The Development of an *In Vitro* Model of Coronary Lesion Thrombosis

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Abstract

Thrombosis is an initiating response to vascular injury. Physiologically, this process aids in the repair and remodeling of the vessel wall. However, if left unchecked, luminal occlusion may rapidly occur. The coronary vascular bed is a life-sustaining environment in which pathological thrombosis can lead to devastating outcomes such as acute coronary syndromes or post-interventional thrombosis. In order to study these coronary thrombotic reactions, it is essential to consider the physical environment in which they occur. We have developed an *in vitro* method for creating pulsatile flows to mimic the coronary hemodynamic setting on a beat-to-beat basis. Flow is generated by accelerating fluid loops about an axis inducing relative wall motion. Using this technique, a variety of oscillating flow patterns can be developed and modulated offering the ability to monitor sensitive, flow-dependent processes with minimal disturbances from pump action and background circuit effects. We provide a detailed discussion of the embodied prototype and test the ability of the device to generate the desired flow conditions. Finally, we consider thrombotic loop occlusion times of endovascular stents as an initial test of biological feasibility, exploring issues of noise, precision, and accuracy.

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CHAPTER 1
Background and Significance

1.1 Coronary Thrombosis

1.1.1 Pathophysiology

The vital coronary arteries transport freshly oxygenated blood to the heart (see Fig. 1.1). These vessels branch off of the aorta, curving over the epicardial surface while bifurcating and providing tributaries to the ever-beating myocardial tissue. Such complex geometries and the pulsing intramural pressures create a high degree of spatial and temporal diversity in the local hemodynamic environment, resulting in a unique vascular setting.

In states of health, the coronary vessels, as all parts of the vasculature, are lined with a protective endothelial barrier that separates the highly reactive vessel wall and contained blood compartments. Traversing through these conduits, changes in the vascular environment are accompanied by changes in the phenotypes of these cells [1]. Gene expression patterns, surface receptor expression, and a slew of other cellular mechanisms are tightly linked to the environment and work to keep this living surface appropriately matched with local needs.

Within the first decades of life, nearly ubiquitous environmental and genetic stresses conspire to create loci of low-grade injury to the protective endothelial surface allowing the accumulation of subendothelial deposits of lipids and lipid-laden macrophages [2-6]. These minor breaches instigate a complex cycle of chronic...
inflammation and immune recruitment through endothelial activation, smooth muscle cell proliferation and migration, extracellular matrix turnover, and the build up of cellular and necrotic debris. Over time, such processes transform into full fledged atherosclerotic disease and luminal stenosis. Figure 1.2 a, b depicts a healthy vessel and one whose lumen has been diminished by atherosclerotic plaque burden.

**a. Healthy**

![Healthy coronary artery with patent lumen](image)

**b. Diseased**

![Atherosclerotic coronary artery with luminal stenosis](image)

Figure 1.2. a. Healthy coronary artery with patent lumen; b. Atherosclerotic coronary artery with luminal stenosis

Typically, the plaques are characterized by a lipid-laden, highly thrombogenic, necrotic core, covered by a fibrous cap region. As the plaques continue to grow, regulatory mechanisms are often able to compensate and uphold the balance between blood supply and myocardial demand. While such progressive, chronic, atherosclerotic processes can eventually lead to significant, uncompensatable disease (> 75% area reduction) characterized by ischemic episodes and stable angina (exertional chest pain), a far more devastating and unpredictable outcome is plaque rupture or endothelial erosion (see Fig 1.3) [3-5, 7, 8].

Under such circumstances, the protective endothelial layer is acutely disrupted, exposing highly reactive subendothelial, atheromatous components. A rapid, thrombotic process ensues which can either exacerbate disease progression or lead to acute coronary syndromes (ACS) such as unstable angina, acute myocardial infarction, or ischemic sudden death.

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*Chapter 1: Background and Significance*
1.1.2 Treatments

Prevention is the preferred treatment option for coronary disease. Epidemiological studies have generated a great deal of information regarding the different genetic and environmental risk factors involved in coronary disease progression [9-11]. Accordingly, appropriate measures can be taken to minimize risk. Currently, our cellular and molecular based understanding of the etiology of atherosclerosis and thrombosis has also allowed the use of relatively safe, long-term drug strategies. Perhaps the most notable example is the widespread use of aspirin in treating populations at risk of coronary disease. By targeting platelet responsiveness (a key component of the arterial thrombotic response), aspirin has offered a 25% relative risk reduction of severe cardiovascular incidence (vascular death, MI, stroke) becoming a nearly ubiquitously used background therapy [12]. A battery of such agents (statins, etc) has shown great risk benefit, with novel drugs, either targeting new mechanisms for reducing the complications of pre-existing ones, constantly emerging [13, 14].

Unfortunately, many cases of coronary disease are brought on unexpectedly (ACS), or are simple too far progressed (severe luminal stenosis), necessitating more intensive strategies. In cases of thrombosis, highly potent anti-thrombotics and fibrinolytic agents (which prevent the build up and promote the dissolution of clots respectively) have proven highly effective if administered within critical windows [15]. Such powerful, systemic agents are not suitable for sustained therapy do to excessive complications, and while these provide immediate, life-saving benefit, the underlying problems of atherosclerosis and plaque instability remain [16, 17]. The supply/demand mismatch accompanying severe stenosis can also often be managed medically with agents such as nitroglycerin which, up to a point, can aid innate autoregulatory mechanisms [18]. While such therapies may provide immediate relief, the chronic need for medication often indicates a need for more invasive revascularization procedures. Coronary bypass arterial grafting (CABG) has long been the standard revascularization procedure [19, 20]. However, the advent of minimally-invasive, ever-improving, catheter-based therapies has transformed the state of coronary disease management. Two wide spread approaches include angioplasty, which mechanically disrupts the atherosclerotic segment through balloon expansion, and stenting, which involves the co-
expansion of a permanent, rigid, foreign structure adding post-procedural, luminal support. Although the benefits offered by such treatments are indisputable, changes in the biological and physical environment accompanying mechanical disruption of the endothelial barrier and insertion of a foreign structure, even as minor as a intravascular wire, can lead to deleterious complications by iatrogenically creating an ACS-like state.

![Diagram](image)

Figure 1.3. Clinical and silent (subclinical) thrombotic outcomes of vascular injury.

Much effort has been placed in understanding and controlling the complex, pathophysiological mechanisms of atherosclerotic disease and its devastating outcome, coronary thrombosis. Still, in westernized societies, such endovascular disease processes account for close to 50% of all deaths [6]. For continued improvement of safe, efficacious management of coronary events (pathological and iatrogenic), it is important to gain further understanding into the mechanisms and events leading up to acute thrombosis within the vital coronary arterial setting.
1.2 The Platelet

While a multitude of biological effectors are involved in vascular thrombosis, we will focus on the platelet as an essential mediator of the biological response to vascular injury. Platelets are circulating cells (1.5-4.5 X 10^8/ml) which bud off from megakaryocytes resident in the bone marrow [21]. Although anucleate, platelets are highly responsive to their environment. A multitude of surface receptor interactions, second messenger pathways, pre-packaged granule release substances (Fig 1.4), and translatable proteins allow these cells to provide constant monitor of the vascular environment and play a key role in initiating biological responses.

The importance of platelets in arterial thrombosis cannot be overstated as attested to by the clinical effectiveness of anti-platelet therapies in minimizing arterial thrombotic risk [22]. Upon binding to an injured wall, they alter their expression of surface molecules and release substances which both attract and adhere to flowing cells, as well as promote the enzymatically driven coagulative response. Accordingly, platelet wall reactions and subsequent component interactions are essential to understand when considering the thrombotic response to vascular injury.

1.2.1 Platelet Reactions

Platelet/wall reactions classically take place in a series of steps, beginning with initial contact, followed by a sequence of activation, firm adhesion, cell spreading, and aggregation (Fig. 1.5).
**Figure 1.5. Sequence of platelet/vessel wall reactions.**

**Tethering/Activation**

The platelet glycoprotein Ib (GPIb; in complex with GPIX, GPV) and von Willebrand's Factor (vWF) are important components in enabling initial platelet/surface contact in arterial, high flow environments [23, 24]. Upon endothelial breach, platelets tether to sub-endothelial matrix vWF (either directly present or adsorbed to matrix collagen from the blood) via fast on-off rate GPIb bonds [25]. The speed of these bonds is an essential quality of this interaction in allowing the platelets to grab onto the surface under high shear conditions [[26-28]]. Several studies have shown that these tethering interactions become increasingly important at shear rates above 300 s⁻¹, becoming essential at shears above 1500 s⁻¹ in reducing the platelet relative wall velocity in a start-stop type dynamic [[26, 29]]. As the platelet slows and translocates, a multitude of other activating responses take place. The GP Ib-vWF bond itself is known to be one of these activating pathways by interacting with internal, second messenger machinery [28, 30-32]. Concurrently, autocrine and paracrine substances, such as ADP, thrombin, thromboxane,
and epinephrine, bind to specific receptors, further potentiating platelet activation [21, 33]. Recently, the importance of the collagen activating receptor GPVI has also been shown to provide a physiologically essential activating interaction with exposed collagen and vWF surfaces [34]. It is an integration of these coalescing, outside-in, activating pathways that dictate the final activation decision.

**Firm adhesion/Spreading**

An essential outcome of platelet activation in supporting shear dependent thrombosis is the modulation of platelet integrin receptors from their low to high affinity state. Integrins are cell surface molecules, composed of an α and β subunit, and support adhesion to particular substrates [35]. The major platelet integrins are αIIbβIII, α2β1, and αVβ3 [[35, 36]]. Upon activation, these receptors support the firm adhesion of platelets onto fibrinogen, collagen, and fibronectin surfaces respectively. However, the existence of other, less prevalent integrins and substrate cross reactivity create redundancy in the adhesion pathways and while providing a safety net given the importance of adequate platelet adhesion, dramatically complicating the mechanistic picture.

After firm adhesion, internal processes that are not fully characterized lead to filipodial extensions and cell spreading [37]. Such cytoskeletal rearrangement allows more complete surface contact, enhanced integrin binding, and increased binding strength of the adherent platelets.

**Aggregation**

Platelet shape change is accompanied by granular exocytosis which helps to transform the local biological milieu by the release of substances such as ADP and thromboxane [[21]]. Free-stream platelets are potentiated and activated in this pro-thrombotic environment. As induced during surface interactions, such activation enables high affinity integrin conformations. Platelet-derived fibrinogen and vWF add to pre-existing, local plasma concentrations of these proteins, enhancing binding to activated αIIbβIII integrin receptors [21, 38]. These protein/receptor interactions mediate inter-platelet cross-linking and aggregation, forming what is commonly referred to as the primary hemostatic plug.
1.2.2 Platelet Interactions

Though platelets play an essential, initiating role in arterial thrombosis, they are far from the only participants. Numerous interactions with other blood-borne cellular and protein components have long been recognized as mediators of thrombotic disease.

Coagulation

When platelets are activated, negatively charged phospholipids, such as phosphotidylcholine and phosphatidylserine, are externally presented, localizing various enzymatic reaction complexes of the coagulation cascade to the platelet surface [21, 33, 39]. This sequestration keeps the reactants in close proximity to one another, while protecting them from other free-stream anti-coagulative mechanisms. Upon association with the platelet membrane, conformational changes in enzyme/substrate complexes also dramatically accelerate the reactions. Such localization and catalysis are essential for the successful progression of coagulation under flow conditions where convective mass transport would otherwise wash away the formed products [21].
Fig 1.6. Classical reactions of the coagulation cascade commencing with the exposure of tissue factor (Extrinsic activation) and/or an appropriate surface (Intrinsic activation), leading to the explosive, proteolytic production of thrombin and fibrin.

While platelets are necessary in supporting coagulative propagation, the products of the coagulation cascade in turn play a critical role in potentiating and stabilizing primary, platelet-driven hemostasis. The key reactions of this proteolytic pathway are shown in Figure 1.6 [21, 39]. Classically, coagulation is initiated via the extrinsic or intrinsic pathways, merging into the common pathway with the eventual production of thrombin which catalyzes the conversion of monomeric fibrinogen to fibrin. While numerous interactions exist, thrombin and fibrin are two of the most important and well characterized links between platelet function and coagulation.

Thrombin is known to be a potent activator of platelets through variety of mechanisms. Protease activated receptors (PAR-1, 4) have been characterized on the surface of platelets [40-42]. Using a unique cleavage mechanism, thrombin causes receptor self-association and activation. Interestingly, the various PARs are functionally activated at different threshold concentrations of thrombin, while additional interactions with the GPIb receptor have been recently recognized to affect platelet activation [42-45].
Such connections allude to the delicate control structures poised between the coagulative network and platelet function.

While the physiological relevance of thrombin-activation has been shown, its catalytic conversion of fibrinogen to fibrin also plays a critical role in platelet-driven thrombosis. Once formed, monomeric fibrin quickly polymerizes into a mesh-like network of filaments. Platelets can adhere to this fibrin mesh via αIIbβ3 receptor linkage, greatly strengthening the cohesiveness of the aggregating, hemostatic plug (Fig. 1.7) [46].

Though these classical platelet/coagulation interactions are key mediators of physiological thrombus formation, they are only representative of a long list of interactions, many of which are characterized, and a great many of which undoubtedly remain to be found.

**Leukocytes**

Leukocytes are the cellular mediators of the inflammatory and immune responses. In blunt categorizations, these responses have often been considered as separate to the thrombotic reactions. However, upon closer examination, the great deal of interactions between these biological processes has grayed the distinction. As with coagulation, a variety of platelet processes are involved in initiating inflammatory responses and vice-versa. Upon vessel wall injury and platelet recruitment, P-selectin is rapidly presented on the surface of the activated platelet layer. Surface expression of this selectin is able to recruit various leukocytic cells. Neutrophils and monocytes initially adhere via P-selectin glycoprotein ligand-1 (PSGL-1), tethering in a manner analogous to the GPIb-vWF interactions of platelets [25, 47]. Again, outside-in signaling causes leukocytic activation and integrin modulation enabling firm adhesion. The importance of platelets in heralding such inflammatory processes is witnessed in the effectiveness of P-selectin blockade or powerful platelet inhibition on limiting the development of chronic vascular

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inflammatory processes such as atherosclerosis or the neointimal formation after interventional treatments [48].

Just as platelets play a critical role in inflammation, the importance of inflammatory mediators in thrombosis is now being recognized. Cytokines produced during inflammatory processes such as tumor necrosis factor (TNF), interlukin-1 (IL-1), monocytic chemoattractant protein-1 (MCP-1), and IL-6, have all been found to upregulate the expression of tissue factor (TF) on endothelial and monocytic surfaces. TF is the key initiating factor in extrinsic activation (see Fig. 1.6), and it is becoming recognized that such cytokine-mediated expression of TF acts as a primary initiator of thrombosis by shifting the vascular environment into a prothrombotic state [49].

Erythrocytes

Erythrocytes (red blood cells; RBCs) are also important in modulating arterial thrombotic events. Being the most numerous blood cell, RBCs dramatically alter the fluidic properties of blood. Flow-dependent gradients in erythrocyte distribution marginalize the platelets to the flow periphery, dramatically increasing local platelet wall concentrations [50, 51]. Further, by increasing the viscous forces of blood, erythrocytes dramatically affect the shear force at a given shear rate which can have significant impact on biophysical processes such as thrombosis.

RBCs are also one of the primary, physiological sources of ADP, a potent activator of platelets. Under conditions of shear, ADP pathways have been shown to greatly enhance platelet activation and adhesion [52, 53]. The relevance of this mechanism in arterial thrombus formation is attested to by the efficacy of ADP receptor blockade via thienopyridine derivatives (i.e. clopidogrel, ticlopidine) in minimizing post-interventional thrombotic risk [53, 54].

In this section, we have described the fundamental processes underlying platelet/wall adhesion, which serves an essential, initiating role in arterial thrombus formation. We
have also introduced some of the classic interactions that platelets undergo with other blood-borne, vascular components. While these interactions only breach the surface of the thrombotic response, they point to the complexity of the biological issue and the need to develop controllable systems where parameters can be sufficiently isolated, allowing tractable, mechanistic studies to be performed.
1.3 The Local Hemodynamic Setting

It is important to recognize that biological interactions both occur in and react to their physical surroundings. In vascular environments, flow is one of the critical physical parameters that helps to maintain vascular diversity and dictate vascular responses. The coronary environment provides a distinctive setting where curving vessels, numerous branch points, geometric pathologies and the time-dependent nature of the driving pressure create highly unique, complex flow patterns (see Fig. 1.8) [1, 55-58].

![LAD Phasic Coronary Blood Flow](image)

Figure 1.8. Typical phasic coronary flow profile in the left anterior descending coronary branch (LAD). In supplying blood to the beating myocardial wall, coronary flow principally occurs during diastolic relaxation, being driven to a halt with rising, systolic, intramural pressures.

These spatially and temporally diverse conditions must be appreciated when considering highly flow-sensitive events such as thrombosis. Here, we introduce some of the mechanisms whereby physical flow parameters may interact with biological factors and the manner by which the local hemodynamic environment can be altered in states of disease.
1.3.1 Local Flow Interactions

Flow can affect biological interactions on variety of levels, from molecular to microscopic to macroscopic scales. Often times these dependencies stem from basic physical concepts such as mass transport and physical forces. However, given the long time scales that these biological mechanisms have been interacting within relatively stable physical environments, selective pressures have also enabled biological systems to evolve sophisticated machinery, allowing them to respond and adapt to their surroundings in a more complex manner.

Molecular Interactions

The mass transport of molecular substances to and from an activating locus plays an important role in thrombus formation. In flowing environments, fleeting conditions are setup as diffusive and convective forces transport substances to and from the reactive site. The mass flux, J, of a substance reacting at a surface has been shown to be:

\[
J(x,t) = \frac{C_0}{k_w + \frac{1}{k_i(x,t)}}
\]

for arteries of diameter \( > 0.1 \text{mm} \) [59]. This relation shows that the flux is dependent on the wall reactivity of the substance, \( k_w \), as well as the mass transport coefficient, \( k_i \). In cases where the wall reactivity is very low compared to the mass transport coefficient, the flux is said to be reaction limited. Alternatively, with low mass transport coefficients, transport dictates the surface flux.

Flow affects this relation implicitly through \( k_i \), which, in the case of large cylindrical arteries, is given by:

\[
k_i = .5K \left( \frac{\gamma D^2}{x} \right)^{\frac{1}{3}}
\]
where $D$ is the diffusion constant, $K$ is a proportionality constant dependent on geometry and boundary conditions, $x$ is the axial distance from the start of the region, and $\gamma$ is the wall shear rate [59]. Thus, when substances are transport limited ($J \rightarrow C_0 k_1$), flow modulates the surface flux. This becomes highly relevant in cases of static or recirculant flows such as those which are often established in the complex coronary vasculature (particularly given the autocatalytic nature of many blood-borne reactions). That the physiological reactions of thrombosis globally function in this flow dependent regime is revealed by the morphological differences in thrombus formation under venous (low) and arterial (high) flow systems and by observations such as the increased likelihood of post-interventional acute thrombotic events in poorly perfused vessels [[60-62]].

**Microscopic Interactions**

**Cellular Transport ($k_m$)**

Just as reactive molecules are transported to and from the reactive surfaces, so are cellular components. However, due to special non-Newtonian properties of blood, the diffusion coefficient, $D$, of cells is itself a function of erythrocyte concentration and flow parameters. As blood flows through the vasculature, RBCs migrate towards the flow axis creating a flow-dependent, radial concentration gradient. As a result, platelets are marginalized to the flow periphery, increasing the shear dependence of platelet mass flux [50].

**Wall Reactivity ($k_w$)**

While cells and molecules must be transported to the wall, they must also be able to react when appropriate, and the wall reactivity, $k_w$, can also be considered a complex function of fluid flow. Local shear stresses and pressure forces have been shown to greatly affect vessel wall morphology. Endothelial cells and wall smooth muscle cells are able to sense these physical forces and respond with directed genotypic and phenotypic changes. When activated, these cells alter their expression of surface adhesion molecules [1, 63]. These, along with numerous other changes (i.e. TF, vWF, tissue plasminogen activator, platelet activating factor-1, thrombospondin, RANTES, monocytic chemoattractant
protein-1), alter the local environment creating a generalized, prothrombotic, proinflammatory state [1, 58, 64-66].

The paradigm of vascular cell adhesion to this reactive wall in flowing environments parallels the scheme outlined for platelet adhesion (recall Fig. 1.5), beginning with the presentation of a particular class of surface adhesion molecules called selectins (or vWF in the case of platelets). Various cell types express various types of selectins. These molecules interact with respective ligands on free-stream cells, thereby recruiting them to the vessel wall. Selectin/ligand interactions are characterized by fast binding constants, and are thus able to catch flowing cells under high shear conditions [25]. While necessary in supporting the initial cell/wall contact under flow, they do not bind tightly, and instead produce a rolling motion as bonds are sequentially broken and formed. During this slowed, translocation, several other pathways cause vascular cell activation, resulting in integrin receptor activation and firm, shear-resistant adhesion to surface ligands [67]. These prototypical flow mediated interactions are representative of vascular cell/wall interactions, with the exact receptor/ligand interactions dependent on the particular cell type and substrate under consideration.

**Macroscopic Interactions**

On macroscopic scales, shear stresses can integrate to create high local forces which can have a significant impact on the pathogenic progression of vascular disease. Atherosclerotic plaques are characterized by atheromatous core regions, covered by a fibrous cap. Acute thrombotic events often occur when such plaques rupture by sudden removal of the cap region and currently there is much interest in studying the strength of the cap as varied biological properties can affect plaque stability [68-71]. However, the final rupture event is predicated on the fact the cap strength is too weak in relation to local fluidic forces.

As cells and molecules aggregate on the reactive wall via the flow-dependent processes outlined above, larger mural thrombus develops and is increasingly subject to macroscopic embolizing forces. As in the case of plaque rupture, embolization is known to be highly dependent on shear rates, with embolic stresses varying linearly with wall shear at thrombus heights less than 0.1 mm and quadratically at larger heights [72, 73].
In either situation, the rheological situation dictates the removal forces that help to determine detachment probability.

### 1.3.2 Local Geometric Environment

Local fluid flow plays an important role in directing the thrombotic response. By prescribing the flow boundary conditions, the local physical geometry is critical to determining the contained flow field, and thus also plays a crucial role. These geometries can be dramatically altered in states of disease or intervention, having great impact on the local hemodynamic environment and the tightly dependent biological outcomes.

**Physiological Geometries**

Physiological vascular properties such as curvature, branching, and tapering are all highly relevant in establishing the local flow environment, particularly in the coronary bed where the arteries coarse over the curved epicardial surface, supplying numerous branches to the demanding myocardial wall (Fig. 1.9) [74]. In purely axial flows, radial and circumferential symmetry ensure purely axial pressure gradients and relatively simple flow profiles. However, in more complex, curved flow streams, adverse axial and orthogonal pressure gradients are established, creating secondary flow fields and zones of recirculation (Fig. 1.10a, b) [56, 75, 76]. These can have significant impacts on local wall shear stresses and through the biophysical connections outlined above, orchestrate local biological responses.

![Angiogram of coronary arteries depicting highly branching, curving geometries overlying and penetrating the heart tissue (image reproduced from Dhond, MK, et. al. Clinical Cardiology. 22(5), 1999).](image)

**Figure 1.9.**
Figure 1.10 Examples of vessel architecture leading to complex flow patterns. a. Vessel branching and axial flow reversal and recirculation; b. Vessel curvature and transverse, secondary flows.

Such responses are essential in vascular remodeling and maintaining healthy vessels. However, when inappropriately harnessed, they can also lead to vascular disease progression. The effect of local, physiological geometries and the resultant flow fields in vascular pathology is well recognized, as indicated by the frequent initiation of atherosclerosis at sites of vessel branching or the increased atherosclerotic burden observed on the inner curvature of coronary vessels [77, 78].

**Pathological Geometries**

While physiological geometries correlate well with disease initiation, local geometries can be significantly altered with disease progression, often compounding the detrimental
effects. As cellular and necrotic debris accumulate within the wall, volume constraints result in either inward (stenotic) or outward (aneurismal) remodeling of the vessel wall. In cases of compensatable stenosis, mass conservation implies increased flow velocities for a given total flow rate, with wall shear rates at times exceeding $10,000 \text{ s}^{-1}$ (typically $< 600 \text{ s}^{-1}$ in the coronary arteries) [51, 52, 79]. Under such pathological conditions, free-stream vascular cell activation and erythrocyte hemolysis can significantly alter volumetric blood status leading to both local and far-reaching systemic effects [79, 80]. When the total flow rate can no longer be maintained due to excessive stenotic flow resistance, severe decompensation can conversely result in dramatically reduced flows. Indeed, when plaques rupture and mural thrombus occludes the lumen, increased resistance can totally eliminate blood flow, quickly potentiating further thrombus growth. Alternatively, under aneurismal conditions, flow rheology can also be significantly affected. As a result of reduced transport and embolic forces in recirculant zones, significant, venous-like mural thrombus formation is often observed, creating a nidus for thromboembolic disease [21, 59].

**Interventional Geometries**

Significant geometry-altering vascular disease is often treated with interventional revascularization techniques which can in themselves affect the local geometry and hemodynamic environment. Stenting involves the placement of an intravascular structure that props open the vessel lumen. The post-interventional luminal shape is determined by the overall profile of the implanted stent device, and has been shown to have an impact on long-term biological outcomes, likely due in part to alterations in fluid flow [81]. Furthermore, in order to provide sufficient radial support, stents are typically made of a mesh work of steel struts on the order of 100 μm thick. While this thickness is relatively small compared to the entire flow diameter (> 2 mm), their juxtaposition next to the vessel wall can significantly alter local wall shear stresses, with complex flow patterns developing in the inter-strut regions [82-85]. Berry et. al. have shown that changes in stent geometry, can dramatically affect these flow fields and resultant mass transport parameters [82].
The effects of stent placement can also be more pronounced depending on implantation technique. Significant over-expansion can lead to aneurismal-like conditions, while insufficient expansion can result in significant flow abnormalities. Indeed, such improper expansions have been associated with the high acute thrombotic rates observed in the early clinical stenting experience [61, 86].

While the placement of intravascular objects can create altered local flow, a more subtle point is that revascularization itself alters the geometry and ‘expected’ flow field of the vessel due to the significant biological adaptations that occur during disease progression. Recently, Richter and Edelman (unpublished observations, 2003) have shown that such acute interventional revascularization can lead to adverse, unexpected outcomes.

We have briefly described some of the key mechanisms by which flow can interact with the thrombotic processes. Furthermore, we have discussed how the physical, geometric environment can have a significant impact on flow parameters under physiological, pathological and interventional conditions. Considering these significant interactions, an attempt to study thrombotic reactions in the coronary setting would hopefully take these physical parameters into account, allowing them to be manipulated and studied in a controlled fashion, along with the pertinent biological components.
1.4 Methods of studying flow dependent thrombosis

A variety of methods have been utilized to investigate thrombotic reactions under appropriate hemodynamic conditions. These methods include both in vivo and ex vivo models, as well as bench-top in vitro setups. Each of these methodologies has offered unique insights by virtue of inherent advantages, though often these same advantages limit the types of information that can be gleaned from their use. It has been a combined use of these techniques, along with an appreciation of their limitations that has contributed greatly to our current understanding of vascular biology. Here, we present various methodologies that have been and are currently being used to study flow-dependent thrombotic reactions and the types of advances they have provided.

1.4.1 In Vivo Models (Realistic)

In vivo models offer a degree of realism and physiological relevance unattainable in bench-top models, and when chosen appropriately, allow thrombotic outcomes to be assessed in relevant hemodynamic conditions. The two most basic types of in vivo study include clinical trials and animal studies.

Human Studies/Clinical Trials

Human studies and clinical trials generate immediately applicable, clinical information, inherently taking into account the complex biological interactions at work in the human body. Such studies allow integrated, relevant, systemic and long-term processes to be observed, offering clear cut advantages over other systems when considering actual disease characterization, patient risk stratification, and the efficacy of therapeutic options. Historically, mechanistic insights have been obtained from observing the human condition, often times becoming evident when systems overtly fail. Indeed, many important processes of the thrombotic response have been initially discovered through clinical presentation (GPVI deficiency, Glanzmann’s thrombasthenia, Bernard-Soulier, GPIb deficiency, vWF disease, etc.) [21]. While such accidental observations are predicated on patients and patience to search the space of biological processes, they are implicitly of great relevance.
Human studies and clinical trials are by definition the most relevant to the human condition. However, as the primary goal is to benefit the immediate patient, making clear scientific progress is difficult at best. The lack of ideal controls and patient variability (particularly given the optimizing nature of medical advance) causes such trials to be expensive and long, oftentimes yielding unclear conclusions. Further complicating the issue is that as trials are performed, the evolving ground of clinical experience often races by, altering the clinical relevance, therapeutic dogmas and potentially, for patient benefit, the clinical trials themselves.

**Animal Studies**

One step removed, animal models of physiology and pathology allow detailed observation of complex biological situations while offering a more controlled setting than human studies. Though ethical considerations and respect must always accompany the experimental use of living organisms, an accepted goal in animal use is the progress of scientific understanding for human benefit, thus allowing intensive, well-controlled, prospective studies to be carried out to completion.

Animal models of thrombosis span the evolutionary ladder, from the use of non-human primates to mice. Architectural similarity and flow conditions in large animals allow highly relevant studies of thrombotic processes under appropriate hemodynamic settings. Alternatively, the ease of manipulation and relatively low cost of small animal studies enable more fundamental questions to be epistatically probed, dramatically accelerating the pace of scientific discovery and understanding. However, the relevance to the human condition varies tremendously as a result of species-specific biological and physical differences and the often crude models used to approximate human vascular disease. Furthermore, while offering greater experimental possibilities than human trials, there are still limitations in the parameters that can be reasonably manipulated in living animals, particularly when considering complex traits such as the hemodynamic environment [79, 87-89].
1.4.2 *In Vitro* Models (Perturbable)

To gain detailed mechanistic insight into the rheological dependence of thrombosis, it is important to have parametric control over the hemodynamic and complex biological environments. *In vitro* models allow powerful manipulations to be performed on isolated biological components under highly consistent, prescribed physical conditions, thus offering a degree of versatility not attainable with *in vivo* settings. Such models can broadly be categorized into systems that maintain physical geometric properties and those that maintain flow conditions.

**Maintaining Geometry**

Geometrically relevant *in vitro* systems maintain realistic vascular dimensions and have been applied towards various issues, from endovascular device thrombogenicity to cellular adhesion in vascular deformities. One limitation in these models has been in developing suitable bench-top mechanisms of flow actuation and control. While some setups have employed gravity to provide a pressure head for generating blood flow, the need for sizable, static holding volumes in these one-pass systems significantly constrains the allowable flow rates and experimental run times [89]. Alternatively, loops partially filled with blood and mounted on a tilted turntable have been employed to create relative wall motion. This method allows the use of small blood volumes, though effects of blood recirculation and the required air-fluid interface must be considered when interpreting results [90-92]. Moreover, the constant flow potential of these gravity-driven systems results in restricted control of the flow environment. To establish more controllable flows, the most basic approach has been to use pumps to drive blood through a model flow circuit [93, 94]. However, even external peristaltic drives have generated excessive background levels of activation, proving too traumatic in setting up arterial-like flow conditions.

**Maintaining Flow**

Strategies that attempt to maintain geometry are typically constrained by the need to create high flow rates to generate arterial-like conditions. In order to study flow effects, laxness in the physical circuit architecture has enabled alternate schemes to be employed.
The use of small circuit geometries such as narrow, parallel-plate flow chambers, allows high shear rates to be developed under relatively low flow conditions. [26, 83, 95-97]. Such in vitro systems allow great control of the biological environment while enabling microscopic visualization of surface-cellular interactions in real time. As a result of their controllability and observability, these studies have greatly enhanced our fundamental understanding of shear-dependent, thrombotic surface reactions.

Techniques such as cone-plate or annular ring devices create relative motion between two fluid-contacting surfaces, thus generating Cuvette-type flow. These models can establish well-described surface and volumetric shear profiles. Furthermore, while these Cuvette-systems dramatically change the physical flow shape, suitable scaling and experimental design have allowed certain geometrically relevant questions to be asked under appropriate flow settings [58, 98].

Chapter 1: Background and Significance
1.4.3 Proposed Flow System

Table 1.1 compares prior model systems that have been employed to study the hemodynamics of thrombosis with respect to the human condition.

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Table 1.1. Comparison of prior methods showing an incomplete niche for experimental strategies supporting the simultaneous parametric analysis of biological and physical systems. (Subjective scale, using clinical trials to reference extremes ratings: +++ → --; Excellent → Poor).

Combining the information obtained from these systems has lead to great leaps in our understanding of acute thrombosis. Ex vivo hybrids, where external sections are placed in-line with a natural circulation, have helped to bridge the gap where in vivo and in vitro models do not meet [51]. Still, cracks remain and alternative models addressing new issues are needed. One particular niche which we hope to address in this work is the need
for systems that concurrently retain significant control of both the biological and physical axes, specifically with regards to the human coronary setting. The key selection criterion we considered when developing the system were:

1.) Generating and controlling physiologically relevant flows
2.) Creating versatile, geometrically relevant circuits
3.) Retaining control of the biological setting
4.) Maximizing the biological signal/circuit background noise ratio

After considering various strategies, we developed a novel, inertial mechanism that could produce highly controllable flows in relevant arterial geometries while maximizing the biological signal to background noise ratio by minimizing the circuit length and surface discontinuities. Briefly, fluid-filled loops are spun about their axis in a prescribed and controlled fashion to modulate relative fluid/wall motions through transmitted shear forces. This method further allows us to maintain low test volumes and cost, enabling reasonable parametric analysis of the biological and physical settings.
CHAPTER 2

Flow System Design

2.1 Theory

2.1.1 Description

In order to create the desired flow profiles, a fluid-filled torus (figure 4) is rotated about its axis. When impulsively started, there is inertial fluid motion relative to the toroid wall. With time, the fluid is accelerated due to momentum transfer into the fluid bulk via shear forces. Lyne has previously analogized such fluid motion to pressure driven flows where, moving in a reference frame with the wall (WRF), the acceleration takes on the driving character of a body force [99]. While the absolute fluid velocity in an inertial reference frame (IRF) may change under pressure driven or accelerating wall conditions, it is the maintenance of the velocity gradients, or shear, that is important in the flow-dependent thrombotic reactions [21, 50, 59]. Rather than fluid being conducted to and from a reactive wall surface, the relevant reaction control volumes are conducted away from the lagging fluid.

Using such a technique, we hoped to create time-varying flows whose characteristics, as defined by the dimensionless mean Reynolds ($\bar{\text{Re}}$; based on an absolute velocity) and Womersley ($\alpha$) parameters were typical of coronary flows, as well as estimate the secondary flow effects of curvature by considering the peak Dean number ($\kappa_{\text{peak}}$) associated with our system (see Table 2.1).
Table 2.1. List of the dimensionless parameters considered, along with customary definitions and typical values found in the left anterior descending coronary branch (LAD) [13]. $V_{\text{fluid}}$ is the mean cross-sectional axial velocity of the fluid, $v$ is the kinematic viscosity, $\omega$ is the flow oscillatory frequency, $a$ is the vessel radius, and $R$ is the radius of curvature of the loop. $\Psi$ This value for $k_{\text{peak}}$ was calculated assuming a peak Re of twice the Re and a aspect ratio, $R/a$, of 10 as suggested by Chang and Tarbell [15].

<table>
<thead>
<tr>
<th>Dimensionless #</th>
<th>Definition</th>
<th>Typical Coronary Values (LAD)</th>
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<tbody>
<tr>
<td>Mean Reynolds # ($Re$)</td>
<td>$\frac{2aV_{\text{fluid}}}{v}$</td>
<td>153±64</td>
</tr>
<tr>
<td>Womersley # ($\alpha$)</td>
<td>$a\sqrt{\frac{\omega}{v}}$</td>
<td>1.5±0.27</td>
</tr>
<tr>
<td>Peak Dean # ($k_{\text{peak}}$)</td>
<td>$\max\left(\frac{2aV_{\text{fluid}}}{v} \sqrt{\frac{a}{R}}\right)$</td>
<td>95$\Psi$</td>
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</table>

Table 2.1 defines these conventional dimensionless parameters [100]. In each of these parameters, a Newtonian approximation for the kinematic viscosity, $v$, is used, as has been shown to be valid for high shear conditions ($> 100$ s$^{-1}$) and often applied when considering coronary flow [76, 78, 101, 102].

### 2.1.2 Analytical, Straight Tube Approximation

**Equations**

Simplifying to a linearly accelerating, straight tube model (Fig. 2.2), streamline curvature effects can be neglected and only the axial ($z$) component of the Navier-Stokes equation for cylindrical coordinates need be considered:
\[
\left( \frac{\partial V_z}{\partial t} + V_r \frac{\partial V_z}{\partial r} + V_\theta \frac{\partial V_z}{\partial \theta} + V_z \frac{\partial V_z}{\partial z} \right) = -\frac{1}{\rho} \frac{\partial p}{\partial z} \frac{d^2 Z}{d t^2} + \nu \left( \frac{\partial^2 V_z}{\partial r^2} + \frac{1}{r} \frac{\partial V_z}{\partial r} + \frac{1}{r^2} \frac{\partial^2 V_z}{\partial \theta^2} \right) + \frac{\partial^2 V_z}{\partial z^2}
\]

where \( V_z, V_r, \) and \( V_\theta \) are the velocity components in the axial \((z)\), radial \((r)\) and tangential \((\theta)\) directions respectively, \( t \) is time, \( \rho \) is the fluid density, \( \delta p/\delta z \) is the axial pressure gradient, and \( d^2 Z/dt^2 \) is the axial wall acceleration.

![Figure 2.2. Theoretical system assuming linear rather than angular motions.](image)

In the special case of flow in a circular pipe of constant cross sectional area, circumferential and axial symmetry allow several dependencies to be eliminated, leaving only an axial velocity component, \( V_z \), dependent on \( r \) and \( t \), and the driving pressure and acceleration terms. These considerations yield the equation:

\[
\frac{\partial V_z}{\partial t} = -\frac{1}{\rho} \frac{\partial p}{\partial z} \frac{d^2 Z}{d t^2} + \nu \left( \frac{\partial^2 V_z}{\partial r^2} + \frac{1}{r} \frac{\partial V_z}{\partial r} \right)
\]

By prescribing periodic functions for the driving terms of the form,

\[
\frac{1}{\rho} \frac{\partial p(t)}{\partial z} = \sum_{n=1}^{\infty} G_n e^{i\alpha n}
\]

**Chapter 2: Flow System Design**
analytical solutions with appropriate wall and centerline boundary conditions ($V_z(R, t)$=0; $dV_z/dr (0,t)$=0; where $R$ is the tube radius) have been derived elsewhere [101, 103-105], yielding:

$$V_z(r, t) = -\frac{(G_0 + A_0)}{4\nu} \left( R^2 - r^2 \right) + \sum_{n=1}^{\infty} \frac{(G_n + A_n)}{in\omega} \left[ J_0 \left( i^{1/2} \frac{\sqrt{n\omega}}{\nu} \right) \right] e^{int}$$

where the axial flow velocity, $V_z(r, t)$ is a function of radius and time, and can be driven by pressure gradients (Eq. 5) or wall accelerations (Eq. 6). $G_n$ and $A_n$ represent the amplitudes for the Fourier components of the nth harmonic of $\omega$ for the pressure gradient and wall acceleration respectively, while $G_0$ and $A_0$ are the steady forcing terms. From this straight tube model we see that identical relative flow profiles can be obtained through either pressure gradients or wall accelerations given the interchangeability of $G$ and $A$.

**Solutions**

Using this linearly accelerating straight tube approximation, we can analytically study the nature of the contained flows under various prototypical forcing conditions. By applying an acceleration impulse of the form:

$$\frac{d^2 Z(t)}{dt^2} = \sum_{n=1}^{\infty} e^{int}$$

Chapter 2: Flow System Design
and solving equation 7 for $V_z(r, t)$ using $R=0.16$ cm (typical of coronary geometries), and a Newtonian approximation for the blood viscosity, $\nu=0.04$ dynes cm/sec (valid for arterial type flows), we obtain the solution shown in figure 2.3.

![Relative Radial Velocity Profile](image)

**Figure 2.3.** Relative radial velocity profile and decay given an impulsive wall acceleration.

We see that there is an initial spike of relative fluid motion which decays as shear forces propagate through the fluid, quickly establishing parabolic, radial velocity profiles. It is important to note that an impulse of wall acceleration leads to a step in wall velocity, and while this means that there continues to be fluid motion in the IRF, relative wall motion is not sustained through constant wall velocities. The impulsive decay is governed by a time constant dependent on the fluid viscosity and square of the tube radius (see Eq. 7) which we find to be equal to $\approx 0.1$ sec (time to $36.7\%$ of peak value). While different amplitudes of acceleration can be used to drive the contained flow, this characteristic time remains unchanged so long as the fluid and tube properties remain constant.

In order to maintain a relative fluid/wall motion, the tube wall must continue to accelerate. For the case of constant acceleration,

$$A_0 = 628\text{cm/sec}^2$$

(9)
we can plot the radial, relative velocity profiles with time in the WRF (solid surface), along with the velocity profiles in an IRF (mesh surface; Fig. 2.4).

Figure 2.4. Parabolic radial velocity profiles due to a constant wall acceleration in both a relative wall (solid) and inertial reference frame (mesh); R=0.16cm; \( v = 0.04 \) dynes cm/sec.

As predicted, the induced, relative, constant velocity profiles are parabolic in nature, paralleling pressure-driven Pousille flows. While such relative flows can be created and maintained through wall accelerations, the continuous acceleration leads to unbounded wall and IRF fluid velocities. More generally, net positive flows require net positive accelerations. Thus any motor used to drive such a system would quickly reach its operating limit.

While this situation poses a problem, the unique coronary flow which we hope to emulate is highly pulsatile, as a systolic rise in intramural pressure drives the flow to a halt (recall Fig. 1.8). This creates a situation where relative wall motions and tube accelerations are reduced to zero. If the time for flow decay is gradual and greater than approximately three system time constants, the flow can be reduced to within 5% of its peak value.
without having to decelerate the tube walls, while slower decay profiles can be quasi-
statically accommodated by modulating the wall motions. On the other hand, rapid flow
decelerations would create flow reversal and more complex flow patterns. Although
identical, repeating acceleration profiles could maintain unidirectional flow pulses, this
situation would lead to impractical, compounding wall velocities in the IRF. Rather,
introducing a one-way valve or allowing bi-directional flow offers two potential
solutions.

By incorporating a one-way valve, the walls can be rapidly brought to a halt once
the contained fluid has reached solid body motion. This action essentially creates a
negative impulsive wall acceleration that would normally drive rapid relative retrograde
flow. However, by virtue of the one-way valve, the fluid can be kept motionless in the
WRF and brought to rest in the IRF. Thus, unidirectional flow pulses can be maintained
without a net compounding wall velocity.

There are various drawbacks to this method. Designing a one-way valve of
sufficiently low profile is difficult, and would itself generate unwarranted resistance and
hemodynamic effects. Even though the flow could be stopped in solid body rotation via a
one-way valve, the imposed pressure wave created in the fluid due to the sudden
deceleration could have consequences on the thrombotic process. As a principle goal of
this system is to reduce noise and minimize external sources of thrombotic activation to
allow sensitive, flow dependent studies, such disruptions are undesirable.

Alternatively, bi-directional flow at one half the frequency ($\omega_{\text{system}}$) of the desired heart
rate ($\omega_{\text{coronary}}$) can be established by following each tube acceleration profile with a
symmetric deceleration. Such an action would create a flow of identical magnitude
though in the opposite direction, at the end of which the tube would have no net change
in momentum. This limits the maximal required speed of the motor to some value
dependent solely of the characteristics of a single pulse, and is minimized by setting the
bounds symmetrically about a zero angular velocity.

To observe the nature of this type of oscillatory flow, we can model wall
velocities assuming a sinusoidal motion.
\[ \frac{dZ(t)}{dt} = \frac{A_1}{\omega_{\text{system}}} e^{i\omega_{\text{system}}t} \quad (10) \]
\[ \frac{d^2Z(t)}{dt^2} = A_1 e^{i\omega_{\text{system}}t} \quad (11) \]

where we only consider one harmonic, \( \omega_{\text{system}} \), which is equal to the frequency of oscillation (heart rate/2=\( \pi \)). \( A_1/\omega_{\text{system}} \) is the amplitude of the wall velocity, and \( A_1 \) gives the corresponding wall acceleration amplitude (200\( \pi \) cm/sec\(^2\)). Again solving equation 7 for the radial velocity profiles, \( w(r, t) \), we obtain the solution shown in figure 2.5.

Figure 2.5. Solid surface depicts the relative radial fluid velocity as a function of time given a sinusoidal wall motion (paralleling the development of those witnesses in sinusoidal, pressure driven flows) while the mesh surface depicts the fluid velocity in the inertial reference frame; \( R=0.16\text{cm} \); \( v=0.04 \text{ dynes cm/sec} \).

Here, we see that oscillatory flows can readily be generated, and moreover, that given the relatively fast system time constants as compared to physiological pulse rates, the fluid/wall motion can be smoothly transitioned from beat to beat, with negligible flow at the beginning of each new pulse. While this example considers a simple, sinusoidal
forcing function, more complex forcing functions (Eq. 6) can be used to generate more complex flows.

### 2.1.3 Curvature Effects

These solutions cover the case of straight tube, axial wall motions. However, both the coronary arteries and our toroidal loop model (adopted for low blood volumes and practical use) have inherent curvature which can have effects on the internal flow. Curving flow streams are affected by centrifugal forces which can establish secondary, off-axis, flow fields when interacting within viscous boundary layers. The magnitudes of these secondary flows are governed by the dimensionless Dean #, $\kappa$, which is a function of the secondary inertial and viscous damping forces, and the main stream centrifugal force. However, the customary definition of $\kappa$ (see Table 2.1) is not applicable in the rotating toroid case, as the relevant velocity term must take into account the fluid's axial velocity in an inertial reference frame ($V_{\text{fluid}}$) which gives rise to the centrifugal forces and a different wall velocity ($V_{\text{wall}}$). It is the difference in these components that gives rise to the relative wall velocity ($V_{\text{rel}}$) and hence, the centrifugal pressure mismatch leading to secondary flows.

To determine a modified $\kappa_{\text{peak}}$ number ($\kappa'_{\text{peak}}$) for comparison with the nominal conditions of table 2.1, we can consider the definition of $\kappa$ as a ratio of the square root of the product of the secondary flow inertial and centrifugal forces to the viscous forces [100], where

\[
\text{Inertial forces} \rightarrow \frac{\rho V_{\text{secondary}}}{2a} \tag{12}
\]

\[
\text{Centrifugal forces} \rightarrow \frac{\rho \left[V_{\text{fluid}}^2 - V_{\text{wall}}^2\right]}{2R} \tag{13}
\]

\[
\text{Viscous forces} \rightarrow \frac{\mu V_{\text{secondary}}}{(2a)^2} \tag{14}
\]
which have been determined from an order of magnitude consideration of the relevant quantities [106]. In these equations, $V_{secondary}$ represents a typical secondary fluid velocity, $\rho$ is the density of the fluid, and $\mu$ is the dynamic viscosity of the fluid. The use of tube and loop diameters ($2a$, $2R$) rather than radii have been used to allow reduction into a quantitatively conventional form. The differential term in the centrifugal relation accounts for the relative centripetal motions in an inertial frame. When both motions are present, $\kappa'_{peak}$ becomes

$$\kappa'_{peak} = \text{Max} \left[ \frac{2a\left(V_{fluid}^2 - V_{wall}^2\right)\sqrt{v}}{2} \left(\frac{a}{R}\right)^{3/2} \right]$$

(15)

When $V_{wall}$ is zero, as in the case of forced flow through a curved, stationary pipe, $\kappa'_{peak}$ reduces to the standard form given in table 2.1

While a thorough, quantitative description of the time-dependent fluid flow profiles within the rotating toroidal case would require more extensive numerical techniques, we satisfied ourselves with the straight tube characterization and the practical goal of matching the critical dimensionless Reynolds, Womersley, and modified peak Dean numbers to those observed in typical coronary flow settings on a beat to beat basis.
2.2 Embodiment

2.2.1 Prototype evolution

In order to create the inertially driven relative flows, we needed to develop a suitable system that could maintain the required toroidal angular motion profiles. Generally, such a system requires a rotor, a suitable strategy of inducing motion and a technique for monitoring the contained flow. In sections 2.2.2-2.2.6, we give a thorough description of the current system embodiment and composite parts. First, we give a brief prototype evolution of the developmental stages.

![Infra-Red Sensors](image)

Figure 2.6 Single fluid loop, infra-red sensing rotor stage. a. Top view; b. Side view.

To verify the inertial method of relative flow induction, we developed a system that could spin a single fluid loop. An Electro-Craft® NEMA42C motor (selected using a similar design rational as detailed in section 2.2.4) rotated a rotor stage through a prescribed motion profile utilizing the control system described in section 2.2.5. The initial prototype utilized a rotor stage with equi-spaced infra-red (IR) sensors and detectors positioned around its perimeter (Fig. 2.6). The stage was designed to allow a fluid loop to pass directly within the IR beam. To detect fluid motion, a small particle of equi-fluid density (typically a fibrin clot) was placed within the fluid loop. With relative fluid motion, the particle would cut the IR beam and its velocity could be determined by
observing sequential deflections of IR detector output. Although this method roughly indicated the suitability and practicality of generating relative flows, the inclusion of a tracer particle with in the actual flow field disturbs both the biological and physical environments and could dramatically affect both the observability and performance of the test system.

![Figure 2.7b](image)

**Figure 2.7b.** Single fluid loop rotor stage with ultrasound flow probe. a. Top view; b. Side view

A second prototype was developed which utilized a rotor stage incorporating an external ultrasound flow probe (see section 2.2.6) to measure relative fluid flows, using the same motor/controller system used for the IR rotor (Fig. 2.7). The external nature of the probe allowed unperturbed flows to be measured with greater granularity and precision than the relatively crude IR technique, while technically verifying the ability to pass the probe signals between rotating and inertial reference frames for this unorthodox use.

These systems have utilized a single rotor stage to create motions within a single fluid loop. In order to perform parametric, mechanistic studies into complex phenomena such as vascular thrombosis, it is important to be able to perform many experiments and developed models and strategies must take this into account. Practically speaking, high throughput systems allow studies to be performed quickly and efficiently. Speed becomes an even greater issue when investigating blood. Upon removal from its natural
vascular environment, it rapidly begins to change. On this shifting ground, studies must be performed soon after withdrawal to maintain study accuracy and temporally close together to maintain study precision. To enhance our ability to probe vascular situations, we developed a multi-rotor system which supported six rotors on a single axis allowing six fluid flops to be spun simultaneously (Fig. 2.8). While the motor needed to be reconsidered to support the increased requirements (section 2.2.4), the single rotor control structures could be maintained.

![Multi-rotor system](image)

**Figure 2.8:** Multi-rotor system. a. Side view showing six, stacked rotor stages with alternating ultrasound flow probes; b. Close-up view of on board probe multiplexer

Using the same evolutionary pressure of study speed and efficiency, we modified the multi-rotor design to enhance modularity and ease of incorporation onto a multi-axis system, thus allowing multiple simultaneous flow profiles to be considered (Fig. 2.9). We now discuss the multi-rotor embodiment in greater detail.

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*Chapter 2: Flow System Design*
Figure 2.9. Current system embodiment with two axes, each holding up to six rotor stages. Much of the electronics have been consolidated into the inertial reference frame, increasing the modularity of system to support future expansions.
System Overview

A system was developed in order to create the inertially driven flow profiles discussed in section 2.1. Figure 2.10 shows the key components of the system, which include a fluid loop, rotor-stage, driving motor, motion controller, and a measurement system.

![System Diagram](image)

Figure 2.10. General multi-axis, multi-rotor flow system schematic.

The blood to be tested is placed into the fluid loop. This torus is then fit onto a rotor platform and is placed in axial alignment with other loops to be tested under the same flow conditions. Although any number of loops is allowed, the embodied system allows six simultaneous runs via six modular rotor platforms per axis. The entire rotor system is then driven through a desired angular motion profile via the motor and controller system.
This motion creates internal flows, which are measured via onboard flow transducers built into the rotor stages. Each transducer sends the flow measurements of a particular fluid loop to the measuring/recording system, which can be used to instantaneously monitor the flow profiles and fluidity of the blood. A detailed description of these components and a rational for their selection follows.

### 2.2.2 Fluid Loops

#### Design

A schematic of the basic fluid torus is shown in figure 2.11. The loops are made of a 24 cm length of 1/8 “ ID::5/32” OD 3350 Tygon silicon tubing. The connecting ends of the tube are squarely cut, orthogonal to the axial dimension to ensure a matching end-to-end fit. This connection is held via a 1.5 cm overlapping segment of S-50-HL Tygon tubing (3/16” ID; 5/16” OD.) The close OD/ID match provides a compression fit and axial alignment of the juxtaposed ends. Further support is provided by a 1 cm elastic band of silicon tubing (Silastic; ¼” ID; 3/8” OD) placed coaxially over the 1.5 cm joiner segment, thus radially compressing the junction.

![Fluid loop design](image)

Figure 2.11. Fluid loop design. a. Loop format with three coaxial sleeves, one for maintaining loop format and two as inlet and outlet ports; b. Coaxial sleeve cross-sectional profile
Two similar structures are slid onto the 1/8" loop at equally spaced 120 degree intervals serving as outlet and inlet ports for the replacement of the loop's contained air by the desired fluid. Briefly, a needle is slid under the outer most elastic sleeve and then driven into the lumen through the middle sleeve and inner loop layers. To minimize possible disturbances to the loop’s inner, fluid-contact surface, small gauge needles are used. While a 26+ gauge needle is suitable for air evacuation, 19 or 20 gauge needles are used for the untraumatic injection of blood components (Fig. 2.12).

Figure 2.12. a. Filling of fluid loop; b. Filled loop. Note the absence of fluid/air interface.

Rational

In settling on these embodied loop dimensions, a primary concern was to match the physiological diameters, typical of a coronary setting (3-3.5 mm; LAD). The 1/8" ID tube was chosen as it fell neatly within this range (3.175 mm), with the 5/32" OD imposed by specifications of the flow probes (discussed below in section 2.2.6).

The overall 24 cm length was determined by balancing the desire to minimize blood contact surface and blood volume (both to maximize the biological signal to circuit noise ratio, while enabling practical biological manipulation) with the need to keep loop curvature at reasonable levels to dimensionally consider secondary flow effects.

The final consideration in tubing selection was the 3350 Tygon silicon tubing chosen for its low bioreactivity and suitable gas permeability. However, given the inertial mechanisms driving the flow, any desirable tube could be used (i.e. rigid vs.
compliant). Furthermore, the tubing can readily be replaced (i.e. vascular graft material, \textit{ex vivo} arterial sections, etc.) or simply coated with a test substance (i.e. proteins, cells, polymers, etc.) whose thrombotic properties are to be specifically studied.

### 2.2.3 Rotors

**Rotor stage design**

The rotors are the platforms upon which the fluid loops are held. In the current embodied system, each axis holds 6 rotors. Figure 2.13 depicts the design of an individual rotor (3 orientation A; 3 orientation B per flow axis). Each rotor was manufactured out of a stock of 3" diameter delrin plastic chosen for its machinability and ease of handling. The key features of the rotor are a resting stage for the fluid loop, an axial hole for alignment of the rotors, a notch for the placement of the flow transducer, and a shaft through which the transducer connections may be passed.

![Rotor Stage Design](image)

Figure 2.13: Schematic drawing of a single rotor-stage. a. Orientation A; b. Orientation B. Note the chirality of the probe notch.

The stage was designed to fit the embodied fluid loops. As such, they snuggly fit the inner most diameter (5.75 mm) of the loop which is outlined by the outer most Silastic
tubing layer of the fluid loop’s inlet, outlet, and junction structures. Only this Silastic band is in contact with the stage, thus suspending the fluid loop. The notch for the flow probe accommodates a Transonic 3CA flow probe (described in further detail in section 2.2.6), and its dimensions were defined by the need to allow the suspended 1/8” ID, 5/32” OD fluid loop to pass through the flow probe’s sensor (Fig. 2.14). As shown in figure 2.13, two chiral rotor conformations exist, based upon notch orientation. This chirality allows the probes to face opposite directions as the rotors are sequentially stacked, thus minimizing asymmetrical, processional loading.

a. 

![Fluid loop positioned on rotor stage. a. Top view showing coaxial positioning; b. Fluid loop passing through the probe’s sensor.](image)

b.

Figure 14. Fluid loop positioned on rotor stage. a. Top view showing coaxial positioning; b. Fluid loop passing through the probe’s sensor.

The shaft for the transducer connections was designed to accommodate a specialized connector (described in section 2.2.6) which relays real-time flow information. A 1/8” hole connects this shaft to the probe’s notch serving as a conduit for essential wires.
Rotor shaft design

The rotor axis is shown in figure 2.15, along with a diagram depicting six stacked, alternating rotor stages. The shaft was machined from a 1” diameter stock of delrin and consists of two sections. The top section has been lathed down to a diameter of ¾”, with a milled ¼” groove running its entire length to accommodate a strip of nylon. This strip protrudes from the shaft and serves as a key to hold the rotor stages in axial alignment via a matching perimeter center-hole in each rotor stage as shown in figure 2.8. The square key design holds each stage in tight tolerance while undergoing angular accelerations. The bottom section remained at the initial stock diameter of 1”. Into it, a centered 1/2” hole allows communication with the driving axis (see Section 2.2.4 for motor selection). To ensure a non-slip junction, a screw key was also placed to grip the motor shaft.

A final caveat that can be observed in figure 2.15 is that the shaft extends past the length of six combined rotor heights. This is critical in allowing for the fit of a cap structure that houses on-board instrumentation for the flow transducers (see Section 2.2.6).

2.2.4 Motor Selection

The driving motor is an Electro-Craft® E643 DC servo-brush motor. The critical design parameters that were considered were the peak torque and maximum operating speed which were estimated from the straight tube, Newtonian fluid approximation outlined in
the previous section, augmented by an impedance factor to account for curvature effects as suggested by Chang and Tarbell [76].

The peak torque was determined from the maximum angular acceleration required to drive the fluid, which in turn was constrained by the desired flow rates. Peak physiological flow rates are around 100 ml/min in the coronary arteries. While the actual profiles are highly pulsatile, the extreme steady flow case (see Fig. 2.4) can be obtained using a constant wall acceleration of 6.28 m/sec$^2$. For the embodied loop diameter of 0.0762 m (3”), this axial wall motion transforms into an angular acceleration ($\alpha; A/2\pi$), of 26.2 sec$^{-2}$. This value can further be augmented by an impedance factor as suggested by Chang and Tarbell [76] to account for curvature effects [100, 104], as well as compounded to allow more extreme flow rates and varying blood conditions (i.e. increased hematocrit), yielding a critical design $\alpha$ of 40 sec$^{-2}$.

The torque, $T$, needed to drive the system at this angular acceleration is given by:

$$T = I\alpha$$

where $I$ is the moment of inertia of the rotor system. $I$ was estimated to be 7.26X10$^{-4}$ kg m$^2$ assuming the rotor was a solid delrin cylinder 0.0762 m in diameter (m~1 kg; $l=1/2mR^2$), resulting in a peak torque of 0.029 Nm.

The maximal operating speed was estimated from the rotor speed required to accommodate one acceleration pulse. With a physiological rate of 60 bpm, we again over-designed our criteria by considering a 2 second pulse duration (30 bpm). This yields a maximum angular velocity of 500 rad/sec (4775 RPM).

The Electro-Craft® E643 model satisfies these criteria, supplying a peak torque of 0.16 Nm and a maximum operating speed of 4800 RPM. A chassis was built to house the motor in a vertical configuration, allowing the rotor system to mount directly on top. It was decided to allow the rotor to communicate directly with the drive shaft, given both the heavy duty nature of the E643 motor and the care taken in axial alignment and minimizing asymmetrical loading. Still, a bearing system to accommodate off-axis loading could be used in future designs.
2.2.5 Control System

Single Axis

In order to control the Electro-Craft® E643 DC servo-brush motor, the control structure shown in figure 2.16 was developed. The components of the motor control system integrate readily and allow specific flow profiles to be generated. These components include a Renco RM15 Encoder, an Electro-Craft® IQ-550 Position Control Module, an Electro-Craft® Max-100 PWM Servo Drive, and a Windows compatible PC terminal running IQ Master software.

![Diagram of control schematic for single axis](image)

Figure 2.16. Control schematic for single axis

The RM15 Encoder is a 1000 line encoder that is placed in axial alignment with the drive, transducing rotor position. This position signal is then sent to the IQ-550 which sends a +/-10 volt analog velocity command signal to control the motion of the motor. The analog signal is dictated by a program written in the IQ Master programming language and stored on the IQ-550’s EPROM. This velocity command is relayed to the MAX-100 drive which serves as a power amplifier, supplying up to +/-3 amps of continuous current to drive the motor.
The rational for choosing these components was their integratability and the ease with which the motor's motion profile could be adjusted via software rather than hardware means, allowing various flow profiles to be readily generated and modified according to the desired experiment. Specific IQ Master programs used to create the various flow profiles used in the current work are given in Appendix A.

**Multiple Axes**

While this control structure allows the control of a single rotor axis and hence one flow profile, we wanted the ability to drive multiple flow profiles simultaneously to increase the precision and accuracy of our model in studying flow dependent processes. The most versatile option would be to have multiple IQ-550 controllers, each driving an individual axis via a specific, implanted IQ program. However, a simple, cost-effective method to

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control multiple flow rates at once is to 'slave' additional motor axes to the controlled, master motor's velocity profile which can be obtained directly from the line-encoder output on the master motor (Fig. 2.17). By using a slaved velocity command signal that is a variable ratio of the master motor's profile, proportional velocities, accelerations, and hence flow rates can be easily generated. For each additional axis, a motor and feedback tachometer is required, along with a driving MAX-100 whose on-board control structures can accept velocity feedback to maintain the slaved velocity command signal.

2.2.6 Measurement/Recording System

In order to measure and record the flow profiles, the outlay shown in figure 2.18 developed. The components of the system are the Transonic 3CA

![Measurement/Recording System Schematic](image)

Figure 2.18. Measurement/Recording system schematic and information flow.
flow probes, probe multiplexer, rotary junctions, 10 volt Tecma power supply, Transonic T106 Flowmeter, and a Windows compatible PC equipped with a National Instruments® LAB-PC A/D interface and LABTECH Version 8.1 software package (Laboratory Technologies Corporation ©)

**Probes**

The Transonic flow probes measure bi-directional flow via an ultrasound transit time method. Typically, four piezoelectric transducers are positioned around the tube. These elements pass ultrasound beams in alternating up- and downstream directions through the fluid, each serving as a transmitter and receiver in turn. The differential, directional transit time can then be used to measure the bulk flow in the tube. The probes were specifically calibrated for use with the 1/8"ID/5/32" OD 3350 Tygon tubing used for the fluid loops, and further modified so that only 2 of the transducers were required (1 upstream, 1 downstream) allowing 4 connections per transducer rather than 8. Accordingly, the total number of connections on a six rotor/six probe axis was reduced from 48 to 24. This reduction, though not necessary, greatly simplified the construction and wiring of the embodied system.

On each rotor stage, the four transducer leads were passed to the connector shaft and soldered onto a given pin on a specially constructed 24 pin connector (Fig. 2.19). Once the proper connections were made, these pieces were press fit into the connector shaft on the corresponding rotor stage. Upon stacking, the male junctions on a given rotor stage communicates with the female junctions on stage immediately below it. Thus, each stacked rotor stage is hardwired to all of the probes via the 24 pin connections. This design allows the stages to be modular for loading and possible future expansion purposes, with the top most stage relaying all probe signals to the probe multiplexer.
Multiplexer

The multiplexer (Fig. 2.20) sequentially passes individual flow signals to a Transonic T106 Flowmeter which outputs a voltage signal, recordable on a computer via the LAB-PC A/D interface and LABTECH software. The trigger to sequentially switch probes is provided after each flow cycle by the high to low or low to high state change of a digital output pin on the IQ 550 controller. In this method, all of the probes’ signals are merged into a continuous waveform. A final signal is passed from the multiplexer to the computer encoding a specific probe label. Therefore, with the waveform and corresponding probe label information, an individual fluid loop can be monitored throughout the time course of a given run. When recording from multiple axes, a similar measurement strategy of cycling between individual probes is applied, with an additional multiplexing layer added to cycle between the various axes.
Figure 2.20. Multiplexing schematic; Six probe signals are merged into one continuous waveform and subsequently decoded using concurrently recorded probe label information.

A rotary electrical coupling interfaces the rotating loop reference frame with the inertial frame. To limit the number of rotary junctions, the probe multiplexing stage is located on the rotor frame itself (Fig 2.18), thus reducing the required number of rotary junctions from 24 to 8 (or 7 in the multi-axes scenario as some functions could be consolidated onto inertial frame circuitry). The onboard multiplexer probe output is wired to four of the rotary couplings. Three additional couplings provide power (+10V, GND) to the multiplexer as well as information on the probe label. A final connection is required to pass the probe switch trigger in cases when multiplexing logic is distributed to individual axes in the rotating reference frames.

Although this measurement system can monitor and record the full flow profiles in the fluid loops, often times only the peak flow values are stored to disk. This data compression is performed in real-time using the LABVIEW software capabilities. To do this, the IQ550 was programmed to send a brief 5V pulse to the PC during peak acceleration, signaling an appropriate, consistent point in each flow pulse to be sampled. Thus, even if the flow is significantly reduced (as in thrombotic loop occlusion), consistent portions of the cycle can still be recorded.
2.2.7 Design Summary

The system described allows an oscillating type flow of a prescribed flow pattern to be produced in fluid loops. The manner in which the flow is produced is atraumatic, thus allowing delicate processes such as thrombosis to be studied in a low noise setting, even under high flow rates. Furthermore, this strategy allows the entire fluid contact surface to be prescribed and minimized, without the use of valves, connectors, or other potential disturbances, further reducing potential sources of background noise. By readily allowing changes in flow profiles, the system also allows the thorough, parametric investigation of how flow may interact with biological processes such as thrombosis.

While the current embodiment allows multiple, 1/8" ID loops to be run under two simultaneous, bidirectional flow rates, various modifications can be readily made to extend the system's applicability. More axes can easily be added (Fig. 2.17), allowing multiple, simultaneous comparisons at several flow rates. By incorporating additional IQ550 Controllers, these flow variations can be extended to include not only flow rates, but profiles as well. Altering rotor and loop characteristics, can also offer a wide degree of variation in the geometric properties. Furthermore, incorporating a one-way valve could be used to allow unidirectional flows, though the impact of such a structure on the hemodynamic loop environment would have to be considered as discussed in section 2.1.2. It is important to recognize that each of these adjustments would also require reconsidering the embodied motor specifications and other aspects of the system design.
3.1 Mechanical validation

To demonstrate the range of system capabilities, a single fluid loop was filled with a 6:4 water/glycerol mix to match the Newtonian approximation blood viscosity under arterial flow conditions (0.04 cm$^2$s$^{-1}$) [78] and placed on a rotor stage. The rotor was driven through a variety of motion profiles to generate impulse, square, triangle, sinusoidal, and coronary-type flows. The full flow profiles, rather than just peak data, were recorded via the LABTECH software.

3.1.1 Flow profiles

The impulsive response of the system was examined after a brief burst of torque was supplied to the rotor. Some points to note in this profile (Fig. 3.1) are the 0-135 ml/min rise time of 0.1 sec and a decay time constant of 0.1 sec, which matches fairly well with the value obtained from our theoretical, linear approximation (Fig. 2.3). The finite rise time was a result of the limitations of rotor inertia, friction, and peak obtainable torque. If a quicker response is required, these parameters can be adjusted through design.

![Flow profile graph](image)

Figure 3.1. Relative fluid flow to impulsive wall accelerations
modifications. The decay time constant on the other hand, being constrained by the vessel radius and kinematic fluid viscosity, is not readily subject to change. Therefore, relative flows decaying to nearly zero (<5% peak) in times less than 3 time constants are not possible without some flow reversal or the incorporation of a directional valve. These parameters help to characterize the realistic impulse response achievable with the current setup, which serves as the limiting building block from which other functions can be composed.

Some examples (square, triangular, sine waves) of other possible flows are shown in figure 3.2 a-c. Here, the periodic, bi-directional nature of the flow is evident. Again, this type of oscillation is necessary in the methodology used to create flow, as no valves were present in the circuit.

Figure 3.2. Various flow profiles depicting the easily alterable flows. a. Square; b. Triangular; c. Sinusoidal profiles.
3.1.2 Coronary-Flow

Since we hope to investigate coronary arterial events, a coronary flow pattern [55, 56] (scaled to a 60 bpm frequency) was approximated with our bi-directional model (Fig. 3.3a). Figure 3.3b gives the driving wall velocities, $V_{\text{wall}}$, required to achieve this coronary-type flow, $V_{\text{rel}}$, and the derived absolute fluid velocity in the inertial frame, $V_{\text{fluid}}$.

![Bidirectional System Flow Profile](image)

**Figure 3.3** Generating coronary type flows. a. Bidirectional bulk flow rate relative to the rotating wall (solid line), superimposed on idealized coronary pulse (dashed line); b. The wall and fluid velocities in the inertial reference frame (blue, magenta lines respectively) in phase with the target, relative, peak fluid velocity (red line; derived from bulk flows assuming parabolic motion, justified for low Wormesley # flows). Prior to a new pulse, there is no fluid motion relative to the wall.
This simulated coronary-type flow is characterized by a \( \overline{Re} \) of 150 (based on \( |V_{rel}| \)), an \( \alpha \) of 2 (based on \( \omega_{\text{coronary}} \), and a \( \kappa'_{\text{peak}} \) of 135 (as defined by equation 1). \( \overline{Re} \) and \( \alpha \), when compared with table 2.1, indicate the ability to achieve coronary-type principle flows while keeping secondary flow effects (as estimated by \( \kappa'_{\text{peak}} \)) to within physiological levels. The relatively low \( \alpha \) (which compares the magnitude of the driving frequency to the system frequency response) indicates that the flows are, for the most part, driven in a relatively quasi-steady manner. This is further indicated experimentally in Figure 5b, which shows that the relative flow velocity is approximately zero by the time the wall velocity begins to initiate a new pulse.

While \( V_{rel} \) essentially resets to zero initial conditions after each unidirectional pulse, we must again point out that the created flows are bi-directional in nature. Though many of the thrombotic protein and cellular surface reactions have been shown to be dependent on shear (Section 1.3.1) which we attempt to approximate by matching beat-to-beat dimensionless parameters, the potential influences of such oscillation on factors such as mass transport [59] and cellular function (adhesion, activation, response) [23] may eventually need to be considered.

Another aspect of the flow that is altered is the secondary flow. Physiologically, such flows have been shown to be up to 5% of the axial flow velocity, shifting the peak of the velocity contour towards the outer wall via half-tube, single vortex flows (recall Fig. 1.7c). Peak wall shear rates due to these flows can be up to 40% of the peak axial wall shear rates [76]. While precise determination of these secondary flow fields through numerical simulation would be interesting and of value in finding the exact time-dependent wall shear rates, we have found that the magnitude of the secondary flows in our system (as determined by \( \kappa'_{\text{peak}} \)) are comparable to those found in a coronary setting of similar axial flows. Qualitatively, a deviation in the secondary flow pattern results from a disconnect between the centrifugal force and the relative wall velocity (which are normally linked in stationary wall flows). During part of the cycle, the bulk fluid velocity is greater than the wall-boundary layer velocity as is typically the case [76]. However, in our rotational system, there are also times when the wall-boundary layer
velocity in the IRF is greater than the bulk fluid’s, resulting in a counter-rotating secondary flow drive. Considering these effects, we see that we can minimize the secondary flows as well as maintain beat-to-beat symmetry by using a zero DC component of $V_{wall}$. If needed, redesigning the fluid loop characteristics to have a larger curvature to tube radius ratio ($R/a$) can further reduce the magnitudes of these flows.

The versatility of the system allows fundamental wave characteristics such as amplitude and frequency to be readily varied as desired. Figures 3.4 a and b depict such profile manipulations as the amplitude is varied by a factor of 2. Furthermore, the system allows for tailoring of more detailed parameters such as the systolic:diastolic ratio if desired.

![Graph of flow rate vs time](image)

Figure 3.4. Changing amplitude of coronary type flow (100, 200, 400 ml/min peak flow).
3.2 Biological validation

We performed an initial assessment of the model’s suitability to study issues of vascular thrombosis by observing the impact of endoluminal stenting on thrombotic outcomes. In this preliminary study, we evaluated loop occlusion times as a straightforward, integrated, measure of thrombosis. This evaluation allowed us to address the system’s repeatability, as well as background levels of circuit thrombotic activation. Together, these qualities play a major role in determining the system’s signal to noise ratio. As a concrete example of this, we compared the loop occlusion times of stents of dissimilar surfaces.

3.2.1 Materials

Blood Source
In this preliminary study, surplus American Red Cross blood products were used to obtain quantities sufficient to allow several experiments to be performed on the same batch of blood for precision testing. Fresh frozen plasma and fresh platelet concentrates (both anticoagulated with 10mmol acid citrate dextrose; ACD) were utilized as these contained the key ingredients of classical thrombosis, neglecting the erythrocytic and leukocytic components in this first level of study. Type AB+ fresh frozen plasma (FFP) with a prescribed storage life of 6 months post-collection was stored at -20 °C. The plasma was thawed in a 37 °C water bath for 45 minutes and then spun down at 10000 G to eliminate debris such as preformed platelet micro-particles. The supernatant was filtered 4 times through a 0.2 μm low protein binding filter to further ensure clean FFP. The platelets (type AB+ PRP) were obtained within one day of collection and stored on a 70 RPM rocker at room temperature. These were used within the first two days post-collection as was justified from adequate functional comparisons with freshly drawn volunteer platelets.

One hour before a planned experiment, the platelets were added to the FFP at a constant ratio of 1:4 PRP to FFP and returned to the rocker for equilibration. Each loop required 2.5 ml of the FFP/platelet mix to ensure proper filling. To reduce experimental
error from mixing and handling variation, the total volume of the suspension for a given run (15 ml; 2.5 X 6) was pooled and prepared in a single tube.

**Stented Loops**

7-9 stainless steel NIR® endovascular stents were obtained from Medinol Ltd. (Jerusalem, ISRAEL). While the platelets were equilibrating in the filtered FFP, the stents were expanded within the 1/8" tubing midway between two coaxial sleeves via a 36mm Maxxum TM 3.5 SCIMED® balloon catheter under a pressure of 12 atm (see Fig. 3.5). The tubes were closed into their loop format ensuring a gapless fit. When the plasma/platelet mix was ready, 5M Ca2+ was added to bring the sample to an additional 10mmol Ca2+ concentration, negating the citrate’s anticoagulant, calcium chelating effect. The stented loops were then filled with the plasma/platelet mix as described above and placed onto the rotors. This process was sequentially performed as rapidly as possible (~15 sec/loop) while ensuring safe handling of the blood components and proper filling of the tubes (no air bubbles). Once complete, the rotors were placed onto the rotor shaft and spun under a coronary-like motion profile (150 ml/min peak flow) within the 37 °C incubator.

Figure 3.5. Picture of stent placement within fluid loop.
3.2.2 Systemic Noise Characterization

Precision/Variance

As a preliminary test of systemic noise, six stents were positioned in their respective fluid loops and run through the described coronary flow protocol. The results obtained after parsing the data into individual loop, peak flow profiles (as discussed in Section 2.2.6) show a constant flow rate followed by a fairly rapid drop to zero flow (Fig. 3.6). The actual initial flows in each loop are identical as they have the same dimensions, fluid properties, and driving motion profiles. Variation in the measured start-up flow rates arises from the hard-wire calibration of the meter for a specific flow probe’s signal while our system employs six different probe signals. To account for this, each signal can be re-calibrated according to these initial deviations where identical fluid conditions are known to exist. The drop-off point indicates when the thrombus is occluding the stent. If a zero-flow condition is taken as the end point, the average occlusion time for this run is $43.1 \pm 6.8$ min (mean ± standard error).

Figure 3.6. System precision. Single run of 6 identical stainless steel stents, showing recorded peak flow profiles and loop occlusion times.
Circuit Background Noise

As another initial test of the system, we compared a trial of three stented tubes to three empty control tubes. The transformed peak flow data for such a run is given in figure 3.7. As above, the initial flow period followed by a drop-off is witnessed in the stented samples, with an average clotting time of 39.1 +/- 1.7min. The stentless controls remained patent for the +2 hour duration of the test, indicating low levels of circuit thrombotic noise.

![Graph showing peak flow tracings for stented and control, non-stented loops.]

Figure 3.7. Background thrombotic noise levels. Peak flow tracings for stented and control, non-stented loops.

3.2.3 Signal to Noise

These results give insight into the important characteristics of the system’s precision and background noise levels. Maximizing precision and minimizing background noise potentiates high signal to noise ratios which enable effective, powerful, parametric studies. We investigated the preliminary effectiveness of our in vitro method in creating...
sufficient signal to noise ratios by exploring the loop occlusion times of 7-9 NIR® stents of dissimilar surfaces properties. Four kinds of stent surface treatments were selected as motivated by clinical and industrial perspectives: untreated stainless steel, standard electropolished stainless steel, gold-coated (7 ± 2 μm), and gold-coated + heat treatment.

**Effects of polishing on loop occlusion times**

We first tested the occlusion times between untreated stainless steel stents with those put through a standard, electropolishing procedure which has been shown to significantly reduce surface roughness. To minimize possible bias due to placement order, 3 polished and 3 untreated stents were loaded in an alternating pattern (#1,3,5 polished; #2,4,6 untreated or vice versa) and run under the prescribed coronary-type flows. Three runs were performed in total, yielding a sample size of 9 untreated and 9 polished stents. To correct for variability in blood samples, all occlusion time values were normalized to the average, polished stent clotting time for each run. Figure 3.8 shows that the non-polished stents took significantly less time to clot than polished stents (unpolished, 0.73 +/- 0.06 normalized mean run steel occlusion time; two-tailed t-test, p <.05). Therefore, when looking at end point, loop occlusion time as a measure of thrombogenicity, the positive effects of polishing on stent thrombotic potential can be seen.

**Effects of gold-coating on loop occlusion times**

Gold-coating is a desirable surface property due to increased radio-opacity and easier stent placement. However, such processing has been shown to alter other surface parameters (i.e. roughness, surface energy, etc.) which can have detrimental side effects [107]. By subsequently heat-treating the coated stents, roughness is significantly reduced to precoating levels. Thus, we tested the loop occlusion times between standard, polished stainless steel with and without gold coating, along with the post-coating, heat processed devices.

Comparisons were performed on the stent types, using the average run, polished steel surface as a normalization control to account for inter-run variability. Stents were loaded (standard vs. gold-coated; standard vs. processed gold) and run in an alternating 1, 3, 5 / 2, 4, 6 pattern as in the preceding trial. From figure 3.8, we see that basic gold-
coating significantly reduced the normalized loop occlusion times (gold, 0.68 +/- 0.03 normalized mean run steel occlusion time; two tailed t-test p-value < .01), while the heat-processing treatment was able negate this pro-thrombotic effect (heat+ gold, 1.0. +/- .05 normalized mean run steel occlusion time ; two tailed t-test p value --). Comparing the normalized, gold-coated pre- and post- treatment results, we see that the treatment effect is significant (two-tailed t-test, p <.02).

![Graph](image)

Figure 3.8. Inter-stent studies. a. Polished vs. Untreated; b. Polished vs. Gold vs. Gold + Heat Treatment. Note that the Polished steel values are by definition 1.

When designing tests, it is important to consider a studies power, which is a function of the differences that are trying to be measured (signal), the inherent variance in the measurement (noise), and the number of trials that must be performed to be relatively certain that observed differences are real rather than merely a consequence of measurement error. At one extreme, clinical trials are plagued with high variance. As a
result, they typically require massive trials of hundreds to thousands of human subjects to sift through this noise for relevant signals. The expense and time needed for such trials creates very real, practical limitations on the types of studies that can be performed. *In vitro* methods are often able to significantly reduce such population variance by offering greater experimental control. In our studies, we see that significant results (as indicated by the p values) could be obtained with relatively small sample sizes (∼10).

Having powerful methods is essential to explore complex biological situations where isolation and directed manipulation of variables with observable outcomes enable epistatic pathway dissection. While sufficient power is only definable in reference to a particular signal of interest, our preliminary case studies of endovascular stent loop occlusion times add confidence that similar techniques will allow for other mechanistic investigations of coronary thrombotic events and processes.

In this section, we have tested our model flow system and found it to be suitable for reproducing coronary-type flow patterns on a beat to beat basis as judged by dimensional consideration. We have further applied this system towards an investigation of endovascular device thrombosis by considering acute loop occlusion times as a measure of the thrombotic response. These preliminary biological results give us confidence that this system will be suitable for other, more directed studies of blood-borne vascular response.
CHAPTER 4
Future Directions/Conclusions

Coronary arterial thrombosis is a highly important vascular phenomenon that results from either normal disease processes or from iatrogenically created states. The components of thrombosis have evolved under complex environmental pressures and it is important to consider the hemodynamic situation in which they interact as such conditions could have profound effects on biological outcomes. In the current thesis, we have recognized the need for observable in vitro systems which allow control over both the physical and biological environments to epistatically probe situational vascular thrombosis. Specifically, we have considered the problem of coronary thrombosis and developed a novel flow system which recreates coronary-like flow rates on a beat to beat basis in relevant arterial geometries. Through mechanical characterization, we have shown the high degree of controllability offered over the physical flow setting. Furthermore, we have begun to explore the utility of the system in studying thrombotic occurrences by looking at the clinically relevant situation of endovascular stenting.

The in-stent occlusion times that we observe are phenomenological findings that occur in our model system. In vivo thrombotic occlusion occurs at a much lower rate and generally takes place subacutely over the course of several days. There are various potential explanations for such observational discrepancies. The clinical situation has both innate and external counter measures, such as a reactive endothelial layer and drug therapies, which help to regulate thrombosis. Furthermore, our system is a closed, in vitro model where produced substances are not cleared. This could not only help to...
explain the regularity of clotting seen within our system, but also the fairly rapid drop to zero flow as confined, explosive reactions could augment rapidly as a result of positive feedback processes.

Due to such differences between our model and the physiological situation, we hope to study not the physical occurrence of end-state occlusion (though this is an important preliminary observation), but the cellular and protein factors leading up to thrombosis. Such dependencies are not only key mediators of occlusive events, but essential in vascular remodeling and sub-clinical responses to injury. In future work, we hope to focus on the platelet as an essential component of vascular thrombosis. Upon binding to an injured wall, platelets alter their expression of surface molecules and release substances which both attract and adhere to flowing cells while promoting the enzymatically driven coagulative response. The richness and importance of this platelet level demands the pursuit of understanding how this component interacts within its hemodynamic environment to promote vascular thrombosis. To accomplish this, methods of observation and control must be developed.

While the current application of the described flow system is to study intravascular thrombosis, its use can be generalized to other situations where carefully controlled, pulsatile flow is required with adjustments (loop dimensions, surface properties, uni/bi-directional flow) being made to suit the different requirements. Such a system would hopefully be of use in helping to better bridge the experimental gap existent between the

\textit{in vitro} and \textit{in vivo} environments.
Appendix A: IQ Master Programs

IQ Master program files for generating rotor motion profiles/flow waveforms. Initial programs were created using general knowledge of how loop accelerations lead to fluid velocities as determined through straight tube approximations. These versions were then fine-tuned through real-time flow observations until the desired flow profiles were generated. The final programs for generating flows are given. Of note, the functions: "Impulse", "Square", "Triangle", "Sine" were applied to tubes using PRP as a test fluid, while the "50", "100", "200", "400" ml/min peak flow functions were applied to whole blood.

IMPULSE FUNCTION

title "impulse"
:assign variables
assign vcity v1
assign accl v2
assign decl v3
assign tc v4

:SET CONSTANTS
reset;
fdr=100; 100% federate
scale=4000; 1 rev=4000 encoder counts
fel=3; following error limit

:start motor at init velocity
accl=500
tc=.3
vcity=50

main:

jaccel=accl
jdecel=accl

forward:
delay=tc
movv=(vcity)
if vel1>vcity jump forward

reverse:
delay=tc
movv=(-vcity)
if vel1>(-vcity) jump reverse

jump main
end
SQUARE FUNCTION

title "square"
:assign variables
assign vcity v1
assign accl v2
assign decl v3
assign vcity2 v4
assign vcity3 v5
assign vcity0 v6
assign scivel v7
assign scltm v8

:SET CONSTANTS
reset;
fdr=100; 100% federate
scale=4000; 1 rev=4000 encoder counts
fcl=.2;
:start motor at init velocity
accl=5

scltm=.5
scivel=1

main:
d=-.08/scltm, v=280/scivel
d=-.055/scltm, v=257/scivel
d=-.18/scltm, v=165/scivel
d=-.13/scltm, v=55/scivel
d=-.02/scltm, v=0/scivel
d=.15/scltm, v=155/scivel
d=.15/scltm, v=220/scivel
d=.2/scltm, v=270/scivel
d=.17/scltm, v=300/scivel
d=.06/scltm, v=280/scivel
d=.055/scltm, v=257/scivel
d=.18/scltm, v=165/scivel
d=.13/scltm, v=55/scivel
d=.02/scltm, v=0/scivel
d=.15/scltm, v=155/scivel
d=.2/scltm, v=220/scivel
d=.2/scltm, v=270/scivel
d=.17/scltm, v=300/scivel
jump main
end
TRIANGULAR FUNCTION

title "triangle"
;assign variables
assign vcity v1
assign accl v2
assign decl v3
assign vcity2 v4
assign vcity3 v5
assign vcity0 v6
assign sclvel v7
assign scltme v8

;SET CONSTANTS
reset;
fdr=100; 100% federate
scale=4000; 1 rev=4000 encoder counts
fel=.2; following error limit
: start motor at init velocity
accl=5
scltme=1
sclvel=1.2

main:
d=-.16/scltme, v=294/sclvel
d=-.11/scltme, v=285/sclvel
d=-.36/scltme, v=225/sclvel
d=-.26/scltme, v=143/sclvel
d=-.15/scltme, v=0/sclvel
d=-.25/scltme, v=200/sclvel
d=-.25/scltme, v=250/sclvel
d=-.5/scltme, v=270/sclvel
d=-1/scltme, v=300/sclvel

d=-.16/scltme, v=294/sclvel
d=.11/scltme, v=285/sclvel
d=.36/scltme, v=225/sclvel
d=.26/scltme, v=143/sclvel
d=.15/scltme, v=0/sclvel
d=.25/scltme, v=200/sclvel
d=.25/scltme, v=250/sclvel
d=.5/scltme, v=270/sclvel
d=1/scltme, v=300/sclvel
jump main
end
SINUSOIDAL FUNCTION

title "sine"
;assign variables
assign vcity v1
assign accl v2
assign decl v3
assign vcity2 v4
assign vcity3 v5
assign vcity0 v6
assign sclvel v7
assign scltme v8

;SET CONSTANTS
reset;
fdr=100; 100% federate
scale=4000; 1 rev=4000 encoder counts
fel=2; following error limit
;start motor at init velocity
accl=5

scltme=1
sclvel=1.2

main:
d=-.1/scltme, v=287/sclvel
d=-.075/scltme, v=270/sclvel
d=-.25/scltme, v=190/sclvel
d=-.18/scltme, v=110/sclvel
d=-.1/scltme, v=0/sclvel
d=-.2/scltme, v=155/sclvel
d=-.2/scltme, v=220/sclvel
d=-.25/scltme, v=270/sclvel
d=-.2/scltme, v=300/sclvel
d=-.1/scltme, v=300/sclvel

jump main
end
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TRIANGULAR FUNCTION (50ml/min peak)

title "50tri" ; 50 ml/min peak triangular wave

assign variables
assign vcity v1
assign accl v2
assign decl v3
assign vcity2 v4
assign vcity3 v5
assign vcity0 v6
assign sclvel v7
assign scltme v8

;SET CONSTANTS
reset;
fdr=100; 100% federate
scale=4000; 1 rev=4000 encoder counts
fel=.2; following error limit

;start motor at init velocity
accl=5
scltme=3.4
sclvel=4.5

main:
o1=off
o2=on
o3=off
d=-.2/scltme, v=320/sclvel
d=-.3/scltme, v=290/sclvel
d=-.3/scltme, v=110/sclvel
d=-.05/scltme, v=0/sclvel
dac1=5
d=-.3/scltme, v=190/sclvel
dac1=0
d=.3/scltme, v=250/sclvel
d=.3/scltme, v=290/sclvel
d=.3/scltme, v=310/sclvel
d=2/scltme, v=320/sclvel
d=.3/scltme, v=330/sclvel
d=.4/scltme, v=330/sclvel

o1=off
o2=on
o3=off
d=-.2/scltme, v=320/sclvel
d=.3/scltme, v=290/sclvel
d=.3/scltme, v=110/sclvel
d=.05/scltme, v=0/sclvel
dac1=5
d=-.3/scltme, v=190/sclvel
dac1=0
d=-.3/scltme, v=250/sclvel
d=.3/scltme, v=290/sclvel
d=.3/scltme, v=310/sclvel
d=2/scltme, v=320/sclvel
d=.3/scltme, v=330/sclvel
d=.4/scltme, v=330/sclvel

jump main
end
TRIANGULAR FUNCTION (100ml/min peak)

title "100tri" ; 100 ml/min peak triangular wave
assign variables
assign velcity v1
assign accl v2
assign decl v3
assign velcity2 v4
assign velcity3 v5
assign velcity0 v6
assign sclvel v7
assign scltime v8

:SET CONSTANTS
reset;
fd=100; ; 100% federate
scale=4000; ; 1 rev=4000 encoder counts
fel=2; ; following error limit

:start motor at init velocity
accl=5
scltime=1.5
sclvel=2

main:
o1=off
o2=on
o3=off
d=-.2/scltime, v=320/sclvel
d=-.3/scltime, v=290/sclvel
d=-.3/scltime, v=110/sclvel
d=-.05/scltime, v=0/sclvel
dac1=5
d=-.3/scltime, v=190/sclvel
dac1=0
d=-.3/scltime, v=250/sclvel
d=-.3/scltime, v=290/sclvel
d=-.3/scltime, v=310/sclvel
d=-.2/scltime, v=320/sclvel
d=-.3/scltime, v=330/sclvel
d=-.4/scltime, v=330/sclvel

o1=on
o2=off
o3=on
d=.2/scltime, v=320/sclvel
d=.3/scltime, v=290/sclvel
d=.3/scltime, v=110/sclvel
d=.05/scltime, v=0/sclvel
dac1=5
d=-.3/scltime, v=190/sclvel
dac1=0
d=-.3/scltime, v=250/sclvel
d=-.3/scltime, v=290/sclvel
d=-.3/scltime, v=310/sclvel
d=-.2/scltime, v=320/sclvel
d=-.3/scltime, v=330/sclvel
d=-.4/scltime, v=330/sclvel

jump main
end
TRIANGULAR FUNCTION (200ml/min peak)

Title “200tri”; 200 ml/min peak triangular wave

Assign variables
assign vcity v1
assign accl v2
assign dec1 v3
assign vcity2 v4
assign vcity3 v5
assign vcity0 v6
assign sclvel v7
assign scitme v8

Set Constants
reset;
fd=100; 100% federate
scale=4000; 1 rev=4000 encoder counts
fel=2; following error limit
accl=5
scitme=0.8
sclvel=1

Main:
o1=off
o2=on
o3=off
d=2/scitme, v=320/sclvel
d=3/scitme, v=290/sclvel
d=3/scitme, v=110/sclvel
d=0.5/scitme, v=0/sclvel
dacl=5
d=3/scitme, v=190/sclvel
dacl=0
d=3/scitme, v=250/sclvel
d=3/scitme, v=290/sclvel
d=3/scitme, v=310/sclvel
d=2/scitme, v=320/sclvel
d=3/scitme, v=330/sclvel
d=4/scitme, v=330/sclvel

o1=on
o2=off
o3=on
d=2/scitme, v=320/sclvel
d=3/scitme, v=290/sclvel
d=3/scitme, v=110/sclvel
d=0.5/scitme, v=0/sclvel
dacl=5
d=3/scitme, v=190/sclvel
dacl=0
d=3/scitme, v=250/sclvel
d=3/scitme, v=290/sclvel
d=3/scitme, v=310/sclvel
d=2/scitme, v=320/sclvel
d=3/scitme, v=330/sclvel
d=4/scitme, v=330/sclvel

Jump main
End
TRIANGULAR FUNCTION (400ml/min peak)

title "400tri" ; 400 ml/min peak triangular wave
.assign variables
assign vcity v1
assign accl v2
assign decl v3
assign vcity2 v4
assign vcity3 v5
assign vcity0 v6
assign sclvel v7
assign scltme v8

.SET CONSTANTS
reset;
frd=100; 100% federate
scale=4000;  1 rev=4000 encoder counts
fe=2; following error limit
: start motor at init velocity
accl=5
scltme=.35
sclvel=.45

main:

o1=off
o2=on
o3=off
d=-.2/scltme, v=320/sclvel
d=-.3/scltme, v=290/sclvel
d=-.3/scltme, v=110/sclvel
d=-.05/scltme, v=0/sclvel
dacl=5
d=-.3/scltme, v=190/sclvel
dacl=0
d=-.3/scltme, v=250/sclvel
d=-.3/scltme, v=290/sclvel
d=-.3/scltme, v=310/sclvel
d=-.3/scltme, v=320/sclvel
d=-.3/scltme, v=330/sclvel
d=-.4/scltme, v=330/sclvel

o1=on
o2=off
o3=on
d=-.2/scltme, v=320/sclvel
d=-.3/scltme, v=290/sclvel
d=-.3/scltme, v=110/sclvel
d=-.05/scltme, v=0/sclvel
dacl=5
d=-.3/scltme, v=190/sclvel
dacl=0
d=-.3/scltme, v=250/sclvel
d=-.3/scltme, v=290/sclvel
d=-.3/scltme, v=310/sclvel
d=-.3/scltme, v=320/sclvel
d=-.3/scltme, v=330/sclvel
d=-.4/scltme, v=330/sclvel

jump main
end

Appendix A
Appendix B: Stented Loop Occlusion Time Data

Stented loop occlusion time data expressed in minutes:

<table>
<thead>
<tr>
<th>Trial</th>
<th>Polished</th>
<th>Unpolished</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 1</td>
<td>33.90365</td>
<td>36.8</td>
</tr>
<tr>
<td></td>
<td>34.60707</td>
<td>32.8</td>
</tr>
<tr>
<td></td>
<td>51.48928</td>
<td>24.8</td>
</tr>
<tr>
<td>Trial 2</td>
<td>32.06226</td>
<td>30.4</td>
</tr>
<tr>
<td></td>
<td>35.77335</td>
<td>25.84</td>
</tr>
<tr>
<td></td>
<td>46.16439</td>
<td>27.36</td>
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<td>Trial 3</td>
<td>45</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>44.41558</td>
<td>29.25</td>
</tr>
<tr>
<td></td>
<td>45.58442</td>
<td>35.55</td>
</tr>
</tbody>
</table>

Table B.1 Comparison of polished and unpolished stainless steel 7-9 NIR® endovascular stents.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Polished</th>
<th>Gold</th>
<th>Gold + Heat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 1</td>
<td>58.75</td>
<td>33.79</td>
<td>41.47</td>
</tr>
<tr>
<td></td>
<td>70.27</td>
<td>36.67</td>
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<tr>
<td></td>
<td></td>
<td>40.51</td>
<td>59.71</td>
</tr>
<tr>
<td>Trial 2</td>
<td>46.27</td>
<td>39.55</td>
<td>62.59</td>
</tr>
<tr>
<td></td>
<td>47.23</td>
<td>43.39</td>
<td>62.59</td>
</tr>
<tr>
<td></td>
<td>70.27</td>
<td></td>
<td>79.87</td>
</tr>
<tr>
<td>Trial 3</td>
<td>38</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>Trial 4</td>
<td>66</td>
<td>36</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>37</td>
<td>68</td>
</tr>
</tbody>
</table>

Table B.2 Comparison of gold-coated and gold-coated+heat treated stainless steel 7-9 NIR® endovascular stents with polished stents of identical geometry.
References


19. Hueb, W.A., et al., The Medicine, Angioplasty or Surgery Study (MASS): a prospective, randomized trial of medical therapy, balloon angioplasty or bypass


