11-9-2005

Effect of blood flow patterns on localized platelet adhesion under physiologic flow conditions using two-dimensional and three-dimensional stent models - an experimental and computational approach

Nandini Duraiswamy
Florida International University

DOI: 10.25148/etd.FI15101251

Follow this and additional works at: <https://digitalcommons.fiu.edu/etd>

Part of the [Biomedical Engineering and Bioengineering Commons](#)

Recommended Citation

Duraiswamy, Nandini, "Effect of blood flow patterns on localized platelet adhesion under physiologic flow conditions using two-dimensional and three-dimensional stent models - an experimental and computational approach" (2005). *FIU Electronic Theses and Dissertations*. 3103.

<https://digitalcommons.fiu.edu/etd/3103>

This work is brought to you for free and open access by the University Graduate School at FIU Digital Commons. It has been accepted for inclusion in FIU Electronic Theses and Dissertations by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

EFFECT OF BLOOD FLOW PATTERNS ON LOCALIZED PLATELET ADHESION
UNDER PHYSIOLOGIC FLOW CONDITIONS USING TWO-DIMENSIONAL AND
THREE-DIMENSIONAL STENT MODELS – AN EXPERIMENTAL AND
COMPUTATIONAL APPROACH

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOMEDICAL ENGINEERING

by

Nandini Duraiswamy

2005

To: Dean Vish Prasad
College of Engineering and Computing

This dissertation, written by Nandini Duraiswamy, and entitled Effect of Blood Flow Patterns on Localized Platelet Adhesion under Physiologic Flow Conditions using Two-dimensional and Three-dimensional Stent Models - An Experimental and Computational Approach, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Nikolaos Tsoukias

Eric Crumpler

James E. Moore

Kalai Mathee

Richard T. Schoephoerster, Major Professor

Date of Defense: November 9, 2005

The dissertation of Nandini Duraiswamy is approved.

Dean Vish Prasad
College of Engineering and Computing

Dean Douglas Wartzok
University Graduate School

Florida International University, 2005

© Copyright 2005 by Nandini Duraiswamy

All rights reserved.

DEDICATION

Foremost, I dedicate this dissertation to my husband Vishwanathan Narayanan. Without his encouragement, support, and love during these long years at school, this work could not have been accomplished. Secondly, I also dedicate this research to Atherosclerotic patients, towards a better stent with reduced restenosis.

ACKNOWLEDGMENTS

I would like to thank all my committee members for their guidance, encouragement, and support. In particular, my major professor and guide, Dr. Richard T. Schoephoerster, was very instrumental in guiding me and my work right from the initial stages. Without Dr. RT Schoephoerster, this work may not have progressed at all at FIU. Thanks to Dr. James E Moore for having left his equipment for me to continue on the work and also for his guidance at every stage of the project. I thank the rest of the committee for their valuable input in preparation of this dissertation. From the experimental standpoint, I got plenty of help from many people. I would like to thank Dr. James Byrne, Bhavani Jayachandran, Qiang Wang, Jose Cesar, and Anna Paola Hegedus for their continuous help from the beginning till the end of this project. I also wish to thank Mike Moreno and Jacqueline C. for their help in getting the right equipment and experimental setup. I would also like to thank Yong He for his help to get me familiarized with the numerical software and Oscar Saenz for his help with the statistical analysis. Plenty of thanks to Ms. Miriam Paret of Cordis for her help in getting me the stents from the company for the experimental testing. Definitely, none of the results could have been published without the help from my blood donors. I wish to thank you all tremendously for your generosity and time.

This research was supported by a grant from the National Heart, Lung, and Blood Institute and the National Institute for General Medical Sciences along with the Minority Biomedical Research Support for Support of Continuous Research Excellence (S06 GM08205) at Florida International University.

ABSTRACT OF THE DISSERTATION

EFFECT OF BLOOD FLOW PATTERNS ON LOCALIZED PLATELET ADHESION
UNDER PHYSIOLOGIC FLOW CONDITIONS USING TWO-DIMENSIONAL AND
THREE-DIMENSIONAL STENT MODELS – AN EXPERIMENTAL AND
COMPUTATIONAL APPROACH

by

Nandini Duraiswamy

Florida International University, 2005

Miami, Florida

Professor Richard Schoepfoerster, Major Professor

This dissertation presents dynamic flow experiments with fluorescently labeled platelets to allow for spatial observation of wall attachment in inter-strut spacings, to investigate their relationship to flow patterns. Human blood with fluorescently labeled platelets was circulated through an *in vitro* system that produced physiologic pulsatile flow in (1) a parallel plate flow chamber that contained two-dimensional (2D) stents that feature completely recirculating flow, partially recirculating flow, and completely reattached flow, and (2) a three-dimensional (3D) cylindrical tube that contained stents of various geometric designs.

Flow detachment and reattachment points exhibited very low platelet deposition. Platelet deposition was very low in the recirculation regions in the 3D stents unlike the 2D stents. Deposition distal to a strut was always high in 2D and 3D stents. Spirally recirculating regions were found in 3D unlike in 2D stents, where the deposition was higher than at well-separated regions of recirculation.

Platelet deposition occurred through convective transport of platelets as defined by the instantaneous streamlines. The instantaneous streamlines were obtained from computational fluid dynamics models of the different stents used with the same experimental flow conditions. Platelet deposition was higher in areas where the blood flow was directed towards the wall and lower in areas where the blood flow was directed away from the wall.

Though the platelet deposition patterns shown in this paper were a result of a short time-scale phenomena, convective transport plays an essential role in the interaction of blood cells with the endothelial or exposed underlying collagen layer, which in turn affects the development of intimal hyperplasia (IH). These results could help in improved stent designs in future that prevent excessive platelet aggregation.

TABLE OF CONTENTS

CHAPTER	PAGE
1 INTRODUCTION	1
1.1 Cardiovascular System of the Human Body	1
1.2 Structure and Function of Vasculature	2
1.3 Composition of Blood and Their Functions	4
1.3.1 Vascular Injury	5
1.4 Atherosclerosis	6
1.5 Stents	10
1.5.1 Vascular Response to Stenting	11
1.5.2 Thrombosis – Role of Platelets in Restenosis	11
1.5.3 Role of Endothelial Cells in Restenosis	13
1.5.4 Stents and Restenosis – A Strong Link	14
1.5.5 Restenosis - Influence of Flow Patterns and Arterial Wall Stress	18
1.5.6 Flow effects on Platelet Deposition	24
1.6 Problem Statement - Study Objectives	25
2 EXPERIMENTAL AND COMPUTATIONAL METHODS	28
2.1 In vitro Experiment	28
2.2 Computational Fluid Dynamic (CFD) Simulation	39
2.2.1 Parametric Comparison of 3D Generic Stented Models	40
2.2.2 Blood Flow Patterns of 2D and 3D Stents used Experimentally	49
3 RESULTS	54
3.1 Platelet Deposition and Computational Results of 2D Stented Models	54
3.2 Computational Results of 3D Generic Stented Models	60
3.3 Platelet Deposition and Computational Results of 3D Stented Models	77
4 DISCUSSION	105
4.1 2D Experimental Stented Models	106
4.2 Generic 3D Parametric Stented Models	113
4.3 3D Experimental Stented Models	121
4.4 General Discussion on Platelet Deposition and Its Relationship to Flow Dynamics	132
4.5 Conclusion and Future work	138
REFERENCES	144
APPENDIX	155
VITA	172

LIST OF FIGURES

FIGURE	PAGE
1. Schematic diagram of the three layers (intima, media, and adventitia) of an artery.....	3
2. Schematic diagram depicting an acute plaque rupture and resultant thrombus formation with major physiological pathways involved in the process.....	6
3. (a) Fully recirculating region, (b) Partially recirculating at higher flow rates and partially reattaching at lower flow rates, and (c) Fully reattached region.....	21
4. Schematic (exploded view) of the in vitro test system.....	28
5. Schematic of the 2D stainless steel stent.	29
6. Schematic of the 3D stented cylindrical flow chamber.....	30
7. Schematic of 4 different 3D stent types that were tested for platelet deposition.....	31
8. Schematic diagram of in vitro flow system..	33
9. Sample image taken between struts of intermediate strut spacing at 3 different time points: (a) time zone 1, (b) time zone 2, and (c) time zone 3	35
10. Sample image taken between struts of Wall stent at 3 different time points: (a) time zone 1, (b) time zone 2, and (c) time zone 3	37
11. (a) computational domain and (b) computational mesh of one of the stent models used in XZ plane (see text).	41
12. Four parametric models of stent struts geometries. (a) $h=1.8\text{mm}$, $f=0.9\text{mm}$, $r=0.45\text{mm}$, (b) $h=1.8\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$, (c) $h=3.6\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$, and (d) $h=3.6\text{mm}$, $f=3.6\text{mm}$, $r=0.9\text{mm}$	43
13. (a) The velocity profile curve (also shows time point t_1 (30 th time point)) and the corresponding WSS for low flow condition and (b) The velocity profile curve (also shows time point t_1 (30 th time point)) and the corresponding WSS for high flow condition.	48
14. (a) Line-probe locations at 0.25H, 0.5H, 1H, 2H, ..., 10H shown for stent model (b) with connector; used for obtaining WSS information along the length of the line-probe at each location. Streamlines at 30 th timestep for $h=1.8\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$ model with connector for (b) low flow and (c) high flow.....	49
15. (a) Computational structured grid domain of stent type (b)	51

16. Mesh convergence for (a) Bx Velocity stent and (b) Wall stent.....	52
17. Simulation showing that an entrance length of 8mm was sufficient for experimental purposes.	53
18. (a) Average normalized platelet deposition at the inlet to first strut plotted as a function of distance (in mm). (b) Path of instantaneous streamlines at minimum flow (Q represents the flow rate) at the inlet to first strut.....	55
19. (a) Average normalized platelet deposition between struts of that smallest strut spacing plotted as a function of distance (in mm). (b) Path of the instantaneous streamlines at minimum flow (Q represents the flow rate) between the struts of smallest spacing.....	56
20. (a) Average normalized platelet deposition between struts of largest spacing plotted as a function of distance (in mm). (b) Path of instantaneous streamlines at minimum flow (Q represents the flow rate) between struts of largest spacing.....	58
21. (a) Average normalized platelet deposition between struts of intermediate spacing plotted as a function of distance (in mm). (b) Path of instantaneous streamlines at minimum flow (Q represents the flow rate) between struts of intermediate spacing. c) Path of instantaneous streamlines at peak flow between struts of intermediate spacing.....	59
22. (a) Average normalized platelet deposition at the outlet to last strut plotted as a function of distance (in mm). (b) Path of instantaneous streamlines at minimum flow (Q represents the flow rate) at the outlet to the last strut.....	61
23. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=1.8\text{mm}$, $f=0.9\text{mm}$, $r=0.45\text{mm}$ model (stent model (a)) with connector under high flow.	64
24. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=1.8\text{mm}$, $f=0.9\text{mm}$, $r=0.45\text{mm}$ model (stent model (a)) without connector under high flow.....	65
25. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=1.8\text{mm}$, $f=0.9\text{mm}$, $r=0.45\text{mm}$ model (stent model (a)) with connector under low flow.	66
26. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=1.8\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$ model (stent model (b)) with connector under low flow.	67
27. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=3.6\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$ model (stent model (c)) with connector under high flow.	70

28. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=3.6\text{mm}$, $f=3.6\text{mm}$, $r=0.9\text{mm}$ model (stent model (d)) with connector under high flow.	71
29. Normalized mean axial WSS between struts in different strut geometries for high and low flow conditions. First two geometries correspond to $h=1.8\text{mm}$ models, third and fourth geometries correspond to $h=3.6\text{mm}$ models.	72
30. Mean transverse WSS between struts in different strut geometries for high and low flow conditions.	73
31. Percentage area between struts where the separation parameter >0.5 in different strut geometries for high and low flow conditions.	74
32. Percentage area between struts where the separation parameter ranged from 0.1-0.5 in different strut geometries for high and low flow conditions.	76
33. Axial wall shear stress variation and separation parameter variation within the stented geometry of Bx Velocity stent.	78
34. Axial wall shear stress variation and separation parameter variation within the stented geometry of NIR stent.	79
35. (a) Platelet deposition at the inlet just before first strut for Wall stent.	82
36. (a) Platelet deposition taken between struts for Wall stent; two geometries within the same stent type is shown.	84
37. (a) Platelet deposition at the outlet just after the last strut for Wall stent.	86
38. (a) Platelet deposition at the inlet just before first strut for Bx Velocity stent.	88
39. (a) Platelet deposition between struts for Bx Velocity stent.	91
40. (a) Platelet deposition at the connector for Bx Velocity stent	93
41. (a) Normalized average platelet intensity at the outlet to concave end of the last strut for Bx Velocity stent; (b) normalized average platelet intensity at the outlet to convex end of the last strut	96
42. (a) Normalized average platelet deposition in the region between struts for Aurora stent.	97
43. (a) Platelet deposition at the connector for Aurora stent	98
44. (a) Platelet deposition at the no-connector region for Aurora stent	99

45. (a) Normalized average platelet deposition at the inlet just before the first strut for NIR stent.....	101
46. (a) Normalized average platelet deposition between the first two struts for NIR stent.....	102
47. (a) Platelet deposition at the connector region for NIR stent.....	104
48. Figure summarizes average platelet deposition based on the flow regime under consideration and the direction of flow of streamlines.....	108
49. Mean wall shear stress distribution between struts of largest strut spacing	135
50. (a) Streamlines (at maximum flow rate) above the proximal portion of the connector have the capability to travel distal to the connector for NIR stent.....	141

1 INTRODUCTION

This section reviews the background information on the some of the main topics involved as part of this research – cardiovascular system, structure and functioning of arteries and blood cells, process of thrombosis and role of blood platelets in thrombosis, formation of atherosclerosis, conventional treatment methods, use of metallic scaffolds called stents with conventional treatment, advancements in stent design, how stent design influences mechanical factors that in turn influence restenosis rates, brief review of all the mechanical factors involved, and the primary goal behind this research.

1.1 Cardiovascular System of the Human Body

The cardiovascular system is comprised of the heart; a four chambered in-series pump, and the connecting vasculature: the arteries, veins, microvasculature, and lymphatic vessels. The primary function of the cardiovascular system is to deliver oxygen, nutrients, and chemical messengers to the respiring cells. In turn, the cardiovascular system removes cellular waste and delivers it to the appropriate organ for elimination or degradation. The cardiovascular system also assists in maintaining normal fluid balance and plays a key role in thermoregulation (Robinson S *et al.*, 1965). Function of the cardiovascular system is regulated by several factors:

- (a) inherent properties of cardiac muscle i.e. the pacemaker potential, gap junctions (Ishii K and Honda K, 1965),
- (b) inherent properties of vascular smooth muscles, i.e. contraction, relaxation, stretch (Karoony P *et al.*, 1998)
- (c) neural control i.e. baroreceptor reflex (Hull D and Seagull MM, 1965),

(d) autonomic nervous system, and endocrine control, i.e. circulating catecholamines, insulin, thyroid hormone etc (Draskoczy PR and Lyman CP, 1967). Homeostatic control of cardiovascular function consists of both long-loop reflexes and local control mechanisms. This arrangement allows for integrated regulation of arterial pressure, cardiac output and fluid balance while simultaneously adjusting perfusion (blood flow) in local circulatory beds. Interestingly the system contains endocrine-like cells that assist in its regulation by secretion of chemical messengers such as atrial natriuretic hormone, nitric oxide (NO), and prostacyclin (Resnik R, 1981; Staszewska-Barczak J, 1978).

1.2 Structure and Function of Vasculature

Arteries, arterioles, and capillaries form the network of vessels through which oxygenated blood is transported from the heart to the body. Consequently, any damage or disease afflicting these vessels can lead to diminished supply of nutrients to the tissue. Therefore the understanding of the structure and diseased states is critical before considering treatment. Arteries are composed of three distinct layers namely the intima, media, and adventitia with connective tissue sandwiched between each layer (Moore DH and Ruska H, 1957; see Fig. 1). Each layer serves in providing strong and elastic mechanical properties that ensures that high pressures can be withstood in the arteries. Arteries and veins have the same three tissue layers, but the proportions of these layers differ. The innermost is the intima; next comes the media; and the outermost is the adventitia. Arteries have thick media, made of smooth muscle cells (SMC) surrounded by connective tissue composed of collagen fibrils, elastic fibers, and structural glycoproteins, to absorb the pressure waves created by the heart's pumping. The smooth-

muscle media walls expand when pressure surges, then snap back to push the blood forward when the heart rests. The smooth muscles in the media can provide vasoconstriction and vasodilation in order to influence pressure and blood flow (Kochemasova NG, 1967).

Figure 1. Schematic diagram of the three layers (intima, media, and adventitia) of an artery and a vein.

As blood enters the capillaries, the pressure falls off. By the time blood reaches the veins, there is little pressure. Thus, a thick media is no longer needed. Surrounding muscles act to squeeze the blood along veins. Valves in veins are used to ensure flow in the right direction. The adventitia is composed primarily of connective tissue with interspersed elastic and collagen fibers (Ragan C, 1952). The intima is composed of a single layer of endothelial cells (EC). The EC layer forms a continuous, selectively

permeable, and thrombo-resistant barrier (Gherdol A and Vales O, 1966). Since blood flows adjacent to the EC, they are responsible for the prevention of thrombosis and homeostasis on the inner surface of the artery. Regulation of thrombus formation by EC takes place through the secretion of certain biochemicals (Higgs GA, 1977).

1.3 Composition of Blood and Their Functions

The components of the blood not only serve as a vehicle to transfer oxygen and nutrients, but also as serve in maintaining a normal functioning vasculature. Adults have up to 7-8 liters of blood in the body. Forty-five percent (45%) consists of cells - platelets, red blood cells (RBC), and white blood cells (WBC - neutrophils, basophils, eosinophils, lymphocytes, monocytes) (Larcan A, 1965). Fifty-five percent (55%) consists of plasma, the liquid component of blood. Plasma is a straw-colored, clear liquid that is 90 percent water, and it is an essential ingredient for human survival. Besides water, plasma also contains dissolved salts and minerals like calcium, sodium, magnesium, potassium, and many microbe-fighting antibodies.

Red blood cells (RBC) perform the most important function of providing nutrients to all the cells. They are devoid of nucleus and have the shape of a biconcave disc with a diameter of 8 microns. There are about 4-6 million RBCs per microliter of blood. Red blood cells are red only because they contain a protein chemical called hemoglobin which is bright red in color. Hemoglobin contains the element Iron, making it an excellent vehicle for transporting oxygen and carbon dioxide (Stich W, 1964). As blood passes through the lungs, oxygen molecules attach to the hemoglobin. As the blood passes through the body's tissue, the hemoglobin releases the oxygen to the cells. The empty

hemoglobin molecules then bond with the tissue's carbon dioxide or other waste gases, transporting it away.

The white blood cells (WBC) are the protectors of the body against infection. They are less numerous than the RBCs, about 5000-7000 per microliter of blood. But they are the largest of cells in the blood, about 10-12 microns in diameter.

Platelets are responsible for maintaining hemostasis and thrombosis through the initiation of blood coagulation as well as the proliferation of vascular cells (Maupin B, 1955). Platelets are round discs of 2-3 microns in diameter and range in concentration between 200,000 to 300,000 platelets per microliter of blood in most healthy people.

1.3.1 Vascular Injury

The interaction of the vasculature and platelets can be seen in the formation of thrombus and in hemostasis during vascular injury (Maupin B, 1955). This process is important to prevent the loss of body fluids. If the endothelial layer of the vessel wall is denuded, the subendothelial layer of collagen is exposed to the blood in the lumen (see Fig. 2). The initial phase of the hemostatic process is vascular constriction. This limits the flow of blood to the area of injury. Next, platelets become activated by thrombin and aggregate at the site of injury, forming a temporary, loose platelet plug (Vassar PS and Spain DM, 1953). The protein fibrinogen is primarily responsible for platelet-to-platelet binding, resulting in clumping. Platelets clump by binding to collagen that becomes exposed following rupture of the endothelial lining of vessels (Wilner GD *et al.*, 1968). Upon activation, platelets release adenosine-5'-diphosphate (ADP) and thromboxine (TXA₂) (which activate additional platelets), serotonin, phospholipids, lipoproteins, and other proteins important for the coagulation cascade. Thrombus is generated through the

activation of enzymes in the coagulation cascade (Macfarlane RG, 1964). In addition to induced secretion, activated platelets change their shape to accommodate the formation of the plug. To ensure stability of the initially loose platelet plug, a fibrin mesh (also called the clot) forms and entraps the plug. Finally, the clot must be dissolved in order for normal blood flow to resume following tissue repair. The dissolution of the clot occurs through the action of plasmin (Alkjaersig N *et al.*, 1959).

Figure 2. Schematic diagram depicting an acute plaque rupture and resultant thrombus formation with major physiological pathways involved in the process. Presence of recirculation regions may increase the degree of plaque formation in that region.

1.4 Atherosclerosis

Arteriosclerosis, a general word for thickening of the arterial wall, remains the most common disease of the cardiovascular system in developed countries.

Atherosclerosis is characterized as the buildup of plaque containing cholesterol and lipids, which reduces the lumen of the artery (Becker GH *et al.*, 1949). Atherosclerosis is a type of arteriosclerosis. It comes from the Greek words *Athero* meaning gruel or paste and *Sclerosis* meaning thickening. It forms mainly in large or medium-sized arteries like the carotids, abdominal aorta, and femoral arteries. Though the actual cause of formation of this plaque in certain arterial locations or in certain people is still unknown, many scientists still believe that atherosclerosis starts because the innermost layer of the artery, the endothelium is damaged (Bjorkerud S and Bondjers G, 1971). The damaged endothelium plays an important role by expressing adhesion molecules that bind and facilitate the transportation of circulating monocytes into the subendothelial layer. These monocytes then become phagocytic macrophages in the intima that engulfs excess cholesterol in the blood (Yutani C *et al.*, 1999). Continual bathing of these cells by plasma lipoproteins provides cholesterol for uptake by macrophages (Yutani C *et al.*, 1999). Cholesterol is stored within the macrophage foam cell, which secretes both growth and chemotactic factors. This results in accumulation of additional macrophages, other leukocytes from the blood, and smooth muscle cells. As more cholesterol is deposited, fibrosis and cell death occurs. As the mass grows, flow is compromised in the arterial lumen. The risk of plaque rupture is dependent on plaque composition and not on plaque size (Falk E, 1992). Inflammatory processes takes over as further thrombosis is triggered when plaque breaks and exposes collagen and extracellular lipids to the blood, which may lead to an additional accumulation of platelets and fibrin. Apart from the response-to-injury theory, there are other theories like “excess low-density lipoprotein overwhelm healthy endothelium”, “lack of nitric oxide production enhance platelet activation”, and

“hemodynamic factors of the arterial flow system determine site of atherosclerosis” (Faergeman O, 1979; Glagov S *et al.*, 1961).

For such atherosclerotic patients, the most non-surgical technique known to open up obstructed arteries is percutaneous transluminal angioplasty (PTA) (Lorimer WS Jr, 1961). ‘Percutaneous’ means opening is made from the exterior, ie., the skin, ‘transluminal’ means procedure performed within the blood vessel, and ‘angioplasty’ means to reshape the blood vessel. PTA uses a balloon to increase the arterial lumen in areas where arteriosclerotic lesions have compromised blood flow (Athanasoulis CA, 1980). The plaque is broken at the point of least resistance and/or redistributed within the intima reestablishing and improving the lumen diameter.

The success in performing PTA is to open up the clogged arterial lumen for free flow of blood. The most common serious complication of PTA is acute occlusion of the dilated vessel due to dissection or thrombosis. This may require ‘bail-out’ stenting or even emergency coronary artery bypass grafting (~2%) and can lead to death (~1%) or non-fatal myocardial infarction (3-4%) (Ellis SJ *et al.*, 1997). Other complications include vascular damage, thromboembolism including stroke, and haemorrhage due to anticoagulant therapy. Nevertheless, the overall procedural mortality is ~1% (Ellis SJ *et al.*, 1997).

The success and complication rate of angioplasty is influenced by many factors including age, sex, clinical presentation, left ventricular function, co-morbidity (e.g. diabetes mellitus), and the experience of the operator (Melkert R *et al.*, 1994). However, the single most important determinant of outcome is the nature of the target lesion. A short, discrete, soft lesion on a straight segment of artery, which does not compromise a major branch, is ideal for PTA (Capek P, 1991). In other words, PTA has a high success

and low complication rate for ideal lesions but a low success and high complication rate for complex lesions.

Most of improvement in luminal diameter following balloon angioplasty results from stretching of the vessel wall by the balloon (Kinney TB *et al.*, 1984). Balloon inflation actually results in overstretching of the vessel wall and partial disruption of not only the intimal plaque but also the media and adventitia, resulting in enlargement of the lumen and the outer diameter of the vessel. Axial redistribution of plaque material also contributes to improvements in lumen diameter (Kinney TB *et al.*, 1984). This may be the reason for vessel recoil after balloon angioplasty and that is how the intravascular stents came to be used more often (since 1990s). Plaque compression is now understood to account for very little of the observed improvement following balloon angioplasty (Kinney TB *et al.*, 1984).

Restenosis is yet another complication introduced post-angioplasty procedures reducing the success rate. Restenosis is reclogging of the arterial lumen as a result of thrombosis and arterial wall remodeling due to smooth muscle cell hyperplasia. Restenosis occurs predominantly within the first 3-6 months, the restenosis rates for successful balloon angioplasty procedures range from 44% to 77% (Escaned C *et al.*, 1999). Restenosis occurs as a direct result of the procedure itself, where there is damage to the arterial wall in the form of endothelial denudation; once the thromboresistant vascular layer is removed, the deeper layers of the artery get exposed to the flow of blood. The endothelial denudation disrupts the regulatory mechanisms of the arterial wall. This results in remodeling through thrombosis and smooth muscle cell migration and proliferation forming intimal hyperplasia and then obstruction of the lumen, thus restenosis (Edelman ER and Rogers C, 1998; Nugent *et al.*, 1999; Rogers C *et al.*, 1999).

Restenosis does not always lead to recurrent symptoms and can potentially be reduced by deploying intravascular stents (Altmann DB *et al.*, 1996; Knight CJ *et al.*, 1997; Serruys PW *et al.*, 1994; Serruys PW *et al.*, 1996; Versaci F *et al.*, 1997).

1.5 Stents

Stents are metallic scaffolds that when placed in an artery after the angioplasty procedure establish the lumen diameter and prevent vessel recoil (Yutani C *et al.*, 1999). Stent implantation is very common and is included in 70-90% of angioplasty procedures. The first stent came to be used by Charles Dotter in 1969. They are usually made of two different material alloys – stainless steel and nitinol (Sigwart U, 1990); other materials like tantalum and biodegradable plastic are also under clinical investigation. Stents fall into two broad range of delivery mechanisms: balloon expandable and self-expanding. Balloon expandable stents are mounted on angioplasty balloons catheter and deployed by inflating the balloon. Self-expanding stents constrained onto a catheter and covered by a sheath. Deployment is accomplished through the withdrawal of the sheath. The stiffness of stainless steel stents is high, whereas nitinol stents are able to change their geometry with change in the vessel geometry as a result of curvature, and/or pulsatile flow (Yachia D and Aridogan IA, 1996). The restenosis rate that develops with intravascular stents after angioplasty are far lower than that with angioplasty alone. (Fischman DL *et al.*, 1994; Pepine CJ *et al.*, 1996). Stents compared to PTA resulted in lower rate of angiographically detected restenosis, improved procedural success rate, improved event-free survival, and less frequent need for revascularization of the original artery (Fischman DL *et al.*, 1994; Pepine CJ *et al.*, 1996). However the presence of acute and subacute

thrombotic occlusions remains a significant problem with 20%-40% of patients receiving stents (Yutani C *et al.*, 1999).

1.5.1 Vascular Response to Stenting

The cause of restenotic occlusion of stented arteries has been mainly neointimal hyperplasia (Edelman ER *et al.*, 1998). There are four phases of vascular response to stenting: (1) thrombosis – remains a feature of every stent; acute thrombosis after stent placement have been treated with anti-thrombotic drug therapy, but has less effect over long-term restenosis; the extent of thrombosis over each stent strut depends on how deep the strut is inside the vessel wall; (2) inflammation – the thrombus that forms at the struts as well as on the vessel wall between struts becomes the site of inflammatory reaction in which monocytes and leukocytes adhere to the internal elastic laminae; the number of adhered leukocytes determine the rate of proliferation within the stented region; after approximately a week, the tissue becomes infiltrated with macrophages; these cells migrate from the luminal surface to the developing neointima to form multinucleated giant cells around stent struts; (3) proliferation – smooth muscle cells and macrophages within the neointima proliferate after a week of stenting; (4) remodeling – increased strain on the arterial wall due to the stent struts causes collagen deposition and fibrosis near the adventitia, with persistent inflammatory processes, to cause arterial shrinkage (Edelman ER *et al.*, 1998).

1.5.2 Thrombosis – Role of Platelets in Restenosis

Thrombosis was determined to play a leading role in neointimal hyperplasia after stenting. It was hoped that interfering with the thrombotic processes would eliminate the

remaining three phases of vascular response to stenting (Rogers C *et al.*, 1993). But studies have shown that restenosis can continue even without thrombus formation (De Scheerder I *et al.*, 1997; Rogers C *et al.*, 1993, 1997). The coagulation process is a cascade of events and the use of the anti-thrombotic medication may have completely bypassed the cascade in the coagulation pathway that could have been involved with restenosis. After vascular injury, the exposed subendothelial collagen leads to rapid adhesion of platelets, through von Willebrand factor binding to the glycoprotein Ib-IX-V complex (Legrand YJ *et al.*, 1983). After adhesion, the platelets get activated, undergo conformational changes, and release various mediators that stimulate the formation of the platelet plug and thus platelet aggregation. These activated platelets help in the formation of the thrombus at the injury site. Studies of neointimal formation in both porcine and rabbit models show that mural thrombus at the injury site is a major determinant of neointimal hyperplasia formation (Miller DD *et al.*, 1996; Wilensky RL *et al.*, 1995). Several chemotactic agents released by platelets such as platelet-derived growth factor, serotonin, histamine, and thromboxine A₂ have stimulatory effect on smooth muscle cell migration and proliferation (Sdringola S *et al.*, 2000). The smooth muscle cells are known to migrate to the intima from the media and change their phenotype. The presence of thrombin and fibrin attracts inflammatory cells like the monocytes or leukocytes/neutrophils to the vessel wall (Welt FGP *et al.*, 2000; Yutani C *et al.*, 1999). Leukocyte adhesion may be the greatest predictor of cellular proliferation rate at the site of injury (Welt FGP *et al.*, 2000). The platelets are responsible for interaction with these cells by release of certain adhesion molecules and chemotactic agents. Later on, macrophages infiltrate through the vessel and concentrate mainly around the stent struts forming giant cells (Komatsu R *et al.*, 1998). These cells probably act as stimulants to

smooth muscle cell proliferation. The early neointima consists of macrophages and spindle shaped smooth muscle cells. After the proliferative phase, these spindle shaped cells change their phenotype to the active state, to form the final neointimal layer around stent struts. In short, many cells in the blood stream interact very closely with the cells of the injured stented arterial wall in the formation of neointima. Though most of the studies carried out on animal models cannot be directly applied to humans, there is considerable evidence that platelets are the foremost cells to be activated after vascular injury and play a key role in neointimal formation and thus restenosis.

1.5.3 Role of Endothelial Cells in Restenosis

Endothelial cells are a monolayer of cells that line the intimal layer of the artery. They are responsible for the anti-thrombotic response of the arterial wall. During PTA, the endothelium is denuded and the underlying collagenous layer is exposed to blood. The eventual restoration of endothelial integrity via ingrowth from neighboring normal vessel segments brings an end to the hyperplastic process (Clowes AW *et al.*, 1983; Fishman JA *et al.*, 1975). Histological staining revealed endothelial cell loss within minutes of stent expansion in arteries without PTA; however in contrast there was some residual endothelium intact after PTA in most cases (Fishman JA *et al.*, 1875; van Beusekom HM *et al.*, 1998). Stent placement after endothelial denudation resulted in two-fold more neointimal hyperplasia than does stent placement without preceding denudation (Rogers C *et al.*, 1996). The endothelial injury was most severe in stent interstices and was least severe adjacent to struts (Rogers C *et al.*, 1996). Factors that contribute to this type of arterial injury may include balloon-wall contact, excess pressure on the wall imposed by blood during stent expansion, eccentric strain on the wall after

stent implantation, or acute alterations in flow (Rogers C *et al.*, 1996). Shear stress has been known to be a mechanical force for modulation of cellular function (Davies PF, 1995; Traub O and Berk BC, 1998). Cellular function could be changed by the release of vasoactive substances, change of gene expression, cell metabolism, and cell morphology. Re-endothelialization of injured artery walls occurs through the migration and proliferation of endothelial cells of the intact surrounding endothelium. Endothelial cell migration rate increased when exposed to high shear stress flow relative to that observed under static no flow or low shear conditions (Sprague EA *et al.*, 2000). The flow restricted stents also had a greater mixture of myofibroblastic and thrombotic material covering the entire region between and on stent struts (Goetz R *et al.*, 1999). Histologic evidence showed that the neointimal thickness of flow restricted stents were significantly higher than that of flow nonrestricted stents (Goetz R *et al.*, 1999). Thus, endothelial cells play a key role in preventing neointimal hyperplasia and thus, maintain vascular homeostasis.

1.5.4 Stents and Restenosis – A Strong Link

Factors such as poor flow, pre-existing intraluminal thrombus, small native vessel size, increased platelet counts, multiple overlapping stents, stent/vessel diameter mismatch and the length of stent used have been suggested as possible sources leading to intimal hyperplasia and the failure of the device (Yutani C *et al.*, 1999). New procedures and stents have been introduced to inhibit neointimal hyperplasia and reduce restenosis rates.

Brachytherapy (radiation therapy) after stenting has proved ineffective due to a greater rate of late total occlusion related to stent thrombosis rather than the occlusive intimal hyperplasia seen with stenting only (Salame MY and Douglas JS Jr., 2001).

Drug-coated Stents

Recently it has been demonstrated that coating stents with antiproliferative drugs is effective in coronary arteries, with reductions in restenosis rates and adverse cardiac events (Lau KW *et al.*, 2004; Morice MC *et al.*, 2002). New stents with drug eluting properties have sought to control intimal hyperplasia and facilitate recovery by administering pharmacologic agents to the surface of the vessel. These drugs (like sirolimus, paclitaxol) target and inhibit smooth muscle cell proliferation.

The restenosis rates for drug-coated stent implanted patients were reduced to single digits, thus quantifying their short-term success (over six months) (SIRIUS or RAVEL study, Morice MC *et al.*, 2002). However, long-term efficacy of such stents is not completely known. Recently, long-term risk of clots has been reported in certain patients with drug-coated stents (Wall street journal, reported October 21st, 2005). In peripheral applications such as femoral and popliteal arteries, the angiographic, hemodynamics, and clinical success of stent placements after one and two years is lower compared to PTA (Cejna M *et al.*, 2001; Grenacher L *et al.*, 2004). In drug-coated stents, anti-proliferative coatings appear to slow the healing response (Virmani R *et al.*, 2003). The long-term failure mode in these arteries may be inadequate healing, marked by thrombus formation and delayed intimal regrowth (Virmani R *et al.*, 2003). Thus, the use of inhibitory drugs in the periphery may not be effective over a long-term period.

Endothelial Cell Antibody-coated Stents

The endothelial cells can be affected by the interaction with the surface of the device as well as with the size and shape of the device it must cover (Simon C *et al.*, 2000). The cell-covered area and migration distance significantly decreased on objects 75 microns thick, and it was nonexistent on objects 250 microns thick. Areas devoid of cells or gaps were largest adjacent to the downflow side of the object, disposed transversely to flow. That is how the possibility for using progenitor endothelial cells for risk stratification was explored (Shirota T *et al.*, 2003). These progenitor endothelial cells circulate in the blood and carry endothelial-specific adhesive markers and also have some similar cellular functions as that of normal endothelial cells. Stents are being tried with coatings of antibodies that attract progenitor endothelial cells that help in endothelial layer repair from angioplasty and stent implantation (Aoki J *et al.*, 2005). Short-term and long-term efficacy is still under trial using EC antibody-coated stent.

What we learn? - Regardless of stent design or coating technology, the initial thrombotic reaction certainly effects the subsequent reestablishment of the endothelium. Thus, a better understanding of the dynamics of platelet adhesion to stents under realistic flow conditions can serve to improve stents of all types in coronary and peripheral applications.

Stent Design Influences Restenosis

One of the strongest factors for incidence of restenosis is the stent design, second only to vessel size (Kastrati AJ *et al.*, 2001). Many clinical studies have shown that failure rates are highly dependent on stent design (Kastrati AJ *et al.*, 2001); restenosis ranged from 20% to 50% depending on the stent type implanted. Changing stent

configuration to reduce strut-strut intersections by 29% while holding diameter, mass, and surface area constant in denuded rabbit iliac arteries reduced vascular injury by 42%, by reducing the radial force exerted on the vessel due to the stent (Rogers C *et al.*, 1995). Surface area coating of the stent with an inert polymer (exact kind unknown from publication) resulted in reduced thrombosis by 69% in the corrugated design compared to the slotted tube design (Rogers C *et al.*, 1995). It was thus demonstrated that stent thrombosis can be limited both by using innovative configuration design to reduce the amount of deep injury to the vessel and by applying a coating at the interface of the metal stent with the blood vessel wall (Rogers C *et al.*, 1995). The polymer coating might attenuate stent thrombosis by reducing the electronegativity and resultant corrosion of the metal, in turn limiting the hemodynamic and chemical stimuli for platelet adhesion and aggregation and enhancing endothelial cell recovery, among other effects (Lahann J *et al.*, 1999). But these coatings do not reduce the vascular injury caused to the vessel wall. Neointimal hyperplasia in a way is a direct effect of the vascular injury caused. Reducing strut-strut intersections by 29% reduced neointimal hyperplasia by 38% (Rogers C *et al.*, 1995). It is possible that stents of different configurations or designs might differ in the extent to which they denude intact endothelium during deployment, and remnant endothelium within the stented artery might enhance regrowth and limit or vary both thrombosis and neointimal hyperplasia. Alterations in secondary flow patterns and flow-induced wall shear stress can modulate intimal hyperplasia via their effects on platelet and inflammatory cell transport toward the wall, as well as direct effects on the endothelium (Eigenthaler M *et al.*, 2003; Weyrich AS *et al.*, 2002). Therefore, differences in hemodynamic conditions produced by different stent designs may affect clinical outcomes of stenting.

1.5.5 Restenosis - Influence of Flow Patterns and Arterial Wall Stress

Many mechanical factors like compliance mismatch, solid mechanical wall stresses, fluid mechanical wall shear stresses, and fluid dynamics play an important role in in-stent restenosis. Some issues are discussed as below.

Role of Other Mechanical Factors - Compliance Mismatch

Stresses may be induced on the arterial wall based on the stent-to-artery diameter expansion ratio (LaDisa JF Jr. *et al.*, 2004). The radial force exerted on the wall by the stent creates large non-physiologic stresses in addition to altering the behavior of the artery from an elastic tendency to a nearly rigid tendency. The abrupt changes to the mechanical properties at the ends of the stent create zones of high stress gradients (Mohammed Z *et al.*, 2000). In order to get back to the physiological levels of stresses, the artery has to increase its thickness; this is possible through smooth muscle cell proliferation and thus neointimal formation. The compliance mismatch caused by a stent is very similar to the mismatch that occurs in graft-artery anastomosis (Ballyk PD *et al.*, 1998). Anastomosis is a term used to describe the interface between the artery and a graft or a vein that is used as a bypass. The stresses are much lower in an end-to-end anastomosis compared to an end-to-side anastomosis. A compliance matching stent (CMS) has been developed to reduce the stress concentration gradients and provide a gradual change in compliance between the non-stented artery and the stented segment; it has been shown that such a CMS reduces the local flow disturbances (Berry JL *et al.*, 2002). It has also been shown recently that CMS attenuates restenosis at the inlet and outlet of the stent (Rolland PH *et al.*, 2004). An end-to-end anastomosis would be an example that one could use for a stented artery. It has been shown that compliance

mismatch alone is not responsible for intimal thickening in an end-to-end anastomotic region (Okuhn *et al.*, 1989). Arterial injury, flow mechanics, stent design, etc are other factors that have a great impact on intimal thickening.

Role of Other Mechanical Factors – Mechanical Stresses

Fluid mechanical shear stresses have a great influence on the vessel. As blood flows through a vessel, it exerts a physical force on the vessel wall. This force can be resolved into two principal vectors: (1) shear stress parallel to the wall, representing the frictional force that the flow of blood exerts at the endothelial surface of the vessel wall; and (2) tensile stress perpendicular to the wall, representing the dilating force of blood pressure on the vessel wall. The dilating tensile stress is experienced by all layers of the vessel wall, but the frictional wall shear stress is experienced only by the inner layer of the endothelial cells of the vessel wall. Low mean wall shear stresses with oscillatory flow have been known to be the cause for atherosclerosis in arteries, while high wall shear stress with laminar flow pattern has been shown to prevent atherosclerosis (Moore JE Jr *et al.*, 1994). In addition, the wall shear stress influence migration and proliferation of endothelial cells (Hsu PP *et al.*, 2001). Analysis of endothelial cell apoptosis (cell death) showed preferential occurrence of apoptosis in the downstream region of the plaque where the shear stress is low and unsteady compared to the upstream part (Tricot *et al.*, 2000). Wall shear stress is known to affect the production of biochemicals by the endothelial cells and the permeability of the arterial wall to macromolecules (Fabregues *et al.*, 1998). Thus the first vascular response to stenting, i.e. formation of thrombus may be influenced by such factors as variations in blood and wall shear stresses and the formation of vortices.

Stent design also affects endothelialization. The doubling time for EC proliferation was higher when the pitch of a coiled stent was much lower, i.e. when the distance between coils was smaller (Akagawa E *et al.*, 2004). The doubling time was also lower for EC proliferation upstream of the stent compared to downstream of the stent. Thus, the flow patterns upstream and downstream of the stent and within the stent influence platelet attachment and aggregation, cell migration and proliferation, and protein and biochemical transport through the wall.

Role of Other Mechanical Factors – Flow Patterns

We need to note here that the flow patterns are dependent on stent design. Fluid dynamics has been visualized since long using video microscopy and computational fluid dynamics modeling (CFD). Fluid dynamic modeling is a useful tool as it helps in the prediction of flow patterns without performing experiments, thus saving time and costs for the investigator. In the next few paragraphs, we will see how CFD modeling has helped us depict flow patterns in two-dimensions and three-dimensions. Two-dimensional (2D) computational fluid dynamic (CFD) modeling of the protrusion of individual stent struts into the flow field showed areas of flow stagnation near the struts (Berry JL *et al.*, 2000). Prior research has shown that flow patterns did not change drastically when using rounded stent struts or rectangular stent struts (Henry FS, 2000). Two types of flow situations can take place in either rounded or rectangular 2D stent struts, a separation region as flow hits the face of the strut and a reattachment region as blood flows past the strut (see Figure 3). In the former, blood flowing down the artery strikes the forward facing strut edge; this inhibits the forward movement of the blood directly adjacent to the leading edge of the strut and therefore creates a separation zone

(see Fig. 3a, Berry JL *et al.*, 2000). In the latter, blood flowing over the top edge, the momentum of the flow washes the blood past the edge of the step therefore creating an area of flow separation adjacent to the trailing edge of the strut (see Fig. 3b, Berry JL *et al.*, 2000).

Figure 3. (a) Fully recirculating region, (b) Partially recirculating at higher flow rates and partially reattaching at lower flow rates, and (c) Fully reattached region. These streamlines were seen at a flow rate lower than the mean flow rate.

The degree of flow separation is usually larger for the trailing edge than for the leading edge. Based on the stent strut spacing (distance between two struts), the flow could vary from being constantly recirculating to partially recirculating to constantly reattaching between the struts (Berry JL *et al.*, 2000). Here, the flow could be characterized just based on one design parameter, ratio of the distance between the struts L (from the center of one strut to the center of the next strut) to the strut height H . With smaller stent wire spacings, less than 6 wire diameters, the stagnation regions from adjacent struts merge together for the entire cardiac cycle, creating one single stagnation

region (see Fig. 3c, Berry JL *et al.*, 2000). With larger stent wire spacings, greater than 12 wire diameters, the flow continuously reattached between the struts. In as little as a few hours, the stent struts can be covered with thrombus therefore changing the ideal geometry into a more complex one (Berry JL *et al.*, 2000). Thus, the use of forward and backward facing steps is only applicable in the acute stages of stent implantation and maybe a few weeks after stent implantation.

Many three-dimensional (3D) models of actual stents have been constructed to study the fluid flow patterns and wall shear stress distribution between the struts. They also provide insight into which geometric properties are most important in influencing the fluid dynamics of the stent. Steady state CFD modeling of Palmaz-Schatz stent showed wall shear stress gradients (WSSG) being the highest around the regions of the stent struts, at the stent inlet and outlet (LaDisa JF Jr. *et al.*, 2004). Theoretically those regions could be susceptible to neointimal hyperplasia (LaDisa JF Jr. *et al.*, 2004). The number, width, and thickness of stent struts imparted distinctive patterns of shear stress along the wall with a realistic stent-to-artery deployment ratio of 1.1:1 (LaDisa JF Jr. *et al.*, 2004). As the number of stent struts were increased, the area between the struts exposed to low wall shear stress was greater (LaDisa JF Jr. *et al.*, 2004). If the number of stent struts were maintained constant, but the stent-to-artery deployment ratio was increased, the area between the struts exposed to low wall shear stress was greater (LaDisa JF Jr. *et al.*, 2004). Increasing strut width may contribute to decrease in the area between the struts exposed to low wall shear stress, but may cause greater vascular damage after deployment. Increase in the stent-to-artery deployment ratio has been shown to alter WSSG, the increase in WSSG was most prominent at the stent inlet (LaDisa JF Jr. *et al.*, 2004). Experimentally, the region of maximal cellular proliferation has been associated

with high wall shear stress gradients (Liu SQ and Goldman, 2001). Smooth muscle cells (SMC) have been shown to migrate to regions of low wall shear stress; they align in the direction of flow in high shear stress regions and align perpendicular to the flow direction in low shear stress regions (Liu SQ and Goldman, 2001). The SMC migration occurred probably through endothelial cells modulation on the surface, which were also sensitive to wall shear stress changes. Endothelial cell migration in a stented surface showed that they migrate along the stent strut first and then orient themselves in the direction of flow after reaching confluency (Walsh PW *et al.*, 2000). Consideration of steady-state simulations corresponding to average blood velocity give a reasonable estimate of the average distribution of shear stress imparted on the wall of the stented vessels during a single cardiac cycle (LaDisa JF Jr. *et al.*, 2004). However full scale time dependent simulations (corresponding to pulsatile blood flow) offer the benefit of visualizing temporal changes in wall shear stress. Polygonal shape for the wall after stent implantation gave rise to higher WSSG compared to circular shape for the wall (LaDisa JF Jr. *et al.*, 2004); polygonal shape of the wall was considered assuming that there was circumferential stretching of the arterial wall after stent implantation. The degree of error in the assumption was dependent on the geometry and the number of struts contained in the stent. Antegrade iliac artery stent implantation in white rabbits showed that neointimal hyperplasia was the greatest in the proximal and distal region of the stent, but much lower in the middle region of the stented artery (LaDisa JF Jr. *et al.*, 2005). It was postulated that this increase in neointimal hyperplasia at the proximal end of the stent is due to increases in temporal WSSG (LaDisa JF Jr. *et al.*, 2005).

1.5.6 Flow effects on Platelet Deposition

Flow patterns differ through differing geometries. So is the platelet deposition at various locations spatially in the geometry under consideration. The study of platelet deposition in an expanded tube showed a peak in platelet adhesion in the vortex in which the number density was appreciably greater than that downstream of the reattachment point where fully developed laminar flow was established (Karino T and Goldsmith HL, 1979). At the reattachment point, platelet adhesion was a minimum. They attributed platelet deposition to convective transport of platelets through the instantaneous streamlines. At the reattachment point, the platelet adhesion was a minimum. This may be a result of the wall reaction rate and wall shear stress. If the wall reaction rate is linearly dependent on the wall shear stress, the platelet flux is maximum downstream of the stagnation point (David T *et al.*, 2001). Aneurysm and stenosis flow models showed greater platelet deposition in the regions of recirculation at the distal end for low Reynolds numbers (Bluestein D *et al.*, 1996, 1997). Lowest platelet deposition has been shown to occur within the smallest strut spacing (Robaina S *et al.*, 2003).

Solid mechanical and fluid mechanical implications need to be considered before designing producing an ideal stent design. Mechanical factors like wall shear stress and circumferential wall stress seems to play a very key role in the restenosis process. Hence, there is an increased need to study the role of mechanical factors in restenosis. Of particular interest in our lab is the role of fluid mechanics within a stented artery. Platelet deposition is strongly influenced by the blood fluid flow patterns. One of the first vascular responses to stenting is thrombus formation (Garasic JM *et al.*, 2000), a process initiated by blood platelets through the action of various enzymes in the coagulation pathway (Macfarlane RG, 1964). The initiation stages of neointimal thickening may be

related to the rate and extent of platelet deposition (Jeong MH *et al.*, 1996). Hence, it is necessary to study this first vascular response with differing stent geometries and the influence of flow patterns formed within differing stent geometries.

1.6 Problem Statement - Study Objectives

Recently, platelet deposition in a stented chamber was shown by our group to be dependent on the stent strut spacing (Robaina S *et al.*, 2003). The lowest platelet deposition was measured in the model with the lowest strut-strut spacing. However, the study did not provide information on the spatial distribution of platelets between the struts as a result of changing flow patterns. No literature is available regarding the effects of blood flow patterns on localized platelet deposition within the stented region either in two-dimensions (2D) or three-dimensions (3D).

We postulate that the medium through which activated platelets attach to the wall are a function of the instantaneous streamlines that depict the fluid flow pattern. Hence, platelet flow and attachment is convection driven. This study focusses on the dynamics of blood flow and its effects on the platelet deposition near stented region in the acute stages of stent implantation. Two dimensional (2D) stent models are selected to simulate cases of constant recirculation of blood flow, constant reattachment of blood flow, and intermittent reattachment of blood flow between individual stent struts. The platelet deposition is quantified at different times between two individual stent struts. The advantage in the 2D study was that stent design could be characterized just based on one single parameter, the ratio of the distance between the strut centers (L) to the strut height (H). The 2D study provides insight on platelet deposition at specific regions of disturbed flow in the stented section. The limitation in the 2D study is that the geometry is simple

and does not simulate the *in vivo* behavior of the artery wall to platelet deposition or the flow dynamics. Hence platelet adhesion studies within 3D stent models (currently marketed stents) is performed to correlate their relationship to localized changes in flow patterns. The dependence of platelet deposition on streamlines in 2D was useful to depict platelet deposition patterns in the 3D study. Some of the research questions that we tried to answer in this study are: How does blood flow dynamics affect platelet adhesion and localization within stented regions in 2D? Is the localization dependent on the time course of blood flow? Does wall shear stress pattern affect platelet deposition in 2D? If so, how? How does blood flow dynamics affect platelet deposition and localization within the stented region in 3D? Are the results and the pattern of localization similar or much different from 2D? Where are the differences, where, how, and why do they exist?

Experimental *in vitro* work using fluorescently labeled platelets (described in detail in Chapters 2 and 3) and computational work using computational fluid dynamics (CFD) software (described in detail in Chapter 2 and 3) are performed in order to answer the above questions. An *in vitro* flow system comprising of a peristaltic pulsatile pump, reservoir, connecting tubes, compliance chamber, and a specially designed 2D and 3D flow chambers (described in detail in Chapter 2 and 4 respectively) is used. Human blood with fluorescently labeled platelets is subjected to physiologic shear stress flow conditions of 10 ± 5 dynes/cm². Thirty-minute recirculation runs are performed. Data is collected using image capture software for later processing. To estimate the possible locations of higher platelet accumulation and deposition in stented arteries, this study focusses on the highly-resolved (~ 1 $\mu\text{m}/\text{pixel}$ or ~ 5 $\mu\text{m}/\text{pixel}$) spatial distribution of platelets in 2D or 3D stented surfaces respectively. CFD geometries are created by digitizing the images obtained from experimental studies and modeled under the

assumption of Newtonian, incompressible fluid with the density and viscosity of blood (1060 kg/m^3 and 0.004 kg/m-s respectively). Flow patterns are extracted from the CFD using the instantaneous streamlines. The platelet deposition at certain locations between the struts of a stented artery is then correlated with these instantaneous streamlines.

The detailed, highly resolved spatial information from this study will elucidate the exact mechanisms behind the variations in platelet adhesion. The results provide insight into how stent design affects fluid mechanical factors, which in turn affect platelet accumulation and attachment. Subsequent reactions of the artery wall, such as inflammatory responses, smooth muscle cell proliferation, and long-term geometric adaptations, while important to the overall development of restenosis, are beyond the scope of this study.

2 EXPERIMENTAL AND COMPUTATIONAL METHODS

2.1 *In vitro* Experiment

A parallel plate flow chamber (Fig. 4) was constructed for study of blood flow over the 2D stent under physiological pulsatile flow conditions using fluorescently labeled blood platelets. The flow chamber includes a lexan top plate, lexan bottom plate, silicone sheet (from Sylgard 184 Elastomer, Dow Corning Corporation, Midland, MI) of ~0.25mm thickness and hydrophilized in 70% sulfuric acid, and a 2D stent model (Fig. 5).

Figure 4. Schematic (exploded view) of the *in vitro* test system

Figure 5. Schematic of the 2D stainless steel stent. Images were taken at the inlet to the first strut, the outlet to the last strut, regions between the 1st and 2nd struts and between the 3rd and 4th struts; these regions were located at least 3mm away from the nearest edge of the strut and are marked on the figure by a darkened rectangle.

The 2D approach was taken so that the stent strut configuration can be defined by only a single parameter, namely the ratio of the strut spacing, L (the distance along the wall in the axial direction from the beginning of one strut to the next), to the strut height, H . The 2D stent, manufactured from electropolished 302 stainless steel (New Jersey Precision Technologies, NJ), was designed such that the effect of blood flow on platelet deposition could be observed in all stent strut spacings simultaneously, in parallel. The number of struts within each configuration was different so that the outlet to the last strut (or downstream of the stent) remained nearly at the same horizontal position along the flow direction. The three strut spacings have the following length to height ratios: 2.53, 4.0, and 7.0. A cylindrical tube flow chamber (Fig. 6) was constructed for study of blood flow over the 3D stent under physiological pulsatile flow conditions using fluorescently labeled blood platelets.

Figure 6. Schematic of the 3D stented cylindrical flow chamber.

The tube was made from silicone (from Sylgard 184 Elastomer, Dow Corning Corporation, Midland, MI) of $\sim 4\text{mm}$ diameter and $\sim 1\text{mm}$ thickness and hydrophilized in 70% sulfuric acid, and 3D stent models (Fig. 7). Sylgard elastomer was chosen in order to simulate the mechanical environment in and around the stented artery. The plain sylgard is elastic. The region of sylgard with an implanted stent becomes rigid. Thus, the compliance mismatch obtained in traversing from an elastic to a rigid section of an artery

can be simulated in our experiments. The 3D stents, manufactured from electropolished 302 stainless steel, were designed by the company and are currently being marketed. All stents when implanted into the sylgard tube produced a stent-to-artery diameter ratio of 1.2:1 (semi-expanded case), except the NIR stent which produced a stent-to-artery diameter ratio of 1.5:1 (over-expanded case).

Figure 7. Schematic of 4 different 3D stent types that were tested for platelet deposition. Thickness of the struts varied from 0.09mm to 0.16mm. In Aurora stent, regions where images were taken are shown.

Note that these are calculated values and do not come from experimental observations. Unlike the 2D approach, the design of these stents were more complex. There were 5 Wall stents, 5 Bx Velocity stents, 2 Aurora stents, and 4 NIR stents available.

Platelets obtained from blood (150ml) of human volunteers were fluorescently labeled with a nucleic acid stain mepacrine (Crewe KH *et al.*, 1986). For 3D experiments, 100ml of blood was sufficient. Blood withdrawal procedures on volunteers (who gave informed consent) followed were in accordance with the ethical standards imposed on human experimentation by the Institutional Review Board (IRB) at Florida International University. 100 microliters of 10mM mepacrine stock was added to 150ml of blood (100ml of blood for 3D experiments). Mepacrine does not affect platelet function at such concentrations (Matsui H *et al.*, 2002). Platelet secretion of mepacrine after platelet adhesion and activation is sufficiently limited in rate and extent that platelet fluorescence does not decrease detectably under our experimental conditions.

An *in vitro* flow system (Fig. 8) comprised of a reservoir, a peristaltic pump connected to a function generator that maintained sinusoidal flow conditions, a capacitance chamber, and the test section. Fluorescently labeled platelet blood was maintained in a reservoir at 37 degree celsius. Blood with fluorescently labeled platelets was pumped from the reservoir to a capacitance chamber that filtered out the high-frequency spikes in flow originating from the peristaltic pump, yet allowed the 1.1 Hz flow pulsatility to pass. The blood pulsated through the test section with a shear stress of 10 ± 5 dynes/cm² (flow rate 1125 ± 562 mL/min (96 ± 50 mL/min for 3D experiments), mean $\pm$ amplitude at 1.1 Hz). The flow rate was maintained by the adjustments on the function generator. For the 3D experiments, the mean flow rate was maintained

consistently, but the amplitude did fluctuate between lowest shear stress of 3 dynes/cm² to highest shear stress of 17 dynes/cm².

Figure 8. Schematic diagram of *in vitro* flow system. The system consists of a reservoir maintained at 37 degree celsius, a peristaltic pump controlled by a function generator, a capacitance chamber to filter out high frequency spikes, a flow probe to measure the actual flow rate through the system, the 2D or 3D test section, and a microscope to observe the fluid patterns and capture images on the computer for further processing.

Depending on the type of experiment being performed, the test section was changed to a 2D parallel plate flow chamber or a 3D cylindrical stented tube. These values of shear stress corresponded to a nominal physiological pulsatile flow condition. The mean Reynolds number was 100 (or 133) for 2D (or 3D) experiments respectively. The flow rate was verified using a transit time ultrasonic flow transducer (Transonic, Ithaca, NY). Flow conditions were imposed for a period of 30-40 minutes approximately.

The movement and adhesion of fluorescently labeled platelets were detected with an inverted microscope (Nikon, FL) equipped with a mercury arc lamp. The inverted lens was set to a magnification of 20x for 2D experiments and 4x for 3D experiments. Realtime images were acquired digitally with a Qimaging camera (Retiga, FL) on a Dell computer using image capture software (Streampix, Version 2.8.1, Norpix, Inc., Quebec, Canada). Because of the inverted lens, the direction of flow also appears inverted in all the images stored in the computer. For each location and time point, 2 or 4 seconds of images were captured at approximately 20 frames/sec or 12 frames/sec respectively. Data collected from the 2D experiments included images from the fully developed flow region, adjacent to the inlet of the first strut, between struts of the lowest spacing, between struts of the intermediate spacing, between struts of the highest spacing, and adjacent to the outlet of the last strut (see Fig. 5); these locations were at least 3mm away from the nearest edge of the strut. Images were taken at various locations of the same strut spacing and also at different time points over the 30-40 minute experimental run time. Sample images taken between the struts of intermediate spacing at 3 different time points are as shown in Figure 9. Data collected from the 3D experiments included images from the fully developed flow region, adjacent to the inlet of the first strut, between struts, between connectors, and adjacent to the outlet of the last strut (see Fig. 7).

Figure 9. Sample image taken between struts of intermediate strut spacing at 3 different time points: (a) time zone 1, (b) time zone 2, and (c) time zone 3.

The stent was placed at a distance of at least 8mm (L) from the inlet to allow for fully developed flow; this distance was calculated based on the formula $L=0.06*Re*diameter$. Though L was theoretically calculated to be 38mm, the experimental value for inlet length was much lower. It has been shown previously that for pulsatile flow, the maximum entrance length was the same as the entrance length for steady peak flow (He X and Ku DN, 1994). Our CFD models showed that an inlet length of 8mm was sufficient for fully developed flow under steady peak flow conditions (see section 2.2 and Chapter 3 for more details). Images were captured at later time points (>20min) over the 30-40 minute experimental run time for all 3D stent types except for Wall stent. This was done because the 2D approach showed that time did not have a significant effect on platelet deposition and moreover, we were always interested in later time points. Sample images taken between the struts of Wall stent at 3 different time points are as shown in Fig. 10. The run time was not extended beyond this time because the adhesion of platelets appeared to plateau after this time (for most conditions, see Chapters 3 and 4).

To reduce the variations in image intensity due to AC lamp fluctuations and experimental section movement from pulsatile flow, the frames in each sequence that were in focus were averaged using image processing software (ImagePro, Version 4.5.1, Media Cybernetics, San Diego, CA). The light intensity from the mercury arc lamp also decayed with increasing radial distance from the center. A custom written Matlab program (Version 6.1, The Mathworks, Natick, MA) provided background correction to the images from the stented region based on the image file from the fully developed flow region upstream from the stent, where platelet deposition should be uniform.

Figure 10. Sample image taken between struts of Wall stent at 3 different time points: (a) time zone 1, (b) time zone 2, and (c) time zone 3.

A 4th order polynomial was fit to the light intensity distribution curve (averaged perpendicular to the blood flow direction) of the image file from the fully developed

region. The background correction was performed by dividing the light intensity at each pixel location of the image file at the stented region by the averaged light intensity at the same horizontal pixel location (along the blood flow direction) within the image but at the fully developed flow region (as fit by the polynomial) at approximately the same time point for the 2D experiments. This resulted in the normalized background corrected data. The normalized background corrected data was then averaged along pixel lines perpendicular to the flow direction (approximately 500 pixels in length). For the 3D experiments, the background correction was performed by dividing the averaged light intensity at each pixel location of the image file (80-100 pixels) at the stented region by the averaged light intensity at the same horizontal pixel location within the image but at the fully developed flow region (as fit by the polynomial). The shape and size of the region chosen in the stented image for background correction was the same as that for the inlet file at the fully developed region. The axial distance (along the flow direction) was calibrated using a hemacytometer glass slide (1mmx1mm squares) placed inverted over the inverted lens of the microscope. Thus, the spatial resolution obtained was $1.056\mu\text{m}/\text{pixel} \times 1.05\mu\text{m}/\text{pixel}$ for 2D experiments and $5.236\mu\text{m}/\text{pixel} \times 5.263\mu\text{m}/\text{pixel}$ for 3D experiments.

The background corrected data at all time points were grouped into 3 different zones: time<10min (zone 1), time=10-20min (zone 2), and time>20min (zone 3) for all 2D experimental data and for 3D Wall stent alone. For the rest of the 3D stent types, we plotted data at time>20min only. The normalized background corrected data from each location measured within a particular time zone were averaged and plotted as a function of axial distance (in mm). Wall stent has two stent geometries within its design as indicated in Fig. 10 and correspondingly platelet deposition in the direction of flow and

perpendicular to direction of flow were plotted from each geometry. From a total of 8 separate 2D experiments, and 16 separate 3D experiments, there were at least 15 samples for averaging in each time zone. The different time zones were created to see if time had a significant effect on platelet deposition.

Using SPSS (SPSS Inc., Chicago, IL, Version 10.0), two-way ANOVA (with respect to time zones and locations along the intensity curve) with a Dunnett C post-hoc test was performed to determine the statistical significance of the results. Differences achieving $p < 0.05$ were considered significant. Quantitative information on percentage of higher or lower platelet deposition was obtained based on significance levels as well as the observed means.

After each experiment, the flow chamber was disassembled; the blood and tubing were removed and discarded as a biological hazard; the chamber, the stent, and the reservoir were cleaned with bleach and soap.

2.2 Computational Fluid Dynamic (CFD) Simulation

We developed generic parametric three-dimensional (3D) models initially to study differences in platelet adhesion in each of those models. But the idea of using currently marketed *in vivo* stents (see Fig. 7) came later. Accordingly, we describe the methods used in the generic 3D simulation models first (since this is published data). Then we could transpose the same to the currently marketed stents, with additional simulations and explanations wherever necessary. Some of the results from generic 3D parametric stent models were helpful for certain explanations with the currently marketed stents.

2.2.1 Parametric Comparison of 3D Generic Stented Models

We developed 3D CFD models to study the blood flow patterns in stented arteries under low and high flow conditions using computational fluid dynamics. Our simulations have provided high-resolution flow data in the near strut region of stented arteries under pulsatile flow conditions. My contribution to this work includes (1) performing reversed flow profile simulations, (2) comparing all mechanical factors in the parametric models between forward and reversed flow profile simulations, and (3) write-up of the paper. Our models allow for a parametric variation in strut geometry, and four different stent designs were examined. Rather than representing specific designs currently on the market, we have chosen to evaluate a “generic” model of a stent in order to provide more generally applicable design criteria. Our evaluation criteria were specifically developed to quantify flow stagnation and WSS, in order to help us interpret platelet adhesion and endothelial cell growth in the near strut region of the arterial wall.

Computational domain: The current state-of-the-art stent designs used in the clinical setting are highly complex 3D “wire-mesh” type structures. The fundamental component of a stent, or the wire from the “wire mesh”, is known as a strut. Each stent design has a specific strut configuration.

Our intent was to simulate the detailed flow characteristics of the complex 3D geometry presented by a stented artery. Our computational domain was chosen based on the following two considerations: 1) we desire to minimize the computational expense of our simulations and 2) we would like to provide the most representative model that best captures the flow characteristics from each stent design. Hence we adopted a 3D Cartesian geometry that signified the near-strut portion of the stented artery (Fig. 11a).

The mainstream flow direction is along the Cartesian Z-axis, and the cross-stream direction is along the Cartesian Y-axis.

Figure 11. (a) computational domain and (b) computational mesh of one of the stent models used in XZ plane (see text).

A full 3D pulsatile computational flow simulation considering the entire stented artery flow domain would be computationally expensive and is not warranted. With that in mind, we have taken advantage of the repetitive strut pattern and the inherent

symmetry built into these patterns. Our model therefore consists of only two adjacent struts along the axial (flow) direction with the circumferential direction restricted by strut pattern symmetry. For simplicity, the computational domain in the cross-stream direction was flat, rather than curved, since the height of the stent strut is ~ 0.15 mm, or 8% of a 1.8mm arterial radius. Furthermore we have emphasized the region closer to the wall of the stented artery. The height of the computational domain above the artery wall was 1.5mm. To achieve fully developed flow (flow without strut influence), and to avoid adverse effects from the boundary conditions, the inlet and outlet lengths proximal and distal to the stent struts were $L_{in}=12\text{mm}$, $L_{out}=3\text{mm}$ for high flow (or $L_{in}=12\text{mm}$, $L_{out}=12\text{mm}$ for low flow). Equal length of inlet and outlet was used because the inlet velocity has negative values under low flow conditions.

The parametric model of the strut considers only three design parameters (strut interspacing (h , or axial strut pitch), spacing between concave to convex portion of the same strut (f , or “amplitude”), and radius of curvature of the strut (r) with the strut cross-section fixed (0.15mm by 0.15mm square) as shown in Fig. 12. Four stent models (models a,b,c,d) were studied (Fig. 12). It should be mentioned that use of existing commercial stent geometries may have allowed direct comparison with human clinical trials. However, it was more desirable to vary the geometry in a parametric fashion for future stent designs. The configurations used in this study do reflect the design of some commercial stents. The domain dimension of W (width of the computational domain) was kept constant; while these three parameters were changed to make new strut designs. Another criterion for design was the presence or absence of a longitudinal connector between the struts in our models. The computational domain was discretized using a structured hexahedral mesh (Fig. 11b). The number of cells ranged from 162924 to

279083, depending on strut design parameters (h , f , and r) and on presence or absence of connector. Mesh independence was determined from convergence ($<4.6\%$) of the axial WSS or the strain rate along the length of the stented model (b).

Figure 12. Four parametric models of stent struts geometries. (a) $h=1.8\text{mm}$, $f=0.9\text{mm}$, $r=0.45\text{mm}$, (b) $h=1.8\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$, (c) $h=3.6\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$, and (d) $h=3.6\text{mm}$, $f=3.6\text{mm}$, $r=0.9\text{mm}$.

Governing equations: The blood flow was modeled as a homogeneous Newtonian viscous incompressible fluid using the 3D Navier-Stokes and continuity equations with blood density of 1060 kg/m^3 and dynamic viscosity of 4.0 mPa s .

Boundary and initial conditions: The artery and strut wall boundary regions were assumed to be rigid and defined with a no-slip velocity boundary condition. Both the side-wall and bottom-wall of the computational domain were treated as symmetric

boundary conditions (normal velocity vanishes at boundary). A fully transient (pulsatile) loading was applied to the computational domain as velocity driven conditions. The inlet boundary condition was described by a spatially uniform and transiently varying velocity profile, as follows:

$$V_x^{\text{Inlet}} = 0, \quad V_y^{\text{Inlet}} = 0, \quad V_z^{\text{Inlet}} = a + b \sin(\omega t) \text{ m/s} \quad (1)$$

The inlet flow parameters (a , b , and ω) were predetermined in order to provide the following flow characteristics observed in arteries (Ku DN *et al.*, 1985; Moore JE *et al.*, 1995): 1) a typical high flow representing a normal level of flow in a relatively straight artery (nominal shear stress of 10 ± 5 dynes/cm² (in SI, 1dyne/cm²=0.1N/m²), mean $\pm$ amplitude); and 2) a low flow condition that may be present at the inner walls of curved sections or in arteries with poor run-off (2 ± 10 dynes/cm²). The frequency of this sinusoidal velocity profile was 1.1 Hz, corresponding to a heart rate of ~70 beats/minute. Note that the low flow condition provides shear stress reversal even in the absence of struts whereas the high flow does not. The WSS of atherosclerotic arteries varies depending on the anatomical location, local geometry, and the distal resistance. In this study, we choose to focus only on the stented section, using different boundary conditions on flow to account for these other variations.

For a heart rate of 70 beats per minute, the resulting inlet velocity parameters under nominal flow can be evaluated as follows: 1) for high flow; $a=12.5$ cm/sec, $b=6.25$ cm/sec, and $\omega=7.331$ rad/sec and 2) for low flow; $a=2.5$ cm/sec, $b=12.5$ cm/sec, and $\omega=7.331$ rad/sec. For the high flow condition, Reynolds number (Re)=240 and Womersley number (Wo)=5. For the low flow condition, $Re=50$ and $Wo=5$.

The outlet flow boundary was described by a spatially and transiently uniform pressure distribution of 0. The computational solutions were fully transient unsteady simulations with the initial conditions for the entire domain determined from a steady state simulation using mean inlet velocity boundary conditions (i.e., 12.5 cm/sec for high flow and 2.5 cm/sec for low flow).

Critical flow solution parameters: In order to examine the effects associated with the varying strut patterns from each stent design, we have evaluated the following two blood flow parameters: 1) a flow separation parameter (ϕ) and 2) a WSS parameter.

The flow separation parameter quantifies the fraction of time, with respect to one period, that flow at some point on the arterial wall is separated from the mainstream flow (i.e., stagnation or re-circulation), as follows:

$$\phi = \frac{T_s}{T} \quad (2)$$

where ϕ is the separation parameter, T_s the amount of time that flow is separated from the mainstream, and T the total time of one period (0.8571 sec). Notice that the separation parameter is only a spatially varying quantity. We have defined flow separation as occurring when the wall shear has the opposite sign from the mainstream flow. For our analysis, only the axial WSS is used for the calculation of separation parameter. Hence flow separation is equally represented by either forward or reverse (based on velocity profile alone) mainstream flow and thus the separation parameter will necessarily vary from 0 to 1. A value of 0 implies no flow separation, and a value of 1 implies constant flow separation throughout the entire flow period (re-circulation or stagnation). The separation parameter for the high flow was calculated as given in above equation (2); the

separation parameter for the low flow was adapted to account for the natural shear stress oscillation as follows:

$$\phi = \frac{T_P(\phi_{pos}) + T_N(\phi_{neg})}{T} \quad (3)$$

where ϕ_{pos} is the separation parameter during the time of forward mainstream flow T_P , ϕ_{neg} is the separation parameter during the time of reverse mainstream flow T_N , T is the total time of the flow cycle ($= T_P + T_N$). WSS is both a spatially and temporally varying quantity. We have normalized the WSS with respect to WSS under nominal flow conditions (i.e., flow without strut influence). The normalized WSS was calculated as follows:

$$WSS_{axial} = \frac{\sigma_{yz}}{\sigma_{yz}^{nominal}} \quad \sigma_{yz} = \mu \left(\frac{\partial V_y}{\partial z} + \frac{\partial V_z}{\partial y} \right) \quad \sigma_{yx} = \mu \left(\frac{\partial V_y}{\partial x} + \frac{\partial V_x}{\partial y} \right) \quad (4)$$

where WSS_{axial} is the normalized axial WSS taken along the direction of flow, σ_{yz} is the axial fluid shear stress, and the nominal σ_{yz} is the normalization factor. σ_{yx} is the transverse WSS perpendicular to the flow direction.

Simulations conducted: Our parametric model was used to examine the flow characteristics of four different stent designs due to variation in their strut patterns, as shown in Fig. 12. The governing equations for the blood flow were discretized using the finite volume method and solved using the CFD-ACE flow solver (CFDRC, Huntsville, AL) with a minimum residual of $1e-18$ and a convergence tolerance of $1e-4$ for the velocity and pressure variables. The upwind scheme (and SIMPLEC algorithm) was used for solving for velocity in most of our models, with a default inertial relaxation coefficient of 0.1 and linear relaxation coefficient of 1.0 for both pressure and velocity.

For solving the stent model (a) with low flow condition, a 2nd order upwind spatial discretization for velocity was used with 20% blending, a pressure correction of 0.2, and a linear pressure relaxation coefficient of 0.8 was used due to difficulty in converging the model with the former solution and relaxation scheme. The time step size was fixed at 8.571 msec, or 100 steps for each cyclic period of the inlet velocity function ($\omega=7.331$ rad/sec). All simulations were carried out from time point 5 to time point 105 in order to obtain one complete flow cycle. Time step independence was determined from convergence (<5%) of the mean axial WSS. At some locations within the stented chamber, the mean WSS deviations were >10%; however the WSS in these regions were very low (of the order of <0.01 dynes/cm² and negative); this was observed only at the time points when the direction of velocity changed. All simulations were carried out until a time periodic flow convergence (<10%) was established between successive oscillatory periods, typically just over 1 flow cycle.

The computational simulations of each of these four stent designs were conducted on a Dell Workstation PWS620 x86 family 6 dual processor, 1GB RAM, and 150 GB hard disk. Each of these simulations typically generated 800 MB of data and required 120 CPU hours to complete. Also note that simulations using each of these stent designs were carried out (a) with and without connector between struts, (b) under high and low flow conditions. Therefore, a total of 16 different simulations were performed. Since we are interested in the flow characteristics adjacent to the wall, we have chosen WSS as our convergence criteria since it is more restrictive than using the primary domain variables (V_x , V_y , V_z , and P).

Axial and transverse WSS from each model were extracted using CFD-VIEW. Data from 5th to 105th time points (including all time points in between) were used to represent

1 complete flow cycle (see Fig. 13) and extracted along the flow axis (in Fig. 14a), at a distance of 0.25H, 0.5H, 1H, 2H, ..., 10H (H is the height of the strut) respectively from the left edge of the connector. The WSS data at 0.25H and 0.5H especially provided quantitative information on flow characteristics in the region next to the connector. Two-dimensional contour plots of mean, maximal, minimal axial and transverse shear stresses, and separation parameter were generated for all models (using Axum 7, Insightful Corp., Seattle, WA). Contour plots included the right half of the connector, which were generated using symmetry.

Figure 13. (a) The velocity profile curve (also shows time point t1 (30th time point)) and the corresponding WSS for low flow condition and (b) The velocity profile curve (also shows time point t1 (30th time point)) and the corresponding WSS for high flow condition.

Figure 14. (a) Line-probe locations at $0.25H$, $0.5H$, $1H$, $2H$, ..., $10H$ shown for stent model (b) with connector; used for obtaining WSS information along the length of the line-probe at each location. Streamlines at 30th timestep for $h=1.8\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$ model with connector for (b) low flow and (c) high flow.

2.2.2 Blood Flow Patterns of 2D and 3D Stents used Experimentally

Pulsatile blood flow in the smallest, intermediate, and the largest stent strut spacings (in 2D experiments) were simulated using CFD-ACE (Version 2002, CFDRC, Huntsville, AL). Details of the methods used and the full results can be found in

references (Robaina S *et al.*, 2003; He Y, Masters Thesis, 2002). In short, the fluid (ie. blood) was considered incompressible and Newtonian, and the velocity profile used was the same as that in Fig. 13a. The Reynolds number (Re) was 100 and Womersley number (Wo) was 2.1. A 3D flow model similar to the 2D stent geometry (i.e. a rectangular parallel plate) used in the experimental setup was used to determine the entrance length from where fully developed flow would occur; the entrance length was 3.8cm as predicted by the simulation. A 3D flow model of a rectangular parallel plate along with the stent was used to estimate the edge effects; a minimum of 3mm distance was maintained from the edge while taking images of platelets between the stent struts in 2D stent (see Fig. 5).

For the 3D stents, the geometrical details were obtained based on digitizing images after the experiment, with the stent in the expanded state. The 3D stent geometry modeled was planar because (1) we were interested only in the near-wall phenomena and (2) the strut height was <8% of the arterial radius. Moreover, it has been observed that there is circumferential and axial stretching of the arterial wall after stent implantation (LaDisa JF Jr. *et al.*, 2005), which further decreases the ratio of strut height to arterial radius. The stent-to-artery diameter ratio was 1:1 for the CFD models. This value is different from those calculated and reported previously because no stagnation zones were observed at the inlet and outlet of the stent during the time of experiments. The stent-to-artery diameter ratio was 1.5:1 for the NIR stent CFD model alone because huge stagnation zones were observed at the inlet of the stent experimentally.

The structured grid was used to mesh the 3D models, for example, see Figures 15a and 15b. Even for the 3D stent models, blood was considered as an incompressible viscous Newtonian fluid and the velocity profile used was the same as that in Fig. 13a.

Figure 15. (a) Computational structured grid domain of stent type (b); (b) computational structured grid domain of stent type (c) as modeled in CFD.

The flow parameters calculated for the 3D stents were $Re = 130$ and $Wo = 2.8$. Inlet and boundary conditions were applied on the model, same as in section 2.2.1. Mesh independence was determined from convergence ($<10\%$) of the axial WSS or the strain

rate along the length of the stented model. See Figures 16a and 16b for mesh independence obtained in Bx velocity stent and Wall stent models. Note that mesh convergence was obtained for most of the length along the stented model, except at very few locations very close to the strut. The spatial resolution of each data point was 0.046mm.

Figure 16. Mesh convergence for (a) Bx Velocity stent and (b) Wall stent.

The 3D models are very complex with curvatures of struts presented at various locations, hence it was necessary to cut short the optimal number of grid points for the models without compromising on the fluid dynamic predictability. The NIR stent model was created by first obtaining mesh convergence on a 1:1 stent-to-artery diameter ratio model and then using the same number of grid points to expand into a 1.5:1 stent-to-artery diameter ratio model. The over-expanded NIR stent model was simulated under steady flow physiological mean shear stress of 10 dynes/cm².

The experimental entrance length of 8mm was verified by simulating steady flow condition of physiological shear stress of 10 dynes/cm² in Aurora stent (see Fig. 17).

Figure 17. Simulation showing that an entrance length of 8mm was sufficient for experimental purposes.

The instantaneous streamlines were extracted from the numerical results using CFD-VIEW for comparison with the platelet deposition in this study.

3 RESULTS

3.1 Platelet Deposition and Computational Results of 2D Stented Models

Platelet deposition patterns and their relationship to flow patterns in the 2D stented region are illustrated in Figures 18-22. Platelet deposition patterns at regions adjacent to the first strut, between struts of smallest spacing, largest spacing, and intermediate spacing, and finally adjacent to the last strut respectively were measured (see Figure 5 for locations). All differences noted in this section are statistically different with $p \leq 0.05$.

Inlet flow leading up to the first strut separates from the bottom wall just before the strut, producing a corner region of recirculation (Figure 18). Platelet deposition was 28.6% higher at the region of recirculation (location B) adjacent to the first strut compared to the region of no flow disturbance upstream (location D), and 26.4% higher than the deposition in the corner flow region (location A). The point of separation (location C) had 50.5% lower deposition compared to the region of recirculation (location B). The point of separation (location C) had 14.6% lower deposition than the region of no flow disturbance (location D), but was not statistically significant. There was no significant difference in platelet deposition between the three different time zones.

For the smallest strut spacing, a region of recirculation between the struts persisted throughout the entire flow cycle (Figure 19). The region of maximum platelet deposition at the center of this recirculation (location B) was 46% higher than regions very close to the struts (with very low platelet deposition, locations A & C). The three different time zones were again not significantly different from one another.

(a)

(b)

Figure 18. (a) Average normalized platelet deposition at the inlet to first strut plotted as a function of distance (in mm). (b) Path of instantaneous streamlines at minimum flow (Q represents the flow rate) at the inlet to first strut. Locations A, B, C, and D correspond to various flow regimes near the wall. Location A is the corner flow region; location B is the center of the recirculation region; location C is the average point of separation between minimum and maximum flow (varies up to an order of 0.035mm); and location D is the region of flow parallel to the wall far upstream.

Figure 19. (a) Average normalized platelet deposition between struts of that smallest strut spacing plotted as a function of distance (in mm). (b) Path of the instantaneous streamlines at minimum flow (Q represents the flow rate) between the struts of smallest spacing. Locations A and C define the corner flow region and location B is the center of the recirculation region.

The largest strut spacing provided ample room for the flow, that had separated off of the upstream strut, to reattach to the bottom wall distal to the upstream strut before forming a separated flow region proximal to the downstream strut (Figure 20). The point of separation (location C) and reattachment (location G) had 24.5% and 41.5% lower deposition compared to the regions of recirculation proximal and distal to the strut respectively (locations B and H). The platelet deposition in the corner flow regions very close to the struts (locations A and I) were 35% (72%) lower than the region of recirculation proximal (distal) to the strut (locations B and H again). Between points D and F the flow remains parallel to the bottom surface and the platelet deposition decreases almost linearly from point F to D in the direction of flow. Platelet deposition at point D was 22.8% significantly lower than the platelet deposition at point F. The three different time zones were once again not significantly different.

The intermediate strut spacing produced a flow that was reattaching at minimum flow, and recirculating at maximum flow (Figure 21). The platelets deposited relatively homogeneously in this scenario, compared to the smallest and largest strut spacing. The platelet deposition at the center (location C) was 35-45% higher than the corner flow regions very close to the struts (locations A and E). The platelet deposition in the predominant distal recirculation region (location D) was 8.7% higher than in location C. In this case alone, the platelet deposition at the third time zone was significantly higher than the deposition at the first and second time zones.

(a)

(b)

Figure 20. (a) Average normalized platelet deposition between struts of largest spacing plotted as a function of distance (in mm). (b) Path of instantaneous streamlines at minimum flow (Q represents the flow rate) between struts of largest spacing. Locations A and I define the corner flow regions very close to the strut; locations B and H are the center of the recirculation regions; locations C and G are the points of separation and reattachment (varies up to an order of 0.04mm and 0.07mm respectively); location E is a point where the flow is parallel to the bottom wall distal to the recirculation regions; and locations D and F are points just upstream and downstream of the separation point and reattachment point respectively.

(a)

(b)

(c)

Figure 21. (a) Average normalized platelet deposition between struts of intermediate spacing plotted as a function of distance (in mm). (b) Path of instantaneous streamlines at minimum flow (Q represents the flow rate) between struts of intermediate spacing. (c) Path of instantaneous streamlines at peak flow between struts of intermediate spacing. Locations A and E denote the corner flow regions very close to the strut; location C is the center between the struts; locations B and D denote the recirculation regions.

Flow separates once again off the last strut, producing a separated recirculating flow region similar to the inlet strut but, of course, opposite in direction (Figure 22). Similar to the other cases, the platelet deposition at the region of recirculation (location D) was 24.8% higher than the point of reattachment (location C) and 98% higher than corner flow region very close to the strut (location E). The platelet deposition downstream of the strut (location A) was 10.7% lower than location B, just downstream of the reattachment; the platelet deposition was decreasing in the direction of flow similar to that found in largest strut spacing. The platelet deposition at the point of reattachment was 19.9% lower than location B, just downstream of the reattachment.

3.2 Computational Results of 3D Generic Stented Models

Many of the principle flow characteristics can be understood by analyzing the instantaneous streamlines. The stent strut geometry caused deviations in near-wall streamlines away from the axial direction that roughly followed the strut shape. However, the degree of deviation depended on the flow conditions. Under low flow conditions, the orientation of the streamlines changed in accordance with the reversed flow velocity profile. There was less change in streamline direction under high flow conditions. Streamlines showing flow disturbance at time point t1 (see Fig. 13a & 13b, refer to the 30th time point) for the stent model (b) with connector, under low flow (Fig. 14b) and high flow (Fig. 14c) conditions are shown (see Chapter 2, section 2.2.1 for these figures). At time point t1, the velocity has already started to increase (from the lowest value, which occurs at the 25th time point). The disturbance in flow was the least in the regions nearest and farthest from the connector, where the streamlines were parallel and in the axial direction.

(a)

(b)

Figure 22. (a) Average normalized platelet deposition at the outlet to last strut plotted as a function of distance (in mm). (b) Path of instantaneous streamlines at minimum flow (Q represents the flow rate) at the outlet to the last strut. Location A defines a region far downstream of the reattached point; location B is the point just downstream of reattachment point; location C is the point of reattachment (varies up to an order of 0.07mm); location D is the center of the recirculation; and location E is the corner flow region very close to the strut.

The streamlines converged at the proximal end of the concave portion of the arc of the strut and diverged from the distal end of the convex portion of the strut. The streamlines within the struts were most parallel (in the direction of flow) for the stent model (d). The streamlines did not vary noticeably between models with connectors and those without connectors.

Under high flow conditions, the mean axial WSS in the *stent model (a) with connector under high flow conditions* was only restored to 50% of the nominal value for much of the area between the struts. The contour plot shows that the mean axial WSS decreased gradually on approaching the first strut (Fig. 23a). The low mean axial shear stress region distal to the first strut was larger than the region proximal to the second strut. This large mean axial shear stress was closer to the second strut. The large mean shear stress region was divided due to the presence of the connector. At a position 0.66 mm proximal to the second strut, the largest mean axial WSS was restored to 80% of the nominal value. The mean transverse WSS was dominant along the oblique parts of the strut, i.e. at regions proximal to the first strut, between struts, and distal to the second strut (Fig. 23b). The struts are at an angle to the main flow and thus cause local changes in flow direction. As a result, the flow near the struts is not limited to the axial direction. The mean transverse WSS was very small near the connector since the connector is aligned parallel to the main flow. Flow separation occurred around the entire length of the struts, most extensively at the distal end of the struts oriented at angles to the main flow direction (Fig. 23c). Recirculating flow for most part of flow cycle (characterized by separation parameter larger than 0.5) existed adjacent to the apex and base of the struts. Separation parameter was <0.1 (approximately 0 in this case) between the struts indicating flow reattachment for most part of the flow cycle.

The axial shear stress for the *stent model (a) without connector under high flow conditions* was continuous between the struts (Fig. 24a). Fig. 24b shows the transverse shear stress variations, similar to that of Fig. 23b. The contour plot of the separation parameter showed much less flow separation distal to the second strut, at regions closer to the base (Fig. 24c).

The largest mean axial shear stress between the struts of *stent model (a) with connector under low flow conditions* was located just proximal to the second strut within 0.42mm (Fig. 25a). This distance was 36% shorter for the model under low flow than high flow. The mean transverse shear stress was largest distal to the strut (Fig. 25b). The contour plot of separation parameter for the model with connector (Fig. 25c) showed recirculating regions at the apex and base of the first and second struts. Flow separation with separation parameter of 0.2-0.3 was distributed proximal and distal to the struts, and along the length of the struts.

The narrow band between the two rows of struts (refer to Fig. 12 for narrow band) in the *stent model (b) with connector under low flow conditions* resulted in more of a reduction in the mean axial shear stress (Fig. 26a). Varying the strut parameters f and r provoked changes in the mean transverse shear stress. At the vicinity of the narrow band, the mean axial shear stress is very low. However, in this region, the mean transverse shear stress is very high (Fig. 26b) and higher than that of the stent model (a) (by ~30% for the region between $4H$ and $7H$). Large mean transverse shear stress existed at regions proximal to the proximal apex, the narrow band between the struts, and distal to the distal base.

Figure 23. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=1.8\text{mm}$, $f=0.9\text{mm}$, $r=0.45\text{mm}$ model (stent model (a)) with connector under high flow. Flow direction is along increasing z .

Figure 24. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=1.8\text{mm}$, $f=0.9\text{mm}$, $r=0.45\text{mm}$ model (stent model (a)) without connector under high flow. Flow direction is along increasing z .

Figure 25. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=1.8\text{mm}$, $f=0.9\text{mm}$, $r=0.45\text{mm}$ model (stent model (a)) with connector under low flow. Flow direction is along increasing z .

Figure 26. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=1.8\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$ model (stent model (b)) with connector under low flow. Flow direction is along increasing z .

Flow separation occurred at the areas near the apex and base of the struts. The separation parameter of 0.2-0.3 was found at steeper sections of the struts (specifically extending from the concave, convex sections of the strut inwards, outwards respectively, see Fig. 26c).

Increasing the longitudinal strut spacing h to 3.6mm increased the degree to which the axial WSS was restored between the struts in *stent model (c)*. For high flow, the largest mean axial shear stress region between struts was divided into two (Fig. 27a). The narrow band was not obvious because of the larger strut spacing. The large mean transverse shear stress regions between struts were connected through a low mean transverse shear stress region of ~ 1.2 dynes/cm² (27% of the largest mean value) for high flow (Fig. 27b), and ~ 0.2 dynes/cm² (12% of the largest mean value) for low flow. The area of flow separation at the apex and base of the struts in this model appeared smaller to that seen in *stent model (b)* (Fig. 27c). Separation parameter (>0.5) in the former was only 30% of the latter.

The strut parameter f was increased to 3.6mm, which caused the connectors connecting the arcs of the struts in *stent model (d)* to be more aligned to the flow axis. A narrow band, narrower than the *stent model (b)*, divided large mean axial WSS into two regions, where the flow had almost recovered (Fig. 28a). Large mean transverse shear stress was located at the narrow band, and their locations were similar to that of *stent model (b)*. For high flow, the flow separation seemed to exist more distal to the struts (Fig. 28c).

The largest mean axial shear stress restored between the struts was at least 80% of the nominal value; hence we are interested in the average of the mean axial shear stress between struts. Fig. 29 shows the average mean axial shear stress between the struts was

largely restored to 67% and 60% of the nominal value under high flow, low flow respectively for the stent model (c) with connector.

The mean transverse shear stress between struts represents the level of interference to flow. Fig. 30 shows that with increase in strut parameter f from 0.9mm to 1.8mm (accompanied with increase in strut parameter r from 0.45mm to 0.9mm), the mean transverse shear stress increased by 14-18% due to the narrow band between the struts as seen in the contour plots. However, when the strut parameter h was increased from 1.8mm to 3.6mm, the mean transverse shear stress decreased by 38-40% due to increased strut interspacing. But when f was increased from 1.8mm to 3.6mm, the mean transverse shear stress did not significantly increase in the presence of the narrow band between struts. It is interesting to note that the mean transverse shear stress for the model without the axial connector is greater by 8% during high flow and is smaller by 8% during low flow.

A greater percentage of the area between the struts had a separation parameter <0.1 , indicating fluid reattachment to the wall during $>90\%$ of flow cycle. For more quantitative evaluation, we analyzed separation parameter >0.5 , which indicates flow recirculation for at least 50% of the flow cycle. When the strut parameter h was increased from 1.8mm to 3.6mm, Fig. 31 reveals a drastic decrease in regions of recirculation, due to greater strut interspacing, by 57-60% under high flow and by 66-68% for low flow. When f was increased from 1.8mm to 3.6mm with the same r , there seemed to be relatively small increase in separation parameter.

Figure 27. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=3.6\text{mm}$, $f=1.8\text{mm}$, $r=0.9\text{mm}$ model (stent model (c)) with connector under high flow. Flow direction is along increasing z .

Figure 28. Contour plot of (a) mean axial WSS, (b) mean transverse WSS, and (c) separation parameter, for the $h=3.6\text{mm}$, $f=3.6\text{mm}$, $r=0.9\text{mm}$ model (stent model (d)) with connector under high flow. Flow direction is along increasing z .

Figure 29. Normalized mean axial WSS between struts in different strut geometries for high and low flow conditions. First two geometries correspond to $h=1.8$ mm models, third and fourth geometries correspond to $h=3.6$ mm models.

Figure 1. Mean transverse WSS between struts in different strut geometries for high and low flow conditions.

Figure 31. Percentage area between struts where the separation parameter >0.5 in different strut geometries for high and low flow conditions.

When the strut parameter f was increased from 0.9mm to 1.8mm (also increasing r from 0.45mm to 0.9mm), the area in which the separation parameter ranged from 0.1-0.5 decreased (Fig. 32), indicating increased fluid reattachment to the wall. When the strut parameter h was increased from 1.8mm to 3.6mm, the area in which separation parameter ranged from 0.1-0.5 further decreased by 50%. When f was increased from 1.8mm to 3.6mm, there was much less change in separation parameter. The area in which the separation parameter ranged from 0.1-0.5 was relatively higher for all the models under low flow compared to high flow, whereas the separation parameter >0.5 was higher for only 2 of the 4 models with connector. From the contour plots, flow stagnation regions occurred at the proximal and distal portion of the apex and base of the strut and separation parameter of 0.2-0.3 were observed along the length of the strut for low flow.

In brief, analysis of strut parameters h , f , and r shows: (a) increasing h or decreasing f increased restoration of axial shear stress between the struts; (b) increasing h decreased the transverse shear stress; (c) increasing r and f reduced restoration of axial shear stress between the struts, and increased the transverse shear stress; (d) regions of recirculation (for most part of flow cycle governed by separation parameter >0.5) were greater for the high flow (except the stent model (b) with connector); under low flow, it remained the same as that under high flow for models (b) and (d); (e) regions of flow reattachment (for most part of flow cycle governed by separation parameter 0.1-0.5) were larger under low flow for all models; (e) models without the axial connector showed increased restoration in axial shear stress and reduced separation parameter compared to models with the axial connector.

Figure 2. Percentage area between struts where the separation parameter ranged from 0.1-0.5 in different strut geometries for high and low flow conditions.

3.3 Platelet Deposition and Computational Results of 3D Stented Models

The mean axial wall shear stress and separation parameter were computed for Bx velocity and NIR semi-expanded stent models and is as shown in the Figures 33 and 34 respectively. Fig. 33 shows that axial wall shear stress was restored around most of the region between the struts except around the connector region. The mean wall shear stress at the laminar flow region before the stent was 9.72 dynes/cm^2 . Regions of stagnation seem to be located mostly within the connector region and distal or proximal to the convex or concave end of the strut respectively. These plots are useful to analyze the regions of stagnation and thus be able to observe carefully at those stagnation regions at the time of the experiment. The depiction of flow patterns within the stented region using instantaneous streamlines can also give information on the stagnation zones. This tool is used in the remaining of the results and the discussion sections.

Platelet deposition patterns and their relationship to flow patterns in the 3D stented region are illustrated in Figures 35-47 (these figures follow with the results as presented in the paragraphs below). For all stent types, the platelet deposition patterns at regions adjacent to the first strut, between struts, between connector regions (if exists), and finally adjacent to the last strut respectively were measured. All differences noted in this section are statistically different with $p \leq 0.05$.

Wall Stent: Inlet flow leading up to the first strut separates from the bottom wall just before the strut, producing a corner region of very low platelet deposition near the intersection of the two struts of Wall stent (Fig. 35).

Figure 33. Axial wall shear stress variation and separation parameter variation within the stented geometry of Bx Velocity stent.

Figure 34. Axial wall shear stress variation and separation parameter variation within the stented geometry of NIR stent.

Platelet deposition was 24% lower at the region of intersection of the struts (location A) compared to a region nearby (location B). Location B had 65% higher platelet deposition compared to the region of no flow disturbance (location C). There is more platelet deposition at the proximal portion of the strut (location D) compared to the distal portion of the same strut (location E) as seen in Figure 35a. Platelet deposition curves showed no significant difference with respect to time. Figure 35c shows the path of streamlines carrying activated platelets just before the first strut; there is mainstream fluid coming in from underneath the strut as well as fluid coming between the wall and the other cross-over strut that reattach; note that there is no region of recirculation here.

Platelet deposition between the struts was plotted both in the direction of flow and perpendicular to the direction of flow (see arrows in Figure 36a). Platelet deposition was 30% lower at regions close to the intersection of the two struts (locations A or D) compared to the center (locations B or C) as seen in Figure 36b. Deposition was higher (location B) closer to the distal portion of the strut by 34% compared to further downstream (location C) and decreased linearly (location C) in the direction of flow. Perpendicular to the flow direction (Figure 36c), the platelet deposition was higher at the center (location B) by 43-80% compared to regions close to the strut (locations A or C). Platelet deposition curves showed no significant difference with respect to time. The path of streamlines in the whole region between struts (Figure 36d) with flow coming in and out of the gaps between the wall and the strut are shown; close-up view of streamlines distal to the struts are similar to streamlines from Figure 35c; close-up view of streamlines proximal to the struts are similar to streamlines from Figure 37c. Based on the inclination of the struts to the flow, two different geometries have been identified

(Figure 36a); platelet deposition curves for geometry 2 was similar to that for geometry 1 and therefore have not been included here.

Figure 35. (a) Platelet deposition at the inlet just before first strut for Wall stent; location D is proximal to strut in the flow field; location E is distal to strut in the flow field; (b) normalized average platelet deposition at the inlet just before first strut for time zones 1 and 2; (c) streamlines observed from the side (at maximum flow rate) directed away from the wall first and then directed towards the gap between the wall and the strut in flow field, causing low platelet deposition in location A; (d) change in direction of streamlines close to the strut on the wall. Location B is just upstream of location A and has increased platelet deposition due to adhesion of platelets proximal to strut in flow field.

(b)

(c)

Figure 36. (a) Platelet deposition taken between struts for Wall stent; two geometries within the same stent type is shown; (b) normalized average platelet deposition between struts in the direction of flow at time zones 2 and 3. Location C (along flow direction) had maximum deposition distal to strut due to streamlines directed towards the wall. Deposition decreased linearly from location C to B as the streamlines get parallel to the wall in (d); (c) normalized average platelet deposition between struts perpendicular to the direction of flow at time zones 2 and 3; (d) streamlines (at maximum flow rate) between struts; note change in direction of streamlines as they approach the struts. Locations A and D (along flow direction) and location C (perpendicular to flow direction) are close to the intersection of the struts and have low deposition due to streamlines directed away from wall.

Platelet deposition at the outlet to last strut was 88% higher (location B) distal to the strut compared to regions close to the strut (location C) as seen in Figure 37. Further distal to the strut, platelet deposition decreases linearly (location A) by 43% compared to location B. Platelet deposition curve at time=10-20min corresponds to data taken at location D (shown in Figure 37a); while the deposition at time>20min corresponds to data taken at location E. In both cases however, the deposition peaks at a point just distal to the intersecting struts. Streamlines distal to the struts at the outlet are shown in Figure 37c; note that the fluid that flows between the wall and the strut reattaches to the mainstream flow distal to the strut.

Figure 37. (a) Platelet deposition at the outlet just after the last strut for Wall stent; (b) normalized average platelet deposition at time zones 1 and 2; deposition pattern at time zone 2 were mainly from region D in (a); deposition pattern at time zone 3 were mainly from region E in (a); (c) streamlines (at maximum flow rate) distal to the last strut. Location C is close to the strut and has low platelet deposition because of the streamlines directed away from the wall. Location B has higher platelet deposition because of streamlines directed towards the wall. Deposition decreases linearly from location B to location A further downstream of the strut.

Bx Velocity Stent: Comparison of platelet deposition (after 20 minutes of experimental run) at the concave and convex ends (locations E and D respectively) just before the first strut is shown in Figure 38a. The platelet deposition at location B was higher by 39% compared to the region of no flow disturbance (location C) and higher by 61% compared to regions close to the strut (location A). This peak in platelet deposition was located just proximal to the strut in Figure 38b and little distance away from

proximal portion in Figure 38c. In Figure 38c, the platelet deposition at location B was higher by 31% compared to the region of no flow disturbance (location C) and higher by 72% compared to regions close to the strut (location A). Figure 38d shows no recirculation region proximal to the convex end of the strut. Figure 38e shows region of recirculation and flow separation proximal to the strut.

(c)

(d)

(e)

Figure 38. (a) Platelet deposition at the inlet just before first strut for Bx Velocity stent; (b) normalized average platelet deposition in region D shown in (a). Location B in (b) has higher deposition and is located very close to the strut because of streamlines carrying platelets to deposit near the convex end of the strut; (c) normalized average platelet deposition in region E shown in (a); (d) streamlines (at maximum flow rate) at the convex end of the first strut; (e) streamlines (at maximum flow rate) at the concave end of the last strut. Location B in (c), just proximal to the flow separation point in (e) has higher deposition compared to location A (region of recirculation); flow separation results in streamlines directing platelets away from the wall. Location C in both cases is in the region of no flow disturbance.

In the region between the struts, the platelet deposition peaked just distal to the proximal strut (location C in Figure 39a) by 87% compared to a point downstream of the proximal strut (location B). This decrease was linear until some point between the struts after which it stabilized (line from location B to A). Regions very close to the strut have low platelet deposition (locations D or A) and location D was 52% lower than the location C. Figure 39b shows streamlines diverging distal to the proximal strut (top view); they start to appear parallel to the wall further downstream. Figure 39c shows streamlines within the region of recirculation and flow reattachment distal to the proximal strut; there is a region of recirculation and flow separation proximal to the distal strut as shown in Figure 39d. Similar to the diverging aspect of the streamlines distal to the proximal strut, the streamlines start to converge proximal to the distal strut.

Platelet deposition within the center connector region (locations C in Figure 40b) was higher by 24% compared to the side connector regions (locations B or E, note location C was not statistically significant from location E) and by 20% compared to the proximal portion of the proximal strut (location F). Deposition at C was higher than D by only 8%, but was not significantly different. Deposition at C was higher than E by 18%, but was not significantly different. The struts in the graph in Figure 40b corresponds to locations F and G of Figure 40a. Streamlines in figure 40d shows the regions of recirculation within each of the connector regions; figure 40c shows streamlines from the main flow that formed these recirculation regions.

(a)

(b)

(c)

Figure 39. (a) Platelet deposition between struts for Bx Velocity stent; (b) top view of streamlines (at maximum flow rate) distal to the proximal strut; streamlines tend to diverge distal to the strut; (c) side view of streamlines distal to the proximal strut. Location C has higher platelet deposition because the streamlines with activated platelets are directed towards the wall distal to the strut. Deposition decreases linearly from C to B as the streamlines orient more parallel to the wall; (d) side view of streamlines (at maximum flow rate) proximal to the distal strut; streamlines tend to converge proximal to the strut. Locations A and D correspond to recirculation regions proximal and distal to strut respectively and have low platelet deposition due to the region being well-separated from mainstream flow as shown in (c) and (d).

(a)

(b)

Figure 40. (a) Platelet deposition at the connector for Bx Velocity stent; (b) normalized average platelet intensity between the struts in the connector region; the two struts shown in (b) correspond to the struts at locations F and G in (a); (c) top view of streamlines (at maximum flow rate) in the connector region; (d) side view of streamlines (at maximum flow rate) in the connector region. Locations B, C, D, and E are recirculating regions but of a spiral nature. Locations C and D have higher deposition due to struts being wider and thus allows for streamlines with activated platelets from the mainstream. Locations B and E allows for mixing of platelets from the mainstream, but their deposition may differ based on the platelet residence time dependent on the number of spirals made in that region.

Deposition distal to the convex end of the last strut was greater by 81% compared to further downstream (location A) and by 122% compared to regions close to the strut (location C), shown in Figure 41b. Deposition distal to the concave end of the last strut was greater by 90% compared to further downstream (location A) and by 90% compared to regions close to the strut (location C), shown in Figure 41a. Deposition distal to the convex strut further downstream seems to be decreasing linearly while the deposition distal to the concave strut further downstream seems to be decreasing exponentially. Figure 41c shows streamlines converging at a point and no region of recirculation distal to the convex end of the last strut, while distal to the concave end of the last strut, there is a region of recirculation and flow reattachment region (Figure 41d).

(b)

(c)

Figure 41. (a) Normalized average platelet intensity at the outlet to concave end of the last strut for Bx Velocity stent; (b) normalized average platelet intensity at the outlet to convex end of the last strut. Location B distal to the last strut has higher deposition compared to location C close to the strut; (c) streamlines (at maximum flow rate) at the concave end of the strut; location B is the point of higher deposition where the streamlines from either ends of the strut meet, as shown in the figure; (d) streamlines (at maximum flow rate) at the convex end of the strut; location B has higher platelet deposition due to streamlines directed towards the wall in the region of reattachment. Location C in both cases has lower platelet deposition. Location A corresponds to a region further downstream of the strut where the deposition is lower due to streamlines directed more parallel to the wall.

Aurora Stent: In the region between the struts, the platelet deposition peaked just distal to the proximal strut (location D in Figure 42a) by 38% compared to a point downstream of the proximal strut (location B). This decrease was linear and slow

between the struts (line from location D to A). Regions C, D, and E were not significantly different from each other, but location D had 5% greater deposition than locations C or E. Location B was not significantly different from location C, but was lower by 23%. Regions very close to the strut have low platelet deposition (locations F or A). Streamlines for this model between struts were similar to Figures 39c and 39d and hence were not shown. Streamlines diverge distal to the proximal strut; they start to appear parallel to the wall further downstream. There are regions of recirculation and flow separation or reattachment, proximal (or distal) to the distal (or proximal strut). Similar to the diverging aspect of the streamlines distal to the proximal strut, the streamlines start to converge proximal to the distal strut.

Figure 42. (a) Normalized average platelet deposition in the region between struts for Aurora stent. Locations C and D have higher platelet deposition than location B due to streamlines directed towards the wall distal to the strut. Locations A and E have low platelet deposition, being close to the struts. Deposition seems to be decreasing linearly from locations C or D to A.

Very low platelet deposition around areas of the connector (locations A and B) in Figure 43a were seen. Fully separated regions of recirculation exist near the connectors, as shown in Figure 43b. Platelet deposition considerably increases (location A) in Figure 44a. Streamlines show spirally recirculating region between the two struts (Figure 44b).

Figure 43. (a) Platelet deposition at the connector for Aurora stent; (b) streamlines (at maximum flow rate) in region B showing recirculation zones. Region A and B have low deposition because they are well-separated from the mainstream and do not allow mixing with fresh platelets.

Figure 44. (a) Platelet deposition at the no-connector region for Aurora stent; (b) streamlines (at maximum flow rate) showing spirally recirculating regions. Region A is spirally recirculating with platelets coming in from flow in both directions and therefore has higher deposition than a location upstream of the proximal strut.

NIR Stent: Platelet deposition at the inlet to the convex end of the first strut and the outlet to the concave end of the last strut seems to be the same as the region of no flow disturbance (Figure 45a and 45b). At about 1mm from the strut, the platelet deposition is higher at the outlet (location A of Figure 45b) compared to that at the inlet (location B of Figure 45a). Streamlines show existence of large recirculation regions at the inlet and outlet to stent (locations D and E respectively in Figure 46b).

Platelet deposition between the first and the second struts of the NIR stent had somewhat uniform deposition as shown in Figure 46a. Platelet deposition was 35% lower at regions close to the strut (locations A and D) compared to a region at the center (location B or C). Streamlines show that there is a region of reattachment between the first two struts (Figure 46b). Location B or C may be present in the vicinity of the reattachment point.

Platelet deposition between another set of struts away from the inlet showed deposition pattern very similar to Fig. 39b. The deposition peaked just distal to the strut and decreased linearly further downstream. Deposition was lower at points close to the struts. Since the deposition pattern was similar, this plot was not shown here.

Platelet deposition within the connector region (location B in Figure 47b) was 21% higher than a region proximal to the proximal strut (location D). The two struts correspond to locations D and E as shown in Figure 47a. Deposition is low around the edges of the connector (Figure 47a). Figure 47c shows streamlines in a spirally recirculating region within the connector zone.

Figure 45. (a) Normalized average platelet deposition at the inlet just before the first strut for NIR stent; (b) normalized average platelet deposition at the outlet after last strut. Location A in (a) shows that the deposition is the same as that at the region of no flow disturbance B due to the large recirculation region at the inlet (corresponds to region D in Fig. 41b); note that NIR stent is an over-expanded stent. Location A in (b) shows higher platelet deposition due to convection geared towards getting more platelets to the recirculation region at the outlet (corresponds to region E in Fig. 41b).

Figure 46. (a) Normalized average platelet deposition between the first two struts for NIR stent; (b) streamlines (at steady flow with mean shear stress of 10 dynes/cm²) showing large recirculation region at the inlet. Location B is the reattachment point, where the deposition seems to be low; deposition decreases linearly from B to A and from B to C, as the streamlines get more parallel to the wall.

(a)

(b)

Figure 47. (a) Platelet deposition at the connector region for NIR stent; (b) normalized average platelet deposition at the connector; the two struts correspond to struts in regions D and E of (a); (c) Streamlines (at maximum flow rate) near the connector for NIR stent. Location B has higher deposition compared to C because of the spiral recirculation zones that entrain more platelets from the mainstream. Location A is distal to the strut where platelet deposition was higher due to streamlines directed towards the wall.

4 DISCUSSION

The purpose of this research was to measure the platelet deposition between stent struts under highly resolved conditions in order to directly relate this deposition pattern to flow dynamics and stent design. Flow patterns are dependent on stent design (Berry JL *et al.*, 2000; Henry FS, 2000; LaDisa JF Jr. *et al.*, 2004, 2005). Stent design affects restenosis rates (Kastrati AJ *et al.*, 2001). Hence, flow patterns were expected to affect restenosis rates. The first vascular response to stenting is formation of thrombus (Garasic JM *et al.*, 2000). This process is initiated by the blood platelets. Therefore platelet adhesion, activation, and aggregation become the foremost processes that occur in restenosis.

We postulated that instantaneous streamlines could be used as a tool to depict flow dynamics within the stented region. We postulated that deposition occurs through the convection of platelets as depicted by these instantaneous streamlines. We first experimented two-dimensional (2D) idealizations of three-dimensional (3D) stents, characterizing them in terms of the ratio of the distance between the strut centers to the strut height. We used three different strut spacings in this case. For 3D stents, four different models of currently marketed stents were used: Bx Velocity stent, Wall stent, Aurora stent, and NIR stent. This chapter first discusses the results separately from the two-dimensional and three-dimensional studies performed previously, for clear understanding and to avoid confusion. Another part of the discussion also includes 3D parametric computational models. Later on, we can see how some aspects of this research could be applied to the experimental 3D stent work. Then we would have a general discussion of some important aspects of this research based on the 2D and 3D stent results obtained.

4.1 2D Experimental Stented Models

The initial thrombotic reaction to the placement of a stent into a diseased artery is a determining factor for subsequent reestablishment of the endothelium. Thus, a better understanding of the dynamics of platelet adhesion to stents under realistic flow conditions can serve to improve stent design and thus eventual clinical outcomes. In our previous study, platelet deposition on the wall in a 2D stented region was shown to vary significantly with different stent strut spacings (Robaina S *et al.*, 2003). The stent model with the closest spacing ($L/H=2.5$) produced the lowest amount of platelet deposition both between stents and on the struts as compared to the intermediate ($L/H=4.0$) and widest spacing ($L/H=7.0$) models. However, the method used to trace platelets, radioactive labeling, did not provide us with direct observation of the attachment of platelets between the struts, or the spatial distribution of platelets between the struts.

Primarily three main mechanisms influence the adhesion of platelets to the wall: (1) platelet transport to the wall (by convection, diffusion, or other means), (2) platelet reactivity with the wall (preceded by platelet activation), and (3) ability to (or lack of ability to) wash the platelet deposits off from the wall (typically via fluid drag). The recirculating flow system utilized in this study, along with the peristaltic pump, make it very likely that platelets are activated and at least moderately reactive with the surface (due to its hydrophilic nature), particularly in the vicinity of the stent. Also, for the geometries considered in this study, recirculation dominates the near wall flow in the stented region, creating large regions of low shear, so washout of platelet aggregates should be relatively low. Therefore, we postulate that the medium through which activated platelets get attached to the wall are the instantaneous streamlines that depict the fluid flow pattern; thus the platelet flow and hence attachment is convection driven.

In all of the stent strut spacing designs utilized for this study, the adhesion of activated platelets was found to coincide with patterns of convective delivery of those platelets to (or from) the wall, rather than the wall shear stress pattern. This is in direct agreement with the findings of Longest PW and Kleinstreuer C, 2003. As indicated in Figure 48, platelet deposition is always higher in areas where there are streamlines with components perpendicular to the wall and flow is directed *towards* the wall (upstream and downstream of separation and reattachment points, and in the center of recirculation regions) than in areas where streamlines are parallel (with no curvature, as in fully developed flow regions and downstream of the proximal recirculation region in the largest strut spacing) to the surface. Conversely, in areas where a component of the streamline is perpendicular to the wall but flow is directed *away* from the wall (separation and reattachment points, and corner regions of the struts), the platelet deposition is always lower than in areas where streamlines are parallel to the wall. This study may hint that flow patterns may play a larger role in restenosis than the wall shear stress.

Recirculation regions allow entrapment of procoagulant agents (with refreshment occurring by entrainment from the main flow during flow pulsatility) and a convergence of streamlines that bring those agents close enough to the wall to react. At the inlet to the first strut, platelet deposition at the region of recirculation (see Fig. 17a, location B) was significantly higher than the region of no flow disturbance (location D). The instantaneous streamlines at the region of no flow disturbance were parallel to the wall (see Fig. 17b); hence only those activated platelets that were carried by instantaneous streamlines very close to the wall got deposited. However in the region of recirculation, the recirculating instantaneous streamlines (which carry platelets with partial mixing

from the mainstream flow) strike the wall surface with activated platelets in increasing numbers as the center of the recirculation is approached.

Figure 48. Figure summarizes average platelet deposition based on the flow regime under consideration and the direction of flow of streamlines.

Another reason would be that the region of recirculation varies in size within the flow cycle; hence platelets get deposited not just at the center but also at points around the center of the recirculating region. This is true for the smallest strut spacing also where platelet deposition is significantly higher around the center, since the smallest strut spacing corresponds to constantly recirculating flow (see Fig. 19a). From Fig. 19b, we can see that the instantaneous streamlines recirculate such that the momentum will cause platelets to strike the wall surface in increasing numbers toward the center. Then the

streamlines start to separate off the wall to complete recirculation, thereby carrying platelets with them away from the wall. This deposition pattern is similarly explained in the region of recirculation proximal and distal to the strut (locations B and H respectively) for the largest strut spacing case (see Fig. 20a).

Regions of recirculation distal to the struts generally showed significantly higher deposition compared to regions proximal to the struts. This is best illustrated in the largest strut spacing case (Fig. 20). At the proximal end of the downstream strut, the instantaneous streamlines of the mainstream flow separate from the wall and traverse away from the wall (see Fig. 20b, location C), hence the number of platelets that gets mixed within the region of recirculation from the mainstream flow is less; therefore the platelet deposition is lower. However, at the distal end of the upstream strut, the instantaneous streamlines of the mainstream flow traverse towards the wall and strike the wall, thus carrying more activated platelets to be deposited distally (location F); therefore the platelet deposition is higher. The platelet deposition at the inlet to first strut and the outlet to last strut could not be compared because of the time difference in images taken at these regions; however the average normalized platelet deposition curves plotted shows that platelet deposition is higher at the outlet to last strut (see Fig. 18a, Fig. 22a). Since the platelets in the system are already in an activated state, these changes in platelet deposition upstream and downstream of the stent are mostly due to convective transport of activated platelets, rather than their activation in a vortex followed by their escape from that vortex to be deposited downstream. The deposition of activated platelets further downstream away from the stented region is beyond the scope of this study.

At the corner regions very close to the strut (proximal or distal to the strut), the platelet deposition was significantly lower when compared to other regions between the

struts. This may be due to two main reasons, and can be gleaned from fig. 19b: (1) the number of instantaneous streamlines closer to the struts on the wall reduces and (2) the instantaneous streamlines closer to the struts on the wall seem to transport the platelets away from the wall in that region (see Fig. 18b, locations A and C). This region of stagnant flow closer to the strut with significantly very low platelet deposition (when compared to other regions) is found in all stent strut spacing cases.

The point of separation (or reattachment) separates the region of recirculation from the mainstream flow. Platelet deposition was consistently lower in this region compared to just upstream or downstream of these locations. The platelet deposition in the reattachment point (see Fig. 20a, location G) is significantly lower than that in the nearby recirculation region (location H) or just downstream of the reattachment point (location F) because the instantaneous streamlines at this point of reattachment near the wall diverge from one another, and thus platelets and procoagulant agents are not carried to these locations (see Fig. 20b, location G). Similarly, the platelet deposition in the separation point (see Fig. 20a, location C) is significantly lower than that in the nearby recirculation region (location B) or just upstream of the separation point (location D). The instantaneous streamlines at the separation point near the wall traverse towards each other from either ends and then traverse away from the wall sharply, thus carrying platelets and procoagulant agents away from the wall at this location (see Fig. 20b, location C). However, there is still some platelet deposition shown in these points of separation or reattachment in the normalized deposition data (see Fig. 18a, Fig. 20a, Fig. 22a). There are two main reasons; (1) the point of reattachment moves slightly due to flow pulsatility; and (2) as time increases, the platelet aggregates grow to eventually cover up the reattachment point, as observed under the microscope; this may be why no statistical

significance was found in platelet deposition at the point of separation (location C) and the region of no flow disturbance (location D) at the inlet to first strut (Figure 19). The point of reattachment distal to the strut showed significantly higher platelet deposition compared to the deposition at the point of separation proximal to the strut (see Fig. 20a, locations G and C respectively). This is similar to the pattern of platelet deposition observed previously between the recirculation regions distal and proximal to the strut (see paragraph 3 of Discussion section). This is because the instantaneous streamlines of the mainstream flow distal to the strut traverse towards the wall and strike the wall, hence there is more deposition distally (see Fig. 20b, locations between E & F, F, E); however the instantaneous streamlines of the mainstream flow proximal to the strut separate from the wall and traverse away from the wall, hence there is less deposition proximally (locations B, C).

Platelet deposition decreases almost linearly from the point of reattachment to the point of separation in the largest strut spacing (from locations little downstream of F to C in fig. 20a). This decrease in platelet deposition is likely due to the decrease in platelet availability near the wall as platelets are deposited. Very little mixing occurs in this region (compared to the recirculation regions), so depletion of available platelets occurs in the flow confined to very near the wall.

The platelet deposition pattern between struts in the intermediate strut spacing has the least spatial variation of the three configurations (Figure 22). The platelet deposition pattern can be thought of as a combination between the deposition patterns in the smallest and largest strut spacing. Distal to the upstream strut, the platelet deposition was significantly higher than at the center (see Fig. 21a, locations D and C respectively). It could be postulated that this may be because (1) during the time points in the flow cycle

when the fluid reattaches to the wall between struts, the distal end of the strut would have more platelet deposition due to instantaneous streamlines striking the wall at an angle with activated platelets (see Fig. 21b, location C and D); (2) during the time points in the flow cycle when the fluid recirculates between struts, the two recirculation regions try to combine partially into a single flow stagnation zone with two slowly turning vortical structures (see Fig. 21c, locations B, C and D); there are no longer any individual separation or reattachment points, however, the platelet deposition pattern may be still similar to that seen above in (1); and (3) in the intermediate strut spacing, the flow remains totally recirculated only 40% of the time, while it remains reattached 60% of the time. If we combine (1), (2), and (3), the result is a platelet deposition pattern as seen in Fig. 22a. The probability of platelet attachment may increase because cells experience the varying flow patterns of one cycle. The reattached fluid entrains more activated platelets, while the slowly turning vortices trap them, increasing the time available for interaction with the wall.

For the intermediate strut spacing, the platelet deposition between struts increased in time zone 3 relative to time zones 1 and 2. It is to be noted that the analysis included normalization of data between struts from each time point with the inlet (or upstream to stent or region of no flow disturbance) data at roughly the same time point. Since we are mostly interested in platelet deposition at later time points, it was interesting to note significance between the latter two time zones (time zones 2 and 3). Also shown in the normalized platelet deposition data (Fig. 21a), the data at each time zone did not follow the same pattern; hence it could be concluded that the platelet deposition in regions between struts of the intermediate strut spacing was increasing in time, faster than at the inlet (or upstream to stent) region.

Platelet deposition patterns were compared to the instantaneous streamlines at minimum flow for almost all models except the intermediate strut spacing. The instantaneous streamlines at maximum flow looked the same as that at minimum flow for these models; this is because the flow pattern is similar throughout the flow cycle for these models. What is actually varying is the point of separation and reattachment if any, during the flow cycle. In the intermediate strut spacing model however, platelet deposition curves were compared to instantaneous streamlines at minimum flow and maximum flow; these instantaneous streamlines represent the two extreme flow patterns that this strut configuration was subjected to, during the flow cycle.

The intermediate strut spacing corresponded to highest platelet deposition in our previous study (Robaina S *et al.*, 2003). The observation in this study that platelet deposition is still increasing between the intermediate struts, compared with upstream, may confirm this previous observation.

4.2 Generic 3D Parametric Stented Models

Flow characteristics of four different stent strut designs were studied using 3D computational fluid dynamics, concentrating specifically on three strut design parameters – strut interspacing (h), spacing between concave to convex portion of the same strut (f , or “amplitude”), and radius of curvature of the strut (r). Four specific strut geometries were considered (see Fig. 12) under high (10 ± 5 dynes/cm²) and low (2 ± 10 dynes/cm²) shear stress flow conditions. The aim was to understand how stent design affects flow patterns, with an eventual aim to improve stent design to minimize restenosis.

Models without the axial connectors seem to have more benefit from the fluid dynamic point of view compared to models with the axial connectors. It is assumed that a

greater degree of restoration of the normal, physiologic WSS is beneficial to the artery wall. The percentage restoration in axial WSS in the models without connector was as much as 11% larger than their counterpart models with connector, for all strut geometries studied (see Fig. 29). The connector divided the symmetric central shear stress region and the shear stress itself was low in the region surrounding the connector. The separation parameter was <0.1 around the connector, indicating fluid reattachment for $>90\%$ of flow cycle. However, area exposed to the separation parameter <0.1 was greater for models without connector compared to those of models with connector. The area exposed to separation parameter >0.5 was less for models without connector under both high and low flows (see Fig. 31). The effect of connectors on the transverse WSS could be considered relatively insignificant under both flow conditions (see Fig. 30). Reducing strut-strut connectors by 29% reduced thrombosis by 69%, and neointimal hyperplasia by 38% (Rogers C *et al.*, 1995). Connectors between struts provide mechanical integrity to stent design and hence cannot be totally neglected; however, they should be used only when required and in parallel to the main flow.

The strut geometry with the largest interstrut spacing ($h=3.6\text{mm}$) seems to be comparatively more beneficial to the artery from its fluid dynamics (see Fig. 29). The mean axial WSS restoration between the struts was the greatest for the stent geometry (c) (67% of the nominal value for the model with connector, 76% for the model without connector). The general tendency in increase in percentage restoration of the average mean axial shear stress between the struts by 42% was observed when the strut parameter h was increased from 1.8mm to 3.6mm. Thus, with larger strut spacing, greater percentage of restoration of shear stress was observed. No significant restoration in WSS was seen when f was increased from 0.9mm to 1.8mm (accompanied by increase in r

from 0.45mm to 0.9mm); however decreased restoration in shear stress was observed when the strut parameter f was increased from 1.8mm to 3.6mm. There existed a narrow band with low mean axial shear stress between struts with increase in strut parameter f ; this band divided the shear stress region, hence lesser percentage restoration in shear stress was observed. For high flow, when strut parameter f was increased from 1.8mm to 3.6mm, the axial shear stress restored was decreased by 16%; however when f was increased from 0.9mm to 1.8mm (accompanied by increase in r from 0.45mm to 0.9mm), the axial shear stress restored was decreased only by 4%. This decreased restoration in axial shear stress is probably due to the combined effect of r and f . This was due to larger interstrut spacing that allowed for increased WSS restoration. Also, the struts were less perpendicular to the flow; hence the transverse shear stress was lower. Regions of recirculation for at least 50% of flow cycle (typified by separation parameters of >0.5) and regions of flow reattachment for most part of the flow cycle (typified by separation parameter 0.1-0.5) were also relatively low for this model under both high and low flows (see Fig. 31, 32). When the strut amplitude f was increased from 1.8mm to 3.6mm, the segments connecting the arcs of the same strut were more parallel to the flow; hence the shear stress and flow separation parameters studied for stent model (d) were close to that of the stent model (c). However, this strut geometry created a narrow band between the struts which causes a sharp local flow disturbance and because of the narrow band, there was a 16% decrease in mean axial WSS restoration. While the effects of such sharp local flow disturbances on intimal hyperplasia are unknown, it is presumably desirable to restore the WSS as much as possible. Thus, strut geometries should be based on increased interstrut spacing and lower intrastrut concave-to-convex arc spacing (i.e. strut geometry corresponding to stent model (c) may provide more benefits).

It is difficult to separate the effect of strut parameter f and r on shear stress and flow separation. The strut parameters f and r define the strut geometry to a large extent, hence the combined effect of both is significant. Increasing r and f reduced restoration of axial shear stress between the struts, and increased the transverse WSS, which was indicative of greater flow disturbance. Increasing r and f increased the area of flow recirculation for most part of the flow cycle characterized by separation parameter >0.5 ; however increasing f with same r did not cause any tremendous change in separation parameters. In all cases studied here, flow separation regions were concentrated near the proximal and distal portion of the apex and base of the strut. The circumferential pitch W of the flow chamber was kept constant while only the strut parameters h , f , and r were varied; if this distance is varied, the same strut parameters used for the simulations will produce different geometries; one could then deduce the effect on shear stress and flow separation from the cases studied.

An interesting observation from the contour plots showed that large mean axial shear stress occurred proximal to the distal strut (0.66mm, 0.42mm proximal to the second strut for high flow, low flow respectively); this means the regions of low WSS and hence the regions of recirculation, if any, are larger distal to the strut. This finding is consistent with other studies of stented artery flow patterns (Berry JL *et al.*, 2000; LaDisa JF Jr. *et al.*, 2003). The presence of stents creates regions of flow recirculation. In regions of complex flow, the relationships between flow and thrombosis are not very clear. Convection patterns and residence time may modulate platelet deposition in separated flows (Wootton DM *et al.*, 1999). Platelets are carried by the streamlines and more number of streamlines strike the arterial surface distal to the strut, hence potentially triggering greater deposition at those regions. The higher residence time in the

recirculation regions may favor platelet deposition (Karino T and Goldsmith HL, 1979). Therefore, platelet deposition may be higher distal to the struts. For the low flow the percentage restoration of mean axial shear stress between struts was lower than that for the high flow by 10-12% (see Fig. 29). Hence there seems to be more number of regions with relatively lower mean WSS under low flow conditions. Since higher WSS have been associated with reduced neointimal hyperplasia, the low flow condition seems to pose a higher potential threat towards restenosis (Hoffman R *et al.*, 1997, 1998; Carlier SG *et al.*, 2003). Regions of reattachment for most part of the flow cycle governed by separation parameter 0.1-0.5 were 82-90% higher under low flow compared to high flow condition; those regions may be sites of higher platelet deposition (Robaina S *et al.*, 2003).

The largest mean transverse shear stresses were 30-50% of the largest mean axial shear stresses. The large mean transverse shear stresses between the struts occurred in the regions where the mean axial shear stresses were low. From the fluid dynamic point of view, the transverse shear stress indicates a directional change in the fluid flow; this is evident from the streamlines. Platelets are sensitive to shear rates. Therefore, the resultant shear stress may have an effect on platelet adhesion. The study into the platelet deposition with high spatial resolution using these stent models may reveal the effects of the altered flow on platelet deposition.

The implications of shear stress on the reaction of the artery wall are not limited to platelet deposition. Endothelial cells responded differently to differing WSS applied in differing directions through mechanotransduction and by altering gene expression (Brooks AR *et al.*, 2002). Most notably, the expression of inflammatory cell adhesion molecules such as ICAM-1, VCAM-1 and E-selectin increases with exposure to low

shear stress (Sampath R *et al.*, 1995). It is clear that an effort to increase WSS after stent implantation would likely be beneficial. It should be noted that the procedure of stent implantation results in denudation of the endothelium, yet it has been shown that remnant endothelial patches remain between the struts (Rogers C *et al.*, 1999). Presumably, these patches of endothelial cells, along with those that migrate or proliferate into the denuded areas, will fare better in the presence of shear stress.

The comparison of the flow characteristics between different strut configurations did not change under high or low flow conditions (Fig. 29, 30, 31, 32), however the figures give a nice visual and quantitative description of variations in WSS and separation parameter of a single strut configuration under high and low flow conditions. For example, all strut design models had descending normalized mean axial WSS for high flow, which followed the same order under low flow (Fig. 29). Therefore, the effects of strut configuration on flow characteristics were basically the same under the high and low flow utilized in the study. However, Fig. 29 also revealed that the percentage restoration in axial shear stress was lower for the low flow by 10-12%. This indicates that regions of already low shear stress will be more easily affected by the stent geometry.

The numerical results are coherent with the experimental results using a square lumen as the stented artery model (Benard N *et al.*, 2003). Three weak WSS regions were identified through particle image velocimetry method. These regions correspond to the proximal and distal regions of the base defined in fig. 12, where flow recirculation occurs. Our simulation results can also give more information about the “critical shearing” zone identified in the experiment owing to the much better spatial resolution of numerical simulations. WSS is restored to a large extent in this zone because of the large inter-strut spacing.

The height of the stent strut is a little higher than those used in the clinical trials (Kastrati AJ *et al.*, 2001). The higher strut may increase the flow disturbance around the strut from the fluid dynamics point of view. The same height was used for all the models in the study. The trends and the comparative results obtained should not be greatly affected by small differences in height.

The mechanical interaction of the stent and the artery wall should also be considered while optimizing stent design. This study focused on the fluid dynamics of stented arteries. The larger inter-strut spacing would allow greater extent of flow restoration. The stents would be weaker in this case because the thickness of the strut is limited too. The vascular tissue may prolapse between struts if the inter-strut spacing is too large. The establishment of an optimal inter-strut spacing would involve consideration of both fluid dynamics and the solid mechanics of stent/artery interaction.

Arteries are always being subjected to pulsatile flow *in vivo*. One of the strengths of this study was the inclusion of pulsatile flow, which had an important influence on the WSS. Figure 13a and 13b show the axial WSS oscillating as a function of time. All time points in the flow cycle can be detected using the time point t1 as a reference in figures 13a or 13b. At time points in the flow cycle with the same velocity but where flow accelerates (i.e. 50th time point) and flow decelerates (i.e. 100th time point), the instantaneous axial WSS between the two time points varied by ~28% and the instantaneous transverse WSS varied by ~23% for the high flow condition. The percentage change in the instantaneous WSS were higher for the low flow condition. The instantaneous WSS also changed around the time point of minimum flow (i.e. 20th and 30th time points) and around the time point of peak flow (i.e. 70th and 80th time points); note that the paired time points (as above) have the same value of velocity. Such changes

in WSS indicate the importance of accounting for pulsatility in analyzing flow in stented arteries.

The present results should be interpreted within the constraints of potential limitations. Accuracy from CFD results depends on computational domain and computational mesh size. The computational domain in the cross-stream direction was flat, rather than curved, since the height of the stent strut is 8% of a 1.8 mm arterial radius. Furthermore, the lumen of the stented artery may have a polygonal shape with each strut serving as a vertex immediate after stenting (Garasic JM *et al.*, 2000). The aim of these simulations was to predict the near-wall flow disturbances of stented arteries and hence the computational domain is justified. There exists prolapse of vascular tissues between struts after stenting. No prolapse was considered in this study. The level of prolapse has been demonstrated to depend on stent design (Prendergast PJ *et al.*, 2003). A large degree of prolapse might have an effect on the flow patterns. Small intimal flaps protruding between stent struts would certainly have a significant effect on the flow patterns demonstrated here. With regard to artery wall motion due to cycling pressure, it is important to note that stents distend the artery wall to a non-physiologic degree, so the artery wall is already highly stretched. This fact, coupled with the relatively close strut spacing, means that the artery wall probably does not undergo large enough deformation over the cardiac cycle to influence the flow patterns. This was confirmed previously in 2D (Henry FS, 2000). Our computational models were shown to be mesh independent based on convergence (<4.6%) of WSS values. Time step independence was also determined from convergence (<5%) of the mean axial WSS values. Increasing mesh size or the number of time points within a flow cycle may have improved our results slightly, but would take an enormous amount of time. Moreover, the convergence in our results is

within acceptable limits along with the rigid wall assumption (LaDisa JF Jr. *et al.*, 2003; Myers JG *et al.*, 2001; Back M *et al.*, 1994). The stent-to artery diameter ratio in the stented section should be larger than one in order for stents to scaffold against the diseased arteries. The use of a larger (1.2:1) deployment ratio has been shown to increase exposure to low WSS by 12-fold compared with stents implanted in a 1.1:1 stent-to-vessel ratio (LaDisa JF Jr. *et al.*, 2004). The present study did not consider the effects of stent-to-artery ratio. The assumption that blood is a Newtonian fluid, while generally valid in the medium to large size arteries that are typically stented, may affect the flow patterns in the low shear regions near the stent struts. Nevertheless, the flow characteristics demonstrated in this 3D unsteady flow study would be expected to be indicative of the *in vivo* flow patterns to the extent that they are used to compare different stent designs.

In conclusion, from the hemodynamic point of view, guidance for stent design optimization could be obtained from the results of this study. The longitudinal distance between adjacent rows of struts should be larger in order to maximize WSS. Additionally, the struts should be wherever possible oriented parallel to the vessel axis to reduce the area of flow recirculation. Longitudinal connectors should be used only as necessary. Further experimental investigations (both *in vitro* and *in vivo*) should be performed to establish more directly the relationship between these 3D CFD results and the processes of restenosis.

4.3 3D Experimental Stented Models

In our previous study, localized platelet deposition on the wall in a 2D stented region was shown to vary significantly with different stent strut spacings (Duraishwamy N

et al., 2005). Platelet deposition varied at all points within the stent struts; for example, deposition was very low at the reattachment or separation point; deposition was higher distal to the strut compared to proximal region of the strut; regions of recirculation distal to the strut had greater platelet deposition compared to regions of recirculation proximal to the strut; etc. However, the geometry of the stent was relatively simple, characterized by only one single design parameter, the ratio of the distance between the centers of two struts (L) to the strut height (H). Real world stents are however far more complex and a single design parameter cannot characterize them. The purpose of this study, using fluorescently labeled platelets, was to measure the platelet deposition between 3D stent struts in order to directly relate this deposition pattern to flow dynamics and stent design and their similarities, if any, with 2D stents studies conducted previously.

We postulate again that the medium through which activated platelets get attached to the wall are the instantaneous streamlines that depict the fluid flow pattern; thus the platelet flow and hence attachment is convection driven.

In Wall stents, platelet deposition was always lower proximal to the intersection of two struts (location A in Figure 35b, Figure 36b, 36c) because (1) the mainstream flow separated from the wall, and (2) the fluid that flows between the wall and the strut in the flow field tries to separate from the wall with the mainstream flow, but finally makes a bend to enter this region (Figure 35c, 36d). This results in less convection of activated platelets to the wall near the intersection of the struts. As indicated in Figure 48, platelet deposition is always higher in areas where there are streamlines with components perpendicular to the wall and flow is directed *towards* the wall (upstream and downstream of separation and reattachment points, and in the center of recirculation regions) than in areas where streamlines are parallel (with no curvature, as in fully

developed flow regions and downstream of the proximal recirculation region) or directed away from the wall (proximal to a strut). Platelet deposition is also always lower distal to the intersection of two struts (location D in Figure 36b) because the fluid that flows between the wall and the strut in the flow field tries to reattach to the mainstream flow by making a bend away from the wall and then curving back towards the wall to join the mainstream flow (Figure 37c). Note that when the streamlines are directed away from the wall, the convection of activated platelets to that region is low. Note here that the streamlines proximal to the strut and distal to the strut, in the region between struts, are the same as that for the inlet proximal to first strut and the outlet distal to last strut respectively. Regions proximal to the strut had 65% larger deposition (location B in Figure 35b) with time compared to region of no flow disturbance; this is due to the existence of the strut in the flow field. The instantaneous streamlines strike proximal to the strut and deposit activated platelets on the strut. As time progresses, more platelets get attached that may form a platelet plug, which may further help in more platelets attaching to the nearby wall. The formation of such platelets plugs could block the flow through the gap between the stent in the flow field and the wall in that region, thus changing the flow dynamics. However, wherever this gap was larger (particularly at the intersection point of the two struts), there seemed to be always flow between the wall and the stent in the flow field. The same is true for platelet deposition proximal to the strut, of the region between struts (geometry 1 or geometry 2 in Figure 36a).

The bend or turn of a streamline is not just directed away proximal to the strut or towards the wall distal to the strut. Figure 35d shows the streamlines entering the gap between the wall and the strut in flow field, directed away from the strut on the wall distally. Thus convection seems to carry platelets away from this strut on the wall. This is

also another reason why there is very less platelet deposition proximal to the strut on the wall compared to the strut in the flow field (Figure 35a, Figure 36a). Note that the platelet deposition in this case was an observation from the images and not any quantitative data.

Platelet deposition (in the direction of mainstream flow) distal to the proximal strut (location C in Figure 36b, location B in Figure 37b, location C in Figure 39a, location E in Figure 42a) was higher than further downstream (location B and C and B and B respectively); this is mainly due to the instantaneous streamlines carrying activated platelets directed towards the wall, distal to the proximal strut. The decrease from location C to B in Figure 36b, from location B to A in Figure 42b, from C to B in Figure 39a, and from E to B in Fig. 42a is linear due to the depletion of platelets on the wall as the streamlines get more parallel to the wall. At time=10-20min in Figure 37b, most of the data was taken concentrating on location D of Figure 37a; however at time>20min, most of the data concentrated on location E of Figure 37a. At time>20min, the decrease in platelet deposition after location B may be caused by a strut (not visible in Figure 37a) in the flow field, that may be causing some flow disturbances in that area leading to the reduction. However, it is important to note that platelet deposition was always higher at the outlet to the last strut compared to the inlet to first strut (see Figures 35a, 37a, 38b, 42a). This was similar in all stent types except NIR stent, which will be discussed later.

Width of the struts in the circumferential direction could influence the platelet deposition greatly. In Figure 42a, the platelet deposition was the maximum at location D, instead of at location E, as was the case with other stent types. Since the struts were very closely associated at the proximal end, more streamlines with activated platelets were directed away from the proximal end and hence, deposition may be higher a little downstream of the proximal strut. The deposition pattern showed that the decrease in

platelet deposition with axial distance was linear but slow in Aurora stent compared to other stent types like Bx Velocity stent (see Fig. 39a also); location D was significantly higher than location B in Figure 42a. Since the axial distance was larger in Aurora stent compared to other stent types, one would think that the platelets would get easily depleted. One possible explanation could be that (1) before the streamlines start to get parallel to the wall, the converging region of the next strut begins; the decrease in platelet deposition we see may be just due to flow separation from the wall in this region rather than depletion of platelets near the wall; (2) ideally platelets would get together when flow converges; in the other models like Bx Velocity stent, the platelets have already depleted when the converging section of the strut begins, so we may be unable to see a major change in platelet adhesion in this section; however, for Aurora stent, platelets may get together at the converging section of the strut and thus further slow the rate of platelet depletion near the wall.

Computationally, there is no significant difference in the fluid flow within geometry 1 and 2 of Wall stent except the difference in the entry and exit of fluid into or from geometry (Figure 36d). Platelet deposition in the direction of mainstream flow has the same pattern of deposition for struts of geometry 1 and geometry 2. Platelet deposition (perpendicular to the mainstream flow) was higher at the center (location B in Figure 36c) due to activated platelets attaching as a result of being carried towards the wall, distal to the proximal strut. However, the regions close to the struts (locations A or C) could have higher or lower platelet deposition depending on the geometry of the struts. In geometry 1, fluid enters from the gap between the strut and the wall on the top and side, hence location C may have slightly higher platelet deposition compared to location A. In geometry 2, fluid enters from the gap between the strut and the wall at the

bottom and side, hence location A may have slightly higher platelet deposition compared to location C.

Platelet deposition curves for two different time zones $\text{time}=10\text{-}20\text{min}$ and $\text{time}>20\text{min}$ was plotted only for Wall stent to see if time had a significant effect on deposition. Figures 35-37 show that time did not have a significant difference on platelet deposition. Hence, for other stent types, deposition patterns for $\text{time}>20\text{min}$ were shown and statistically analyzed.

All stent types except Wall stent have convex and concave ends of the first strut subjected to flow. The words convex and concave have been used subjectively. Here, they are defined based on the orientation of the strut to the mainstream flow. The platelet deposition patterns can be very different near both ends. Platelet deposition was higher just proximal (tip) to (of) the convex end of the first strut (location D in Figure 38a, location B in Figure 38b) compared to the region of no flow disturbance; the instantaneous streamlines with activated platelets strike the strut that was obstructing the flow field and deposits platelets on the strut or nearby the wall (Figure 38d). As the fluid travels within the stent, towards the concave end, the deposition was lower near the end of the strut (location A in Figure 38c, also true for Aurora stent); this was due to the small region of recirculation that is strictly separated and does not receive many platelets from the mainstream flow (see Figure 38e). The deposition was higher (location B) some distance away from the proximal end; this region corresponds to the flow separation from the wall, which means that deposition would not be significantly different from the region of no flow disturbance (location C). However the deposition closer to the struts (inclined portions, or connectors of concave and convex ends) was higher, which may have increased the platelet intensity after processing at location B. Note that for Aurora stent,

this may not be true as the struts were more inclined in the direction of flow and less platelet deposition may be seen in a similar zone. However, the above statement cannot be told for certain at this point since only two experiments were done with Aurora stent. Analysis of data from more experiments of this type would show if this would really be the case. This statement is supported by the 3D computational simulations that show the more parallel the connector between the convex and concave ends of the strut to the mainstream flow, the less separation near that connector (see Figures 23-28 or see He Y *et al.*, 2005).

The above observation of deposition being higher at the convex end of the strut was made only at the inlet just before the first strut and was usually not present in the region between struts. It may be possible that the platelets interact a foreign object for the first time at the inlet and hence, there is more deposition at the inlet just before first strut. As time progresses, more platelets may be getting adhered and activated at the inlet compared to a similar geometrical location within the stent. As said earlier, more experiments have to be done to evaluate the effect of platelet deposition near the connector of convex and concave end of the strut and relate to its inclination to the flow.

For Bx Velocity stent, the distance between the struts was ~3mm (see location B to A in Figure 39a), this was large enough to allow for deposition to plateau (similar to the region of no flow disturbance). This plateau in platelet deposition was due to the depletion of platelets as the streamlines get more parallel to the wall distal to the proximal strut. This is also expected to be observed at the outlet to the stent for all stent types.

Regions of recirculation proximal to the strut in 3D stents have very low platelet deposition (see location A in Figure 39a, location A in Figure 42a). Again, this was

because of flow separation from the wall proximal to the strut and a tight region of recirculation that remained fully separated such that too much mixing of platelets from the mainstream flow did not occur (Figure 39d). The region of recirculation distal to the strut in 3D stents also has very low platelet deposition (location D in Figure 39a, location F in Figure 42a). One would have expected at least the recirculation regions distal to the strut to have more platelet deposition because of flow reattachment distal to the strut that gets more activated platelets to the wall. This may be again because of a tight region of recirculation (see Figures 39b and 39c) and the geometry of the strut.

Regions of recirculation near the connector region of two struts (locations A and B in Figure 43a) of Aurora stent, had very low platelet deposition due to the well-separated recirculation region (as shown by the streamlines in Figure 43b). The previous statement is based on purely qualitative data from the experimental images obtained and from previous observations of low platelet deposition in recirculation regions of other stent types. Image processing would result in averaging the pixels where the connector was present between the struts and therefore would not be appropriate for analysis.

Flow reattachment points and flow separation points were not visible in 3D stents, because of the low (4x) magnification of the lens used for image capture. However, the reattachment points and separation points were visible in Aurora stent as seen in Figure 43a. Note the platelet deposition was low in these regions as the flow was directed away from the wall.

Platelet deposition at the outlet, distal to the concave and convex ends of a strut were different. The peak in platelet deposition close to the concave end of the last strut (location B of Figure 41a) was caused by a combination of the lack of recirculation region distal to the strut and the intersection of streamlines from either sides of the strut

(see Figure 41c). The deposition seemed to be decreasing exponentially further downstream and this may be due to the lack of a flow separation region distal to the last strut; streamlines are more parallel to the wall after the intersection point near the concave end of the last strut and thus platelet deposition may be decreasing exponentially. The peak in platelet deposition at the convex end of the last strut (location B of Figure 41b) was located distal to the region of recirculation (see Figure 41d) due to streamlines carrying activated platelets directed towards the wall; the platelet deposition decreased linearly further downstream due to the existence of a flow separation region; as the streamlines further downstream get more parallel to the wall, the platelets get depleted and hence, the deposition decreases linearly. The above was observed in Bx Velocity and Aurora stents. As stated earlier, recirculation regions have very low platelet deposition (location C of Figure 41b).

Each stent type has a different design for a connector between the struts. Platelet deposition within the connector (location C of Figure 40b, location B of Figure 46b) of Bx Velocity stent was significantly higher than in other locations of the connector (like locations B or E in Figure 40b) and regions proximal to the first strut (location F of Figure 40b, location C of Figure 46b). This may be because (1) of the existence of a spirally recirculating region (see Figure 40c and 40d, within location C or D, and see Figure 46c, within location B) that provides for adequate mixing of platelets from the mainstream of flow; (2) some locations (like locations C and D of Bx Velocity stent, location B of Aurora stent) are wider than others (for example, locations B or E of Bx Velocity stent); and (3) the spiral nature of the flow may help in increasing the residence time of the platelets on the wall. Note that at location B, the fluid generally does not undergo more than one complete recirculation (see Figure 40d); this is not the same in

location E. One could then propose that the decrease in residence time of the platelets in location B may cause decrease in platelet deposition over time in this region compared to location E. Thus the spiral nature of the flow tends to deposit more platelets on the wall. Again, platelet deposition within the region between two non-connecting struts of Aurora stent (see Figure 44a) was higher than in the region proximal to the first strut; this was due to the existence of spiral flow between the two struts that allowed entrainment of more activated platelets from the mainstream flow.

All of the four stent designs studied had connectors between all stent struts. Previous 3D computational simulations between four different parametric stent design types showed that the lack of a connector between stent struts increased the percentage of wall shear stress restoration and decreased the level of flow separation near the wall or the struts (see Fig. 29, 32 or see He Y *et al.*, 2005). Hence, one should consider the advantages of having a connector between the struts at the time of the design. We have also seen from the images previously that the more complicated the design of the connector, the more complex the flow mechanics in that region. Hence, one should consider the advantages of having a certain design criteria for the connector.

In the semi-expanded stents (where the stent-to-artery diameter ratio was less than or equal to 1.2:1), the outlet distal to last strut had much higher platelet deposition compared to the inlet proximal to first strut. However, in an over-expanded stent, for example in NIR stent, the platelet deposition at the inlet and outlet to stent were the same as that of the region of no flow disturbance (see Figures 45a, 45b). At the inlet, there is a large recirculation region, the reattachment point of which exists at a point between the first and the second struts (see Figure 46b); as a result, the platelets were probably deposited just before the reattachment point in the recirculation zone and therefore

trapped between the struts. Thus, the inlet just before first strut may be having less convection of activated platelets and hence, lesser deposition. However, at the outlet, the recirculation region is small, but the streamlines may be convecting more platelets to that recirculation region and hence, less deposition just distal to the last strut. Since platelet deposition has been found to be greater distal to the strut; here also we see slight increases in platelet deposition in location A compared to location B of Figure 45b.

The platelet deposition peaked distal to the proximal strut and decreased linearly further downstream, in the region between struts of other stent types. For the over-expanded NIR stent, the deposition was greater at the center compared to regions close to the strut (see Figure 46a). Between locations B and C was a small decrease in platelet deposition and this region may be associated to the reattachment point (see Figure 46b). Deposition was slightly greater proximal to location C and decreased linearly towards the proximal strut. Deposition was also slightly greater distal to location B and decreased linearly towards the distal strut. Increase in deposition proximal to location C or distal to location B was because of the streamlines that were directed towards the wall and then as they get more parallel to the wall, the platelets were depleted and the deposition decreased linearly. Hence, deposition was higher at the center and lower closer to the struts.

Just as we see variation in platelet deposition axially along the wall, interestingly we also see variation in platelet deposition circumferentially along the wall. See Figures 42a and 43a. Note that circumferentially along the wall, there are alternative regions of lower and higher platelet deposition. This was mostly a qualitative observation, based on quantitative data from other observations stated previously. This must be very common in

most stent types that have a stent design similar to that of Bx Velocity stent and Aurora stent. The repercussions of this observation over long-term restenosis are unknown.

4.4 General Discussion on Platelet Deposition and Its Relationship to Flow Dynamics

This research study presents dynamic flow experiments with fluorescently labeled platelets to allow for spatial observation of wall attachment in inter-strut spacings of stents (three-dimensional – currently marketed *in vivo* stents, two-dimensional – idealized versions of three-dimensional stents) to investigate their relationship to flow patterns. Human blood with fluorescently labeled platelets was circulated through an *in vitro* system that produced physiologic pulsatile flow in a flow chamber that featured different stent designs. Analysis and comparisons are made between 2D stented models and 3D stents. It is important to keep in mind that 2D stented models were very simple characterized on a single design parameter, while the 3D stents were more complex characterized by several different design parameters.

Spatially resolved distribution of platelets was obtained by imaging fluorescently labeled platelets between the struts. Platelet deposition was higher in areas where flow is directed towards the wall, and lower in areas where flow is directed away from the wall. Fully separated regions of recirculation both proximal and distal to the strut were very small and exhibited very low platelet deposition in 3D stents. This was because of less mixing of blood from the mainstream flow, thus low amount of activated platelets were available for deposition. Contradictory to this, it was statistically proved that regions of recirculation distal to the strut had more platelet deposition in 2D stent models. The flow detachment and reattachment points could not be easily deciphered using the low spatial

resolution in 3D stents. But in 2D stents, these deattachment and reattachment points were very obvious both from the images and the background corrected image intensity variation plots.

Deposition at the connector region depended on the connector geometry; spirally recirculating regions had more deposition compared to fully separated regions of recirculation proximal and distal to the strut. These spirally recirculating regions were observed only for our 3D stents and not from the 2D idealization models. The spirally recirculating regions had more complex fluid dynamics than fully separated recirculation regions proximal or distal to the strut. The use of connectors between stent struts and the design of the connector is critical towards fluid dynamics and platelet deposition. Therefore connector design requires careful review.

Circumferentially along the wall in 3D stents, alternative regions of higher and lower platelet deposition was found. In 2D stent models however, platelet deposition was evenly distributed all along the direction perpendicular to the flow.

We have seen that the decrease in platelet deposition with axial distance is mostly a function of how fast the streamlines get parallel to the wall. More number of struts placed circumferentially like in Aurora stent resulted in depletion of platelets distal to the proximal strut in the flow direction at a lower rate compared to lesser number of struts placed circumferentially like in Bx Velocity stent. It is important to note that increasing strut number circumferentially increases the number of connectors, connecting the concave and convex ends of the same strut, oriented parallel to the flow direction. This would imply reduced regions of recirculation around the struts, but increase in the number of regions with alternating higher and lower platelet deposition circumferentially. Hence, one cannot imply that lesser number of struts would result in less platelet

deposition. Lesser number of struts definitely cause less vascular injury, but platelet deposition is driven largely by the fluid dynamics within the stented region. Other design factors like axial and circumferential distance between struts, connector design, and orientation or strut design (that act in tandem) are important for quantifying overall platelet deposition.

Semi-expanded stents into the artery wall had more platelet deposition at the outlet compared to over-expanded stent. Over-expanded stents had large regions of recirculation primarily at the inlet to the stent, combined with small regions of recirculation at the outlet. The variation in data was much lower in the over-expanded NIR stent. The reason for this is that the platelet deposition at the inlet just before over-expansion was more uniform and higher compared to other stent types. This pattern of platelet deposition is related to the presence of large recirculation region in the region around the over-expansion. The use of this data for background correction of images at the stented region resulted in lesser variation in the results.

What is then the relationship of wall shear stress to platelet deposition? Fig. 49 shows the variation of wall shear stress in the largest strut spacing and the corresponding platelet deposition variation. Proximal to the distal strut and distal to the proximal strut, there are regions of recirculation and the wall shear stress is low (see Fig. 49b). However, platelet deposition is higher distal to the proximal strut and lower proximal to the distal strut (see Fig. 20a). Between these regions of recirculation, the wall shear stress increases and decreases along the flow direction (see Fig. 49b). But the platelet deposition continuously decreases linearly between the struts (see Fig. 20a). Therefore, a relationship between wall shear stress variation and platelet deposition was not seen from this study. Platelet deposition was hence purely attributed to convective transport of

activated platelets through the action of instantaneous streamlines. This does not mean that wall shear stress is not important in the restenosis problem. It definitely is, but could play a more prominent role in the migration and proliferation of cells. Migration and proliferation of endothelial cells would have an effect on platelet adhesion *in vivo*. But platelet adhesion, activation, and aggregation occurs much faster (few minutes to hours) than the formation of a healthy endothelial lining (few days) (Haudenschild CC and Schwartz SM, 1979). Therefore, the results from this research are valid for the acute stages of stent implantation.

$$\sigma_{mean} = \frac{WSS_{stented}}{WSS_{unstented}}$$

$$WSS_{axial} = -\mu \frac{\partial u}{\partial y}$$

Figure 49. Mean wall shear stress distribution between struts of largest strut spacing; locations A to I represent the same locations as in Fig. 20a for comparisons.

The limitations of this study include the simplified 2D stent geometry, the constitutive model of the blood used in the CFD, and the fact that the phenomena being modeled here are limited to the acute stages of stent implantation. The limitation in the

2D stented study is that the geometry is simple and may not simulate the 3D stented *in vivo* behavior. However, experiments were performed under pulsatile flow conditions with physiologically relevant shear stresses and the analysis allowed for just one single stent design parameter, L/H. Later on, currently marketed 3D *in vivo* stents were used for experimentation and analysis of platelet deposition. Platelet adhesion and aggregation is the first step in the restenosis process. Therefore measurement of platelet deposition in the acute stages of stent implantation is valid.

In the CFD study, blood was considered to be incompressible and Newtonian fluid (Perktold K and Rappitsch G, 1995. Blood may show non-Newtonian behavior at low shear rates especially in the capillaries. The wall reaction to flow in the stented region has long been shown to be dependent on wall shear stress (Carlier SG *et al.*, 2003; Gijssen JH *et al.*, 2003). This factor would be important for determining the long-term effects of intimal hyperplasia or restenosis. But as a result of this research, we have been able to relate platelet deposition to flow patterns in the acute stages of stent implantation. Though the causes of long term restenosis using stents are still unknown, this research may help us to design stents that would avoid excessive platelet aggregation. Recirculation within stents is unavoidable, however future stents could be designed to avoid increasing number of recirculation regions so that flow disturbance and platelet aggregation can be reduced and this may help reduce restenosis rate.

Arteries near branches, curvatures, and stenoses are subjected to complex flow phenomena. We modeled the flow in a straight section of the artery in this study. This approach was good as it revealed the mechanics behind platelet deposition to fluid dynamics. More complex stented geometries could be modeled in future for better understanding of the platelet adhesion process.

Our results suggest that convective transport plays an essential role in the interaction of blood platelets with the endothelial or exposed underlying collagen layer. An exposed collagen layer naturally attracts platelets, which enhance endothelial cell migration. This process also affects the development of intimal hyperplasia (IH). The development of the IH may change the surface contours with the stented region, thus disturbing the flow pattern; this may in turn result in a different convective transport pattern of cells. There is limited literature on the 2D and 3D stent studies on platelet deposition in stented regions; hence, it is possible that some of the findings of 2D and 3D stents as a result of our research may be applicable to other natural cylindrical stent models available in the market today. It has been demonstrated that slight changes in geometry of 3D stents can cause significant changes in flow patterns and wall shear stress (He Y *et al.*, 2005).

To analyze the applicability of the 2D stent results in 3D stents, real 3D marketed stents were used to study platelet deposition and see the similarities and differences in both studies; they were summarized previously. One of the major limitations of the 3D stented study was the use of lower magnification lens to capture images. This did not give us the spatial resolution as high as that in 2D stented models (1 μm). However, many of the distribution patterns in platelet deposition in 3D could be characterized based on analysis of streamline information from computational fluid dynamics (CFD) and their relationship to deposition patterns in 2D (see Fig. 48). Higher spatial resolution and concentration of specific locations in the 3D stents need to be investigated further experimentally. This is possible by use of a 2D idealization model of a 3D stent for experimentation. One could then transpose these results to other 3D stented models.

Computational modeling of 3D stent geometries assumed circumferential and axial stretching of the vessel wall and the strut height was $<8\%$ of the arterial radius, hence the 3D geometry was planar. In real stented arteries, this assumption is very true. The artery wall is assumed to be rigid both for computational and experimental purposes. The effect of wall compliance on flow patterns needs to be investigated further. It has been shown recently that the temporal wall shear stress gradients are significantly different in a polygonal stented artery compared to a circular stented artery (LaDisa JF Jr. *et al.*, 2005). Also the streamlines obtained from the model for over-expanded NIR stent and its relationship to platelet deposition patterns at inlet and between first and second struts proved that the assumption was not insufficient; real-time movies of platelets between first and second struts of NIR stent showed existence of reversed flow regions and possible reattachment points. Hence, the streamline information obtained as a result of modeling the 3D geometry as planar is accurate in the context of this study.

A question that arises as a result of this research – what would be an optimal stent design? A simple answer would be something that has maximum number of struts oriented circumferentially to maintain a circular geometry after implantation, minimum of struts along the axial flow direction, simple geometry for the connector (mainly oriented along flow direction), and to have connectors only where necessary.

4.5 Conclusion and Future work

We have identified distinct flow regions within struts, such as recirculation, reattachment, and separation, and correlated these flow regions with localized platelet deposition in a highly resolved scale. Previous measurements of platelet deposition in an expanded tube (Karino T and Goldsmith HL, 1979) showed a peak in platelet adhesion in

the vortex in which the platelet density was appreciably greater than that downstream of the reattachment point where fully developed laminar flow was established. They attributed platelet deposition to convective transport of platelets through the instantaneous streamlines. Distal to the tubular expansion, ie. just downstream of the reattachment point, the platelet attachment was lower than in the region of recirculation because of the decreased velocity of platelets striking the wall. The geometry of the tubular expansion was different from that of the stented region considered here, and the shear stresses in the tubular expansion geometry were much higher. However, at the reattachment point, platelet adhesion was a minimum. This may be a result of the wall reaction rate and wall shear stress. If the wall reaction rate is linearly dependent on the wall shear stress, the platelet flux is maximum upstream of the stagnation point (David T *et al.*, 2001). Aneurysm and arterial stenosis flow models showed greater platelet deposition in the regions of recirculation at the distal end for low Reynolds numbers (Bluestein D *et al.*, 1996, 1997). Results obtained in the regions of recirculation, separation and reattachment in our research (Chapter 3 and 4: sections 4.1, 4.2, 4.3, 4.4) confirm these previous findings.

The use of streamlines for quantification of platelets in each location between the stent struts is a valuable tool. Based on inferences made from both 2D and 3D stent studies so far, it may be possible to guess the platelet deposition in a region not analyzed experimentally. For example, in NIR stent, images of platelet deposition at locations A (proximal to connector in Figure 50a) and B (distal to connector) were not taken; what would be the degree of platelet deposition within these locations? Assume that we are looking at deposition far away from the recirculation region at the inlet. The instantaneous streamlines from CFD show that there is a region of separation away from

the wall proximal to the connector while no flow separation occurs distal to the connector (see Figures 50b, 50c). Apart from streamlines that carry activated platelets from location A to location B, Figure 50a shows that streamlines from the center of the struts also carry activated platelets down to location B. Based on the above, platelet deposition is expected to be lower in location A and higher in location B. For example, in Bx Velocity stent, there was more platelet deposition at the inlet near the connector of the concave and convex ends of the first strut at the inlet (see Figure 38a); this was similar to the 3D stented geometry (see Fig. 23c) where a separation parameter was used to characterize regions of the wall susceptible to platelet deposition; separation parameter existed all along the inclined portions of the connector. However, in Aurora stent, there was no platelet deposition near the connector of the concave and convex ends of the first strut. This difference in platelet deposition is related to the degree of stent strut orientation in the flow field; the connectors connecting the concave and convex end of the strut in Aurora stent were oriented in the direction of flow and hence no deposition was seen. This observation is similar to the 3D stented geometry (see Fig. 28c) where separation parameter existed at the apex and base of the struts. 3D CFD evaluations of other stented geometries were helpful to analyze current experimental results also. The above example showed that the more inclined the strut to the flow direction, the greater the platelet deposition along the length of the connector. Thus, CFD analysis can be very useful in relating geometrical changes to platelet deposition. Further experimental investigations should be performed under higher spatial resolutions to confirm platelet deposition patterns in and around complex recirculation regions and also at the converging and diverging junctions of the struts.

Figure 50. (a) Streamlines (at maximum flow rate) above the proximal portion of the connector have the capability to travel distal to the connector for NIR stent; (b) streamlines proximal to the connector undergo flow separation; (c) streamlines distal to the connector do not undergo flow separation. One could then expect that the platelet deposition at region E would be greater than at region D.

Quantification of relative rates of platelet deposition requires more complex modeling of particle-wall interactions in non-parallel flow domains (Longest PW and Kleinstreuer C, 2003). Many arteries may be subjected to reversed flows; the platelet

deposition could vary spatially due to these reversed flows. In 2D stents, we think that reversed flow may cause platelet deposition to be evenly distributed within the stent struts. In 3D stents, the deposition pattern may be more complex. Additional computations performed based on generic parametric 3D geometries and their comparison showed that regions of partial recirculation or partial reattachment were greater in reversed flow compared to forward flow (see Fig. 32). Further investigations of fluorescent platelet tracking could be done to reveal the exact mechanisms involved in platelet deposition under reversed flow conditions.

This study has demonstrated the effects of geometric configuration of stents on the flow characteristics and platelet deposition under physiological shear and pulsatile flow conditions in the acute stages of implantation. There are likely other portions of the restenosis process that can be modulated by changes in stent geometry. Such information can be used to improve stent design. For example, stent designs like the compliance matching stent have been shown to restrict local restenosis *in vivo* (Rolland PH *et al.*, 2004). One of the main aims of this study was to show that fluid dynamics plays an important role in platelet deposition in stented regions. Further experimental investigations (both *in vitro* and *in vivo*) should be performed to correlate platelet deposition with other factors involved in restenosis - (1) many of the processes of thrombus and neointimal formation were not considered as part of this research; (2) stent-to-wall interactions involved in arterial remodeling were neglected and could form the basis for another research study; (3) changes in the arterial geometry due to existence of already formed plaque were omitted; (4) circumferential mechanical stresses exerted on the wall due to stent design were not considered; and (5) existence of tissue prolapse and arterial compliance on the arterial wall strain and flow mechanics is unknown. Before we

have answers to these processes mentioned above, it would not be appropriate to say if we have an optimal stent design.

REFERENCES

1. Akagawa E, Ookawa K, and Ohshima N. Endovascular stent configuration affects intraluminal flow dynamics and *in vitro* endothelialization. *Biorheol.* 2004; 41:665-680.
2. Alkjaersig N, Fletcher AP, and Sherry S. The mechanism of clot dissolution by plasmin. *J Clin Invest.* 1959; 38:1086-1095.
3. Altmann DB, Racz M, Battleman DS, Bergman G, Spokojny A, Hannan EL, and Sanborn TA. Reduction in angioplasty complications after the introduction of coronary stents: results from a consecutive series of 2242 patients. *Am Heart J.* 1996; 132:503-507.
4. Aoki J, Serruys PW, van Beusekom H, Ong AT, McFadden EP, Sianos G, van der Giessen WJ, Regar E, de Feyter PJ, Davis HR, Rowland S, and Kutryk MJ. Endothelial progenitor cell capture by stents coated with antibody against CD34: the HEALING-FIM (Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth-First in Man) Registry. *J Am Coll Cardiol.* 2005; 45:1574-1579.
5. Athanasoulis CA. Percutaneous transluminal angioplasty: general principles. *AJR An J Roentgenol.* 1980; 135:893-900.
6. Back M, Kopchok G, Mueller M, Cavaye D, Donayre C, and White RA. Changes in arterial wall compliance after endovascular stenting. *J Vasc Surg.* 1994; 19:905-911.
7. Ballyk PD, Walsh C, Butany J, and Ojha M. Compliance mismatch may promote graft-artery intimal hyperplasia by altering suture-line stresses. *J Biomech.* 1998; 31:229-237.
8. Becker GH, Meyer J, and Necheles H. Fat absorption and atherosclerosis. *Science.* 1949; 110:529.
9. Benard N, Coisne D, Donal E, and Perrault R. Experimental study of laminar blood flow through an artery treated by a stent implantation: characterisation of intra-stent wall shear stress. *J Biomech.* 2003; 36(7):991-998.
10. Berry JL, Manoach E, Mekkaoui Choukri, Rolland PH, Moore JE, and Rachev A. Hemodynamics and wall mechanics of a compliance matching stent: *In vitro* and *in vivo* analysis. *J Vasc Interv Radiol.* 2002; 13:97-105.
11. Berry JL, Samtamarina A, Moore JE, Roychowdhury S, and Routh WD. Experimental and computational flow evaluation of coronary stents. *Ann Biomed Eng.* 2000; 28:386-398.

12. Bjoekerud S and Bondjers G. Arterial repair and atherosclerosis after mechanical injury. I. Permeability and light microscopic characteristics of endothelium in non-atherosclerotic and atherosclerotic lesions. *Atheroscl.* 1971; 13:355-363.
13. Bluestein D, Niu L, Schoepfoerster RT, and Dewanjee MK. Fluid mechanics of arterial stenosis: Relationship to the development of mural thrombus. *Ann Biomed Eng.* 1997; 25:344-356.
14. Bluestein D, Niu L, Schoepfoerster RT, and Dewanjee MK. Steady flow in an Aneurysm model: Correlation between fluid dynamics and blood platelet deposition. *J Biomech Eng.* 1996; 188:280-286.
15. Brooks AR, Lelkes PI, and Rubanyi GM. Gene expression profiling of human aortic endothelial cells exposed to disturbed flow and steady laminar flow. *Physio Genomics.* 2002; 9:27-41.
16. Capek P, McLean GK, and Berkowitz HD. Femoropopliteal angioplasty. Factors influencing long-term success. *Circ.* 1991; 83:170-180.
17. Carlier SG, van Damme LCA, Blommerde CP, Wente JJ, van Langehove G, Verheye S, Kockx MM, Knaapen MWM, Cheng C, Gijzen F, Duncker DJ, Stergiopoulos N, Slager CJ, Serruys PW, and Krams R. Augmentation of wall shear stress inhibits neointimal hyperplasia after stent implantation: Inhibition through reduction of inflammation? *Circ.* 2003; 107:2741-2746.
18. Cejna M, Thurnher S, Illiasch H, Horvath W, Waldenberger P, Hornik K, and Lammer J. PTA versus Palmaz stent placement in femoropopliteal artery stenoses: results of a multicenter prospective randomized study (REFSA). *J Vasc Interv Radiol.* 2001; 12:23-31.
19. Clowes AW and Clowes MM. Kinetics of cell proliferation after arterial injury, I: Smooth muscle cell growth in the absence of the endothelium. *Lab Invest.* 1983; 49:327-335.
20. Crewe KH and Feuerstein IA. Platelet adhesion to fibrinogen-coated glass at an abrupt tubular expansion viewed under fluorescent-video microscopy. *Biorheol.* 1986; 23:443-52.
21. David T, Thomas S, and Walker PG. Platelet deposition in stagnation point flow: An analytical study and computational simulation. *Med Eng Phys.* 2001; 23:299-312.
22. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev.* 1995; 75:519-560.
23. De Scheerder I, Wang K, Wilczek K, Meuleman D, Van Amsterdam R, Vogel G, Piessens J, and Vande Werf F. Experimental study of thrombogenicity and foreign body reaction induced by heparin-coated stents. *Circ.* 1997; 95:1549-1553.

24. Draskoczy PR and Lyman CP. Turnover of catecholamines in active and hibernating ground squirrels. *J Pharmacol Exp Ther.* 1967; 155:101-111.
25. Duraiswamy N, Jayachandran B, Byrne J, Moore JE Jr., and Schoephoerster RT. Spatial distribution of platelet deposition in stented artery models under physiologic flow. *Ann Biomed Eng.* 2005; accepted.
26. Edelman ER and Rogers C. Pathobiologic responses to stenting. *Am J Cardiol.* 1998; 81:4E-6E.
27. Eigenthaler M, Schwarz UR, Schinke B, and Kobsar A. Activated platelets induce migration, proliferation, and gene expression in human vascular endothelial cells. *J Throm Haem.* 2004; 1:12-18, PO164.
28. Ellis SG, Weintraub W, Holmes D, Shaw R, Block PC, and King SB. Relation of operator volume and experience to procedural outcome of percutaneous coronary revascularisation at hospitals with high interventional volumes. *Circ.* 1997; 95: 2479-2484.
29. Escaned J, Cortes J, Alcocer MA, Goicolea J, Alfonso F, Fernandez-Ortiz A, Hernandez R, Banuelos C, and Macaya C. Long-term angiographic results of stenting in chronic total occlusions: Influence of stent design and vessel size. *A Heart J.* 1999; 138:675-680.
30. Fabregues S, Baijens K, Rieu R, and Bergeron P. Hemodynamics of endovascular prostheses. *J Biomech.* 1998; 31:45-54.
31. Faergeman O. Atherosclerosis and the low density lipoprotein of plasma. *Scand J Haematol Suppl.* 1979; 34:116-118.
32. Falk E. Why do plaques rupture? *Circ.* 1992; 86:III30-III42.
33. Fischman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, Detre K, Veltri L, Ricci D, Nobuyoshi M, Cleman M, Heuser R, Almond D, Teirstein PS, Fish RD, Colombo A, Brinker J, Moses J, Shalnovich A, Hirshfeld J, Bailey S, Ellis S, Rake R, and Goldberg S. A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. *New Engl J Med.* 1994; 331:496-501.
34. Fishman JA, Ryan GB, and Karnovsky MJ. Endothelial regeneration in the rat carotid artery and the significance of endothelial denudation in the pathogenesis of myointimal thickening. *Lab Invest.* 1975; 32:339-351.
35. Fung YC, (1993). Biomechanics: mechanical properties of living tissues; *Springer-Verlag.*

36. Garasic JM, Edelman ER, Squire JC, Seifert P, Williams MS, and Rogers C. Stent and Artery Geometry Determine Intimal Thickening Independent of Arterial Injury. *Circ.* 2000; 101:812-8.
37. George J, Herz I, Goldstein E, Abashidze S, Deutch V, Finkelstein A, Michowitz Y, Miller H, and Keren G. Number and adhesive properties of circulating progenitor endothelial cells in patients with in-stent restenosis. *Arterios Throm Vasc Biol.* 2003; 23:e57-e60.
38. Gherdol A and Vales O. Study of the intima of the pulmonary arteries in normal rabbits. *Rev Soc Argent Biol.* 1966; 42:50-55.
39. Gijssen JH, Oortman RM, Wentzel JJ, Schuurbiers JCH, Tanabe K, Degertekin M, Ligthart JM, Thury A, de Feyter PJ, Serruys PW, and Slager CJ. Usefulness of shear stress pattern in predicting neointimal distribution in Sirolimus-eluting stents in coronary arteries. *Am J Cardiol.* 2003; 92:1325-1328.
40. Glagov S, Rowley DA, and Kohut RI. Atherosclerosis of human aorta and its coronary and renal arteries. A consideration of some factors that may be related to the marked differences in atherosclerotic involvement of the coronary and renal arteries. *Arch Pathol.* 1961; 72:558-571.
41. Goetz R, Palmaz J, Noeldge G, and Tio F. Relationship between blood flow, thrombus, and neointima in stents. *J Vasc Inter Radiol.* 1999; 10:598-604.
42. Grenacher L, Saam T, Geier A, Muller-Hulsbeck S, Cejna M, Kauffmann GW, and Richter GM. PTA versus Palmaz stent placement in femoropopliteal artery stenoses: results of a multicenter prospective randomized study (REFSA). *Rofo* 2004; 176:1302-1310.
43. Haudenschild CC and Schwartz SM. Endothelial regeneration. II. Restitution of endothelial continuity. *Lab Invest.* 1979; 41:407-418.
44. He X and Ku DN. Unsteady entrance flow development in a straight tube. *J Biomech Eng.* 1994; 116:355-360.
45. He Y, Duraiswamy N, Frank AO, and Moore JE Jr. Blood flow in stented arteries: A parametric comparison of strut design patterns in 3D. *J Biomech Eng.* 2005; 127:637-647.
46. He Y. Computational analysis of blood flow in a stented chamber under physiologic flow condition. *Master's Thesis* 2002; Florida International University, Miami.
47. Henry FS. Flow in stented arteries. In: Intra- and extracorporeal cardiovascular fluid dynamics, edited by P. Verdonck and K.Perktold. Boston: WIT, 2000; 33-364.

48. Higgs GA, Moncada S, and Vane JR. Prostacyclin (PGI₂) inhibits the formation of platelet thrombi induced by adenosine diphosphate *in vivo*. *Br J Pharmacol*. 1977; 61:137P.
49. Hoffman R, Mintz GS, Mehran R, Pichard AD, Kent KM, Satler LF, Popma JJ, Hongsheng WU, and Leon MB. Intravascular ultrasound predictors of angiographic restenosis in lesions treated with Palmaz-Schatz stents. *J Am Coll Cardiol*. 1998; 31:43-49.
50. Hoffman R, Mintz GS, Mehran R, Satler LF, Pichard AD, Popma JJ, and Leon MB. Serial intravascular ultrasound predictors of restenosis at the margins of Palmaz-Schatz stents. *Am J Cardiol*. 1997; 79:951-953.
51. Hsu PP, Li S, Li YS, Usami S, Ratcliffe A, Wang X, and Chien S. Effect of flow patterns on endothelial cell migration into a zone of mechanical denudation. *Biochem Biophys Res Commun*. 2001; 285:751-759.
52. Hull D and Segall MM. Sympathetic nervous control of brown adipose tissue and heat production in the new-born rabbit. *J Physiol*. 1965; 181:458-467.
53. Ishii K and Honda K. The effects of electrical stimulation of the carotid sinus nerve on shivering, blood pressure, and respiration in rabbits. *Tohoku J Exp Med*. 1965; 86:191-200.
54. Jeong MH, Owen WG, Staab ME, Srivatsa SS, Sangiorgi G, Stewart M, Holmes DR Jr., and Schwartz RS. Porcine model of stent thrombosis: platelets are the primary component of acute stent closure. *Cathet Cardiovasc Diagn*. 1996; 38:38-43.
55. Karino T and Goldsmith HL. Adhesion of human platelets to collagen on the wall distal to a tubular expansion. *Microvasc Res*. 1979; 17:238-262.
56. Karoon P, Knight G, and Burnstock G. Enhanced vasoconstrictor responses in renal and femoral arteries of the golden hamster during hibernation. *J Physiol*. 1998; 512:927-938.
57. Kastrati A, Mehilli J, Dirshinger J, Pache J, Ulm K, Schuhlen H, Seyfarth M, Schmitt C, Blasini R, Neumann FJ, and Schomig A. Restenosis after coronary placement of various stent types. *Am J Cardiol*. 2001; 87:34-39.
58. Kinney TB, Chin AK, Rurik GW, Finn JC, Shoor PM, Hayden WG, and Fogarty TJ. Transluminal angioplasty: a mechanical-pathophysiological correlation of its physical mechanisms. *Radiology*. 1984; 153:85-89.
59. Knight CJ, Curzen N, Groves PH, Patel DJ, Goodall AH, Wright C, Clarke D, Oldershaw PJ, and Fox KM. Stenting for suboptimal results following balloon angioplasty significantly reduces restenosis: results of a single centre randomised trial [abstract]. *Circ*. 1997; 96:1_709.

60. Kochemasova NG. Electrical and mechanical reactions of vascular smooth muscles under the effect of noradrenaline. *Fiziol Zh Sssr Im I M Sechenova*. 1967; 53:1476-82.
61. Komatsu R, Ueda M, Naruko T, Kojima A, and Becker AE. Neointimal tissue response at site of coronary stenting in humans – macroscopic, histologic and immunohistochemical analyses. *Circ*. 1998; 98:224-233.
62. Ku DN, Giddens DP, Zarins CK, and Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation: Positive correlation between plaque location and low oscillating shear stress. *Arterios (Dallas)*. 1985; 5:293-302.
63. LaDisa JF Jr., Guler I, Olson LE, Hettrick DA, Kersten JR, Warltier DC, and Pagel PS. Three-dimensional computational fluid dynamics modeling of alterations in coronary wall shear stress produced by stent implantation. *Ann Biomed Eng.* 2003; 31:972-980.
64. LaDisa JF Jr., Meier HT, Olson LE, Kersten JR, Warltier DC, and Pagel PS. Antegrade iliac artery stent implantation for the temporal and spatial examination of stent-induced neointimal hyperplasia and alterations in regional fluid dynamics. *J Pharmacol Toxicol Meth*. 2005; 51:115-121.
65. LaDisa JF, Jr., Olson LE, Guler I, Hettrick DA, Audi HS, Kersten JR, Warltier DC, and Pagel PS. Stent design properties and deployment ratio influence indexes of wall shear stress: a three-dimensional computational fluid dynamics investigation within a normal artery. *J Appl Physiol*. 2004; 97:424-430.
66. LaDisa JF, Jr., Olson LE, Guler I, Hettrick DA, Kersten JR, Warltier DC, and Pagel PS. Circumferential vascular deformation after stent implantation alters wall shear stress evaluated with time-dependent 3D computational fluid dynamics models. *J Appl Physiol*. 2005; 98:947-957.
67. Lahann J, Klee D, Thelen H, Bienert H, Vorwerk D, and Hocker H. Improvements of hemocompatibility of metallic stents by polymer coating. *J Material Sci*. 1999; 10:443-448.
68. Larcen A, Steiff F, Peters A, and Genetet B. The components of blood viscosity. *Pathol Biol*. 1965; 13:648-659.
69. Lau KW, Johan A, Sigwart U, and Hung JS. A stent is not just a stent: stent construction and design do matter in its clinical performance. *Singapore Med J*. 2004; 45:305-312.
70. Lau KW, Mak KH, Hung JS, and Sigwart U. Clinical impact of stent construction and design in percutaneous coronary intervention. *Am Heart J*. 2004; 147:764-773.

71. Legrand YJ, Karniguian A, Fauvel F, and Gutman N. The molecular interaction between platelet and vascular wall. *Blood Cells*. 1983; 9:263-274.
72. Liu SQ and Goldman J. Role of blood shear stress in the regulation of vascular smooth muscle cell migration. *IEEE Trans Biomed Eng*. 2001; 48:474-483.
73. Longest PW and Kleinstreuer C. Comparison of blood particle deposition models for non-parallel flow domains. *J Biomech*. 2003; 36: 421-430.
74. Lorimer WS Jr. Internal carotid artery angioplasty. *Surg Gynecol Obstet*. 1961; 113:783-784.
75. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature*. 1964;202:498-499.
76. Matsui H, Sugimoto M, Mizuno T, Tsuji S, Miyata S, Matsuda M, and Yoshioka A. Distinct and concerted functions of von Willebrand factor and fibrinogen in mural thrombus growth under high shear flow. *Blood*. 2002; 100:3604-3610.
77. Maupin B. Current concepts regarding the role of blood platelets in hemostasis and thrombosis. *Angiologie*. 1955; 7:8-11.
78. Melkert R, Violaris AG, and Serruys PW. Luminal narrowing after percutaneous transluminal coronary angioplasty. A multivariate analysis of clinical, procedural, and lesion related factors affecting angiographic outcome in the PARK study. *J Invasive Cardiol*. 1994; 6:160-171.
79. Miller DD, Karim MA, Edwards WD, and Schwartz RS. Relationship of vascular thrombosis and inflammatory leukocyte infiltration to neointimal growth following porcine coronary artery stent placement. *Atheroscl*. 1996; 124:145-155.
80. Mohammed Z, Moore JE, Rachev A, Berry JL, and Manoach E. Stress concentration reduction in stented arteries using compliance transitioning. *Int J Cardiovasc Med and Sci*. 2000; 3:1-7.
81. Moore DH and Ruska H. The fine structure of capillaries and small arteries. *J Biophys Biochem Cytol*. 1957; 3:457-462.
82. Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnar F, and Falotico R. A randomized comparison of the sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med*. 2002; 346:1773-1780.
83. Morton AC, Arnold ND, Crossman DC, and Gunn J. Response of very small (2mm) porcine coronary arteries to balloon angioplasty and stent implantation. *Heart*. 2004; 90:324-327.

84. Morton AC, Crossman D, and Gunn J. The influence of physical stent parameters on restenosis. *Pathologie Biologie*. 2004; 52:186-205.
85. Myers JG, Moore JA, Ojha M, Johnston KW, and Ethier CR. Factors influencing blood flow patterns in the human right coronary artery. *Ann Biomed Eng*. 2001; 29:109-120.
86. Nugent HM, Rogers C, and Edelman ER. Endothelial implants inhibit intimal hyperplasia after porcine angioplasty. *Circ Res*. 1999; 84:384-391.
87. Okuhn SP, Connelly DP, Kalakos N, Ferrell L, Pam MX, and Goldstone J. Does compliance mismatch alone cause neointimal hyperplasia? *J Vasc Surgery*. 1989; 9:35-45.
88. Pepine CJ and Homes DR. Coronary artery stents: ACC expert consensus document. *Am J Cardiol*. 1996; 28:782-94.
89. Perktold K and Rappitsch G. Computer simulation of local blood flow and vessel mechanics in a compliant carotid artery bifurcation model. *J Biomech*. 1995; 28:845-856.
90. Prendergast PJ, Lally C, Daly S, Reid AJ, Lee TC, Quinn D, and Dolan F. Analysis of prolapse in cardiovascular stents: a constitutive equation for vascular tissue and finite-element modelling. *J Biomech Eng*. 2003; 125: 692-9.
91. Ragan C. The physiology of the connective tissue (loose areolar). *Annu Rev Physiol*. 1952; 14:51-72.
92. Resnik R. The endocrine regulation of uterine blood flow in the nonpregnant uterus: a review. *Am J Obstet Gynecol*. 1981; 140:151-156.
93. Robaina S, Jayachandran B, He Y, Frank A, Moreno M, Moore JE Jr., and Schoepfoerster RT. Platelet adhesion to simulated stented surfaces. *J Endovasc Ther*. 2003; 10:978-986.
94. Robinson S, Meyer FR, Newton JL, TS'ao CH, and Holgersen LO. Relations between sweating, cutaneous blood flow, and body temperature in work. *J Appl Physiol*. 1965; 20:575-582.
95. Rogers C and Edelman ER. Endovascular stent design dictates experimental restenosis and thrombosis. *Circ*. 1995; 91:2995-3001.
96. Rogers C, Karnovsky MJ, and Edelman ER. Inhibition of experimental neointimal hyperplasia and thrombosis depends on the type of vascular injury and the site of drug administration. *Circ*. 1993; 88:1215-1221.

97. Rogers C, Kjelsberg MA, Seifert P, and Edelman ER. Heparin-coated stents eliminate mural thrombus deposition for days without affecting restenosis. (Abstr.) *Circ.* 1997; 9:I-710.
98. Rogers C, Parikh S, Seifert P, and Edelman ER. Endogenous cell seeding – remnant endothelium after stenting enhances vascular repair. *Circ.* 1996; 94:2909-2914.
99. Rogers C, Tseng DY, Squire JC, and Edelman ER. Balloon-artery interactions during stent placement: A finite element analysis approach to pressure, compliance, and stent design as contributors to vascular injury. *Circ Res.* 1999; 84:378-383.
100. Rogers C, Tseng DY, Squire JC, and Edelman ER. Balloon-artery interactions during stent placement. *Circ Res.* 1999; 84:378-383.
101. Rolland PH, Mekkaoui C, Vidal V, Berry JL, Moore JE Jr., Moreno M, Amabile P, and Bartoli JM. Compliance matching stent placement in the carotid artery of the swine promotes optimal blood flow and attenuates restenosis. *Eur J Vasc Endovasc Surg.* 2004; 28:431-438.
102. Salame MY and Douglas JS Jr. The restenosis story: is intracoronary radiation therapy the solution? *Cardiol Rev.* 2001; 9:329-338.
103. Sampath R, Kukielka GL, Smith CW, Eskin SG, and McIntire LV. Shear stress mediated changes in the expression of leukocyte adhesion receptors on human umbilical vein endothelial cells. *Ann Biomed Eng.* 1995; 23:247-256.
104. Sdringola S, Assali A, Anderson HV, and Smalling RV. Restenosis: Relationship with thrombosis. *Curr Int Cardiol Rep.* 2000; 2:285-292.
105. Serruys PW, de Jaegere P, Kiemeneij F, Macaya C, Rutsch W, Heyndrickx G, Emanuelsson H, Marco J, Legrand V, Paterne P, *et al.* A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. Benestent Study Group. *N Engl J Med.* 1994; 331:489-95.
106. Serruys PW, Emanuelsson H, van der Giessen W, Lunn AC, Kiemeney F, Macaya C, Rutsch W, Heyndrickx G, Suryapranata H, Legrand V, Goy JJ, Materne P, Bonnier H, Morice MC, Fajadet J, Belardi J, Colombo A, Garcia E, Roygok P, de Jaegere P, and Morel MA. Heparin-coated Palmaz-Schatz stents in human coronary arteries. Early outcome of the Benestent-II Pilot Study. *Circ.* 1996; 93:412-22.
107. Shirota T, Ysui H, and Matsuda T. Intraluminal tissue-engineered therapeutic stent using endothelial progenitor cell-inoculated hybrid tissue and *in vitro* performance. *Tissue Eng.* 2003; 9:473-485.
108. Sigwart U. Coronary endoprostheses (stents). *Herz.* 1990; 15:319-328.

109. Simon C, Palmaz JC, and Sprague EA. Influence of topography on endothelialization of stents: Clues for new designs. *J Long-Term Eff Med Impl.* 2000; 10:143-151.
110. Sprague EA, Luo J, and Palmaz JC. Endothelial cell migration onto metal stent surfaces under static and flow conditions. *J Long-Term Eff Med Impl.* 2000; 10:97-110.
111. Staszewska-Barczak J. Role of renal prostaglandins in circulatory homeostasis. *Contrib Nephrol.* 1978; 11:179-188.
112. Stich W. Physiology and pathology of heme synthesis. *Folia Haematol (Frankf).* 1964; 9:197-216.
113. Tominaga R, Kambic HE, Emoto H, Harasaki H, Sutton C, and J Hollman. Effects of design geometry of intravascular endoprostheses on stenosis rate in normal rabbits. *Am Heart J.* 123:21-28, 1992.
114. Traub O and Berk BC. Laminar shear stress – mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscl Throm Vasc Biol.* 1998; 18:677-685.
115. Tricot O, Mallat Z, Heymes C, Belmin J, Leseche G, and Tedgui A. Relation between endothelial cell apoptosis and blood flow direction in human atherosclerotic plaques. *Circ.* 2000; 101:2450–2453.
116. van Beusekom HM, Whelan DM, Hofma SH, Krabbendam SC, van Hinsbergh VW, Verdouw PD, and van der Geissen WJ. Long-term endothelial dysfunction is more pronounced after stenting than after balloon angioplasty in porcine coronary arteries. *J Am Coll Cardiol.* 1998; 32:1109-1117.
117. Vassar PS and Spain DM. Platelet thrombosis syndrome. *Circ.* 1953; 8:664-671.
118. Versaci F, Gaspardone A, Tomai F, Crea F, Chiariello L, and Gioffre PA. A comparison of coronary-artery stenting with angioplasty for isolated stenosis of the proximal left anterior descending coronary artery. *N Engl J Med.* 1997; 336:817-822.
119. Virmani R, Kolodgie FD, Farb A, and Lafont A. Drug eluting stents: are human and animal studies comparable? *Heart.* 2003; 89:133-138.
120. Walsh PW, Berkani F, and Moore JE Jr. Stented flow chamber for endothelial cell migration studies. *Eur Soci Biomech.* 2000.
121. Welt FGP, Edelman ER, Simon DI, and Rogers C. Neutrophil, not macrophage infiltration, precedes neointimal thickening in balloon-injured arteries. *Arterioscl Throm Vasc Biol.* 2000; 2553-2558.

122. Weyrich AS, Prescott SM, and Zimmerman GA. Platelets, endothelial cells, inflammatory chemokines, and restenosis; Complex signaling in the vascular play book. *Circ.* 2002; 106:1433-1435.
123. Wilensky RL, March KL, Gradus-Pizlo I, Sandusky G, Fineberg N, and Hathaway DR. Vascular injury, repair, and restenosis after percutaneous transluminal angioplasty in atherosclerotic rabbits. *Circ.* 1995; 92:2995-3005.
124. Wilner GD, Nossel HL, and LeRoy EC. Aggregation of platelets by collagen. *J Clin Invest.* 1968; 47:2616-2621.
125. Wootton DM and Ku DN. Fluid mechanics of vascular systems, diseases, and thrombosis. *Annu Rev Biomed Eng.* 1999; 1: 299-329.
126. Yachia D and Arigodan IA. Comparison between first-generation (fixed-caliber) and second-generation (self-expanding, large caliber) temporary prostatic stents. *Urol Int.* 1996; 57:165-169.
127. Yutani C, Imakita M, Ishibashi-Ueda H, Tsukamoto Y, Nishida N, and Ikeda Y. Coronary atherosclerosis and interventions: Pathological sequences and restenosis. *Pathol Int.* 1999; 49:273-290.

APPENDIX

Appendix A - 2D Flow Chamber – CAD Drawing

Bottom Plate - Front View

Bottom Plate - Top View

Top Plate - Top View

Appendix B – Stock Solution Preparation

Heparin

Ingredients:

- 50 mg of heparin sodium
- 166.5 ml of 0.9% NaCl

Mix together and pass through a 22 μ m membrane filter. Solution should be stored at 4°C. Using 5ml of Heparin solution corresponds to 270 units of Heparin.

Mepacrine

Ingredients:

- 47 mg of mepacrine
- 10 ml of distilled water

Mix together and store stock at 4°C. Using this, we prepare a 10 mM mepcarine stock.

Appendix C – Procedure of Blood Collection

Syringe Preparation for Blood Withdrawal

1. Take three 60 ml syringes.
2. Extract 5 ml of Heparin solution (270 units) into each syringe.
3. Give all three syringes to the phlebotomist for blood withdrawal.
4. Collect 50 ml of blood into the syringes therefore filling to a total of 55ml in each.
5. Place syringes on rocker or maintain at 37 degree celsius until use.

Only 2 syringes (total of 100ml blood) were required for 3D experiments.

**EFFECT OF BLOOD FLOW PATTERNS ON PLATELET DEPOSITION
IN 2D AND 3D STENTED ARTERIES
– AN EXPERIMENTAL AND COMPUTATIONAL APPROACH**

RESEARCH OBJECTIVES:

Introduction and Background: Percutaneous transluminal coronary angioplasty was introduced to treat occlusive vascular disease, but was associated with 40% or more restenosis rate. Now-a-days, vascular stents form an effective treatment for occlusive vascular disease. The use of stents after balloon angioplasty maintains the lumen diameter and therefore prevents vessel recoil. However 20% to 30% of patients receiving endovascular stents develop significant restenosis. Restenosis is related to patient-, and procedure-specific factors. Patient-specific factors such as disease-specific or gene-specific cannot be influenced by any means; procedure-specific factors that influence restenosis include implantation technique, complexity of the artery, and stent design and material make-up. Many researchers have sought to address these problems by modulating stent surface characteristics, by treating patients with a variety of antiplatelet therapy, by coating stents with drugs such as sirolimus, paclitaxel, or actinomycin D, and by manufacturing stents of different designs (currently, 55 kinds of stents are available in the market). However, the success of each of these procedures used to reduce restenosis rate has been limited. The success has been limited by an increase in cases of severe

bleeding and hemorrhaging with pharmacological treatments; drug coated stents reduce smooth muscle cell proliferation, but have shown good short-term success only; different stent designs have still not helped in reducing the restenosis rate drastically.

Both in vitro and in vivo studies have revealed that stent design influences thrombus accumulation between struts and restenosis. The presence of a thrombogenic metallic surface, along with the denudation of endothelium creates favorable conditions for platelet adhesion to the vessel wall. New techniques in coating stents with non-thrombogenic materials and evidence that variations in geometric configuration of stents lead to reduced endothelial injury have sought to address the issue. Major determinants of the stent profile include shape and height of strut, number of intersections, and metal-to-artery ratio. In fact, stents designed with fewer strut-strut intersections were reported to lead to significantly less neointimal hyperplasia. Reducing strut-strut intersections by 29% while keeping diameter, mass, and surface area constant in denuded rabbit iliac arteries reduced vascular injury by 42%, thrombosis by 69%, and neointimal hyperplasia by 38%. The use of a thin-strutted stent was associated with a 43% reduction in the restenosis rate at six months compared to a thick-strutted stent. Clinical studies have shown stent design to be the second greatest risk factor for determining restenosis, second only to vessel size. Mechanical factors influenced by stent design include stress concentration on the artery wall and the disruption of blood flow imposed by stents.

The protrusion of individual stent struts into the flow field affects the flow adjacent to the artery wall, creating areas of flow stagnation near the struts. Low mean wall shear stresses associated with these areas of stagnant flow, along with high particle residence times, and non-laminar flow, have all been shown to create favorable conditions for intimal thickening. The first step to intimal thickening may be related to

the rate and extent of platelet deposition near stented regions. The study of platelet deposition in an expanded tube showed a peak in platelet adhesion in the vortex in which the number density was appreciably greater than that downstream of the reattachment point where fully developed laminar flow was established. At the reattachment point, platelet adhesion was a minimum. This may be a result of the wall reaction rate and wall shear stress. If the wall reaction rate is linearly dependent on the wall shear stress, the platelet flux is maximum downstream of the stagnation point. Further, as stated previously, the simple inclusion of a gap between individual stent struts has been shown to produce significantly less neointimal formation than stents with no gaps between stent struts. Numerous computational fluid dynamics simulations have shown that the creation of areas of flow recirculation (where the wall shear stress is usually low) and reattachment (where the wall shear stress is usually high) between individual stent struts throughout the cardiac cycle are dependent on stent strut spacing. The study of the Wallstent wire mesh stent revealed that a strut spacing less than six wire diameters lead to constant flow separation zones between the struts where as a strut spacing greater than six vessel diameters lead to flow reattachment between the struts. Thus blood flow patterns strongly depend on stent strut spacing, thereby affect platelet deposition between struts.

The influence of stent design on solid mechanics conditions inside the stented artery may also play an important role in stent failure and restenosis. A compliance matching stent has been designed to reduce the abrupt changes in mechanical properties (compliance) at the ends of the stent and the arterial wall that create zones of high stress concentration. The higher stress in the stented region may cause the endothelial layer of the arterial wall to denude and thus the artery wall must adapt to accommodate these new

stresses by the generation of additional tissue, which may eventually lead to restenosis. Researchers have shown that the arterial injury produced by stents creates a strong stimulus for smooth muscle cell proliferation and neointimal hyperplasia. Such injury to the artery attracts platelets for repair. Lowest platelet deposition has been shown to occur within the smallest strut spacing. However, no research is available regarding the effects of blood flow patterns on localized platelet deposition within the stented region and their relationship to wall shear stress variations either in 2D or 3D.

This study will focus on the dynamics of blood flow and its effects on the platelet deposition near stented region, of particular interest within different stent strut spacings, in the acute stages of stent implantation. Two dimensional stent models will be selected to simulate cases of constant recirculation of blood flow, constant reattachment of blood flow, and intermittent reattachment of blood flow between individual stent struts. The platelet deposition will be quantified at different times between two individual stent struts and the deposition pattern will be compared to computational wall shear stress patterns for each of the stent models. The limitation in the 2D study is that the geometry is simple and will not simulate the in-vivo behavior of the artery wall to platelet deposition due to the flow dynamics and the geometry. Hence platelet adhesion studies within three dimensional stent models will be performed to correlate their relationship to localized changes in wall shear stress pattern. However, the 2D study will provide insight on platelet deposition at specific regions of disturbed flow in the stented section; this information will be useful to depict platelet deposition patterns in the 3D study but there may be other concerns that will be observed only on 3D experimentation.

- The theoretical goal of this research is to study the effect of blood flow dynamics and time course on platelet adhesion in stented arteries and the relationship to the

computational wall shear stress pattern. The empirical goal of this research is to be able to address the role of some mechanical factors in restenosis and thereby minimize it with a better stent design.

- The involvement of the research subject(s) helps us to carry out the research project as needed and be able to prove the theoretical and empirical goals of the research project. By minimizing the effects of restenosis, patients (who get stents) will feel and perform better on a daily basis. This invokes a sense of satisfaction and achievement for all subjects who involve in this research.

SUBJECT RECRUITMENT:

Information: Recruitment refers to the contact and the communication that takes place between the investigator and a potential participant. Recruitment occurs prior to the introduction of the consent process. Recruitment methods include but are not limited to verbal exchanges and advertisement. Recruitment processes should avoid the appearance of coercion or undue influence.

- Subjects to be included in this research are normal volunteers (i.e. people with no physiological diseases or disorders of any kind, non-smoking, non-alcoholic, and taking no medications that alter blood function); they are estimated to be 30 in total number.
- The source of potential subjects will be mostly students who work or study at the FIU engineering center and/or their relatives.
- Potential subjects will be approached personally; they will be contacted through phone or through their department at FIU engineering center. Advertisements will

also be put up wherever needed so that subjects could get in touch with the principal investigator or the faculty advisor directly. Prior to the study, the main research goals will be explained to the subject and a promise to keep their identity confidential will be made prior to obtaining consent. There will be no direct risk or benefit to the subjects involved.

- All normal volunteers can be included in the study. We reiterate that volunteers should not smoke and should not have consumed alcohol over a period of 24 hours. Volunteers should not have consumed drugs like aspirin over a period of 48 hours.

METHOD AND PROCEDURES:

- There are no direct risks involved with the study protocol. Minimal risks if any, are the same with this study protocol as it would be with the blood donation procedure. 150ml of blood will be drawn from the subject through venipuncture. A licensed phlebotomist, Bhavani Jayachandran (Undergraduate advisor, Biomedical Engineering, FIU) will be present to withdraw blood from the subject, thus minimizing all risks to the subject. All needles, bottles used are disposable and will be used only one time. Hence, the risks to the subject are minimal. There may be slight bruising at the point of the venipuncture, which will be covered using cotton and bandaid pads; these bruises usually disappear in a matter of hours on the same day of blood donation.
- Research will be conducted only at the Cardiovascular Engineering Center, Biomedical Engineering, FIU, Miami.
- Expected start date is 20th November, 2004. Expected end date is 20th November, 2005.

- No tests or examinations are done on the subject. All normal volunteers who can donate blood are eligible; their blood will not undergo any kind of processing before the experiment.
- There are no alternative procedures or treatments that the subject may be able to consider.
- The procedure is very simple and standard to that of any blood donation procedures and hence no particular treatment is needed since the amount of blood to be withdrawn is very minimal.
- The heparinized blood collected by the phlebotomist will be used by the PI for the experiments. The heparinized blood will be stained by mepacrine (a nucleic acid stain) to stain the platelets and the leucocytes; these stained cells could then be observed under the fluorescent microscope (Nikon TS100 with mercury arc lamp) in dark as the blood flows in the stented region with a pulsatile pump. The reservoir where the blood collects will be maintained at 37 degree celsius. No testing will be performed with the blood.
- Data to be collected will consist of image files showing fluorescent platelets inside the stented region. Data will be collected over a 30 minute time span, using image capture software called streampix as tif images. Each image captured at one time point will consist of several frames. Selected number of frames of each image will be averaged using image processing software called imagepro to reduce error from light intensity changes due to AC lamp fluctuations. The averaged frame will be then used for light intensity background correction (using a custom program written in matlab). The image file at the no flow disturbance region will be used for background correction of the actual image file at the stented region. Variations in platelet

deposition between struts will be then compared to wall shear stress variations from computational fluid dynamics (CFD). Data will be analyzed in Excel or SPSS software.

- No cost or compensation is involved as a result of participation in this research. In order to help the subject regain strength after the donation, a pack of cookies and juice is provided for free.

BENEFITS:

- There are no direct benefits to the subject, but the research itself provides anticipated benefits to the society in general. If this research helps in providing information on designing a stent better than the current designs, it has benefited the patient by reducing the restenosis rate. As part of this research, the subject may be able to gain knowledge of the specific discipline.
- The level of major risk in relation to anticipated benefits is null. As stated before, the risks as part of this study if any are the same as that for any blood donation procedure; these risks are however minimal such as discomfort of the needle or small bruising.

RISKS TO SUBJECTS:

- There are no long-range risks associated with the procedure. Some immediate risks include discomfort due to the needle and slight bruising. Discomfort of the needle usually disappears if one maintains their calm and takes a deep breath. Discomfort may also disappear if the position of the needle is changed slightly within the vein (done by the phlebotomist). Slight bruising is characteristics due to the needle prick.

Cotton and bandaid will be used to cover the wound to prevent infection and in a matter of hours, the bruising will disappear. The risk of infection is very minimal because only disposable needles will be used for blood withdrawal. The confidentiality of the subject will be maintained and hence, no legal risk is involved.

- No medical or psychological care is available as part of this research as the operating procedure is simple and does not require one. Psychologically, care will be provided by the principal investigator and the phlebotomist at the time of blood withdrawal to keep the subject at ease. To augment the physical condition of the subject after the blood withdrawal procedure, some cookies and juice will be provided.
- There is no scientific necessity of the experimental aspects that lead to these risks. No legal risks are involved as the confidentiality of the subject will be maintained.
- There are no known risks to the subject. The risk level is very minimal in this study as mentioned above since only 150ml of blood is withdrawn and are not greater than if the same procedure is faced in normal blood donation scenario (where more than 500ml of blood is withdrawn usually).

INFORMED CONSENT:

- Informed consent form has been prepared with the idea that the person giving consent for blood withdrawal for this research is able to understand the relevant information about the research and understands clearly the benefits and risks if any.
- Informed consent will be obtained before blood withdrawal and after giving a brief explanation of the research project by the principal investigator, free of coercion or any undue influence.

- The subject will be welcome to clarify or ask further questions about the informed consent to the principal investigator or the faculty advisor, before giving consent.
- Adult consent only will be used.
- A copy of the written informed consent form is attached to the application.

CONFIDENTIALITY OF DATA:

- The consent forms and records will be maintained in a separate location (file cabinet) that will always remain locked; the keys will be accessible only to the PI; the PI will also have information on all people from the department who have access to the keys. The consent forms will be maintained on file for at least 3 years from the start of the research and disposed (by shredding) later on. This is how subject data will be safeguarded.
- Other than the consent form, there is no place elsewhere in the research where the subject name or other details regarding the subject will be used (or appears).
- Gender is not a criteria for this research; hence this information also does not appear and thus remains confidential.

INFORMED CONSENT

EFFECT OF BLOOD FLOW PATTERNS ON PLATELET DEPOSITION IN STENTED ARTERIES

I freely and voluntarily consent to be a participant in the research project referenced above to be conducted at Florida International University during the 2004-2005 academic year, with Nandini Duraiswamy as Principal Investigator. I have been told that the blood collection procedure will last approximately 10 minutes.

I understand that the purpose of the research is to gain further understanding of the role of blood flow patterns in platelet deposition in stents (devices used to treat cardiovascular diseases).

I understand that the procedures will be as follows: 150 milliliters of blood will be collected by venipuncture from the forearm into three 50 cc syringes containing heparin as an anticoagulant. This blood sample will then be used in blood flow experiments designed to measure platelet deposition.

I understand that there are no known benefits involved in my participation. I understand the venipuncture presents a minimal risk of infection, discomfort and possible bruising. I have been told that individual data regarding the blood sample will be kept confidential. The data will be coded and the key to the code will be kept in a locked cabinet with access only available to the PI.

I understand that I may withdraw my consent and discontinue participation in this research project at any time with no negative consequences. I have been given the right to ask questions concerning the procedure, and any questions have been answered to my satisfaction.

I understand that if I desire further information about this research I should contact Nandini Duraiswamy (Principal Investigator of this research) or Dr. Richard Schoepfoerster (Faculty Advisor) at (305) 348-6950. I understand that if I have questions regarding the rights of research subjects, I should contact Dr. Jonathan G Tubman (Chair of the University Research Committee) at (305) 348-5290. I have been offered a copy of this informed consent form.

I have read and understand the above.

Participant's signature

Date

Print name

I have explained and defined in detail the research procedure in which the participant has agreed to participate, and have offered him/her a copy of this informed consent form.

Principal Investigator's signature

Date

VITA

Nandini Duraiswamy

1993-1997	Bachelor of Engineering, Medical Electronics BMS College of Engineering, India
1994-1996	Diploma in Information Technology National Institute of Information Technology, India
1997-1998	Software Engineer, Tata Infotech Ltd., India
1998-2000	Masters in Science, Biomedical Engineering Texas A&M University, TX
1999-2000	Research Assistant Cardiovascular Biomechanics Laboratory Texas A&M University, TX
2000-2002	Biomedical Engineer Biotronik, Inc., Portland, OR
2002-2005	Doctoral Candidate, Biomedical Engineering Florida International University, FL
2002-2005	Research Assistant Cardiovascular Engineering Center Florida International University, FL

PUBLICATIONS AND PRESENTATIONS

Duraiswamy N, Jayachandran B, Byrne J, Moore JE Jr., and Schoepfoerster RT. Spatial distribution of platelet deposition in stented artery models under physiologic flow. *Annals of Biomedical Engineering* 2005; accepted.

He Y, Duraiswamy N, Frank AO, and Moore JE Jr. Blood flow in stented arteries: A parametric comparison of strut design patterns in 3D. *Journal of Biomechanical Engineering* 2005; 127(4):637-47.

BMES and AEMB Conference, Houston, 2000 – Presented paper on ‘Mechanical multiaxial properties and their characterization in porcine hypertensive carotid arteries’.

BMES Conference, Baltimore, 2005 – Presented paper on ‘Spatial distribution of platelet deposition in 3D stented artery models under physiologic flow’.