S. W. Kim. "Antithrombogenic Surfaces - Characterization and Bioactivity of Surface Immobilized Pgl-Heparin Conjugate." In *Journal of biomedical materials research*, 611. Hoboken, NJ: John Wiley Sons, 1989.
- Jesty, J., W. Yin, P. Perrotta, and D. Bluestein. "Platelet Activation in a Circulating Flow Loop: Combined Effects of Shear Stress and Exposure Time." In *Platelets*, 143. Edinburgh: Churchill Livingstone, 2003.
- Jones, M. I., I. R. McColl, D. M. Grant, K. G. Parker, and T. L. Parker. "Protein Adsorption and Platelet Attachment and Activation, on Tin, Tic, and Dlc Coatings on Titanium for Cardiovascular Applications." In *Journal of biomedical materials research*, 413.

- Hoboken, NJ: John Wiley Sons, 2000.
- Kanazawa, R., S. Kominami, Y. Yoshida, S. Kobayashi, and A. Teramoto. "Middle Cerebral Artery Thrombolysis through the Contralateral Internal Carotid Artery - Case Report." *Neurologia Medico-Chirurgica* 44, no. 7 (2004): 372-75.
- Keller, K. H. "Effect of Fluid Shear on Mass Transport in Flowing Blood." *Federation Proceedings* 30, no. 5 (1971): 1591-&.
- Kleinstreuer, C., M. Nazemi, and J. P. Archie. "Hemodynamics Analysis of a Stenosed Carotid Bifurcation and Its Plaque-Mitigating Design." *Journal of Biomechanical Engineering-Transactions of the Asme* 113, no. 3 (1991): 330-35.
- Lee, D., and J. J. Chiu. "Intimal Thickening under Shear in a Carotid Bifurcation - a Numerical Study." *Journal of Biomechanics* 29, no. 1 (1996): 1-11.
- Leonard, E. F., V. T. Turitto, and Grabowsk.Ef. "Role of Convection and Diffusion on Platelet Adhesion and Aggregation." *Annals of the New York Academy of Sciences* 201, no. OCT27 (1972): 329-&.
- Mann, K. G. "Thrombin Formation." In *Chest*, 4S: American College of Chest Physicians, 2003.
- McKenzie, S. B., Textbook of Hematology. Williams and Wilkins. Baltimore, MD, 1996.
- Nagai, M., Y. Koizumi, J. Tsukue, and E. Watanabe. "A Case of Extravasation from a Cerebral Aneurysm During 3-Dimensional Computed Tomography Angiography." *Surgical Neurology* 69, no. 4 (2008): 411-13.
- Nazemi, M., C. Kleinstreuer, J. P. Archie, and F. Y. Sorrell. "Fluid-Flow and Plaque-Formation in an Aortic Bifurcation." *Journal of Biomechanical Engineering-Transactions of the Asme* 111, no. 4 (1989): 316-24.
- Panteleev, M. A., N. M. Ananyeva, F. I. Ataullakhanov, and E. L. Saenko. "Mathematical Models of Blood Coagulation and Platelet Adhesion: Clinical Applications." *Current Pharmaceutical Design* 13, no. 14 (2007): 1457-67.
- Platt, W. R., Color Atlas and Textbook of Hematology. Lippincott, Philadelphia, PA, 1979.
- Ramstack, J. M., L. Zuckerman, and L. F. Mockros. "Shear-Induced Activation of Platelets." *Journal of Biomechanics* 12, no. 2 (1979): 113-25.
- Schaub, Richard D. "Assessing Acute Platelet Adhesion on Opaque Metallic and Polymeric Biomaterials with Fiber Optic Microscopy." In *Journal of biomedical materials research*, 460. Hoboken, NJ: John Wiley Sons, 2000.
- Song, X., A. L. Thockmorton, H. G. Wood, J. F. Antaki, and D. B. Olsen. "Computational Fluid Dynamics Prediction of Blood Damage in a Centrifugal Pump." In *Artificial organs*, 938. Malden, MA: Blackwell Science, 2003.

- Sorensen, E. N., G. W. Burgreen, W. R. Wagner, and J. F. Antaki. "Computational Simulation of Platelet Deposition and Activation: II. Results for Poiseuille Flow over Collagen." *Annals of Biomedical Engineering* 27, no. 4 (1999): 449-58.
- Sorensen, E. N., G. W. Burgreen, W. R. Wagner, and J. F. Antaki. "Computational Simulation of Platelet Deposition and Activation: I. Model Development and Properties." In *Annals of biomedical engineering*, 436. Cambridge, Eng.: Blackwell Scientific Publications, 1999.
- Stiene-Martin, A. E., Lotseich-Stheininger, C. A., Koepke, J. A., Clinical Hematology: principles, procedures, correlations. Lippincott-Ravenm Philedelphia, PA, 1998.
- Strong, A. B., G. D. Stublely, G. Chang, and D. R. Absolom. "Theoretical and Experimental-Analysis of Cellular Adhesion to Polymer Surfaces." *Journal of Biomedical Materials Research* 21, no. 8 (1987): 1039-55.
- Tritton, D. J., 2003, Physical Fluid Dynamics, Oxford University Press, New York, NY, Chap. 16, 17.
- Turitto, V. T., and H. R. Baumgartner. "Effect of Temperature on Platelet Interaction with Subendothelium Exposed to Flowing Blood." *Haemostasis* 3, no. 4 (1974): 224-36.
- Turitto, V. T., and H. R. Baumgartner. "Platelet Deposition on Subendothelium Exposed to Flowing Blood - Mathematical-Analysis of Physical Parameters." *Transactions American Society for Artificial Internal Organs* 21, (1975): 593-601.
- Turitto, V. T., R. Muggli, and H. R. Baumgartner. "Physical Factors Influencing Platelet Deposition on Subendothelium - Importance of Blood Shear Rate." *Annals of the New York Academy of Sciences* 283, no. FEB10 (1977): 284-92.
- Untaroiu, A., H. G. Wood, P. E. Allaire, A. L. Thockmorton, S. Day, S. M. Patel, P. Ellman, C. Tribble, and D. B. Olsen. "Computational Design and Experimental Testing of a Novel Axial Flow Lvad." In *ASAIO journal*, 702. Hagerstown, MD: Published for the Society by J.B. Lippincott Co., 2005.
- Wagner, C. T., M. H. Kroll, T. W. Chow, J. D. Hellums, and A. I. Schafer. "Epinephrine and Shear Stress Synergistically Induce Platelet Aggregation Via a Mechanism That Partially Bypasses Vwf-Gp Ib Interactions." *Biorheology* 33, no. 3 (1996): 209-29.
- Wagner, W. R., J. A. Hubbell, L. V. McIntire, and K. G. Mann. "Importance of Thrombin Synthesis in Mural Thrombosis on Collagen-Coated Glass." *Arteriosclerosis* 8, no. 5 (1988): A672-A72.
- Wootton, D. M., C. P. Markou, S. R. Hanson, and D. N. Ku. "A Mechanistic Model of Acute Platelet Accumulation in Thrombogenic Stenoses." *Annals of Biomedical Engineering* 29, no. 4 (2001): 321-29.
- Zydney, A. L., and C. K. Colton. "Augmented Solute Transport in the Shear-Flow of a

Concentrated Suspension." *Physicochemical Hydrodynamics* 10, no. 1 (1988): 77-96.

Appendix A Implementation into Fluent

The following is a case specific guide to loading and solving the Concentric Cylinder case file in Fluent 6.3.2.

1. Read the .cas file.
2. Check the grid
3. Scale the grid – I used 1e-3 for both x and y.
4. Choose the correct solver: Define – Models – Solver
 - a. Unsteady
 - b. All others are default
5. Turn species transport on: Define – Model – Species – Transport and Reaction
 - a. Species Transport
 - b. Volumetric
 - c. Wall Surface
 - d. Mass Deposition Source
6. Compile and Load the UDF: Define – User Defined – Functions – Compiled
 - a. Click Add
 - i. Select your C-code file from the list and click OK
 - b. Library Name is the name of the folder that will be created and contain the compiled UDF files. A directory of the same name should already exist in the working directory.
 - c. Click build
 - i. A prompt will appear to make sure the C-code file and the mesh or case file are in the same directory. Make sure this is true and click OK
 - ii. If it builds correctly, the Fluent screen should look like this:

```
FLUENT [2d, pbns, lam]
File  Grid  Define  Solve  Adapt  Surface  Display  Plot  Report  Parallel  Help

Building...
  grid,
  materials,
  interface,
  domains,
  zones,
    default-interior
    topwall
    bot_wall
    outs
    Skipping zone outs_shadow (not referenced by grid).
    fluid
  shell conduction zones,
Done.
  1 file(s) copied.
(system "copy c:\fluent.inc\fluent6.3.26\src\makefile_nt.udf libudf\win64\2d\mal
  1 file(s) copied.
(chdir "libudf")()
(chdir "win64\2d")()
cc_code_1.c
c:\fluent.inc\fluent6.3.26\src\machine.h(114) : warning C4005: 'stdout' : macro
C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(160
c:\fluent.inc\fluent6.3.26\src\machine.h(115) : warning C4005: 'stderr' : macro
C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(161
c:\fluent.inc\fluent6.3.26\src\machine.h(116) : warning C4005: 'stdin' : macro
C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(159
d:\fluent\cc\libudf\src\cc_code_1.c(307) : warning C4700: uninitialized local v
# Generating udf_names.c because of makefile cc_code_1.obj
udf_names.c
c:\fluent.inc\fluent6.3.26\src\machine.h(114) : warning C4005: 'stdout' : macro
C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(160
c:\fluent.inc\fluent6.3.26\src\machine.h(115) : warning C4005: 'stderr' : macro
C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(161
c:\fluent.inc\fluent6.3.26\src\machine.h(116) : warning C4005: 'stdin' : macro
C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(159
# Linking libudf.dll because of makefile user_nt.udf udf_names.obj cc_code_1.ob
Microsoft (R) Incremental Linker Version 8.00.50727.42
Copyright (C) Microsoft Corporation. All rights reserved.

  Creating library libudf.lib and object libudf.exp

Done.
```

- d. Click Load
 - i. If it works correctly, the fluent screen should look something like this:

```
FLUENT [2d, pbns, lam]
File Grid Define Solve Adapt Surface Display Plot Report Parallel Help

c:\fluent.inc\fluent6.3.26\src\machine.h(114) : warning C4005: 'stdout' : macro
      C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(160)
c:\fluent.inc\fluent6.3.26\src\machine.h(115) : warning C4005: 'stderr' : macro
      C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(161)
c:\fluent.inc\fluent6.3.26\src\machine.h(116) : warning C4005: 'stdin' : macro
      C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(159)
d:\fluent\cc\libudf\src\cc_code_1.c(307) : warning C4700: uninitialized local va
# Generating udf_names.c because of makefile cc_code_1.obj
udf_names.c
c:\fluent.inc\fluent6.3.26\src\machine.h(114) : warning C4005: 'stdout' : macro
      C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(160)
c:\fluent.inc\fluent6.3.26\src\machine.h(115) : warning C4005: 'stderr' : macro
      C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(161)
c:\fluent.inc\fluent6.3.26\src\machine.h(116) : warning C4005: 'stdin' : macro
      C:\Program Files (x86)\Microsoft Visual Studio 8\VC\INCLUDE\stdio.h(159)
# Linking libudf.dll because of makefile user_nt.udf udf_names.obj cc_code_1.ob
Microsoft (R) Incremental Linker Version 8.00.50727.42
Copyright (C) Microsoft Corporation. All rights reserved.

    Creating library libudf.lib and object libudf.exp

Done.
"d:/fluent/cc"

Opening library "libudf"...
Library "libudf\win64\2d\libudf.dll" opened
    adjust
    cell_viscosity
    rp_source
    ap_source
    adp_source
    txa2_source
    pt_source
    thrombin_source
    at_source
    Diffusivity
    rp_init
    ap_init
    pt_init
    at_init
    Surface_Reactions

Done.
```

7. Set Function Hooks: Define – User Defined – Function Hooks
 - a. Click edit next to Adjust
 - i. select “adjust:libudf” and click add. Then click OK.
 - b. Click the drop down arrow next to Surface Reactions
 - i. Select surface_reactions::libudf
8. Set User Defined Memory allocations: Define – User Defined – Memory
 - a. 17 – this number must be exactly correct or you will get errors when you attempt to iterate.
 - i. This is the number of memory allocation set in the UDF

9. Define the material: Define –Material all of the commands below are in the Materials panel
- a. Material Type – mixture
 - i. Change Density to volume-weighted-mixing-law
 - ii. Click Change/Create
 - b. Material Type – Fluid
 - i. Copy liquid water from the Fluent Database
 - ii. Change the name to blood
 - iii. Delete the Chemical Formula
 - iv. Density – 1056
 - v. Click Change/Create and Overwrite H2O
 - c. Now enter each individual species (rp, ap, dp, adp, txa2, pt, th, at) with their molecular weights. The Material Type should be Fluid for all species
 - i. Enter the species name in the Name box e.g. rp
 - ii. Enter the molecular weight from Table 5
 - iii. Click Change/Create and don't overwrite
 - iv. Repeat
 - d. Material Type – Mixture
 - i. Mixture Species – Edit
 1. Move the species from the Available Materials box to the Selected Species box in the following order : rp, ap, ADP, txa2, pt, th, at, blood (this must be the same order they are listed the UDF code)
 2. remove any other species that are in the Selected Species box
 3. Move dp to the Selected Solid Species box.
 4. click OK
 - ii. Reaction – Edit
 1. Use arrows to change values in this menu
 2. Total Number of Reactions – 4
 3. Reaction Type – Wall Surface
 - a. Make sure to click the Wall Surface radio button for all four of the reactions.
 4. Enter the following stoichiometry reactions in the given order. All Rate Exponent values should be zero.
 - a. Reaction 1
 - i. Reactants: 1 rp
 - ii. Products: 1 dp
 - b. Reaction 2
 - i. Reactants: 1rp
 - ii. Products: 1dp
 - c. Reaction 3

- i. Reactants: 0 ap
 - ii. Products: 0ap + 1 txa2
 - d. Reaction 4
 - i. Reactants: 1 pt
 - ii. Products: 1 th
 - e. All Arrhenius Rate values should remain as defaults
- 5. Click OK
 - iii. Viscosity – User Defined
 - iv. Mass Diffusivity – User Defined
 - v. Click Change/Create and Close the Materials Window
- 10. Set Boudary Conditions: Define – Boundary Conditions
 - a. Bot wall
 - i. Species Tab
 - 1. Check the Reaction box
 - b. Fluid
 - i. Reaction box should be checked if it is not already
 - ii. Check the Source Terms box
 - iii. Source Terms Tab
 - 1. Click the Edit box next to the species you wish to add a source term to.
 - 2. Select the source term for the appropriate species given in the list below
 - a. rp – rp_source and rp_init
 - b. ap – ap_source and ap_init
 - c. adp – adp_source
 - d. txa2 – txa2_source
 - e. pt – pt_source and pt_init
 - f. th – thrombin_source
 - g. pt – at_source and at_init
 - 3. click OK
 - c. Outs
 - i. Change the Periodic Type to Rotational
 - d. Topwall
 - i. Click the Moving Wall radio button
 - ii. Click the Absolute and Rotational radio buttons
 - iii. Enter the desired rotational speed in rad/sec
 - 1. For high rotational speeds and shear rates, it can help the stability of the problem to start at a low rotational speed, solve the velocity field, and then raise the rotational speed and continue solving. Sometimes many small steps like this are necessary to avoid a divergent solution.

2. For a shear rate of $10,000 \text{ s}^{-1}$ a rotational speed of 220 rad/sec is used.

iv. Species Tab

1. Check the Reaction check box
- e. Close the Boundary Condition Window
11. Set Solution Controls: Solve – Controls – Solution
 - a. Different computers require different Under-relaxation factors and Pressure-velocity coupling. I have had mixed results using the PISO and SimpleC couplers. Sometimes they work great and other times the solution diverges. But when they do work, they solve much faster than Simple. It is a good idea to lower the Momentum, rp , and ap Under-relaxation factors to about half of their default values.
 - b. Click OK
12. Initialize the problem: Solve – Initialize – Initialize
 - a. Compute From – all zones
 - b. For the given rotational speed of the outer wall or topwall determine the average steady state velocity and enter this in Y-velocity box
 - c. Click Init and Close the Initialization Window
13. Set the Residuals: Solve – Monitors – Residual
 - a. Set continuity, x-velocity, y-velocity and rp to $1e-5$. Set ap to $5e-5$.
 - b. Click OK
14. Solve the Problem: Solve - Iterate
 - a. Click the Adaptive Time Step radio button
 - b. Enter the desired number of time steps
 - c. I have not had good luck trying to start the simulation with a time step larger than $1e-5$.
 - d. Change the Max Iterations per Time Step to 200
 - e. Leave the Adaptive Time Step Parameters as default
 - f. Click Iterate
15. When the solution has completed, perform the necessary post processing.

Appendix B Some notes on Compiling the UDF

On a 64-bit machine running Windows x64, the full version of Visual Studio is needed to compile a UDF. Visual Studio 2005 Standard Edition was used for the current research. On a 32-bit machine, Visual Studio Express can be used and is a free download from Microsoft. Fluent must be opened in the Visual Studio command prompt (x64 Win64 command prompt for 64-bit machines) that is located in the Visual Studio Tools.

Appendix C Source Code

```
/*User-Defined Functions*/

#include "udf.h"

/* global variables */

/* physical constants, misc.*/
int wall1_ID = 5;
int wall2_ID = 6;

int interior_ID = 8;
real flow_rate = 0;
real visc = 0.00325;
int partition = 2;

/* platelet info */
real plt_count = 300.;
real minf = 6.0e10;
real test;

/* kinetic variables */
int thrombus_count = 1;
real krs = 0.0000025;
real kas = 0.0035;
real kt = 0.0035;
real tact = 1.0;
real temb = 120.0;

/* global time for initial concentrations */
real delt_int = 0.00001;

/* User-Defined Memory Allocations. This stores the data to access it later. */
int M=0;          /*C_UDMI(c,t,0)=M(x,t), total platelet surface coverage (plt/m^2)*/
int Sa=1;         /*C_UDMI(c,t,1)=Sa(x,t), unitless, % available surface area (x,t)*/
int rpadh=2;      /*C_UDMI(c,t,2)=flux of unact. plts to the surface (plt/m^2-s)*/
int apadh=3;      /*C_UDMI(c,t,3)=flux of act. platelets to the surface (plt/m^2-s)*/
int upemb=4;      /*C_UDMI(c,t,4)=flux of act. pits embolizing (plt/m^2-s)*/
int delthu=5;     /*C_UDMI(c,t,5)=delta thrombus, unitless, change in vol% of grid
                  cell changing to unactivated thrombus */
int delth=6;      /*C_UDMI(c,t,6)=delta thrombus, unitless, change in volume% of grid
                  cell changing to thrombus */
```

```

    int thrombus=7; /*C_UDMI(c,t,7)=thrombus growth, unitless, % of grid cell that is
thrombus () */
    int gridid=8; /*C_UDMI(c,t,8)=grid cell type (bulk fluid, thrombus, etc.,
unitless () */
    int tcount=9; /*C_UDMI(c,t,9)=thrombus_count, unitless, identifies the cell as part of a
numbered thrombus () */
    int area=10; /*C_UDMI(c,t,10)=wall surface area (m^2) */
    int pltact=11; /* C_UDMI(c,t,11)=platelet activation, calculated from weighted agonist
concentrations, kg/m^3 */
    int gama=12; /*C_UDMI(c,t,12)=maximum local shear rate in cells adjacent to surface
(1/s) */
    int Xact=13; /*C_UDMI(c,t,13)=fraction of thrombus/wall that is act. platelets*/
    int actime=14; /*C_UDMI(c,t,14)=time at which a grid cell becomes active (s)*/
    int Ma = 15; /*C_UDMI(c,t,15)=Ma(x,t), act. platelet surface coverage (plt/m2)*/
    int Matime = 16; /*C_UDMI(c,t,16)= time at which the surface of the cell becomes active*/
/* end global variables */

```

```

/* Adjust Function keeps track of thrombus*/
/* growth, platelet adhesion, etc.*/

```

```

static int last_ts = -1; /* this global variable is used to identify the first iteration in a time
step*/

```

```

DEFINE_ADJUST(adjust,d)
{
Thread *t, *tc1, *ft, *tc0;

cell_t c, c1, c0;
face_t f;
real deltt,curr_t,A[ND_ND],total_force[ND_ND], adhesion_area;
int curr_ts, n, th, new, old,p,neighbor;
new = 0;
old = 0;
curr_ts = RP_Get_Integer("time-step");
deltt = RP_Get_Real("physical-time-step");
curr_t=RP_Get_Real("flow-time");

```

```

/*The following if loop ensures that the adjust function is calculated only once per
time step */

```

```

if (last_ts != curr_ts)
{

```

```

last_ts = curr_ts;
thread_loop_c(t, d)
{

    if (FLUID_THREAD_P(t)) /* applies adjust function only to fluid threads*/
    {

/* Initialization performed only on the first time step*/
        if(curr_t==delt)
        {
            thrombus_count = 1; /*initialize the thrombus ID number*/
            begin_c_loop_int(c,t) /*the int applies the loop to cells that are
inside the partition*/
            {
                C_UDMI(c,t,Sa)=1.; /*initialize Sa(t)*/
                C_UDMI(c,t,area) = 0.0; /* initialize area calculation*/
                c_face_loop(c,t,n)
                {
                    f=C_FACE(c,t,n);
                    ft=C_FACE_THREAD (c,t,n);
                    if(THREAD_ID(ft)==wall1_ID ||
THREAD_ID(ft)==wall2_ID)
                    {
                        F_AREA(A,f,ft);
                        C_UDMI(c,t,area) += NV_MAG(A);
                        C_UDMI(c,t,actime)=0.;
                    }
                }
            }
            end_c_loop_int(c,t)
        }
/*end first step initialization*/

/* General cell loop to determine thrombus growth, max shear, grid cell type (i.e. thrombus),
and to identify individual thrombi numerically */

        begin_c_loop(c,t)
        {
/*calculate the cumulative thrombus growth */

            C_UDMI(c,t,thrombus)=C_UDMI(c,t,thrombus)
(C_UDMI(c,t,delth)+C_UDMI(c,t,delthu)) * delt;
            if(C_UDMI(c,t,thrombus)>=1. )
                C_UDMI(c,t,thrombus)=1.;
+

```

```

/* calculate the shear rate in all cells */
    if (abs(C_DUDX(c,t)) > abs(C_DUDY(c,t)) && abs(C_DUDX(c,t)) >
abs(C_DVDX(c,t)) && abs(C_DUDX(c,t)) > abs(C_DVDY(c,t)))
        C_UDMI(c,t,gama) = abs(C_DUDX(c,t));
    if (abs(C_DUDY(c,t)) > abs(C_DUDX(c,t)) && abs(C_DUDY(c,t)) >
abs(C_DVDX(c,t)) && abs(C_DUDY(c,t)) > abs(C_DVDY(c,t)))
        C_UDMI(c,t,gama) = abs(C_DUDY(c,t));
    if (abs(C_DVDX(c,t)) > abs(C_DUDY(c,t)) && abs(C_DVDX(c,t)) >
abs(C_DUDX(c,t)) && abs(C_DVDX(c,t)) > abs(C_DVDY(c,t)))
        C_UDMI(c,t,gama) = abs(C_DVDX(c,t));
    if (abs(C_DVDY(c,t)) > abs(C_DUDY(c,t)) && abs(C_DVDY(c,t)) >
abs(C_DVDX(c,t)) && abs(C_DVDY(c,t)) > abs(C_DUDX(c,t)))
        C_UDMI(c,t,gama) = abs(C_DVDY(c,t));

```

```

/* identify cells as
(0) interior fluid;
(1) wall fluid;
(2) wall thrombus;
(3) interior thrombus;
(4) interior fluid with thrombus neighbor; and
(5) wall fluid with thrombus neighbor */

```

```

    C_UDMI(c,t,gridid) = 0;
    if (C_UDMI(c,t,thrombus)==1.)
    {
        C_UDMI(c,t,gridid) = 3;
        if(C_UDMI(c,t,actime)==0. && C_UDMI(c,t,gama)>0.)
            C_UDMI(c,t,actime)=
curr_t+4.0e6*pow(C_UDMI(c,t,gama)*0.035,-2.3);
        if(curr_t >= C_UDMI(c,t,actime) || C_UDMI(c,t,pltact)>0.)
            C_UDMI(c,t,Xact)= 1.;
    }
    c_face_loop(c,t,n)
    {
        ft=C_FACE_THREAD(c,t,n);
        f=C_FACE(c,t,n);
        c0 = F_C0(f,ft);
        tc0 = F_C0_THREAD(f,ft);
        c1 = F_C1(f,ft);
        tc1 = F_C1_THREAD(f,ft);
        if (PRINCIPAL_FACE_P(f,ft))
        {
            if(THREAD_ID(ft)==wall1_ID
THREAD_ID(ft)==wall2_ID)
            {

```

```

        if (C_UDMI(c,t,thrombus) == 1.)
        C_UDMI(c,t,gridid) = 2;
        if (C_UDMI(c,t,thrombus) < 1.)
        {
            if(C_UDMI(c,t, gridid)!=5 )
            C_UDMI(c,t,gridid) = 1;

/* calculate the new M(x,t) value: M = M+(jr+ja-Memb)*delt */

        C_UDMI(c,t,M)=C_UDMI(c,t,M)+(C_UDMI(c,t,rpadh)+C_UDMI(c,t,apadh)-
C_UDMI(c,t,upemb))*delt;

        if (C_UDMI(c,t,M) > minf)
        C_UDMI(c,t,M) = minf;

/* calculate the new fraction of activated platelets value*/
        C_UDMI(c,t,Ma)  =  C_UDMI(c,t,Ma)  +
C_UDMI(c,t,apadh)*delt;

/*calculate the new Sa(t) value: Sa=1-M/minf*/
        C_UDMI(c,t,Sa)=1.-C_UDMI(c,t,M)/minf;
        if (C_UDMI(c,t,Sa)<0.)
        C_UDMI(c,t,Sa)=0.;
        if(C_UDMI(c,t,Ma)time)==0.          &&
C_UDMI(c,t,gama)>0.)
        C_UDMI(c,t,Ma)time)                  =
4.0e6*pow(C_UDMI(c,t,gama)*0.035,-2.3);
        if(curr_t  >=  C_UDMI(c,t,Ma)time)  &&
C_UDMI(c,t,Ma)time)>0.)
        {
            C_UDMI(c,t,Ma)=C_UDMI(c,t,M);
            if(C_UDMI(c,t,actime)==0.        &&
C_UDMI(c,t,gama)>0.)
            C_UDMI(c,t,actime)                =
4.0e6*pow(C_UDMI(c,t,gama)*0.035,-2.3);
            if(curr_t  >=  C_UDMI(c,t,actime)
&& C_UDMI(c,t,actime)>0.)
            C_UDMI(c,t,Xact)=1.;
        }
    }
}
if (THREAD_ID(ft)==interior_ID)
{
    if  (C_UDMI(c0,tc0,thrombus)  ==  1.  &&
C_UDMI(c,t,gridid) == 0 && C_UDMI(c,t,thrombus)<1.)
    {

```

```

C_UDMI(c,t,gridid) = 4;

C_UDMI(c,t,tcount)=C_UDMI(c0,tc0,tcount);
/*C_UDMI(c,t,tcount) is the thrombus*/
    }
    if (C_UDMI(c1,tc1,thrombus) == 1. &&
C_UDMI(c,t,gridid) == 0 && C_UDMI(c,t,thrombus)< 1.)
    {
        C_UDMI(c,t,gridid) = 4;

C_UDMI(c,t,tcount)=C_UDMI(c1,tc1,tcount);
    }
    if (C_UDMI(c0,tc0,thrombus) == 1. &&
C_UDMI(c,t,gridid) == 1 && C_UDMI(c,t,thrombus)< 1.)
    {
        C_UDMI(c,t,gridid) = 5;

C_UDMI(c,t,tcount)=C_UDMI(c0,tc0,tcount);
    }
    if (C_UDMI(c1,tc1,thrombus) == 1. &&
C_UDMI(c,t,gridid) == 1 && C_UDMI(c,t,thrombus)< 1.)
    {
        C_UDMI(c,t,gridid) = 5;

C_UDMI(c,t,tcount)=C_UDMI(c1,tc1,tcount);
    }
    }
}
/*end of the face loop and if principle_f */
}
end_c_loop(c,t) /* End of the general cell loop*/

```

/* Verify that each existing thrombus has a unique identification */

```

/* to avoid duplication, each partition is evaluated serially */
for(p=0; p <= partition-1; p++)
{

```



```

{
C_UDMI(c,t,tcount)=thrombus_count;
thrombus_count=thrombus_count + 1;
/*Message ("new thrombus value assigned");*/
}
}
}
end_c_loop(c,t)
}
PRF_GSYNC(); /* global synchronization to make the for loop work across
partitions */
} /*end for loop*/

/* calculate the force on thrombi, and determine the probability of embolization */

for(th=1; th <= thrombus_count-1; th++)
{
NV_VS(total_force, =, total_force, *, 0.);
test = 0.;
adhesion_area=0.;
begin_c_loop(c,t) /*loops over the cells in the fluid thread*/
{
if(C_UDMI(c,t,tcount)==th)
{
if (C_UDMI(c,t,gridid)>= 2)
{
c_face_loop(c,t,n)
{
ft=C_FACE_THREAD(c,t,n);
f=C_FACE(c,t,n);
c0 = F_C0(f,ft);
tc0 = F_C0_THREAD(f,ft);
c1 =F_C1(f,ft);
tc1 = F_C1_THREAD(f,ft);
if (THREAD_ID(ft)==interior_ID)
{
/*if thrombus neighbor, calculate the net force on the thrombus*/
if (C_UDMI(c,t,gridid) == 4 ||
C_UDMI(c,t,gridid)==5)
{
if (C_UDMI(c0,tc0,thrombus) == 1.)
{
F_AREA(A,f,ft);
NV_V_VS(total_force, =,
total_force, +, A, *, F_P(f,ft));

```

```

/*Message ("pressure at c0 face is %d\n",F_P(f,ft));*/
    }
    if (C_UDMI(c1,tc1,thrombus) == 1.)
    {
        F_AREA(A,f,ft);
        NV_V_VS(total_force,    =,
total_force, -, A, *, F_P(f,ft));
        /*Message ("pressure at c1 face is %d\n",F_P(f,ft));*/
    }
} /*end if thrombus neighbor*/

/*check thrombi for adjacent thrombi and merge them*/
if (C_UDMI(c,t,gridid) == 2 ||
C_UDMI(c,t,gridid) == 3)
{
    if (C_UDMI(c0,tc0,tcount) <
C_UDMI(c,t,tcount) && C_UDMI(c0,tc0,tcount) != 0)
    {
        old = C_UDMI(c,t,tcount);
        new =
C_UDMI(c0,tc0,tcount);
        C_UDMI(c,t,tcount) =
C_UDMI(c0,tc0,tcount);
        /*Message ("neighbor found c0 %d,%d,%d\n",old, new,
thrombus_count);*/
        /*begin_c_loop(c,t)

        {
            if (C_UDMI(c,t,tcount) == new)
                C_UDMI(c,t,tcount) = old;
        }

        end_c_loop(c,t) new = 0;
        old = 0;*/
    }
    if (C_UDMI(c1,tc1,tcount) <
C_UDMI(c,t,tcount) && C_UDMI(c1,tc1,tcount) != 0)
    {
        old = C_UDMI(c,t,tcount);
        new =
C_UDMI(c1,tc1,tcount);
        /*Message ("neighbor found c1 %d,%d,%d\n",old, new,
thrombus_count);*/
        C_UDMI(c,t,tcount) =
C_UDMI(c1,tc1,tcount);
        /*begin_c_loop(c,t)
        {

```

```

(C_UDMI(c,t,tcount) == new)
= old;

if
C_UDMI(c,t,tcount)

}
end_c_loop(c,t)
new=0;
old = 0;*/
}
/*end if thrombus*/
}
}
}
/*begin_c_loop(c,t)
{
if (C_UDMI(c,t,tcount) == new)
C_UDMI(c,t,tcount) = old;
}
end_c_loop(c,t)
new = 0;
old = 0;
if (new != 0)
C_UDMI(c,t,tcount)=new;
new = 0;*/
}
}
if (C_UDMI(c,t,tcount) == th && C_UDMI(c,t,gridid)==2)
{
adhesion_area=adhesion_area+C_UDMI(c,t,area);
}
}
end_c_loop(c,t)
PRF_GRSUM1(adhesion_area);
test = NV_MAG(total_force);
PRF_GRSUM1(test);

test = test*0.1/adhesion_area;
if(test>0.0)
{
Message ("thrombus id = %d\n", th);
Message ("total force (dyne/cm^2) = %g\n", test);
Message ("adhesion area = %g\n", adhesion_area);
Message ("neighbor found %d\n",new);
}
if (test > 500.)
{
begin_c_loop(c,t)

```

```

        {
        if (C_UDMI(c,t,gridid) == 5 || C_UDMI(c,t,gridid) == 2 ||
C_UDMI(c,t,tcount) == th)
        {
            C_UDMI(c,t,gridid) = 1;
            C_UDMI(c,t,M) = 0.;
            C_UDMI(c,t,thrombus) = 0.;
            C_UDMI(c,t,tcount) = 0;
        }
        if (C_UDMI(c,t,gridid) == 4 || C_UDMI(c,t,gridid) == 3 ||
C_UDMI(c,t,tcount) == th)
        {
            C_UDMI(c,t,gridid) = 0;
            C_UDMI(c,t,M) = 0.;
            C_UDMI(c,t,thrombus) = 0.;
            C_UDMI(c,t,tcount) = 0;
        }
        }
        end_c_loop(c,t)
    }
    /* end for loop*/
}
if (new !=0)
{
    begin_c_loop(c,t)
    {
        if (C_UDMI(c,t,tcount) == new)
            C_UDMI(c,t,tcount) = old;
    }
    end_c_loop(c,t)
    new = 0;
    old = 0;
}
}
}
}
}

```

/* Define Viscosity of the blood/thrombus */

```

DEFINE_PROPERTY(cell_viscosity,c,t)
{
    real mu_lam;
    /* set viscosity to 100 if the cell is thrombus*/
    mu_lam = visc;
    if (C_UDMI(c,t,thrombus)>=1.)
        mu_lam = 100.;
}

```

```

return mu_lam;
}

/* Define Inlet Velocity Profile*/

/*DEFINE_PROFILE(inlet_profile,thread,position)
{
real x[ND_ND], y;
face_t f;
begin_f_loop(f,thread)
{
F_CENTROID(x,f,thread);
y=x[1];
/*v = 2Q/A(1-(r/R)^2)*/
/*F_PROFILE(f,thread,position)= flow_rate*0.1179*(1-y*y/9.0e-8);
}
end_f_loop(f,thread)
}*/

/* Source Term for Unactivated Platelets */

DEFINE_SOURCE(rp_source,c,t,dS,eqn)
{
real source,omega,area,thrombus,A[ND_ND];
int n;
face_t f;
cell_t c1, c0;
Thread *ft, *tc1, *tc0;

/* calculate bulk platelet activation */

omega=528000.*(2.357 *C_YI(c,t,2)+9.362*C_YI(c,t,3)+6.01*C_YI(c,t,5));
if (omega > 1)
C_UDMI(c,t,pltact)=omega/tact*C_YI(c,t,0)*1056.; /* kg/m^3*/
else
C_UDMI(c,t,pltact)=0.;

/* calculate source term for each cell */

if (C_UDMI(c,t,gridid)==0)
source = - C_UDMI(c,t,pltact); /* kg/m^3*/
if(C_UDMI(c,t,gridid)==1 || C_UDMI(c,t,gridid)==5)
{
/*initialize so grid cells with two wall sides can be correctly counted*/ area=0.;

```

```

thrombus = 0.;

c_face_loop(c,t,n)
{
    ft=C_FACE_THREAD(c,t,n);
    f=C_FACE(c,t,n);
    c0 = F_C0(f,ft);
    tc0 = THREAD_T0(ft);
    c1 =F_C1(f,ft);
    tc1 = THREAD_T1(ft);
    if (PRINCIPAL_FACE_P(f,ft))
    {
        if(THREAD_ID(ft)==interior_ID)
        {
            if (C_UDMI(c0,tc0,gridid) == 2 || C_UDMI(c0,tc0,gridid) == 3)
            {
                F_AREA(A,f,ft);
                area = NV_MAG(A);
                thrombus = thrombus +
C_UDMI(c0,tc0,Xact)*kt*C_YI(c,t,0)* 1056*area/C_VOLUME(c,t); /*kg/m^3*/
            }
            if (C_UDMI(c1,tc1,gridid) == 2 || C_UDMI(c1,tc1,gridid) == 3)
            {
                F_AREA(A,f,ft);
                area = NV_MAG(A);
                thrombus = thrombus +
C_UDMI(c1,tc1,Xact)*kt*C_YI(c,t,0)* 1056*area/C_VOLUME(c,t); /*kg/m^3 */
            }
        }
        if(THREAD_ID(ft)==wall1_ID || THREAD_ID(ft)==wall2_ID)
        {
            F_AREA(A,f,ft);
            area = NV_MAG(A);
            thrombus = thrombus +
C_UDMI(c,t,Ma)/minf*kt*F_YI(f,ft, 1 )* 1056*area/C_VOLUME(c,t); /* kg/m^3 */
        }
    }
} /* end face loop*/

source = -C_UDMI(c,t,pltact) - thrombus; /* kg/m^3*/
C_UDMI(c,t,delthu) = thrombus*0.00472/0.8; /*retums % thrombus, assuming 20%
void volume*/
/* .0047 comes from vol/mass of platelet: 10.2 fL and 2.12 pg*/
}

if (C_UDMI(c,t,gridid)==2 || C_UDMI(c,t,gridid)==3)
    source = 0.;

```

```

if (C_UDMI(c,t,gridid)==4)
{
    area = 0.;
    thrombus = 0.;
    c_face_loop(c,t,n)
    {
        ft=C_FACE_THREAD(c,t,n);
        f = C_FACE(c,t,n);
        c0 = F_C0(f,ft);
        tc0 = THREAD_T0(ft);
        c1 = F_C1(f,ft);
        tc1 = THREAD_T1(ft);
        if (PRINCIPAL_FACE_P(f,ft) && THREAD_ID(ft) == interior_ID)
        {
            if (C_UDMI(c0,tc0,gridid) == 2 || C_UDMI(c0,tc0,gridid) == 3)
            {
                F_AREA(A,f,ft);
                area = area + NV_MAG(A);
                thrombus = thrombus +
C_UDMI(c0,tc0,Xact)*kt*C_YI(c,t,0)* 1056*area/C_VOLUME(c,t); /* kg/m^3*/
            }
            if (C_UDMI(c1,tc1,gridid) == 2 || C_UDMI(c1,tc1,gridid) == 3)
            {
                F_AREA(A,f,ft);
                area = area + NV_MAG(A);
                thrombus = thrombus +
C_UDMI(c1,tc1,Xact)*kt*C_YI(c,t,0)*1056*area/C_VOLUME(c,t); /* kg/m^3 */
            }
        }
    }

    source = -C_UDMI(c,t,pltact) - thrombus; /* kg/m^3 */
    C_UDMI(c,t,delthu) = thrombus *0.00472/0.8; /*returns % thrombus,
assuming
    20% void volume, see above*/
}
dS [eqn] = 0.0;
return source;
}

/* Source Term for Activated Platelets */
DEFINE_SOURCE(ap_source,c,t,dS,eqn)
{
    real source,thrombus,area,A[ND_ND];
    face_t f;

```

```

cell_t c1, c0;
int n;
Thread *ft, *tc1, *tc0;

/* calculate source term for each cell */

if (C_UDMI(c,t,gridid)==0)
    source = C_UDMI(c,t,pltact);
if(C_UDMI(c,t,gridid)==1 || C_UDMI(c,t,gridid)==5)
{

    /*initialize so grid cells with two wall sides can be correctly counted*/
    area = 0.;
    thrombus = 0.;

    c_face_loop(c,t,n)
    {
        ft=C_FACE_THREAD(c,t,n);
        f=C_FACE(c,t,n);
        c0 = F_C0(f,ft);
        tc0 = THREAD_T0(ft);
        c1 = F_C1(f,ft);
        tc1 = THREAD_T1(ft);
        if (PRINCIPAL_FACE_P(f,ft))
        {
            if(THREAD_ID(ft)==interior_ID)
            {
                if (C_UDMI(c0,tc0,gridid) == 2 || C_UDMI(c0,tc0,gridid) == 3)
                {
                    F_AREA(A,f,ft);
                    area = NV_MAG(A);

                    thrombus = thrombus +
C_UDMI(c0,tc0,Xact)*kt*C_YI(c,t,1)*1056*area/C_VOLUME(c,t);
                    /*kg/mA3 */
                }
                if (C_UDMI(c1,tc1,gridid) == 2 || C_UDMI(c1,tc1,gridid) == 3)
                {
                    F_AREA(A,f,ft);
                    area = NV_MAG(A);

                    thrombus = thrombus +
C_UDMI(c1,tc1,Xact)*kt*C_YI(c,t,1)*1056*area/C_VOLUME(c,t); /*kg/mA3 */

```



```

    }
    }
    if(THREAD_ID(ft)==wall1_ID || THREAD_ID(ft)==wall2_ID)
    {
        F_AREA(A,f,ft);
        area = NV_MAG(A);
        thrombus =
+C_UDMI(c,t,Ma)/minf*kt*F_YI(f,ft,1)*1056*area/C_VOLUME(c,t); /* kg/m^3*/
    }
    }
    } /* end face loop*/
    source = C_UDMI(c,t,pltact) - thrombus; /* kg/m^3*/
    C_UDMI(c,t,delth) = thrombus*0.00472/0.8; /*returns % thrombus, assuming
20% void volume*/
    /* .0047 comes from vol/mass of platelet: 10.2 fL and 2.12 pg*/
    }

    if (C_UDMI(c,t,gridid)==2 || C_UDMI(c,t,gridid)==3)
    {
        C_UDMI(c,t,delth) = 0.;
        source = 0.;
    }

    if (C_UDMI(c,t,gridid)==4)
    {
        area=0.;
        thrombus = 0.;
        c_face_loop(c,t,n)
        {
            ft=C_FACE_THREAD(c,t,n);
            f = C_FACE(c,t,n);
            c0 = F_C0(f,ft);
            tc0 = THREAD_T0(ft);
            c1 =F_C1(f,ft);
            tc1 = THREAD_T1(ft);
            if (PRINCIPAL_FACE_P(f,ft) && THREAD_ID(ft) == interior_ID)
            {
                if (C_UDMI(c0,tc0,gridid) == 2 || C_UDMI(c0,tc0,gridid) == 3)
                {
                    F_AREA(A,f,ft);
                    area = area + NV_MAG(A);
                    thrombus = thrombus
+C_UDMI(c0,tc0,Xact)*kt*C_YI(c,t,1)*1056*area/C_VOLUME(c,t); /* kg/m^3*/
                }
                if (C_UDMI(c1,tc1,gridid) == 2 || C_UDMI(c1,tc1,gridid) == 3)
                {

```

```

        F_AREA(A,f,ft);
        area = area + NV_MAG(A);
        thrombus = thrombus + C_UDMI(c1,tc1,Xact)*kt*C_YI(c,t,1)*
1056*area/C_VOLUME(c,t); /*kg/m^3 */
    }
}
}
source = C_UDMI(c,t,pltact) - thrombus;
C_UDMI(c,t,delth) = thrombus*0.00472/0.8;
}
dS[eqn] = 0.0;
return source;
}

```

/* Source Term for Adenosine DiPhosphate (ADP) */

```

DEFINE_SOURCE(adp_source,c,t,dS,eqn)
{

```

```

    real source;

```

```

    source = .0046*C_UDMI(c,t,pltact);

```

```

    dS [eqn] = 0.0;

```

```

    return source;

```

```

}

```

/* Source Term for Thromboxane A2 (TxA2) */

```

DEFINE_SOURCE(txa2_source,c,t,dS,eqn)
{

```

```

    real source;

```

```

    if (C_UDMI(c,t,gridid)==0 || C_UDMI(c,t,gridid)==4 || C_UDMI(c,t,gridid)==1 ||
C_UDMI(c,t,gridid)==5)

```

```

        source = 0.164*C_YI(c,t,1)-17.002*C_YI(c,t,3);

```

```

    if (C_UDMI(c,t,gridid)==2 || C_UDMI(c,t,gridid)==3)

```

```

        source = 0.15;

```

```

    dS[eqn] = 0.0;

```

```

    return source;

```

```

}

```

/* Source Term for Prothrombin (pT) */

```

DEFINE_SOURCE(pt_source,c,t,dS,eqn)
{

```

```

    {

```

```

    real source;

```

```

if (C_UDMI(c,t,gridid)==0 || C_UDMI(c,t,gridid)==1 || C_UDMI(c,t,gridid)==4)
    source = -4.792e9*C_YI(c,t,4)*(3.69e-6*C_YI(c,t,1)+6.5e-7*C_YI(c,t,0));
if (C_UDMI(c,t,gridid)==2 || C_UDMI(c,t,gridid)==3)
    source = -1.765e4*C_YI(c,t,4)*C_UDMI(c,t,Xact);
dS [eqn] = 0.0;
return source;
}

/* Source Term for Thrombin (th) */

DEFINE_SOURCE(thrombin_source,c,t,dS,eqn)
{
    real source,generation,loss;
    if (C_UDMI(c,t,gridid)==0 || C_UDMI(c,t,gridid)==4 || C_UDMI(c,t,gridid)==1)
    {
        generation = 4.792e9*C_YI(c,t,4)*(3.69e-6*C_YI(c,t,1) + 6.5e-7 * C_YI(c,t,0)) *0.508;
        loss = 0.047*C_YI(c,t,6)/(3.5e-9+2.885e-3*C_YI(c,t,5) + 5.96e-4*C_YI(c,t,6) +
491.423*C_YI(c,t,6)*C_YI(c,t,5))*C_YI(c,t,5)* 1056.;
        source = generation - loss;
    }
    if (C_UDMI(c,t,gridid)==2 || C_UDMI(c,t,gridid)==3)
    {
        generation = 8.973e3 *C_YI(c,t,4)*C_UDMI(c,t,Xact);
        loss = 0.047*C_YI(c,t,6)/(3.5e-9+2.885e-3*C_YI(c,t,5) + 5.96e-4*C_YI(c,t,6) + 491.423
*C_YI(c,t,6)*C_YI(c,t,5))*C_YI(c,t,5) * 1056.;

        source = generation - loss;
    }
    dS[eqn] = 0.0;
    return source;
}

/* Source Term for antithrombin HI (at) */
DEFINE_SOURCE(at_source,c,t,dS,eqn)
{
    real source;
    source = -84.077*C_YI(c,t,6)/(3.5e-9+2.885e-3*C_YI(c,t,5)+5.96e-4*C_YI(c,t,6)+491.423
*C_YI(c,t,6)*C_YI(c,t,5)) *C_YI(c,t,5);
    dS [eqn] = 0.0;
    return source;
}

```

```

/* Diffusivity Definition */

DEFINE_DIFFUSIVITY(Diffusivity, c, t, i)
{
  real source;
  if (i==0)
  {
    source = 6.0e-14*C_UDMI(c,t,gama)+1.58e-13;

  }
  if (i==1)
  {
    source = 6.0e-14*C_UDMI(c,t,gama)+1.58e-13;

  }
  if (i==2)
  {
    source = 2.57e-10;

  }
  if (i==3)
  {
    source = 2.14e-10;

  }
  if (i==4)
  {
    source = 6.0e-14*C_UDMI(c,t,gama)+3.32e-11;

  }
  if (i==5)
  {
    source = 6.0e-14*C_UDMI(c,t,gama)+4.16e-11;

  }
  if (i==6)
  {
    source = 6.0e-14*C_UDMI(c,t,gama)+3.49e-11;

  }
  /*if (i==7)
  {
    source = 6.0e-14*C_UDMI(c,t,gama)+3.49e-11;

  }
  */
  Message("i%d", i);
}

```

```

Message("mass_diff%e", source);*/

return source;

}

/*Source Terms to Generate Initial Concentrations of Species*/

DEFINE_SOURCE(rp_init,c,t,dS,eqn)
{
    real source;
    real curr_int = RP_Get_Real("flow-time");
    source = 0;
    if (curr_int==delt_int)
    {

        source = 0.0006;
        dS [eqn]=0.0;

    }
    return source;
}

DEFINE_SOURCE(ap_init,c,t,dS,eqn)
{
    real source;
    real curr_int = RP_Get_Real("flow-time");
    source = 0;
    if (curr_int==delt_int)
    {

        source = 0.000006;
        dS [eqn]=0.0;

    }
    return source;
}

DEFINE_SOURCE(pt_init,c,t,dS,eqn)
{
    real source;
    real curr_int = RP_Get_Real("flow-time");
    source = 0;
    if (curr_int==delt_int)
    {

```

```

        source = 0.000075;
        dS [eqn]=0.0;

    }
    return source;
}

DEFINE_SOURCE(at_init,c,t,dS,eqn)
{
    real source;
    real curr_int = RP_Get_Real("flow-time");
    source = 0;
    if (curr_int==delt_int)
    {

        source = 0.000167;
        dS [eqn]=0.0;

    }
    return source;
}

/* Surface Reaction Rates */
DEFINE_SR_RATE(Surface_Reactions, f, t, r, mw, yi, rr)
{
    Thread *t0=t->t0;
    cell_t c0=F_C0(f,t);

    /*Reaction 1: Unactivated platelet adhesion
        rp --> dp
        rate = Sa*kps*Xrp*rho/mwplt
        to get plt/m^2: kps*Xrp*rho/massplt (hence 4.774e17)*/

    if (STREQ(r->name, "reaction-1"))
    {
        C_UDMI(c0,t0,rpadh) = C_UDMI(c0,t0,Sa)*krs*yi[0]*4.774e17; /* plt/m^2-s*/

        /*Memb = M*(1-exp(-.000309*gama))/temb, units in platelet/m^2
        in the exponent, 0.0095*0.00325 (visc)/0.1(conversion to dynes/cm2) */
        C_UDMI(c0,t0,upemb)= C_UDMI(c0,t0,M)*(1.-C_UDMI(c0,t0,Xact))*(1.-
exp(.000309*C_UDMI(c0,t0,gama)))/temb; /* returns plt/m^2-s */

        *rr =C_UDMI(c0,t0,Sa)*krs*yi[0]* 1056./mw[0] - C_UDMI(c0,t0,upemb)/6.023e26; /*
returns rate in kmoles/m^2-s*/
    }
}

```

```

/*Reaction 2: Activated platelet adhesion
ap --> dp rate = Sa*kas*Xap*rho/mwplt */

if (STREQ(r->name, "reaction-2"))
{
*rr = C_UDMI(c0,t0,Sa)*kas*yi[1]*1056./mw[1]; /* returns rate in kmoles/m^2-s */

C_UDMI(c0,t0,apadh) = C_UDMI(c0,t0,Sa)*kas*yi[1]*4.774e17; /* plt/m^2-s*/

}

/*Reaction 3: Release of TxA2 from deposited platelets dp --> dp + txa2 rate = M*St =
M(plt/m^2)*(9.5e-21molTxa2/plt-s) leave the stoichiometry at 0 for dp, 1 for txa2 */
if (STREQ(r->name, "reaction-3"))
{
*rr = C_UDMI(c0,t0,M)*C_UDMI(c0,t0,Xact)*9.5e-24; /* returns rate in
kmoles/m^2-s */
}

/*Reaction 4: Release of Thrombin from deposited platelets
dp + pT --> dp + th
rate = M*fi*pT = M(plt/m^2)*3.55e-14(kg-blood/plt-s)*Xi[pT]/mwpT stoichiometry for the
reaction: 1 for pT and th; 0 for dp */

if (STREQ(r->name, "reaction-4"))
{
*rr = C_UDMI(c0,t0,M)*C_UDMI(c0,t0,Xact)*3.55e-14*yi[4]/mw[4];
/*returns rate in kmoles/m^2-s */
}
}

```

Appendix D Standard Operating Procedure for Blood Collection

Scope

To describe the procedure for collection and storage of whole blood. This procedure is designed to maintain the physiological properties of blood. The blood can then be used in experiments of hemolysis, coagulation, or other properties of blood. This procedure is meant to be scaled depending on quantity of blood required.

Requirements

Materials:

1. Large container (1000ml beaker – 6qt cooking pot)
2. Ice chest
3. Ice
4. Storage container (PETG bottles – PVC blood bag)
5. ___ ft² of PVC sheet
6. Heparin (or other anticoagulant)

Equipment and Instruments:

Scale

Heat sealer

Procedures

Collection of Blood from Slaughter House:

1. Determine amount of blood required.
2. Determine the large collection container. The container should have a volume 3 times the amount of blood required, and should also be round. Because the blood will never come in contact with this container, material is not a consideration.
3. Cut two pieces of PVC film in the same shape. The shape should be twice as tall as the collection container and be wider than the diameter of the container.
4. Place the PVC shapes on top of each other. Using the heat sealer, create a seal about ½" thick on three sides of the PVC. This makes a pouch with one open end. This open end is where the blood will come into the pouch.

5. Place the PVC pouch inside the large collection container and fold the top of the pouch over the side of the collection container. Secure with clips or rubber bands if necessary.
6. Measure the amount of blood required and mark it on the PVC pouch.
7. Measure the amount of heparin needed and place in the PVC pouch. The recommended amount of heparin is 1.5U/ml. This can be changed based on individual needs. The activity of a particular heparin should be given on the bottle in U/mg. Other anticoagulants can be used in a similar fashion but with different quantities.
8. When using a commercial butcher, have the large collection container with the PVC pouch inside and the pre-measured anticoagulant inside the PVC pouch all prepared. Hand this to the butcher and they will hand it back to you filled with blood.
9. If a large quantity of blood is being collected (>1L) the blood should be gently mixed to ensure the anticoagulant is evenly distributed.
10. Remove the PVC pouch from the large container. Pour the blood into storage container such as PETG bottles or PVC blood bag. Try to avoid foaming the blood while pouring. While the PVC pouch can be used to store the blood, it is recommended that a different bag be used for storage because of sanitation issues.
11. Place the storage container that is now containing blood on ice for transport.
12. Label storage container as "Bovine Whole Blood" Also label with the date of collection and date to be discarded. The date to be discarded can be up to 60 days after date of collection. After 60 days, the blood has lost all physiological properties.
13. The blood can now either be used or stored between 2-4 C for a few days. Precise time when blood is no longer good is dependent of the experiment being performed on the blood.

Appendix E Standard Operating Procedure (SOP) for Concentric Cylinders Test Stand

This device is intended to expose a fluid to a controlled shear rate. This is done by controlling both the gap between the cylinders and the rate at which the outer cylinder rotates. The speed at which the outer cylinder rotates is controlled by a DC motor and power supply. Please follow the startup procedure below whenever using this device.

Equipment and Instrumentation

- 1) Concentric Cylinder test stand including DC motor
- 2) power supply
- 3) frequency counter

Procedure

- 1) Center the inner cylinder in the outer cylinder
 - a) Make sure the pillow blocks of the outer cylinder are tight.
 - b) Loosen all the fasteners for the inner cylinder. This includes: the collar around the 1.5 stainless steel, and both of the fasteners on the 90 degree, 0.5 in post holder.
 - c) Place the brass sleeve in the outer cylinder, then place the inner cylinder in the brass sleeve. The brass sleeve acts as a physical centering device for the cylinders.
 - d) Tighten the fasteners of the inner cylinder being careful to maintain the best possible alignment.
 - e) Once all three fasteners are tightened, loosen the fastener holding the post that is directly connected to the inner cylinder. Pull the post directly up, removing the inner cylinder from the outer cylinder and the brass sleeve. The post should raise with minimal resistance. If you feel resistance, that means the cylinders are misaligned and you should begin from step (a) again.
 - f) Remove the brass sleeve.
 - g) Replace the inner cylinder into the outer cylinder. The two cylinders should be concentric now.
- 2) Place the fluid in the test section.
- 3) Expose the fluid to shear stress.
 - a) Turn on the power supply. It is recommended that the power supply be turned to 0 V when you turn it on.

- b) Turn up the voltage until the desired RPM is reached.
 - i) The Counter reports revolutions/sec, so unit conversions may be necessary.
 - c) If the cylinders are misaligned, fluid will spray or foam in the test section if high shears are induced. It is recommended that you cover the device while in operation.
- 4) The fluid and cylinders can now be examined depending on the current experimental needs.
- 5) The cylinders should be thoroughly washed before the next experiment.

Appendix F Drawing Package for Concentric Cylinder Test Stand

Part #	Part name	Drawing name	Vendor
7173628	.75x1.625 in deep groove bearing	bearings	MSC
p6	6" mounting post	thor_p6	thor labs
p14	14" mounting post	thor_p14	thor labs
pb1	mounting post base	thor_pb1	thor labs
c1525	post clamp and 4.5x4.5 in mounting surface	thor_c1525	thor labs
c1501	mounting post clamp	thor_c1501	thor labs
ra90	right angle post clamp	thor_ra90	thor labs
c1500	compact p-series post clamp	thor_c1500	thor labs
c1498	slip on post clamp	thor_c1498	thor labs
tr2	1/2" post	thor_tr2	thor labs
1LWK8	DC Motor, 12v, 1/35 HP, 2350 RPM, 3.8 A, TENV	-	Gainger
Rig1	Bearing block	bearingblock	in-house
Rig2	Base Plate	base plate	in-house
Rig3	Inner cylinder	inner cylinder	in-house
Rig4	Outer Cylinder	outer cylinder	in-house

Table E.1: List of parts in the concentric cylinder test stand. Part# references the vendor part # and corresponds to the annotations in the complete assembly drawing. Drawing name in the name of the file found on the media.

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		DIMENSIONS ARE IN INCHES
		TOLERANCES:
		FRACTIONAL: $\pm$
		ANGULAR: MACH $\pm$ BEND $\pm$
		TWO PLACE DECIMAL $\pm$
		THREE PLACE DECIMAL $\pm$
		MATERIAL
NEXT ASSY	USED ON	FINISH
APPLICATION		DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	CDC	
CHECKED		
ENG APPR.		
MFG APPR.		
G.A.		
COMMENTS:		

Complete annotated concentric cylinder test rig		
SIZE	DWG. NO.	REV.
A	complete assembly	
SCALE: 1:5	WEIGHT:	SHEET 1 OF 1

UNLESS OTHERWISE SPECIFIED:		NAME		DATE	
DIMENSIONS ARE IN INCHES		DRAWN		CHECKED	
TOLERANCES:		ENG. APPR.		MFG. APPR.	
FRACTIONAL		Q.A.		COMMENTS:	
DECIMAL		MATERIAL		Aluminum	
ANGULAR: MATCH		FINISH		USED ON	
HOLE: 2		NEXT ASSY		APPLICATION	
TWO PLACE DECIMAL		DO NOT SCALE DRAWING		5	
THREE PLACE DECIMAL		1		2	
INTERPRET GEOMETRIC TOLERANCING PER:		3		4	
TITLE:		SIZE		DWG. NO.	
Test Rig Base		A		base plate	
SCALE: 1:5		WEIGHT:		SHEET 1 OF 1	

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THORLABS [®] PD BOX 366			
TITLE INTERLOCKING POST COLLAR			
TOL. XXXXX+/-0.005 XXXXX+/-0.005 ANGULAR +/-30°			
SURF. FINISH 32 MICROINCHES UNLESS OTHERWISE NOTED			
DRAWN	ENGINEER	APPROVED	
DATE 04/17/00	REV 1	DATE 04/17/00	REV 1
PART NO. 3001-EC01	C1498		

Ø1.00 KNOB PROVIDES SUFFICIENT
TORQUE FOR CLAMPING OF MOUNTS

4 HOLES IN SIDE OF KNOB
ALLOW USER TO INSERT A 5/64" HEX KEY
FOR ADDITIONAL TORQUE

MOUNTS TO Ø1.50 P-SERIES POST

#8-32 MOUNTING SCREW

CLAMPING SCREW HOLDS
MOUNT SECURELY TO POST

THORLABS PO BOX 366 NEWTON NJ	
TITLE COMPACT POST CLAMP	
TOL 1.0000" 1.0000" 1.0000" 1.0000" 1.0000"	
DATE 07/07/99	
DRAWN 18/15/99	
CHECKED 18/15/99	
APPROVED	
MATERIAL N/A	
REV	DATE
1	07/07/99
PART NO. C1500	
WHERE USED	
MECHANICAL	
DRAWING	

INFORMATION ONLY, NOT FOR MANUFACTURING

Technical drawing of the Thorlabs C1501 component, showing three views: top, side, and front.

Top View Dimensions:

- Overall width: 4.51"
- Overall height: 1.500"
- Central hole diameter: 0.36"
- Mounting hole diameter: 0.86"
- Distance from center to mounting hole: 1.75"
- Distance from edge to mounting hole: 2.50"

Side View Dimensions:

- Overall height: 0.48"
- Base width: 4.50"

Front View Dimensions:

- Overall width: 4.50"
- Overall height: 0.25"
- Mounting hole diameter: 0.36"
- Distance from center to mounting hole: 1.75"
- Distance from edge to mounting hole: 2.50"

Component Details:

- 1/4"-20 MOUNTING HOLE (STACKABLE POST SYSTEM)
- THORLABS ITEM# C1501
- THORLABS ITEM# P6A
- THORLABS ITEM# P6B
- 1/4"-20 MOUNTING HOLE (4 PLACES)
- 1/4"-20 MOUNTING HOLE (1 PLACES)
- DRILLED FOR 1/4"-20 CAP SCREW (4 PLACES)
- DRILLED FOR #8-32 CAP SCREW (6 PLACES)

C1525

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P6

THORLABS PD. BOX 366 NEWTON NJ			
TITLE 6" POST			
TOL. XXXX+/--XXXX XXXX+/--XXXX	ANGULAR +/- .50°		
MATERIAL	WHERE USED		
303 STAINLESS STEEL	ENGINEERING		
DRAWN	ENGR.	APPROVED	SUPPORT
JW	AC	AC	DRAWING
DATE	REV	SIZE	
6/11/95	B	A	1 OF 1
DWG. NO.	PART. NO.		
0677-E01	P6		

REV. 2: 10/11/95
REV. 1: 10/11/95
REV. 0: 10/11/95

P14

THORLABS[®]			
PO BOX 366 NEWTON, NJ			
TITLE 14' POST			
MATERIAL 303 STAINLESS STEEL			
FINISH POLISHED			
DATE 6/11/95			
REV. 0: 10/11/95			
REV. 1: 10/11/95			
REV. 2: 10/11/95			
PART NO. 0086-E01			
PART NAME P14			
WHERE USED ENGINEERING SUPPORT DRAWING

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P-SERIES PIST BASE			
MATERIAL: 303 STAINLESS STEEL			
REV		DATE	
02/18/06		1 OF 1	
PST		PST	
0264-006		PST

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DATE: 01/11/02	
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MATERIAL: 6061-T6 ALUMINUM	
FINISH: ANODIZED	
TOLERANCES: DECIMALS: .005 FRACTIONS: 1/16 ANGLES: .5°	
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**Appendix G Standard Operating Procedure for PT and
APTT tests from Stiene-Martin, Clinical Hematology,
pg. 638-639**

tube with diatomite is attached to the needle. When blood starts to flow into the tube, the first stopwatch is started. The tube is filled, mixed, and placed in the heat block. The procedure is repeated with the second tube, and the second stopwatch is started. After 60 seconds, the first tube is observed by tilting it at 5-second intervals until a clot is formed, at which time the second tube is observed using the same procedure. The appropriate stopwatch is stopped at the first appearance of a clot in each tube. The duplicates should agree within 10 seconds. The average time is reported.⁴⁹

Reference Range. The reference range is 75 to 120 seconds. The target range during heparin therapy is 140 to 185 seconds.

Interpretation. Prolongation of the ACT is indicative of one or more factor defects in the intrinsic or common pathways or the presence of a circulating anticoagulant such as heparin.

Comments. Temperature control is critical: all testing must be maintained at 37°C. The primary use of the ACT is during procedures requiring extracorporeal circulation, in which frequent testing and rapid turnaround time are required.⁵³ In fact, the ACT has enjoyed considerable popularity of late as a "point-of-care" test (*i.e.*, a test performed at the patient's bedside).⁹⁷ Another recent use of the ACT is in monitoring therapy with hirudin (a direct thrombin inhibitor derived from leaches).¹⁷

Other than the duplicate testing, there is no suitable method of quality control for this procedure.

PARTIAL THROMBOPLASTIN TIME

Several researchers observed that blood samples from classic hemophiliacs have a prolonged clotting time, but that when tissue thromboplastin is added, the plasma clots just as normal plasma does.⁷⁴ Expanded studies showed that thromboplastins, which are lipoproteins (protein + phospholipid), may be classified as complete or partial (only phospholipid). From these conclusions, investigators developed the partial thromboplastin time (PTT). This test is more sensitive to abnormalities in the early stages than were previous tests of the intrinsic system. An important refinement of the PTT was the addition of negatively charged activators to the system, resulting in significantly shorter clotting times.⁸⁷ This modification is now used exclusively. It is the test of choice to screen for factor deficiencies of the intrinsic and common pathways and also for monitoring heparin therapy. It also is the basis for assays of factors of the intrinsic system.

The PTT reagent consists of two components: a platelet substitute (phospholipid), prepared from brain or plant phospholipids, and an activator. Kaolin, Celite, micronized silica, or ellagic acid are used as activators, depending on the manufacturer.

Principle. The PTT measures all factors except VII and XIII. Maximum activation of the contact factors is accomplished by addition of the activator. Phospholipid is supplied to substitute for platelet factor 3 (PF3). From this point, the PTT is essentially the same as a recalcification time of plasma.

Specimen Requirements and Reagents. Citrated platelet-poor plasma should be collected according to the guidelines in Chapter 2. Reagents include phospholipid with activators (PTT reagent) and 0.025 M CaCl₂ (or as recommended by reagent manufacturer).⁹³

Controls. Commercial lyophilized controls are available in normal, midrange, and extended ranges.⁶⁶ It is recommended that a normal control and at least one abnormal control be used.⁹⁴ In-house preparations of pooled/frozen plasma may be used as controls. Each laboratory must specify when controls are to be tested, what the satisfactory control limits are, and if duplicates are run, how closely the values should agree.

Equipment. For the manual method, 12 × 75 mm glass tubes, a heat block, and pipettes are needed. For the fibrin strand method, an instrument for electromechanical fibrin strand detection and appropriate cups and pipettes, as recommended by the manufacturer, are required. For the photo-optical method, the specialized instrument and appropriate accessories as listed by the manufacturer, are needed.

Procedure. Platelet-poor plasma (0.1 mL) is added to 0.1 mL of PTT reagent and incubated at 37°C for the period of time specified by the reagent manufacturer (approximately 3–5 minutes). After incubation, 0.1 mL of warmed CaCl₂ is added, and the time for clotting to occur is recorded.

Reporting, Reference Range and Interpretation. The PTT is reported in seconds, to the nearest tenth, along with the reference range.⁹⁴

Reference ranges differ according to the reagent, method, and instrument used. The reference ranges may extend from a lower limit of 20 seconds to an upper limit of 45 seconds.

When properly performed, the PTT is an excellent screening test for the intrinsic and common coagulation pathway factors. Most commercial reagents will demonstrate a prolonged PTT if any factor measured by the PTT is less than 40% to 50% of normal. A prolonged PTT in the absence of heparin indicates a factor deficiency, an acquired circulating anticoagulant such as the lupus inhibitor, or an antibody to a specific factor such as factor VIII.

Comments. Sources of error in the PTT may be grouped into three categories: sample collection and preparation, reagent preparation, and instrumentation. Improper collection and processing of specimens may significantly affect the results. The anticoagulant volume should be adjusted for individuals with hematocrits greater than 0.55 L/L to avoid error caused by an incorrect anticoagulant-to-plasma ratio. Specimens with hematocrits greater than 0.55 L/L will have anticoagulant excess in the collection tube. The extra sodium citrate in the collection tube chelates the calcium in the PTT test system and may result in spuriously abnormal results. Sodium citrate volume should be adjusted in the collection tube to keep the recommended citrate-plasma volume correct.⁹⁴ Theoretically specimens with hematocrits less than 0.20 L/L need more anticoagulant in the collection tube to compensate for the increased plasma volume of the specimen. In practice, however, low hematocrits do not seem to cause problems with spurious results. Correction for citrate volume is not required for these specimens.⁹⁴ Hemolysis may cause a falsely shortened PTT. Platelets in the plasma sample may cause erratic results or falsely shorten the PTT. Unexpected heparin contamination (*e.g.*, from heparin locks) can falsely lengthen the PTT.

Reagents may be affected by improper storage, water impurities, or incorrect dilution. Reagent systems should be tested for sensitivity to factor deficiencies by performing the PTT on serial dilutions of plasma.

A failing light source, fluctuations in temperature, loss of calibration of tubing, or contamination will cause instrument error. A good quality control program should reveal any instru-

mental or reagent-related error. Refer to Chapter 58 for a discussion of instruments and quality control.

ROUTINE TESTS FOR THE EXTRINSIC AND COMMON PATHWAYS

PROTHROMBIN TIME

Early experiments by the French physiologist de Bainville³ showed that blood could be made to clot quickly by adding tissue factors. The discovery that oxalate and citrate inhibit coagulation¹¹⁹ led to the assumption that clotting was dependent on calcium.

The one-stage prothrombin time (PT), as described by Dr. Armand Quick in 1935,¹¹² was believed to be an indirect measure of prothrombin in plasma, dependent on the presence of fibrinogen. Subsequent discoveries of factors V, VII, and X showed that the PT is a reflection of the activities of several factors.^{102,111} Thus, the PT used in the modern laboratory screens for deficiencies of factors I, II, V, VII, and X.

Although the PT is no longer considered a measure of prothrombin itself, it is still the test of choice for monitoring anticoagulant therapy by vitamin K antagonists (Chap. 54).^{83,84,134,142} Three of the five factors measured by the PT (II, VII, X) are sensitive to and depressed by these anticoagulants.¹⁰⁹ Only factor IX, the other factor depressed by vitamin K antagonists, is not detected by the PT.

Principle. When tissue extract or thromboplastin¹⁰³ is added to PPP along with calcium, it reacts with factor VIIa to convert factor X to Xa. Factor Xa, along with factor Va, phospholipid, and Ca^{2+} converts prothrombin to thrombin. Thrombin subsequently converts fibrinogen to fibrin. The time from the addition of thromboplastin/ CaCl_2 to the formation of a clot is reported.^{55,56}

Specimen Requirements, Reagents and Equipment. Citrated platelet-poor plasma is collected according to the guidelines in Chapter 2. Thromboplastin/ CaCl_2 (PT reagent) and controls (see discussion under PTT controls) are needed. Equipment is the same as that used for the PTT.

Procedure and Reference Range. Aliquots of control and patient plasma are warmed according to the method being used. The thromboplastin reagent is warmed by incubating it at 37°C for 3 to 5 minutes, and 0.2 mL of PT reagent is added to 0.1 mL of plasma (patient or control). The clotting time is recorded to the nearest tenth of a second.

Normal values differ with the reagent and method. These values may range from 10 to 12 seconds in some photo-optical systems and from 12 to 14 seconds using manual methods.

Reporting. Prothrombin times may be reported in several ways: (1) patient time (in seconds) with the reference range²⁴; (2) patient time with the control time (in seconds); (3) prothrombin ratio (the PT of the patient divided by the mean of the reference range and multiplied by 100—rarely used in the United States); (4) percent activity (outdated and not recommended); and (5) the International Normalized Ratio (INR), which has been proposed as the standard method of reporting.^{54,80,95} The INR is discussed in Chapter 54.

Interpretation and Comments. Prolongation of the PT indicates an abnormality of one or more common or extrinsic coagulation factors. This abnormality may be inherited or acquired. Prolongation may also occur with factor inhibitors. Using most commercial reagents, the PT is sensitive to factor deficiencies of less than 40% to 50% of normal.

Reporting of percent activity is no longer recommended because the dilution curve used to determine percent activity dilutes all factors, not just those affected by anticoagulant therapy, and is therefore an inaccurate representation of therapy. The most common method of reporting in the United States at the present time is patient time in seconds along with the reference range. The INR is gaining popularity in an effort to standardize PT reporting.⁹¹ Portable instruments capable of determining PT and calculating the INR are available.¹⁴⁷ The sources of error in this test are similar to those discussed for the PTT.

OTHER TESTS

Two other laboratory determinations related to the extrinsic and common pathways are the Stypven time and prothrombin-proconvertin time.⁷¹ Neither test has enjoyed widespread popularity. The reader is referred to textbooks dedicated to hemostasis and the indicated references for details on these rarely performed tests.^{68,96,104}

OTHER ROUTINE TESTS

FIBRINOGEN

Many methods for the measurement of fibrinogen have been described. Five are most commonly performed.

1. Precipitation or denaturation methods,^{44,60,128} in which heat, salt, or another agent is used to denature fibrinogen to an insoluble state. The suspended fibrinogen precipitate may be measured turbidimetrically, or the precipitated fibrinogen may be isolated and measured by colorimetric or ultraviolet protein quantitation. These methods generally are semiquantitative and are subject to many technical problems.
2. Turbidimetric or fibrin clot density procedures²⁸ are based on photo-optical measurement of the change in turbidity of plasma as fibrinogen is converted to fibrin by thrombin. The kinetics of this conversion have been used to measure the fibrinogen concentration.^{58,141} Turbidimetric procedures are the basis for some automated fibrinogen methods that have been introduced.^{130,149} Modifications of the original procedure have improved sensitivity at lower fibrinogen levels and have eliminated heparin interference.^{122,123,130}
3. Coagulable protein assays are performed by clotting the plasma sample using thrombin or CaCl_2 , isolating the fibrin clot, and then measuring the fibrin by weight, chemically, or by ultraviolet absorbance.^{60,114,128} Ancrod, a thrombin-like enzyme that is specific for fibrinogen and not influenced by heparin, also has been used to clot plasma.³² The coagulable protein assay is considered the reference procedure for fibrinogen measurement. The principal disadvantage of this type of assay is the time required to perform the test.¹²⁸
4. Immunologic assays utilize antibodies to fibrinogen in assays such as radial immunodiffusion,^{14,19,24} kinetic latex agglutination,² measurement of turbidity,³² or