# CHAPTER 1

## 1 INTRODUCTION AND LITERATURE REVIEW

### 1.1 Motivation

Atherosclerosis is a degenerative disease in which occlusive developments, or ‘plaques’, form over many years within the vessel wall and may ultimately restrict blood flow, cf. Fig. 1.1.1. Advanced arterial lesions, or stenoses, resulting from atherosclerosis are generally localized in medium to large arteries, such as the coronary, carotid or femoral, and generally consist of accumulations of fat, layers of collagen like fibers, and excessive migration and proliferation of vessel-wall smooth muscle cells (Davies, 1994). The localization of plaques is most likely determined by hemodynamic forces acting on the vessel surface (Kleinstreuer et al., 2001).

Focusing on occlusive developments in the lower peripheral arteries, e.g., femoral and/or popliteal (cf. Fig. 1.1.2a), a common surgical remedy to restore blood flow is vascular bypass grafting (cf. Fig. 1.1.2b). Saphenous vein is typically the conduit of choice with a graft success rate at 5 years of 81%; however, synthetic PTFE grafts must often be employed, resulting in a 48% success rate (Archie, 1994). Graft failures are predominately due to restenosis resulting from intimal hyperplasia and/or thrombosis. Intimal hyperplasia, or tissue overgrowth, is characterized by an abnormal accumulation of cells and extracellular matrix within the intima of the vessel wall and is often viewed as an accelerated form of atherosclerosis (Ross, 1993). As with atherosclerosis, hemodynamic factors are a primary culprit in the development of intimal hyperplasia. The specific site of graft failure is typically the distal anastomosis (Fig. 1.1.2b), where hyperplasia formation localizes at the toe, bed, and heel (Madras et al., 1981; Sottiurai, 1999), i.e., distal anastomotic intimal hyperplasia (DAIH), cf. Fig. 1.1.3. A common bypass failure scenario consists of a moderately occluded graft-to-artery anastomosis. Thrombosis (platelet adhesion and aggregation) may then occur in the region of constricted flow, resulting in total vessel occlusion (Davies, 1994), cf. Fig. 1.1.1.

While the pathogenic mechanisms responsible for the development of DAIH remain elusive, certain hemodynamic parameters, or 'indicators of disturbed flow events', have been qualitatively linked to intimal thickening in arterial bypasses and other branching blood vessel configurations. These parameters include low mean and oscillatory wall shear stress, large spatial and temporal gradients in wall shear stress magnitude, and gradients in wall shear stress vector direction (Kleinstreuer et al., 2001). Other factors that have been implicated with DAIH formation include excessive intramural wall stress, graft-to-vessel compliance mismatch, blood particle stasis, and blood particle deposition (Kleinstreuer et al., 2001; Sottiurai, 1999; Ku, 1997). The methodology for branching blood vessel design via hemodynamic parameter minimization, and its underlying assumptions, have been outlined by Kleinstreuer and Hyun (2000) and are illustrated in Fig. 1.1.4.

The relative importance of the individual hemodynamic parameters is not known; however, current studies and hypotheses suggest that blood particle deposition plays a predominate role in DAIH formation. Excessive blood particle interactions with a dysfunctional vascular surface trigger and sustain a cascade of biological processes which may result in rolling or static adhesions (Henry and Chen, 1993; Ross et al., 1993). Adherent monocytes may penetrate the vascular surface at endothelial junctions, migrate into the intima and differentiate into macrophages (Ross, 1993). Mitogenic factors released by adherent monocytes and macrophages contribute to smooth muscle cell migration and proliferation resulting in general intimal thickening (Ziats and Robertson, 1981; Ross, 1993). The release of mitogenic factors by adherent platelets may also contribute to intimal thickening (Davies, 1994). Therefore, a strong relationship exists between critical blood particle interactions with the vascular surface and the development of vascular diseases, such as atherosclerosis, thrombosis, and hyperplasia.

A majority of my research focuses on improving the prediction of near-wall blood particle motion and deposition, as well as developing a parameter to quantify the likelihood of particle deposition in local regions, in terms of the near-wall residence time (NWRT) concept. This research proposes that focal regions with both a high probability for blood particle deposition and a dysfunctional endothelial layer, as indicated by the WSS-based hemodynamic parameters, may be the most likely sites for DAIH development. Results

indicate that this hypothesis compares favorably with actual sites of DAIH development in experimental studies. In the application phase, the NWRT parameter in conjunction with more established hemodynamic wall parameters are employed to analyze femoral graft-to-artery anastomoses with respect to the potential for restenosis and other post-operative complications.

## 1.2 Research Objectives

A considerable amount of emphasis has been placed on identifying which WSS-based hemodynamic parameters, e.g., low WSS, OSI, high spatial WSSG, initiate and sustain DAIH. However, due to a lack of experimental evidence, quantitative correlations have yet to be established for end-to-side distal arterial anastomoses. Other experiments have found that critical blood elements, e.g., platelets and monocytes, interacting with the vascular surface play a critical role in general intimal thickening, included DAIH. Researchers often imply that low WSS directly correlates to regions of prolonged blood-particle wall interaction. This assumption may not necessarily be true considering that (a) platelets often adhere in high shear regions, and (b) convection and diffusion of particles play critical roles in defining regions of elevated particle residence time. A better approximation of the potential for blood particle interaction with the vascular surface is needed, particularly for applications to complex branching vessels.

Novel contributions of this work include the development of a local residence-time based hemodynamic parameter to quantify blood particle interactions with the vascular wall and the probability for deposition. This model will be validated in geometries for which blood particle deposition data is available and will then be applied to the femoropopliteal bypass configuration in order to evaluate sites of likely DAIH. Calculation of a near-wall residence time parameter in complex 3-D geometries will require evaluation of a number of particle point-force model terms that have previously not all been included in a model for blood particle transport. The *contributions* of a particle based hemodynamic parameter computed using a comprehensive point force model, its validation, and its application to the evaluation of intimal thickening in multiple vascular geometries, including the femoropopliteal bypass, are summarized next.

## Outline of Research Objectives

A. Fundamental Research

i. A literature review outlining: (a) the role of critical blood particles (i.e., platelets and monocytes) in the development of pathologic vascular conditions such as hyperplasia, atherosclerosis, and/or thrombosis; (b) biophysical mechanisms for critical blood particle interactions with the vascular surface including deposition; (c) available models for the study and simulation of adhesion, aggregation, and embolism; (d) mechanisms and models for red blood cell induced dispersion.

ii. A computational investigation regarding blood particle motion particularly as a particle approaches a boundary, e.g., evaluation of the near-wall drag (or lubrication forces), lift force, and pressure gradient terms as well as *modeling* of biochemical interactions - The resulting particle dynamics model will be validated using empirical results of particle motion and deposition patterns as found in the literature. The model will be developed for and applied to two groups of critical blood particles, i.e., platelets (≈ 4 μm) and monocytes (≈ 10 μm), in both far- and near-wall regions. Furthermore, a blood particle collision sub-model for bioparticle transport will be considered, i.e., a feasible and experimentally sound description will be selected and incorporated.

iii. Development and testing of a novel approach for simulating the probability for 'bioparticle deposition' in terms of prolonged near-wall residence times and concentrations (NWRT model) - This approximate method should be particularly useful and necessary given that physico-biochemical factors such as vessel surface roughness, actual blood particle shape, and nano-scale bond formations responsible for possible cell attachment, rolling or re-suspension, cannot feasibly be included in simulations involving large-scale geometries. In conjunction with item *ii*, this approach for simulating prolonged

particle-wall interactions will be evaluated and compared with experimental evidence.

iv. A theory for incorporating a particle-hemodynamics parameter, such as the NWRT, with the now classic wall shear stress based parameters (which are related to endothelial activation and reactivity) will be proposed and evaluated with respect to qualitative and quantitative *in vivo* deposition and intimal thickening results.

B. Applications

i. Current femoral graft-to-artery junctions will be evaluated considering key hemodynamic wall parameters (cf. iv) to indicate sites susceptible to arterial diseases. Anastomotic variables of interest include:

   a. Graft-to-artery diameter ratio
   b. Effect of graft-end cut on final anastomotic configuration (straight, concave, and S-shaped graft-end cuts)

ii. Potential geometric alternatives intended to reduce DAIH formation will be investigated in terms of key hemodynamic wall parameters, including the NWRT model. Current surgical remedies (e.g., the Miller cuff) as well as realistic virtual prototypes will be considered.

## 1.3 Description of Vascular Diseases

Intimal thickening, mainly caused by local smooth muscle cell proliferation inside the vessel wall, creates a progressive reduction of the cross sectional area at critical locations in medium to large arteries and veins. These stenotic developments may be the result of late stage arteriosclerosis lesions or due to a more rapid response to injury referred to as hyperplasia.

From a clinical viewpoint, vessel occlusion has two phases. The first stage is the development of intimal thickening, which may result from intimal hyperplasia or arteriosclerotic plaque, both of which involve smooth muscle cell proliferation and lipid

accumulation within the intima. This primary process can cause sufficient plaque growth to encroach on the arterial lumen, causing a chronic obstruction to flow and producing, for example, stable exertional angina. Figure 1.1.1 illustrates this phase of moderate lesion development as it progresses. As illustrated in Fig. 1.1.1, intimal hyperplasia development may continue, providing the final mechanism for vessel occlusion. Alternately, and most frequently, thrombus formation superimposed on, and in, a stenotic development accounts for the ultimate mode of vessel occlusion. This second phase leads to the acute ischemic syndromes of myocardial infarction, unstable angina, sudden ischemic death, and cerebral infarction. These clinical symptoms depend on the location of the occlusion, as illustrated in Fig. 1.3.1. In the second stage, the hemostatic mechanism and the intimal thickening process are linked to produce thrombotic occlusions. There is, however, also clear evidence of the involvement of platelets and other critical blood elements in smooth muscle proliferation, which is an integral part of the growth of intimal thickening formations, although not as important in their actual initiation.

Intimal thickening is influenced by hemodynamic factors (Kleinstreuer et al., 2001), blood particle depositions and particle wall interactions (Ziats and Robertson, 1981), as well as intramural wall stresses (Thubrikar and Robicsek, 1995). Similarly, thrombosis depends on the local hemodynamic environment surrounding an aggregate, induced stress on the aggregate, blood particle attachment, the transport of blood particles and related chemical species, and a cascade of biochemical reactions.

### 1.3.1 Hyperplasia

Intimal hyperplasia is the rapid abnormal continued proliferation and overgrowth of smooth muscle cells in response to endothelial injury or dysfunction (Chervu and Moore, 1990). Endothelial injury or dysfunction often occurs at sites of bypass graft anastomoses, locations of balloon angioplasty, and stented vessels. At such locations, hemodynamic factors may be a primary source of, or may contribute to, endothelial dysfunction. Hyperplasia is often viewed as an accelerated form of atherosclerosis due to the similarities in the lesions. Ross (1986) advanced the response to injury hypothesis to propose that intimal hyperplasia may be an early lesion on the pathway to atherosclerotic plaque. However, in similar sized plaques *those developing due to hyperplasia tend to have a higher*

*concentration of smooth muscle cells* and a lower concentration of lipid accumulation than do atherosclerotic lesions.

The work of Fry (1968) was first to clearly demonstrate acute endothelial changes could occur from either extremely high shear stress or turbulence. Other sources concur with and expand the observations of Fry (1968) by stating that endothelial cell modification can be induced by severe hemodynamic conditions or by surgical processes such as the creation of an anastomosis. Clowes et al. (1983) were first to clearly demonstrate that acute injury to the intima and media can produce hyperplasia. Subsequent studies have shown that such injury produces smooth muscle cell proliferation, which occurs at a rate proportional to the degree of the injury (Chervu and Moore, 1990). The growth of intimal hyperplasia may or may not subside with the endothelial layer is reestablished. Idu et al. (1993), in animal bypass studies, demonstrated that smooth muscle cell proliferation could continue beneath apparently intact endothelium. The injury model, then, does not require that endothelial cells be denuded, but that they be disturbed, by hemodynamic conditions or surgical procedure, to a point that results in smooth muscle cell proliferation. *Platelets and other critical blood elements that adhere to the dysfunctional endothelium may be a major factor in stimulating this smooth muscle cell proliferation.*

### 1.3.2 Atherosclerosis

Based on cell culture and *in vivo* studies over the past decade, a working model of atherogenesis has been developed. Initiation of atherosclerosis involves monocyte migration through an intact endothelium (Ross, 1986, 1993, 1999). Advanced intimal proliferation lesions of atherosclerosis may occur by at lest two pathways described by Ross (1986) and illustrated in Fig. 1.3.2. In Fig. 1.3.2,

> the pathway demonstrated by the clockwise (long) arrows to the right has been observed in experimentally induced hypercholesterolemia. Monocytes attach to endothelium (B), which may continue to secrete growth factors (short arrow). Subendothelial migration of monocytes (C) may lead to fatty-streak formation and release of growth factors such as PDGF (short arrow). Fatty streaks may become directly converted to fibrous plaques (long arrow from C to F) through release of growth factors from macrophages or endothelial cells or both. Macrophages may also

stimulate or injure the overlying endothelium. In some cases, macrophages may lose their endothelial cover and platelet attachment may occur (D), providing three possible sources of growth factors – platelets, macrophages, and endothelium (short arrows). Some of the smooth muscle cells in the proliferative lesion itself (F) may form and secret growth factors such as PDGF (short arrows).

An alternative pathway for development of advanced lesions of atherosclerosis is shown by the arrows from A to E to F. In this case, the endothelium may be injured but remain intact. Increased endothelial turnover may result in growth factor formation by endothelial cells (A). This may stimulate migration of smooth muscle cells from the media into the intima, accompanied by endogenous production of PDGF by smooth muscle as well as growth factor section from the "injured" endothelial cells (E). These interactions could then lead to fibrous-plaque formation and further lesion progression (F) (Ross, 1986).

Initially, there is no endothelial denudation injury and so platelets interaction with the intact endothelium. That is not to say that the endothelial function is normal, but simply that no adhesion of aggregates is present (Davies, 1994). There is ample evidence of functional endothelial abnormality at this stage atherosclerosis (DiCorleto and Chisholm, 1986). As the atherosclerotic lesion progresses, there are gaps in the endothelial surface within which lipid-filled monocytes are impacted, and the presence of foam cells in peripheral blood suggests that some are emigrating from the intima. Platelets then adhere to the exposed connective tissue matrix underlying the endothelial defects, and this is the stage in which a true thrombogenic dimension is involved in atherosclerosis. *The attached platelets play a major role in plaque growth by stimulating smooth muscle cell migration and proliferation even though they may not significantly influence initiation.*

### 1.3.3 Thrombosis

Thrombosis is the formation of a blood clot, called a thrombus, inside an artery or vein. Thrombosis develops by the same mechanisms that control hemostasis, the clotting system which prevents blood loss in the event of vessel injury. The thrombus (Fig. 1.3.3a) is primarily composed of platelets and red blood cells bound together by molecules in the cell

membrane of the platelets, called membrane glycoproteins (GPs), by other proteins in the blood or inside the platelets, and by a network of polymerized plasma protein called fibrin (Colman et al., 1994). An arterial thrombus, as found in high shear regions, is composed primarily of platelets, with some fibrin and trapped red cells, which are typically found distal to the platelet-rich part of the thrombus. An arterial thrombus is usually found superimposed on an atherosclerotic plaque which has fissured (or ruptured) to expose subendothelium plaque components to the blood, cf. Fig. 1.3.3b from Davies (1990). Arterial thrombosis may also develop on artificial surfaces such as vascular grafts, heart valves and stents, as well as in aneurysms or injured arteries. An acute arterial thrombosis is often followed by a fibrin "tail" of trapped red blood cells, which extends downstream (Davies, 1990).

Thrombi formed in the low shear environments typical of the venous system are composed mostly of red blood cells which are held together by a fibrin mesh, such as a fibrin tail. Low shear regions in which these thrombi are found include regions of flow stasis and recirculation which commonly occurs within branching blood vessels. The observable difference in thrombus composition due to environment is an indication that shear stress plays a primary role in thrombus formation.

A general view of the currently understood mechanisms of arterial thrombosis is provided in Fig. 1.3.4. In normal flow, platelets collide with the vessel wall due to both convective transport and the diffusive motion imposed by collisions with red blood cells. The convergent flow of a stenosis, stagnation locations, and reattachment points are all areas of high platelet wall interactions. Some of these colliding platelets may adhere to the vessel wall, especially in the presence of tissue factor, a chemical mediator present when the endothelium is injured. Platelets do not normally adhere to healthy endothelium. Platelet deposition at dysfunctional sites increases with increasing shear rate, however; there is a limit to this relationship. The adhesion of a small number of platelet layers may significantly contribute to smooth muscle cell proliferation and intimal thickening. The platelet may be activated which makes adhesion, regulated by membrane glycoproteins and proteins in the vessel wall, more permanent. Activation also permits the aggregation of platelets, which may form a platelet plug that occludes the vessel, or may embolize (break free) totally or in part. Simultaneously, thrombin is produced at the injury site due to tissue factor exposure.

Thrombin causes coagulation of fibrin into a polymer network that may reinforce a platelet plug and incorporate red blood cells into the clot. During early hemostasis, as platelets are activated, more thrombin is released accelerating the aggregation process (positive feedback mechanism). Healthy endothelium cells trigger a negative feedback mechanism that limits the propagation of the clot to the region of injury, reduces the sustained rate of clotting, and breaks down the clot. Other mechanisms which control thrombus growth rates, such as reduction by embolization, are poorly understood.

### 1.3.4 Vessel Occlusion

Vessel occlusion often occurs due to rapid thrombus formation in the vicinity of significant intimal thickening. Davies (1994) describes two distinct patterns by which occlusive thrombosis may occur. Approximately one quarter of observed thrombi are superimposed on stenotic plaques. The plaque itself does not undergo dramatic change. This form of occlusive thrombosis typically occurs near a high-grade stenosis and is likely the result of endothelial denudation.

In contrast, three quarters of occlusive thrombi are related to plaques that have undergone a deeper injury, typically extending into the core of the plaque. The process of plaque tearing or disruption is referred to as fissuring, ulceration, or rupture. Once the plaque cap has ruptured a large amount of collagen is exposed to the blood providing a major stimulus for thrombosis. The thrombus itself forms initially within the interior of the plaque and may or may not advance to a degree that fills the remaining lumen. Figure 1.3.3 is an example of a thrombus that has occluded the lumen as a result of plaque cap rupture.

Determining the biomechanical properties of atherosclerotic plaque is a difficult task that some researchers have begun to investigate. Atherosclerotic plaque exhibits a wide range of material properties that are associated with its multiple components and variable composition. Its mechanical properties can be classified as nonlinear and inelastic. Salunke and Topoleski (1997) have recently reviewed the mechanical properties of atherosclerotic plaque including conditions under which plaque cap rupture may occur. Due to a wide range of plaque cap structures and the long-term cyclic loading of the hemodynamic forces, definitive conditions for plaque cap rupture have not been established. From coronary artery

studies (Richardson et al., 1989), it was observed that a majority of plaques that rupture and result in occlusive thrombosis are eccentrically situated and have a lipid pool that does not have an internal lattice of collagen supporting the cap. Such a structure will lead to a concentration of circumferential wall stress on the plaque cap in systole because of the inability of the pool to carry a load that is redistributed to the vessel wall. Recent studies have also found that a factor contributing to the vulnerability of the plaque is infiltration of the cap tissue with macrophages (Salunke and Topoleski, 1997).

Fig 1.3.5 illustrates the possible thrombosis events upon plaque cap rupture (Davies, 1994). As indicated in the figure, a majority of tears through the plaque cap occur at the junction with the intima (Davies, 1994). Clearly, a variety of outcomes are possible once the plaque has been disrupted ranging from occlusive thrombosis to a healed fissure with no increase in stenosis. Clinically, it is important to know the outcome of a plaque rupture. If a model of occlusion risk were available, it could be combined with a model for plaque rupture risk to decide which patients are good candidates for surgical treatment and which patients can be managed medically (Wootton and Ku, 1999). Currently, only stenosis severity is used, and very often inappropriately, to determine the necessity of surgical intervention (Wootton and Ku, 1999).

## 1.4 Role of Blood Particle Deposition in Vascular Diseases

All arterial plaques have the connective tissue proteins produced by smooth muscle cells, e.g., collagen and elastin, as major constituents. In addition, plaques contain a variable amount of lipid derived originally from the plasma. The lipid may be contained within foam cells or be free within the intima. Any hypothesis concerning the initiation and growth of plaques must therefore explain both lipid accumulation within the intima and intimal smooth muscle cell migration and proliferation. A majority of atherosclerotic investigations focus on the accumulation of lipids in the lesion (cf. Kleinstreuer et al., 2001 for a thorough review). However, smooth muscle cell migration and proliferation is of importance in atherosclerotic lesions and of primary importance in hyperplasia. The migration of smooth muscle cells into the intima and proliferation therein, in general, can be stimulated by a number of processes, one of which is platelet and *attachment* and the release of mitogenic factors, i.e., thrombosis.

Other critical blood borne elements, such as monocytes, also have a widely documented influence on intimal thickening development.

### 1.4.1 Critical Blood Particle Influence on Smooth Muscle Cell Proliferation

It has been well documented that adherent monocytes may penetrate the vascular surface and differentiate into macrophages, i.e., a key constituent of atherosclerotic lesion (Ross, 1993). Mitogenic factors released by adherent monocytes and macrophages contribute to smooth muscle cell migration and proliferation resulting in general intimal thickening (Ziats and Robertson, 1981; Ross, 1993; Sottiurrai et al., 1999). Furthermore, as described by the atherosclerosis model of Ross (1986), adherent monocytes and macrophages may stimulate or injure the endothelium thereby providing an indirect pathway for platelet attachment and possible aggregation. Platelets may also attach directly to transmigrating monocytes or exposed macrophages.

When platelets interact with or adhere to subendothelial connective tissue or attached monocytes, they are stimulated to release their granule contents, which include growth factors (Baumgartner, 1972). Endothelial cells normally prevent platelet adhesion because of the non-thrombogenic character of their surface and their capacity to form anti-thrombogenic substances. There may be a spectrum of endothelial injury, ranging from alterations in cell-surface constituents to increased turnover (non-denuding injury) or, after extreme injury, to loss of endothelial cover. It is possible that any of these forms of endothelial injury may promote platelet adherence and release of platelet constituents, although it is not clear that platelet adherence to “modified” endothelium is a common event (Ross, 1986).

The most studied growth factor is one that was originally isolated from platelets and is referred to as platelet derived growth factor (PDGF), but it is now known to be released from a wide range of cell types including monocytes (Funayama et al., 1998). The name PDGF is misleading, but there is no doubt that release of this growth factor from platelets and other critical blood particles will be a very potent stimulus to smooth muscle proliferation in any circumstance where these particles are deposited on the vessel wall. Researchers that have attributed the misguided release of PDGF from platelets to the development of intimal hyperplasia in graft anastomoses include LoGrefo et al. (1983) and

Sottiurai et al. (1988). Other sources of PDGF include damaged endothelium, smooth muscle cells, and activated macrophages (Davies, 1994).

PDGF may be of particular importance in intimal thickening because it is both chemotactic (Grotendorst et al., 1982) and mitogenic (Kohler and Lipton, 1974). Therefore, PDGF may be responsible for the early migration of smooth muscle cells from the media into the intima and the subsequent late stage proliferation of these cells, which results in a bulk of the stenosis. In support of this relationship are studies on pigs with von Willebrand disease, which prevents platelet adhesion (Fuster et al., 1978 and Griggs et al., 1981). Homozygous pigs with von Willebrand disease (which prevents platelet adhesion) were fed a high-cholesterol diet and showed a degree of lipid accumulation similar to that of controls but had less smooth muscle proliferation. Cross-transplantation of aortas from normal to von Willebrand pigs confirms that the latter develop less intimal thickening. *Thus, in an animal model of von Willebrand disease there is significant suppression of smooth muscle proliferation, providing support of the view that platelet-derived growth factors from adherent platelets are important in vivo.*

With regard to hyperplasia formation, several investigators have demonstrated that if platelets are absent from sites of endothelial injury induced by an indwelling catheter (Moore, 1976) or de-endothelialization caused by a balloon catheter (Friedman, 1977), or if platelet interactions can be pharmacologically prevented, then the intimal proliferative lesions that usually accompany such injury will not occur. However, none of these studies has yet demonstrated which of the growth factors in platelets are responsible for this proliferative response *in vivo*. Nevertheless, PDGF is a principle mitogen released by platelets and other cells that most likely has a direct effect on hyperplasia proliferation (Davies, 1994).

### 1.4.2 A Current Alternative Theory of Intimal Thickening

As established, the predominant theory of intimal thickening maintains that platelets and monocytes play necessary supporting roles. In contrast to the ‘modified response to injury’ hypothesis is the theory that atherosclerotic plaques develop from organizations of mural thrombi, which form in regions of hemodynamic or hemorheologic abnormalities (Sloop, 1999). This view partially explains the intermittent and unpredictable progression of

atherosclerotic plaques. Leu et al. (1988) has shown that circulating mononuclear cells can perform the entire task of generating an atherosclerotic plaque without any contribution from the underlying media. Leu et al. (1988) observed that shortly after thrombus formation, mononuclear cells within the clot differentiate into macrophages, endothelial cells, fibroblasts, and smooth muscle cells capable of synthesizing matrix.

Sloope et al. (2002) has presented the model of a synthetic arteriovenous graft in support of the theory that intimal thickenings originate from mural thrombi structures with a limited contribution from the underling media. Clearly, the ability of a synthetic surface to respond to 'injury' and aggravating effects is limited. Therefore, Sloope et al. (2002) concluded that the prevalent atherosclerotic plaque-like lesions within these grafts developed from mural thrombi. Hence, with the mural thrombi model, platelets and other circulating blood particles are elevated from a necessary supporting role in lesion occurrence to the only necessary components for significant atherosclerotic plaque-like formations.

### 1.4.3 Interrelation Between Critical Blood Particle Deposition, Thrombosis, Intimal Thickening, and Vessel Failure

In summary, intimal thickening is influenced by hemodynamic factors (Kleinstreuer et al., 2001), blood particle depositions and particle wall interactions (Ziats and Robertson, 1981), as well as other factors including intramural wall stresses (Thubrikar and Robicsek, 1995). Similarly, thrombosis depends on the local hemodynamic environment surrounding an aggregate, induced stress on the aggregate, blood particle attachment, the transport of blood particles and related chemical species, and a cascade of biochemical reactions.

The process of thrombosis is composed of three general components, i.e., platelet adhesion, activation and aggregation. The former two mechanisms, adhesion and activation, may describe platelet attachment to dysfunctional endothelium and adherent monocytes which may have a general impact on smooth muscle cell proliferation and continued hyperplasia development. The role of attached platelets is supported by *in vivo* models, in which limited platelet attachment resulted in a reduced amount of intimal hyperplasia. Similarly, general critical blood cell interaction with the vascular endothelium may play a critical role in intimal thickening and continued development (Ziats and Robertson, 1981;

Funayama et al., 1998). Once an occlusion has developed, hemodynamics then regulate the transport and, to some extent, the attachment of platelets in the throat. Hemodynamically induced cyclic stress may also induce a rupture of the plaque cap which separates the thrombogenic stenotic contents from the blood. Once the plaque cap is ruptured, the revealed contends of the atheroma stimulate the blood clotting reaction, which is one possible mechanism for vessel failure. Alternately, occlusive thrombi may develop in a high-grade stenosis that has not undergone significant change, but has lost a portion of its endothelial cover. Given variations in plaque constituents and structure, as well as the cyclical loading conditions, it may not be feasible to effectively simulate plaque cap rupture. However, the location of the rupture in relation to particle transport characteristics may affect the development of a thrombus and determine whether or not the vessel will become fully occluded. In summary, critical blood particle interaction with the vascular surface provides a necessary stimulus for intimal thickening initialization and progression, as well as a means for thrombosis formation and subsequent vessel failure.

## 1.5 Mechanisms for Blood Particle Deposition

Blood particle deposition in a disturbed flow field is governed solely by hydrodynamic transport (including collisional effects) up to the point that biochemical interactions begin to play a role. Blood particle motion is then controlled by both hydrodynamic transport mechanisms and molecular forces, which may firmly *bind* the particle to the wall or allow for rolling. Figure 1.5.1 illustrates that the distance required for molecular interaction is one order of magnitude less than the vessel wall roughness height, and two orders of magnitude less than the diameter of a monocyte. The formation and dissociation of ligand-receptor bonds is often viewed as a stochastic event and, as such, has recently been approximated using probability theory and Monte Carlo simulations (Zhu, 2000). Similar to monocyte ligands, activated platelets extend pseudopods that may make first contact with a reactive surface (or come within at least 1 nm). *This physical proximity, or contact, is required before a bio-reactive force comes into play*. In general, biochemical forces don't display an attraction component *per se*. Instead, hydrodynamic forces transport blood particles to within the range necessary for biochemical interaction of occur. This interaction then has a

*binding characteristic* resulting in firm *adhesion* or *rolling*. Further details regarding platelet and monocyte adhesion and rolling are described below.

### 1.5.1 Deposition and Aggregation of Platelets

*Adhesion*

Thrombosis is triggered when a thrombogenic surface is exposed to blood. Thrombogenic surfaces include subendothelial tissue (exposed due to endothelial injury) and exposed atheroma (due to plaque cap rupture) which contain collagen and absorbed proteins such as von Willebrand factor (vWF) and fibrinogen. Artificial surfaces such as grafts and stents are also thrombogenic (Fernadez-Ortiz et al., 1994).

Vascular surfaces differ in structure and composition and may differ in reactivity with platelets. Healthy endothelial and smooth muscle cells within the vessel wall produce prostacyclin, which is a potent inhibitor of platelet attachment and aggregation. Subendothelial tissue, exposed due to an injury, is composed of a variety of thrombogenic components. Amorphous basement membrane-like material is the main structure at the surface, usually covering the other components. Superficial endothelial injury may also expose collagen fibrils which, like the basement membrane, are highly reactive. The rupture of an atherosclerotic plaque may expose a large amount of collagen to flowing blood. However, other components of the atheromatous core, such as the lipid core (Fernandez-Ortiz et al., 1994), may be more thrombogenic than the exposed collagen (Colman, 1993).

Despite the complex biochemical reactions that regulate the conditions for which adhesion may occur, there also appears to be a dependence on shear stress (or shear rate) in conjunction with convective platelet transport. A direct increase in platelet adhesion and accumulation rates with shear rate has been observed *in vitro* for platelets deposition on subendothelium (Turitto and Baumgartner, 1975) and collagen-coated surfaces (Alevriadou et al., 1993; Badimon et al., 1986; Sakariassen et al., 1988; Sixma and de Groot, 1994). For example, Sixma and de Groot (1994) have quantified the percent attachment of platelets to a purified protein-coated surface *in vitro* for shear rates ranging from 100 $s^{-1}$ to 2250 $s^{-1}$. At shear rates up to at least 2250$s^{-1}$, purified collagen supports platelet adhesion. Platelet adhesion on purified vWF increases with shear rate up to a shear rate of at least 2250 $s^{-1}$ and

vWF can support significant amounts of adhesion up to shear rates of at least 6000 $s^{-1}$ (Savage et al., 1996). The effect of shear rate has also been demonstrated in human (Barstad et al., 1996; Sakariassen et al., 1990), baboon (Markou et al., 1993), and porcine (Badimon et al., 1986) *ex vivo* experiments.

With regard to the initial adhesion location, shear appears to affect where platelets are deposited. Platelet adhesion on collagen-containing stenotic surfaces is highest at the stenotic throat, where shear rate is the highest (Badimon et al., 1986; Markou et al., 1993) for peak shear rates ranging from 1,300 to greater than 20,000 $s^{-1}$. On smooth artificial surfaces, by contrast, platelet adhesion may be depressed in high shear regions (Bluestein et al., 1997; Schoephoerster et al., 1993). Permanent platelet adhesion has been shown to also depend largely on convective forces, the adherent surface, as well as the state of activation that the platelets are in (Wurziger and Schmid-Schonbein, 1990).

*Activation*

Activation is a change in platelet function triggered by chemical or physical agonists (stimuli). Chemical agonists include ADP, thrombin, Thromboxane $A_2$ ($TxA_2$), fibrillar collagen, platelet activation factor and serotonin (Colman, 1993). Wurzinger and Schmid-Schonbein (1990) state that by far the major agonists are thrombin and ADP presumably leaking from damaged cells. The primary physical agonist is shear stress (Hellums, 1994). Platelets may also be activated by biomaterials via the complement system (Gemmell et al., 1996; Kalman et al., 1991). Chemical and physical agonists have an interrelated effect. For example, shear stress exposure appears to sensitize platelets to many chemical agonist including ADP and thrombin (Goldsmith et al., 1994; Goldsmith et al., 1975).

The role of shear stress activation remains in question. Hellums (1994) mapped out the threshold shear stress required for activation, as a function of exposure time. Depending on exposure time, the threshold for activation varies from $10^3$ to $10^7$ $s^{-1}$. Boreda et al. (1995) quantified activation by fitting experimental data to a platelet stimulation function (PSF) such that $PSF = \tau t^{0.452}$ where $\tau$ is the shear stress in dynes per $cm^2$ and *t* is the exposure time in seconds. Boreda et al. (1995) found the threshold for shear induced activation to be $PSF \geq 1000$. Boreda et al. (1995) calculated the PSF for platelets passing *in vivo* through

75% and 95% area reduction stenoses and found that the residence time was insufficient by an order of magnitude for shear induced platelet activation. However, activation is not necessary for platelet adhesion in which case residence time is sufficient and a more permanent linking may take place. Furthermore, recirculation behind a partially formed platelet plug may allow sufficient residence time for activation thereby accelerating the thrombus growth.

The history of shear stress exposure may change the threshold of platelet activation by chemical agonists (Goldsmith et al., 1994; Goldsmith et al., 1975). The rate at which shear stress increases may also effect activation of platelets as observed in stenotic flows (Holme et al., 1997). Wurzinger and Schmid-Schonbein (1990) observed that shear stress exceeding 500 dyn/cm$^2$ leads to irreversible damage of platelets within intervals in the range of $10^{-1}$ s.

Activation causes shape change with pseudopod extensions, which increases the strength and duration of adhesion, as well as aggregation rates. Activated platelets contract, consolidating loose cells and fibrin into a compact thrombus, and release granular contents (Colman, 1993). These activated platelets release ADP and thrombin for activation of other platelets (positive feedback mechanism). Thrombin production also increases fibrin concentration, which traps red blood cells in the thrombus.

*Aggregation*

Once the initial adhesion of a platelet monolayer to a surface has occurred, the thrombus grows due to platelet-platelet attachment, i.e., aggregation. Alternatively, platelet aggregates may form in flowing blood as a result of collisions. At all shear rates, platelets approaching a thrombus may be activated by passing through an agonist 'cloud' of ADP, thrombin, or $TxA_2$ before interacting with an adherent thrombus (Hubbell and McIntire, 1986; Richardson, 1973). Alternatively, unactivated platelets could adhere to the thrombus via vWF binding, followed by chemical and physical activation and more permanent binding.

Microscopic monitoring of thrombosis on collagen shows that small thrombi form around attached platelets causing a naturally rough thrombus to form on a small scale (Adams and Feuerstein, 1980). Platelets either adhere preferentially to the downstream side of the thrombus, or the thrombus may deform downstream as it grows, due to increasing

stress (Sakariassen et al., 1988). Platelets aggregation has not been visualized dynamically at higher shear rates, so it is not known if newly added platelets interact with the upstream end of the thrombus and stop on the far side after some degree of activation has occurred.

*Coagulation*

Thrombogenic surfaces contain tissue factor, collagen, or absorbed proteins such as vWF, all of which rapidly lead to thrombin generation (coagulation cascade) and the production of fibrin. In normal hemostasis, injury exposes tissue factor. Tissue factor is also found at high concentrations in the necrotic core of the atheroma and may be exposed by plaque cap rupture (Wilcox et al., 1989). Artificial surfaces are also thrombogenic (Colman, 1993). Thrombin is one of the most potent platelet agonists, causing activation, release of granular contents, and irreversible aggregation (Colman, 1993) in thrombi that last longer than 10 to 15 minutes (Hanson et al., 1988; Hanson et al., 1993; Kelley et al., 1991). At the same time, thrombin cleaves fibrinogen into fibrin monomers that polymerize to form fibrin, which traps red blood cells in the clot and supports platelet adhesion. Thrombin is generated by a complex series of biochemical reactions collectively called the coagulation cascade.

*Embolization*

Thrombosis growth may be limited by embolization, which is the removal of parts of the thrombus due to shear stress. Current models of embolization are missing quantitative data on the stress required for platelet removal from a surface. More recent studies on adhesive platelet properties, however, may soon make such a model more feasible (Haga et al., 1998; Zhu, 2000). Embolization rates may differ significantly depending on the properties of the wall material such as collagen (Markou et al., 1993), damaged artery (Badimon et al., 1989), and polymers (Schoephoerster et al., 1993).

*Summary*

The adhesion of platelets to a surface (as well as aggregation) in flowing blood may involve the platelet membrane, the substrate surface, plasma cofactors, and other agents present in the plasma or the blood cells themselves. In general, it is believed that platelet adhesion and platelet-platelet interactions involve the glycoprotein complexes GP Ib-IX and

GP IIb-IIIa, exposed on the surface membrane of activated platelets; various vessel wall collagens and possibly other structures; and the adhesive proteins, such as vWF, fibronectin, and fibrinogen, which are found in the plasma, the platelet granules, and the vessel wall. At present, the relationships among platelet membrane glycoproteins, surface substrate, and adhesive proteins have not been fully elucidated.

Fibronectin and vWF have been identified in the subendothelium of human tissue and have been implicated in platelet adhesion (Turitto and Baumgartner, 1994). Both proteins are high molecular weight glycoproteins that are also found in platelets and can be absorbed from plasma. Both glycoproteins have been found in subendothelial collagen and may mediate the adhesion of platelets to this material as well. The precise role of surface bound proteins in the vessel wall is unclear. The absence of these proteins, however, has been repeatedly observed to dramatically reduce and often eliminate platelet adhesion to subendothelial tissue (Turitto and Baumgartner, 1994). Platelet activation is a factor influencing adhesion to subendothelium; however, it is not required for platelet attachment. Recently, Savage et al. (1996) have shown that attachment to vWF, as absorbed in subendothelial tissue and exposed collagen, may occur very rapidly in high shear regions. This type of binding allows for slow rolling of the platelet, which maintains continuous contact with the surface even at shear rates in excess of 6,000 $s^{-1}$. The high shear environment of the attached platelet and exposure to mitogenic factors results in platelet activation and, consequently, static irreversible attachment.

Platelet interaction with the vessel wall may be described using one of three categories depending on the state of platelet activation and the reactivity of the vascular surface.

1) An *unactivated* platelet rolling over *healthy* endothelium (in the absence of adhesive proteins) is not influenced by biochemical forces. The mechanics of a deformable ellipsoid contacting a rough wall propelled by hydrodynamic transport will determine the rolling velocity.
2) An *unactivated* platelet will interact with *disturbed* endothelial cells (which may have exposed collagen fibers) that have absorbed adhesive proteins. This

will result in a slower rolling velocity and increase the possibility for activation (Savage et al., 1996).

3) An *activated* platelet is more likely to firmly attach to a number of thrombogenic surfaces included adhesive proteins absorbed by disturbed endothelial cells, artificial surfaces, and exposed atheroma due to plaque cap rupture (Fernandez-Ortiz et al., 1994).

### 1.5.2 Attachment of Monocytes

*Mechanisms*

A large number of molecules (selectins, immunoglobulins, integrins and their corresponding ligands) are involved in the adhesion and transmigration of monocytes (Springer, 1995). *In vitro* and *in vivo* experiments often use a cytokine of some kind, e.g., TNF-∝, to activate the endothelium and induce leukocyte interaction (Rinker et al., 2001). Activated endothelium typically expresses E-selectin, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion modlecule-1 (VCAM-1). Initial biochemical attachment and subsequent rolling of monocytes results from binding of E-selectin to monocyte ligands. ICAM-1 and VCAM-1 binding to integrins on monocytes produces rolling and firm adhesion (Hinds, 1998).

The adhesion of monocytes to the vessel wall and the subsequent interaction is hypothesized to take place in four distinct stages, as shown in Fig. 1.5.2 (O'Brien and Chait, 1994). First, leukocytes are transported to the endothelium by hydrodynamic forces to with in a range of 10 nm. Biochemical attachment may then occur in two steps, i.e., stages two and three. Stage two requires that the leukocyte encounter an activated endothelium. In this stage leukocytes continue to roll, but it is now tethered to the endothelium via receptor-ligand bonds that continuously form and dissociate. This form of adhesion results in a rolling velocity that is an order of magnitude less than the corresponding velocity of an unencumbered cell (Zhu, 2000). While rolling, if the leukocyte encounters activation signals in the presence of VCAM-1 and ICAM-1 receptors firm adhesion will result. When driven

by chemotactic gradients, the final phase is the transmigration into the vessel wall through receptor-ligand interactions and the active shape deformation of the leukocyte.

*Adhesive Molecule Expression*

It would be most beneficial to know what, if any, hemodynamic conditions are responsible for the expression of adhesion molecules *in vivo*. Davies (2000) associated endothelial cell turnover and transient changes in the cell-cell junctions, which are linked to the hemodynamic environment, to increased lipoprotein permeability. Malinaskas et al. (1995) suggested that white blood cells and LDL localize in regions of secondary flow that are not necessarily associated with replicating endothelial cells. *In vitro*, an inverse relationship between VCAM-1 expression and shear stress has been established (Sampath et al., 1995; Gimbrone et al., 1997). *In vivo* results have partially confirmed this. Specifically, Wapola et al. (1995) examined the influence of altered shear in rabbit carotid arteries. Expression of VCAM-1 increased dramatically in low shear regions and moderately in high shear regions. Hence, the *in vivo* results indicate that any variation in system conditions results in an increase in adhesive molecule expression. Recently, Truskey et al. (1999) have shown a correlation between VCAM-1 expression in cholesterol-fed rabbits and the integrated plasma cholesterol level. Additionally, they demonstrated a positive correlation for the co-localization of VCAM-1 and intimal macrophages near the celiac. By extension, this suggests that the secondary flows also play a role in the expression of adhesion molecules. With regard to actual blood cell deposition, Buchanan et al. (1999) demonstrated a correlation between segmental averages of intimal white blood cell densities and spatial shear stress gradients. Buchanan (2000) established local correlations between elevated areas of macrophage densities and the wall shear stress gradient parameters, WSSG and WSSAG.

## 1.6 Models of Blood Particle Deposition and Embolism

### 1.6.1 Thrombosis and Platelet Adhesion Models

Thrombosis models are designed to allow observation and/or manipulation of some aspect of thrombosis. Experiments with thrombosis models have been conduced over the last

40 years resulting in a wealth of information; however, all aspects of thrombosis are not fully understood. A complete understanding of thrombosis is complicated by differences between the models. Furthermore, thrombosis models differ in the interactions that are to be observed, e.g., activation, adhesion and/or aggregation, as well as the experimental setup, e.g., *in vivo*, *in vitro*, and *ex vivo*.

*In vivo* models are fully contained within the body of a subject. These models typically use local injury and manipulation of an artery, e.g., denuded stenosis, to stimulate thrombosis. Historically, these models have been somewhat arbitrary and difficult to interpret due to a lack of control over substrate composition, geometry, and flow field characteristics. These models have rarely been used to study thrombosis.

*In vitro* models are conduced using blood or a platelet mixture supply and an artificial conduit not connected to a subject. These models allow the most control over the geometry, flow condition, and substrate. They can be used to dynamically monitor thrombosis over a long period of time. However, purified substrates are often used, e.g., collagen, which may not accurately represent deposition on a ruptured plaque. *In vitro* models often use flow loops to reticulate a limited amount of blood. As the blood recirculates, platelets are exposed to reactive conditions multiple times, which may enhance activation, adhesion, and aggregation. Furthermore, *in vitro* models require some degree of anticoagulation, which prevents clotting throughout the experimental chamber. Anticoagulants reduce platelet aggregation, but do not necessarily affect platelet adhesion. The fact that many *in vitro* models use strong anticoagulants, such as citrate, renders then inapplicable to the development of a model of occlusive thrombosis (Baumgartner et al., 1980). It has been shown, however, that models using the anticoagulant heparin demonstrate only a minor reduction in aggregation (Badimon et al., 1991).

*Ex vivo* models direct blood flow from an animal into an experimental apparatus. The blood is then either returned to the animal at a remote location or collected. *Ex vivo* models are used because anticoagulation is not always required, hemodynamics can be precisely controlled, and blood that is recirculated goes back to the animal's systemic circulation, where it is diluted and activated platelets may be removed by the spleen. *Ex vivo* models are

the dominant source of reliable information regarding advanced stages of aggregation and occlusive thrombus formation.

Experimental models of thrombosis, which either are historically relevant or might be useful in a numeric model, are now discussed. As introduced, thrombosis models are categorized according to the experimental characteristics, e.g., *in vivo*, *in vitro*, and *ex vivo*. Mathematical models which approximate both early adhesion as well as thrombus progression are then reviewed.

*Experimental Models*

Geometries used for *in vitro* models of thrombosis are typically either a capillary tube, an annulus, a parallel plate flow chamber, a stenotic tube, or some type of aggregometer or viscometer. The former models are mostly used for studying platelet deposition and aggregation on a surface while aggregometers and viscometers are used to study platelet activation and attachment in stirred blood.

Baumgartner (1973) developed an *in vitro* concentric flow chamber in which rabbit subendothelium is inverted on a mandrill and exposed to annular flow. This model had a controllable well defined shear rate. Baumgartner (1973) used citrate anticoagulated blood and measured the percent of platelet attachment, aggregation, spreading, and fibrin coverage via straining and morphometry. Turitto et al. (1980) used the same experimental apparatus with citrated blood and discussed an analytic approximation of platelet flux at the wall. The analytic analysis of platelet transport to the wall was originally put forward by Friedman (1970) who discussed diffusion limited and reaction limited platelet attachment conditions, as described in Levich (1962). Baumgartner et al. (1980) showed that, for the annular flow chamber, results for native blood exhibit localized thrombus formation as opposed to the even platelet coating observed with citrated blood.

Despite the use of anticoagulants, the studies of Friedman (1970) and Baumgartner (1973) were among the first to illustrate a direct relationship between shear rate and platelet deposition (independent of flow rate). This relationship is due to (a) enhanced diffusion of platelets in whole blood resulting from fluctuations in local fluid velocities due to rotation, deformation and migration of red cells induced by fluid shear (historically, Blackshear et al.,

1966); and (b) enhanced platelet reactivity (or activation) due, in part, to shear stress exposure (cf. Hellums, 1994).

Interestingly, Karino and Goldsmith (1979a & b) considered platelet adhesion to collagen and aggregation in the complex environment of an annular vortex downstream of a sudden tubular expansion, as might occur distal to an arterial stenosis. Platelet adhesion was at a local minimum in the region of the reattachment point and peaked to a maximum on either side of the reattachment streamline. The peaks of deposition increase with shear rate (diffusion controlled attachment) up to a point at which attachment is *likely limited by the reaction rate*. Karino and Goldsmith (1979a & b) speculate that along a streamline near the reattachment point, the flux of incoming platelets may be low, however, collision efficiency (the proportion of collisions resulting in adhesion) may be high because of the relatively long platelet-collagen fiber contact time. Along other streamlines, platelet flux may be higher but at the expense of shorter contact times resulting in similar adhesion efficiencies.

Many investigators have used parallel-plate chambers to measure platelet deposition on slides coated with thrombogenic material. Sixma and de Groot et al. have widely used this system to measure platelet deposition on collagen types I – VIII and various other plasma proteins, e.g., vWF and fibronectin (Sixma and de Groot, 1994). However, they are quick to point out that subendothelial tissue and atheromatous plaques are complex combinations of these purified proteins (Sixma and de Groot, 1994).

McIntire et al. have used a parallel-plate chamber with fluorescently labeled platelets to monitor deposition on collagen and other materials dynamically (cf. Hubbell and McIntire, 1986). These studies typically use heparin anticoagulated blood. Hubbell and McIntire (1986) provided details on thrombi growth with time and observed that platelets add preferentially to the downstream end of growing thrombi. As discussed previously, when platelets are activated they generate and release additional platelet activation substances. Among the more important of these are adenosine diphosphate (ADP), thromboxane $A_2$ ($TxA_2$), and thrombin. The generation and release of these species from surface activated platelets is important in the mechanism of mural thrombosis. Hubbell and McIntire (1986) provide a consolidated reference of the amount of these chemicals released by platelets.

They also model chemical concentrations around a growing thrombus, and speculate on the activation of incoming platelets.

In a normal parallel-plate chamber, shear rate is constant at the slide surface. Savage et al. (1996) have modified the epifluorescence technique by using a Hele-Shaw flow parallel-plate chamber, in which shear rate is a linear function of axial position. Savage et al. (1996) found that unactivated platelets can readily bind to immobilized vWF (as would be absorbed by subendothelium, collagen, or other attached platelets). Under high shear, this initial bond allows for low velocity platelet rolling until activation occurs resulting in immediate and permanent platelet attachment for shear rates in excess of 6,000 $s^{-1}$ (180 $dyn/cm^2$). Savage et al. (1996) provide velocities for platelets rolling on vWF; Frenette et al. (1995) provide velocities of platelets rolling on stimulated endothelium.

For *in vitro* models of stenoses, and an aneurysm, Schoephoerster et al. (1993) measured deposition of radiolabeled platelets under various steady flow conditions. This model was successful in proving that platelet deposition is increased in certain areas due to the enhanced convective transport of platelets and blood cells to the vessel wall along locally curved streamlines with velocity components perpendicular to the wall. However, the model predicts a minimum degree of deposition in the stenotic throat, which is in direct contrast to clinical observations and other *ex vivo* studies. The lack of deposition is likely due to the fact that a physiological irrelevant surface material (Lexan) has been used from which platelets embolize at low shear rates. Blood was also anticoagulated with citrate and recirculated multiple times.

Hellums et al. have developed a quantitative method for assessing aggregation by using a cone-plate viscometer and applying a population balance mathematical analysis (Belval and Hellums (1986); Huang and Hellums, 1993a-c), which was first derived in the field of chemical engineering (Hulburt and Katz, 1964). Several prior workers have also used population balance equations to analyze platelet aggregation, however, the work of Hellums et al. is representative and the most current. A basic hypothesis of this work is that studies of platelet activation and aggregation reactions in a controlled shear field, coupled with population balance equations, can yield parameters of significance in elucidating the mechanics of platelet aggregation.

Huang and Hellums (1993a – c) have used a cone-plate viscometer to estimate collision efficiencies and breakage rate coefficients and to determine how these parameters are affected by shear rate, exposure time, and concentration. With knowledge of these coefficients, a population balance model may be numerically solved to predict a distribution function for aggregate size for a particular group of platelets, e.g., for a specific packet of platelets moving in a flow field.

Activation results in the release, through granules or directly, of thrombin, ADP, and $TxA_2$, which may activate other platelets, as well as the release of PDGF, which may contribute to intimal thickening. Shear stress has been shown to be a physical agonist of platelet activation (in the presence of vWF, ADP, and $Ca^{++}$) in many *in vitro* experiments, however, the applicability of these results to flowing blood is questionable. Hellums (1994) mapped out the threshold shear stress required for activation, based on serotonin release, as a function of exposure time taken from a number of *in vitro* experiments using diverse apparatus, Fig 1.6.1. There appears to be a remarkable consistency in the findings of the various workers, which prompted Boreda et al. (1995) to devise the platelet stimulation function (PSF) parameter for activation.

Other *in vitro* experiments have illustrated the limitations of the relation expressed in Fig. 1.6.1. Goldsmith et al. (1994) showed that the threshold curve shifts dramatically to lower exposure times and lower stress levels in a dose-dependent way in response to added platelet agonists such as ADP. Fig. 1.6.1 represents results with relatively unreactive solid surfaces. The situation is entirely different for reactive surfaces. For example, when platelets adhere to collagen, there is typically significant activation as demonstrated by the release of serotonin (Olson et al., 1989). Alternately, the platelet may become activated as it passes through a “cloud” of platelet agonists (such as ADP or thrombin) diffusing from the site of surface reaction (Hubbell and McIntire, 1986). Finally, the data for Fig. 1.6.1 was derived for a mixture of platelets in plasma in the absence of red blood cells. *In vitro* studies have shown that red blood cells also release ADP and modify platelet collisions (Alkhamis et al., 1990).

*Ex vivo* models have been used for the last three decades to measure platelet deposition on a variety of surfaces as well as mural thrombus growth and embolism. Some

of the more recent models use an arteriovenous shunt to circulate blood through an inline tube, stenosis or flow chamber. These models are often implemented in the absence of anticoagulation.

Historically, Petschek et al. (1968) developed one of the first *ex vivo* models of thrombosis by shunting flow from a canine carotid artery directly into an axisymmetric stagnation flow device. Platelet adhesion onto glass and plastic was measured dynamically using dark field microscopy. Shortly thereafter, Friedman et al. (1970) developed an *ex vivo* model which directed canine blood through a U-shaped flow chamber with an exposed flat biomaterial or artificial surface. Friedman et al. (1970) developed an analytical model to illustrate the concepts of diffusion limited and reaction limited attachment. Based on these experimental results, Friedman et al. (1970) found that platelet attachment is either a diffusion limited or, depending on the shear rate, an intermediate kinetic process.

Hanson et al. (1985) developed a baboon *ex vivo* model of thrombosis using a femoral arteriovenous non-thrombogenic (silicone) shunt. A tube with a thrombogenic surface is inter-positioned in the shunt and the junctions are maintained smooth. Platelets are labeled with $^{111}$In-oxine and accumulation is measured using a scintillation camera, which captures a 2-D image of platelet radio emissions.

Markou et al. (1993) used the *ex vivo* baboon model of Hanson et al. to determine the role of shear on thrombus formation *in vivo*. Straight tubes of 2, 3, and 4 mm internal diameter and axisymmetric, sinusoidal-shaped stenoses of 50, 75, and 90 % area reduction were coated in Type I collagen and exposed to blood flow for two hours. Steady flow of 100 ml/min was maintained in the *ex vivo* model using a downstream peristaltic roller pump resulting in an upstream Reynolds number of 130. The high shear throat region of all stenosis models had much higher platelet depositions than the upstream region of the inlet or the downstream region of recirculation. The 3 mm and 4 mm tubes and the 50 % stenosis remained patent for the two hour duration of the experiment. For the 2 mm tube, the 75 and the 90 % stenosis occluded about 25 minutes into the study. Figure 1.6.2 illustrates platelet deposition at the throat of the stenosis versus time for a 4 mm upstream diameter. Platelet deposition has been normalized to initial surface area of detection (i.e., not surface area of the growing thrombus). The direct relationship between shear rate and platelet deposition

prompted Siegel et al. (1994) to numerically investigate steady wall shear rates in the model stenoses used by Markou et al.

The *ex vivo* experiment of Markou et al. (1994) revealed a time course of platelet accumulation in tubes as represented in Fig. 1.6.3. Wootton and Ku (1999) divided this experiment into three phases: (I) an accelerating phase, lasting for about five minutes; (II) an acute phase, lasting approximately 50 minutes; and (III) a slow phase, which extends to the end of the experiment, and is characterized by a lower accumulation rate than the acute phase.

Badimon et al. (1986) constructed an *ex vivo* model by establishing an arteriovenous shunt between the carotid artery and jugular vein of a pig. This model was used evaluate platelet deposition on subendothelial tissue (as exposed due to a mild injury) and on collagen Type I (such as in disrupted advanced plaque) in tubes of 1 and 2 mm internal diameter. On the de-endothelialized vessel wall, platelet attachment increased with shear rate reaching a maximum value between 5 and 10 minutes and then began to decrease indicating that some platelets were embolized by the flow. Collagen induced a progressive accumulation of platelets following a power type curve of aggregate growth with exposure time, without reaching a saturation level for the time considered. For 35, 55, and 80 % area reduction stenoses, Badimon et al. (1989) locally quantified platelet deposition on stripped tunica media (which mimics severe vessel wall damage). *This study suggests that the severity of the thrombotic complication of plaque rupture, i.e., whether or not occlusion occurs, will depend on the location of the rupture with relation to the apex of the plaque.*

Sakariassen et al. (1990) provided data on thrombus height, frequency, and volume for platelet deposition on Type III collagen in a parallel plate flow chamber for a perfusion period of five minutes. *This study points out that thrombin and fibrin accelerate and stabilize growth of platelet plugs and mural thrombi, respectively, at the site of severe vascular injury*. Fibrin deposits on the thrombi covered 6 % of the surface irrespective of the shear rate, indicating that some of the deposited platelets accelerated the deposition of fibrin. Sakariassen et al. (1990) also noticed a correlation between the deposition of leukocytes and coverage with fibrin, suggesting a role for these cells in the deposition of fibrin in low shear environments.

In an earlier *in vitro* study, which used blood that had been anticoagulated with citrate, Sakariassen et al. (1988) correlated the number of deposited platelets with the volume of a resultant thrombus per unit area. Despite the use of citrated blood, this correlation has been applied in later works and may prove useful in developing a mathematical model of mural thrombosis.

Wootton and Ku (1999) complied all available deposition data from *ex vivo* experiments into a figure similar to Fig. 1.6.4. This figure represents average platelet accumulation over a period in time taken from experiments using the baboon (Markou et al., 1993), pig (Badimon et al., 1986), and human (Barstad et al., 1996; Sakariassen et al., 1990) thrombosis models. Platelet accumulation rate on collagen I is averaged over 15 minutes, measured in tubes (•), stenoses (+) (Markou et al., 1993) and U-channels (■) (Badimon et al., 1986). Platelet accumulation rate on collagen III, averaged over five minutes (∗, ◊) (Sakariassen et al., 1990; Barstad et al., 1996), is estimated from thrombus volume by a linear correlation of data published for the same system (Sakariassen et al., 1988), i.e., platelets/thrombus volume is equal to $9 \times 10^9$ platelets/ml.

Figure 1.6.4, which was originally generated by Wootton and Ku (1999), illustrates that the rate of platelet accumulation on fibular collagen, for the acute phase only, increases for shear rates from 100 to at least 10,000 $s^{-1}$. In these experiments, aggregation was significant so that the resulting average rate of attachment represents both initial deposition onto the collagen surface as well as adhesion to the developing thrombus. At low shear rates, there is a direct relation. At higher shear rates there appears to be a divergence from this trend, for deposition onto collagen. Barstad et al. (1996) found a decrease in deposition rate between 10,000 and 32,000 $s^{-1}$, whereas Markou et al. (1993) shows an increase in deposition rate between 4,300 and 20,000 $s^{-1}$.

Embolism and the roughness of the thrombus surface affect the probability that total occlusion will occur. Relevant experimental data on these components of thrombosis is not available. Local hemodynamics around a developing thrombus may also limit the acute growth phase. It is the duration of this phase that determines if a thrombus will occlude a vessel.

*Mathematical Models of Arterial Thrombosis*

To organize the available literature on thrombosis models, a distinction can first be drawn between models that focus solely on chemical reactions and models that also include the effects of hydrodynamic transport. An example of the former type is the model developed by Cooper et al. (1986), which computes protein deposition, platelet deposition, and embolization over the course of several hours. Each mechanism has an arbitrary constant controlling an associated rate, e.g., there exists a platelet deposition rate constant. The model is used to examine the effects of changing the arbitrary rate constants with respect to one another. Similarly, the Hellums et al. model for platelet aggregation doesn't directly consider platelet transport effects.

As engineers, we are interested in thrombogenesis as well as in the transport mediated events of thrombosis. Transport of blood platelets is a two-phase flow process while convection and diffusion of thrombosis related chemical species is governed by multi-component flow. The distinction is made here that multi-component flow is a subset of two-phase flow in which the various species are mixed at the molecular level, share the same velocity and pressure fields, and mass transfer takes place by convection and diffusion. Transport related math models of thrombosis can be classified as *separated flow models* or *flow mixture models*. Generally, *separated flow models either* track discrete particles, e.g., platelets, using the Euler-Lagrange approach or employ the continuum approach for two interacting media, while the *multi-component* approach treats platelets as chemical species or accounts for concentrations of chemical agonists in the flow.

A theoretical model describing the effect of blood velocity on the growth rate of a single hemispherical thrombus, using separated flow concepts, was developed by Richardson (1973). He used the theory of gradient coagulation (Levich, 1962) to compute the number of platelets that encounter a thrombus and postulated that a delay time elapses before the platelets are activated. Platelets that encounter the thrombus adhere only if they remain close for the time required to induce activation. Hydrodynamic interactions between the platelet and the thrombus are ignored and the velocity field is assumed to have a constant shear rate, which is not effected by the presence of the thrombus. Gradient coagulation theory, which assumes linear trajectories for the platelets, predicts the local flux of incoming platelets to be

$$J = n_o\, u \qquad (1.6.1)$$

where $n_o$ is the number of platelets per unit volume and $u$ is the local velocity of blood. This simple flux calculation is not strictly a separated flow technique; however, this model is included here due to the discrete particle nature of the particle residence time activation condition used.

Based on these very simple transport and adhesion components, the model of Richardson (1973) predicts thrombus behavior consistent with the *in vivo* measurements of Begent and Born (1970). The rate of thrombus growth is proportional to the thrombus volume, leading to an exponential growth of the thrombus with time. The growth rate constant increases linearly with shear rate at low shear rates, then decreases exponentially with shear rate above some threshold. This shear rate threshold is the point where the interaction time for some platelets begins to exceed the time required for binding to the thrombus. Both of these features match the experiment. The model predicted times on the order of 0.1 second for activation by ADP from comparison to the Begent and Born (1970) *in vivo* model. Richardson (1981) amends this observation with the fact that ADP activation delay times are likely shortened in the event of high shear stress exposure.

Ruckenstein et al. (1977) extended the Richardson (1973) model of a 2-D semi-cylinder thrombus by considering molecular attraction and repulsion forces and a simplified treatment of fluid interaction effects. The effect of the growing thrombus on flow is considered to be between the extremes of: (1) a constant flow rate while blood flow velocity increases as the thrombus grows; and (2) a constant pressure gradient at the most constricted point resulting in a reduction of flow rate. The Ruckenstein et al. (1977) model predicts two of the experimentally known features of thrombus formation, cf. Fig. 1.6.3: (1) the growth of the accumulation is approximately exponential during most of the formation (or acceleration period); and (2) the growth curve reach a plateau when the thrombus exceed a certain volume. The plateau reached by the growth curve is due to the transport effects captured by this model. In the case of a constant flow rate, the *reduction in platelet residence time* as the thrombus matures is responsible for the decrease in growth rate. With the assumption of a constant pressure gradient, competing effects result. The *platelet residence time near the thrombus is increased as the thrombus grows*, however, the flow rate is decreasing lowering

the platelet flux. The net result is again a leveling off of thrombus growth, however, this occurs at a time later than with the previous flow condition.

Fogelson (1984) treated platelets in a very narrow vessel (≈ 50 μm) as a discrete phase immersed in Stokes flow. The effect of platelet aggregates on the continuous phase is accounted for by the inclusion of a force density term in the Stokes equation. Individual platelets are assumed to follow fluid elements with the exception of a random dispersion term used to approximate collisional effects. Cohesion between platelets and adhesion of platelets to the injured wall are modeled by creating elastic links. Aggregates are modeled as a finite number of fluid points. The elastic links within the aggregates generate internal particle forces that influence the nearby fluid motion. This effect is included in the Stokes equation through the force density term thereby eliminating the need for internal material boundaries in the two-phase flow. This method is referred to as an *immersed boundary* technique and was first introduced by Peskin (1977) for studying blood flow in the heart. Once a flow field has been generated, the use of a distribution of points can represent particle rotation, translation and deformation.

Fogelson (1984) assumes individual platelets to be initially unactivated or non-cohesive. According to this model, only if platelets are stimulated by a certain concentration of ADP or by contact with an assumed injured region of the vessel wall will they become cohesive and secrete more ADP into the flowing blood. Values of ADP required for activation as well as platelet secretion amounts and secretion rates were gleaned from the literature or estimated. Transport of the ADP chemical species was handled using a multi-component model.

The immersed boundary method implemented by Fogelson (1984) and refined by Fogelson and Peskin (1988) is limited to microvessel applications with around 1,000 platelets due to the use of the Stokes equation and the computational cost. Fogelson et al. have only generated solutions for non-inertial Stokes flow using the discrete particle model. Furthermore, Fogelson (1984) suggests that modeling ADP transport using a multi-component model introduces a relatively large amount of numerical diffusion due to the dominant convection terms.

To improve the simulation of ADP transport in a microvessel, Fogelson (1992) developed a particle-method solution for convection dominant (Pe >> 1) 2-D convection-diffusion equations that contain localized source terms. In this method, discrete particles are used to track the evolution of the chemical species concentration field. These particles are convected at the local fluid velocity and undergo a random walk to simulate diffusion. Each particle caries with it point values of the gradient of the concentration field. The concentration field may be recovered from the particle location and gradient values by integration. This technique introduces no numerical diffusion and automatically concentrates computational effort near steep gradients.

Fogelson (1993) developed a similar particle method for the solution of convection dominant convection-diffusion equations in which particles carry information about the solution function, rather than gradient information. Smoothing functions are then used to generate concentration contours, eliminating the need for integration.

Fogelson (1992) extended the immersed boundary method to a continuum model approach that treats platelet concentration as a chemical species. Nevertheless, this model is classified here as a separated flow model due to the fact that the effect of discrete platelet aggregates on the flow field is accounted for locally and dynamically. In this model, platelets are described using population density functions allowing for the simulation of a large number of particles. Furthermore, the continuous field solution includes inertia so that this model can be applied in larger scale vessels ($\geq$ 1 mm). Again, force density terms are included in the continuous phase equations to account for the elastic links in aggregates. Wang and Fogelson (1999) apply the continuum model for platelet aggregation including chemical activation and elastic link breakage effects. At present, platelet wall interaction and the growth of wall bound aggregates has not been considered. Furthermore, the complexity of these models appears to limit them to simple geometries.

Tandon and Diamond (1997) extended the population balance model of Huang and Hellums (1993a - c) to include the effects of hydrodynamic interaction between two aggregates. With the Hellums et al. model, the collisional efficiency contained all the platelet receptor biology and hydrodynamic effects. Tandon and Diamond (1997) predict collisional efficiency by numerically accounting for the effects due to hydrodynamics

(beyond simple shear rate dependence) and receptor interactions. The aggregates are treated as solid spheres isolated in a shear field that is uniform far from the two particles. *Particles are bound together if enough time is available based on a particular receptor reaction rate.*

The salient feature of a multi-component deposition flux model is that platelet concentration is modeled as a chemical species and is transported in the flow by convection and diffusion. An enhanced diffusive motion of platelets results from local fluctuations in fluid velocity induced by rotation, deformation and migration of red blood cells in shear flow. Deposition flux models provide an approximate analytic solution for the flux of platelets attaching to the wall or the amount of surface coverage over time. These models typically use shear dependent parameters, determined from parallel experimental studies, to account for platelet diffusion and the rate of platelet adhesion. The surface boundary condition is typically based on the initial adhesion of platelets to a relatively clean surface (less than 50% platelet coverage by area), which severely limits their application to the study of platelet aggregation beyond a monolayer. Nevertheless, diffusion and reaction rate parameters from these models have wide applicability. *In general, deposition flux models indicate that platelet attachment is governed by the diffusion of platelets up to some critical shear stress, above which platelet-surface reaction rate controls the process.*

Friedman et al. (1970) complemented an *ex vivo* flow chamber experiment with an appendix that detailed an analytic expression for platelet flux as determined by assuming platelets to be a transported chemical species. Using dimensional analysis, it was apparent that initial platelet adhesion could be interpreted as either a diffusion-limited or an intermediate kinetic process, depending on shear rate.

In a series of studies, Turitto and Baumgartner (1973) and Turitto et al. (1980) investigated a chemical species model of platelet deposition on subendothelium with comparisons to the *in vitro* annular model of Baumgartner. The boundary condition for mass transfer analysis in this model allowed for a higher degree of surface coverage than in previous models. Platelet deposition was more extensive on the subendothelium tissue compared to the artificial surfaces used in previous experiments. These studies showed trends with enhanced diffusivity, in the range of $10^{-7}$ to $10^{-6}$ $cm^2/s$, and platelet attachment kinetic rates on the order of $10^{-4}$ to $10^{-3}$ cm/sec. Turitto et al. (1980) report, for citrated blood,

platelet deposition was diffusion controlled below a shear rate of 350 $s^{-1}$. For wall shear rates above 800 $s^{-1}$, platelet deposition was more reaction-controlled.

Wootton et al. (1998) applied the chemical species model of Turitto et al. (1980) to determine deposition flux in an axisymmetric collagen coated stenosis. A shear rate dependent platelet diffusivity and a constant reaction rate parameter were used. Upstream of the stenosis (which was also coated in collagen) the deposition rate dropped as a mass transfer boundary layer with a depleted platelet concentration developed. The deposition rate increased dramatically as the stenosis narrowed. Distal to the throat, a region of low shear recirculation reduced the transport rate. Quantitatively, these numeric results match the *ex vivo* experiment of Markou (1993) very well except for in the recirculation region where significant discrepancies arise.

Wootton and Ku (1999) developed a mathematical model of stenosis occlusion due to thrombosis using a platelet flux value at the apex of a stenosis as determined from the above chemical species model. They then assembled other parameters such as lumen diameter and a thrombus shape factor to generate a crude algebraic model of the expected time to vessel occlusion (Wootton et al., 2001).

Basmadjian (1990) developed a similar multicomponent thrombosis model that also considered platelets to be a chemical species controlled by convection and diffusion. Basmadjian (1990) introduced a lumped mass transfer coefficient $k_L(x,t)$ in the convection-diffusion equation and retained the reaction rate constant $k_w$ of the Turitto et al. model. The lumped mass transfer coefficient is reported to change drastically in regions of highly disrupted flow (Basmadjian, 1990) limiting the applicability of results derived from tubular flow experiments. Moreover, species transport models, in general, depend *on boundary layer assumptions* to determine the platelet flux expression. In regions where boundary layer assumptions are not valid, e.g., in separated flow, these models will typically fail, as was the case with Wootton et al. (1998 & 2001).

There are several other difficulties with the multicomponent model. The main problem is that platelets are not a chemical species, and are subject to hydrodynamic interaction with the thrombus and other platelets. The binding of platelets to a surface is also much more complicated than a simple rate of adhesion and rate of detachment. Platelet

adhesion involves receptors on the platelet membrane, plasma proteins in solution form or absorbed on the platelet surface or thrombus, and platelet-surface contact mechanics. Furthermore, platelet dispersion is more complicated than diffusion, and is still not well understood.

Most recently, David et al. (2001) considered the multicomponent model with respect to the experimental results of Affeld et al. (1995) for platelets depositing in a stagnation point flow chamber. David et al. (2001) found that a multicomponent model with a constant wall reaction rate was incapable of simulating platelet deposition patterns in the stagnation flow device. This is surprising considering that researchers included Turitto et al. (1980) through Wootton (2001) have applied the multicomponent platelet flux model using a constant reaction rate. David et al. (2001) were somewhat successful in improving the multicomponent model by using a wall reaction rate dependent on shear stress.

*Math Models of Embolization*

Platelet deposition histories often reveal a peak in deposition after which the number of attached platelets drops by some degree, to a plateau. This peak is interpreted as the point at which embolization begins to overtake the rate of deposition. Basmadjian has developed a theoretical model for embolization in steady and pulsatile flows (Basmadjian, 1989) based on models of drag on an idealized small protrusion from a tubular wall in steady (Basmadjian, 1984) and pulsatile Basmadjian (1986) flows.

For steady flow, Basmadjian (1984) analytically considered drag, shear, torque and tensile stress on small and idealized thrombi in tubular flow. This study is based on the creeping flow assumption, which limits the particle (or protrusion) Reynolds number to

$$\mathrm{Re}_p = \frac{H_p v_p}{\nu} \quad \leq \quad 0.1 \tag{1.6.2}$$

In the above equation, $H_p$ is the height of the idealized thrombus and $v_p$ is the free stream approach velocity at a height $H_p$ away from the wall. This study is also restricted to very small thrombi such that the ratio of thrombus height to tube diameter remains less than 0.05, i.e.,

$$\frac{H_p}{D_t} < 0.05 \tag{1.6.3}$$

In a complimentary study, Basmadjian (1986) relaxed the steady flow assumption and investigated the forces on a very small idealized thrombus in tubular flow for $\mathrm{Re}_p \leq 0.1$ . This study applied an empirically modified Basset-Boussinesq-Oseen equation similar to the form introduced by Odar and Hamilton (1964). With the aforementioned assumptions, in conjunction with a Stokes number less than 0.1, Basmadjian (1986) drops the history and inertial terms in the modified BBO-equation and predicts drag force, shear stress, and torque acting on an attached thrombus.

Basmadjian (1989) again considered the empirically modified BBO-equation. The inertial and history terms were evaluated in this case using estimates to maximize their value and, hence, provide a conservative estimate of embolization stress. In this manner, the assumptions of small particle Reynolds number, thrombus height, and Stokes number could be relaxed. Basmadjian (1989) used these results to develop a model that predicts zones of low, transitional, or high probability of embolization, based on the maximum wall shear rate and thrombus height. This model predicts that certain combinations of shear rate and thrombus height in a sufficiently large lumen should allow a vessel or stenosis to remain patent due to embolization.

Basmadjian makes several important observations about drag and lift on a thrombus. The stress on the thrombus depends on the particle Reynolds number. For small thrombi ($\mathrm{Re}_p < 1$), stress is 4 to 5 times the wall shear stress of the approaching flow, for an isolated thrombus. For larger thrombi, stress becomes a function of thrombus height, and stress increases rapidly as the thrombus grows. The stress from the transient terms varies from being negligible at low particle heights and shear rates to doubling the stress for large thrombi at high shear rates. For rough surfaces and thrombi, negative lift can be generated, which could help a platelet or aggregate adhere to the surface, at least in steady flow. The main limitations to the analysis are that quantitative data on the force required for platelet removal from a surface are not available, and the effect of multiple thrombi, which mutually influence the flow field, are not considered. Basmadjian does not use the model to make any predictions about the critical diameter and initial shear rate required for graft patency.

### 1.6.2 Monocyte Attachment and Rolling Models

Compared to platelets, relatively few experimental models have been constructed to study monocyte adhesion in flowing blood. Studies available in the literature have been conducted in simplistic step-type *in vitro* geometries and typically implement U937 cells as a reliable substitute for monocytes. Several researchers have also attempted to numerically model the adhesion of individual monocytes.

*Experimental Models*

Pritchard et al. (1992, 1995) examined the rolling velocity and adhesion patterns of monocytes and U937 cells in a cylindrical geometry that contracted gradually into a throat followed by a sudden expansion. The flow was steady ($Re_d = 200$ at the inlet) and of relatively low shear stress by *in vivo* standards. Adherent cells were only noted in regions where the wall shear stress was less than 0.4 dyne/cm$^2$ and in these regions the cell rolling velocity directly correlated with wall shear stress. The surface of the geometry was biologically inert such that quantitative results cannot be extended to *in vivo* conditions. As with Karino and Goldsmith (1979), Pritchard et al., (1992, 1995) observed a minimum in monocyte adhesion at the site of flow reattachment flanked on either side by two peaks in adhesion.

Barber et al. (1998) examined the interaction of U937 cells with activated and unactivated endothelial cells in a step expansion geometry. Video microscopy was applied to characterized cell adhesion near the flow reattachment point. As with previous studies, Barber et al. (1998) observed a minimum in cellular adhesion near the reattachment point. Barber et al. (1998) compared their experimental results with a computational point force model of cellular motion. It was found that drag and gravity terms were insufficient to compute near-wall particle motion.

Hinds et al. (2001) implemented the geometry used by Pritchard et al. (1992, 1995) with a vertical orientation and E-selectin coating. Adhesion studies were conducted for two different steady flows at Reynolds numbers of 100 and 140 and a transient flow with a Womersley parameter of 3 and a mean Reynolds number of $Re_d = 107$ at the inlet. For both steady and transient conditions, the geometry was perfused for two hours and the adhesion patterns were recorded. For steady flow, cell density appeared to correlate with the inverse

of shear stress in the tapered throat region only. No such correlation was observed in the expansion region. As expected, transient flow served to equalize particle attachment. Furthermore, Hinds et al. (2001) reported that U937 cells could adhere to E-selectin at shear stresses on the order of one dyne/cm$^2$. However, do to low receptor density in the experiment, qualitative extrapolation to *in vivo* conditions is not possible.

*Mathematical Models*

The adhesion of monocytes to the vascular surface depends on biophysical interactions between reactive molecules. The likelihood, strength, and duration of adhesion are functions of the kinetics of molecular interactions and the mechanics of the surrounding environment. To mathematically describe the phenomenon of cellular adhesion, the concept of an adhesion energy density was first proposed (cf. Zhu, 2000). The adhesion energy density was defined as the mechanical work required to separate a unit area of the adherent surfaces. According to Zhu (2000), this thermodynamic model failed to appropriately relate the necessary mechanical and thermodynamic variables, possibly due to the infrequency and significance of individual bond formation.

Given that molecular bond formation occurs on a random basis, reaction kinetics theory has been applied to model interactions between single receptors and ligands. Subsequent reaction rate constants have been incorporated into models for rolling monocyte interactions. The use of a computational technique that combines a fluid mechanical analysis of particle motion and a Monte Carlo simulation of bond formation and breakage between molecular pairs has been referred to as *adhesive dynamics* (Hammer and Apte, 1992). Several reaction kinetics and adhesive dynamics models are reviewed below.

Hammer and Apte (1992) originated the adhesive dynamics model to simulate the interaction of a single cell with a liquid-coated surface driven by flow. The cell was idealized as a hard sphere covered with adhesive springs to simulate microvilli. Kinetic reaction rates provided the necessary probability density functions such that bond formation and dissociation were simulated using a Monte Carlo-type technique. Cell velocity was a function of several interrelated influences such as hydrodynamic interactions and bond strength. The bonding force is simply the sum of individual contributions from each

receptor-ligand pair. The model of Hammer and Apte (1992) was found to recreate the entire range of adhesive phenomena including firm adhesion, rolling, and transient attachment.

With the Hammer and Apte (1992) model, the receptor-ligand separation distance was considered to be the only variable factor that affected the forward reaction rate. Other factors, such as diffusivity, size and orientation of the binding site, and influences of the surrounding solution, are combined into a single parameter referred to as the intrinsic rate of reaction. Hence, these calculations did not consider the effect of convection on the transport of ligands and receptors. As an improvement to this method, Chang and Hammer (1999) incorporated a convective-diffusive approximation for the transport of adhesive molecules. Furthermore, Chang and Hammer (1999) introduced a 'cut-off' separation distance to define a reactivity region. Cell surfaces beyond the cut-off distance were not considered reactive.

Dong and Lei (2000) as well as Lei et al. (1999) investigated the deformation and adhesion of leucocytes to endothelial cells using an *in vitro* side-view flow chamber and a two-dimensional Adhesive Dynamics model. Cell rolling velocity was based on an energy balance. The rate of energy provided by the surrounding fluid was assumed to influence both the energy dissipation due to bond separation and energy loss due to cell deformation. The primary finding of both the *in vitro* and the adhesive dynamics investigations was that cell deformation increased dramatically with shear stress, i.e., cell-surface contact area doubled under high shear stress (20 dyn/cm$^2$) compared to low shear stress (0.5 dyn/cm$^2$) conditions.

## 1.7 Collision Models for Blood Particle Transport

Blood can be considered a mixture of a Newtonian solution and highly deformable liquid-like cells slipping past one another or aggregating, depending on the local shear rate. Moreover, local concentrations of blood cells are also shear rate dependent as evident in the near-wall plasma layer. The presence of red blood cells at volume concentrations from 40 – 50% considerably disturbs the motion of other cells, such as platelets and white blood cells. The continued collisions and hydrodynamic interaction between, and deformations of, the red blood cells in flowing blood induces an alternate form of mixing, similar on a macroscopic scale to the intermolecular collision which result in Brownian motion. Due to the extremely low Stokes number and a fluid-to-particle density ratio near unity, a dimensionless parameter

analysis has shown that actual blood particle contact occurs to a low degree. However, fluid dynamic interactions between blood cells cannot be ignored due to the high volume fraction and will be referred to as *mixing*. For platelets and white blood cells, the movement induced by this mixing, results in a persistent dispersion, and is often modeled with effective diffusion coefficients which are two to three orders of magnitude greater than those due to Brownian motion. This mixing motion becomes a major mechanism for permitting cells and possibly some of the larger proteins to interact with the wall. In very small vessels, and at low shear rates, radial dispersion coefficients for platelets and other cells have been measured (cf. Goldsmith and Turitto, 1986). However, the dispersion of blood cells due to collisions at high shear rates ( $\dot{\gamma} > 440\ s^{-1}$ ) is determined by the local velocity gradient, cell concentration, and cell deformation. *Therefore, collision induced blood cell dispersion should not be evaluated using a constant diffusion coefficient.*

To determine the motion of a critical blood particle in a variable concentration of red blood cells, all collisions, cellular deformations, and inter-particle velocity effects would need to be included and fully resolved. Such a simulation is not computationally feasible for a system of, say, 100 particles, whereas there are more than $10^9$ particles per $mm^3$ of blood. Approximations are therefore necessary to account for the motion of a single particle in a red blood cell suspension under the action of variable shear and volume fraction fields. The motion of a single particle in such an environment is influenced by a streamwise impedance (Soo, 1990) and a lateral dispersion or drift (Eckstein and Belgacem, 1991). The streamwise resistive force exerted on a single particle in a cloud of other particles might be expressed as a modified drag using empirical coefficients such as those described by Crowe et al. (1998). Alternatively, Fan and Zhu (1998) describe a collision-based linear resistive force in terms of an effective particle shear stress or an effective viscosity. In this type of model, regions of high particle interaction, which correspond to regions of high red blood cell concentration, induce a linear resistance to particle motion via an increased effective fluid viscosity. Interestingly, a simple shear based rheological model, such as the Quemada, correlates viscosity to particle concentration, e.g., regions of high shear indicate regions low volume fraction and correspondingly low viscosity. To a first approximation, then, the use of the continuum assumption to determine blood viscosity and the subsequent use of this property

to determine blood particle motion (as done by Buchanan, 2000) may account for a collision induced linear resistance.

For sufficiently dense particle suspensions, lateral mass transport may be augmented by two mechanisms: (1) particle rotation, which causes local mixing in the surrounding fluid, and (2) shear-induced collisional diffusion where interactions and/or collisions between particles produce net lateral displacements and associated motions in the continuous (fluid) phase. The remainder of this section deals with approximations for the lateral transport of critical blood particles (which, for platelets may be viewed as a solute) in constant and varying concentrations of spheres and red blood cells.

### 1.7.1 Measured Radial Particle Dispersion Coefficients

A classic rheology concept is that the shearing action in a tubular suspension flow forces particles to interact continuously and that such interaction leads to lateral particle motion (Zydney and Colton, 1988). By analogy with Brownian translational diffusion, a radial particle dispersion coefficient, $D_p$, obtained from the mean square radial distance $\overline{\Delta r^2}$ traveled by an observed particle in a time interval $\Delta t$ is given by

$$D_p = \frac{\overline{\Delta r^2}}{2\Delta t} \tag{1.7.1}$$

This does not imply that the lateral motions are considered to be random, as in Brownian diffusion. The fluctuations are the result of multi-body collisions determined by *the local velocity gradient, cell concentration, and cell deformation.* Goldsmith (1971b) used a traveling microtube apparatus to track the motions of platelet sized latex beads and platelets in low shear-rate blood flows. With measurements of the change in lateral position over a fixed time interval, and Eq. (1.7.1) for the diffusive motion of an unbiased random walk, Goldsmith (1971b) computed a coefficient for augmented diffusion. At local shear rates varying from 5 to 20 $s^{-1}$ and red blood cell volume concentrations varying from 20% to 70%, the values of the calculated radial dispersion coefficient ranged between $10^{-12}$ and $2 \times 10^{-11}$ $m^2/s$ for red cells and 2 μm latex microspheres. Similar values were observed for leukocytes and platelets (Goldsmith and Karino, 1977).

The use of a constant particle dispersion coefficient ignores the obvious dependence between cellular interactions and the interrelated occurrences of variable shear rate, cellular concentrations, and particle deformation. For example, with increasing shear rate, red blood cells exhibit drop-like deformation, align with the flow direction and induce a decreasing effective blood viscosity (Goldsmith and Turitto, 1986). The influence of shear rate on the radial dispersion coefficient, as well as cellular concentration, has been investigated by several researchers. Turitto et al. (1972) used Taylor's method to measure the effective lateral diffusion coefficient in blood flow through a 200 μm tube. For low wall shear rates, the measurements of Turitto et al. (1972) compare well with the findings of Goldsmith and Karino (1977). However, Turitto et al. noted a direct relationship between $D_p$ and shear rate as the shear rate increased, i.e., a variable dispersion coefficient.

Eckstein et al. (1977) tracked the radial position of a platelet-like radioactive particle in a suspension of polystyrene spheres in a Couette viscometer. Eckstein et al. found that the dispersion coefficient scaled linearly with shear rate and particle radius, i.e.,

$$D_p = 0.03 a^2 \dot{\gamma} \quad for \; 0.2 < \alpha_p < 0.5 \tag{1.7.2}$$

Leighton and Acrivos (1987a, b) found significantly higher values of $D_p$ compared to previous studies, and proposed that this discrepancy was caused by the presence of wall effects in the Eckstein et al. (1977) experiment.

An important finding of the earlier work of Turitto et al. (1972), was that platelets became non-uniformly distributed when the wall shear rate was above 440 $s^{-1}$ ($\tau \approx 13.2$ dyn/$cm^2$). This concentration gradient cannot be explained with the use of a simple random (Gaussian) particle dispersion coefficient. As discussed in Sect. 2.4.3, due to different cellular sizes and compliance, red blood cells tend to migrate further toward the tube axis than do platelets resulting in a stratified blood particle concentration profile. Clearly, particles in regions of higher local cellular concentration will experience an increased number of collisions and tend to move to a region of lower particle concentration (or persistent non-random motion).

Sources of persistent motion are described in detail for a system of rigid spheres in a widely referenced paper by Leighton and Acrivos (1987a). Leighton and Acrivos presented

an approximate correlation for dispersion in the presence of a concentration gradient of rigid spherical particles, i.e.,

$$\frac{D_p}{a^2\dot{\gamma}} = \frac{1}{3}\alpha_p{}^2\left[1 + 0.5\exp(8.8\alpha_p)\right] \qquad (1.7.3)$$

which predicts a $D_p$-value that is two orders of magnitude higher than the previous low shear rate studies.

Eckstein and Belgacem (1991) referred to persistent motion as drift or drift flux and accounted for it in the convective diffusive equation with a term directly proportional to $D_p$ and the local platelet concentration. Buchanan and Kleinstreuer (1998) extended this idea to non-uniform geometries. Hofer and Perktold (1997) accounted for persistent particle diffusion using the arguments of Leighton and Acrivos, via Phillips et al. (1992).

### 1.7.2 Effective Solute Diffusion Coefficient

Both particle rotation and shear induced collisions contribute to an enhanced diffusivity of solutes (which may include oxygen, platelet agonist, or platelets themselves) present in the continuous phase. Theoretical analyses of augmented solute transport in sheared suspensions have been based on the particle rotation mechanism. Investigators such as Keller (1971) have evaluated the augmentation arising from the convection flow field around a single rotation sphere using an approximate analysis similar to mixing length theory.

A result of some *in vitro* platelet deposition experimental models has been an estimate of the enhanced diffusion of platelets modeled as a chemical species or solute. Grabowski et al. (1972) estimated platelet diffusivity by fitting a species transport model of platelet deposition to experimental measurements of canine platelet deposition on glass surfaces. Other investigators used the same approach with different experimental data. Turitto and Baumgartner (1975) used *in vitro* measurements of rabbit platelet deposition on rabbit subendothelium in an annular chamber, and Feuerstein et al. (1975) used *in vitro* measurements of platelet deposition on rotating collagen-coated glass rods. Enhanced diffusivity was fit to a power law

$$D_e = a\,\dot{\gamma}_w^n \qquad (1.7.4)$$

where $\dot{\gamma}_w$ is the shear rate at the surface and $D_e$ is the effective solute diffusion coefficient. The shear rates in these experiments ranged from 10 to 832 $s^{-1}$. The power that best fit the experimental data was usually around 0.5 to 0.6. The main difficulty with these experiments is the interdependence between the estimated diffusivity and the effective kinetic rate of platelet deposition, which is probably also a function of shear rate (Turitto et al., 1980).

Wang and Keller (1985) have measured shear-enhanced solute transport in red blood cell suspensions using an electrochemical technique in a Couette viscometer, for shear rates of 200 to 4,000 $s^{-1}$. The augmentation of diffusivity $A$ is defined as

$$A \equiv \frac{D_e - D_s}{D_s} \tag{1.7.5}$$

where $D_e$ is the effective diffusivity in the sheared blood and $D_s$ is the diffusivity in stationary blood, or the thermal diffusivity. Augmentation is correlated to the particle Peclet number

$$Pe \equiv \frac{a^2 \dot{\gamma}}{D_s} \tag{1.7.6}$$

where $a$ is the red blood cell equivalent radius (2.75 μm for human red blood cells) and $\dot{\gamma}$ is the shear rate. The data for various hematocrits and chemical species were fit to a power law

$$A = \alpha Pe^{\beta} \tag{1.7.7}$$

The power ranges from 0.71 to 1.06 and $\alpha$ ranges from 1.8 to 14.2 (Zydney and Colton, 1988).

### 1.7.3 Unifying Particle Dispersion and Enhanced Solute Diffusion

Zydney and Colton (1988) proposed that augmented solute transport in a concentrated suspension under shear arises primarily from the shear induced particle motion. They correlated enhanced diffusion in concentrated suspensions to particle Peclet number and concentration, based on a wide survey of the literature, most of it related to transport in blood. The work uses a theoretical argument to tie together measurements of enhanced solute or platelet transport, and enhanced particle or red blood cell dispersion, in shear flow. Particle volume fractions and velocity components are decomposed into a volume-averaged mean and a fluctuating component. When the continuity equation with decomposed values is

averaged over a volume, a term that is analogous to the eddy diffusivity in turbulent transport appears where the diffusive flux would be found in the transport equation. This term is used to empirically define the particle diffusivity. Similarly, the transport equation can be decomposed into mean and fluctuating components. Under the assumption of small gradients in the concentration of particles, and assuming that particle rotation does not contribute much to solute transport, the effective solute diffusivity is equal to the sum of the particle diffusivity and the thermal diffusivity

$$D_e = D_p + D_s \tag{1.7.8}$$

where, as above, $D_e$ is the effective or shear-enhanced diffusivity, $D_p$ is the particle dispersion coefficient, under the influenced of shear, and $D_s$ is the thermal diffusivity of the solute, i.e., platelets through the suspension.

A functional fit for the nondimensional particle dispersion coefficient is derived

$$\frac{D_p}{a^2\dot{\gamma}} = k\alpha_p\left(1-\alpha_p\right)^n \tag{1.7.9}$$

with $k$ = 0.15 +/- 0.003 and $n$ = 0.8 +/- 0.3, where $a$ is the red blood cell major radius and $\alpha_p$ is the hematocrit. This function fits the experimental values of self-dispersion of red blood cells and ghost cells only qualitatively; there is quite a bit of scatter in the experimental data.

When experimentally measured solute diffusivity augmentation is compared to particle dispersion coefficients using Eq. (1.7.9), the model is within about one order of magnitude for a physiologic hematocrit (40%), over a range of almost five orders of magnitude of augmentation and solute Peclet number. The scatter is somewhat higher for lower hematocrit (10%). The platelet transport data has the most scatter from the model, and the highest augmentation.

Aarts et al. (1986) have calculated platelet diffusivity over a wide range of shear rates and included the effect of hematocrit. Using the *in vitro* experimental arrangement of Turitto and Baumgartner (1975), Aarts et al. (1986) estimated platelet diffusion by measuring platelet deposition on subendothelial tissue over a shear rate range of 200 $s^{-1}$ to 1300 $s^{-1}$, i.e., the region of diffusion controlled deposition. The maximum mean hematocrit studied was $\bar{h}$ = 0.6. The resulting correlation was

$$D_e = 1.05\times10^{-9}\,\dot{\gamma}_w^{\,0.297+1.29\bar{h}-0.90\bar{h}^2} \qquad (1.7.10)$$

where $\bar{h}$ is the mean hematocrit and $\dot{\gamma}_w$ is the local wall shear rate. The resulting values for platelet diffusivity ranging from 0.4 to $1.3\times10^{-7}$ cm$^2$/s at $\bar{h}$ = 0.4 as illustrated in Fig. 1.7.1.

Although the Zydney and Colton (1988) and Aarts et al. (1986) models theoretically explain a relationship between particle dispersion and enhanced solute diffusion in dense suspensions of deformable particles, they are somewhat limited. Correlation (2.61) is based on data that may or may not include the drift effect depending on the shear rate. Furthermore, these models do not consider the effect of local particle concentration or shear rate. *Accordingly, Eqs. (1.7.9) and (1.7.10) are not capable of accounting for the local variation in the dispersive motion that results in the experimentally observed phenomena of variable concentration profiles.*

### 1.7.4 Particle Dispersion in a Non-Uniform Concentration Field

As described above, at a certain shear rate, particle dispersion in a high concentration suspension exhibits a persistent motion. Leighton and Acrivos (1987a) suggest that in the presence of a concentration gradient, there is a non-uniform random particle *drift* across streamlines that can arise from the level of particle interaction, among other things. For any given particle, the rate of interaction with other particles is proportional to the number of particles (i.e. the particle concentration); thus, the particle will tend, on average, to undergo a greater number of displacements from high to low concentration than from low to high. In addition, the gradient in concentration gives rise to a gradient in suspension viscosity, which also leads to a non-random (or directional) motion from high to low concentration. Thus, in the presence of a concentration gradient, irreversible particle interactions give rise to both a non-random particle drift and a diffusive flux arising from random particle migrations. Although the non-random particle drift is not a true diffusive flux, it is proportional to the concentration gradient, and can be expressed as such.

*Drift-Flux Modeling*

Eckstein and Belgacem (1991) have added a drift term to the convective diffusive equation for platelet transport to account for persistent collision induced motion. The goal of the Eckstein and Belgacem model was to simulate the development of platelet concentration profiles in capillary tube flow. In the Eulerian sense, the drift term acts like a potential that drives up platelet concentration in the near-wall region. The drift term is introduced in a single component platelet transport equation as a way to include more information about events such as collision frequency and local viscosity. Eckstein and Belgacem (1991) derive the constitutive drift term as proportional to platelet thermal diffusivity and the fully developed platelet concentration profile. This development is somewhat cyclic, i.e., experimental knowledge of the fully developed platelet concentration is required *a priori*. The model is then used to calculate the fully developed platelet concentration in tubular flow. There is no theoretical basis for extending the drift term of Eckstein et al. to a system other than the one for which it was derived, i.e., shear rate, platelet concentration, hematocrit, and geometry, cannot be varied. Interestingly, Eckstein and Belgacem (1991) did convert the Eulerian drift term into a position dependent biasing factor in a Lagrangian random walk scheme.

Buchanan and Kleinstreuer (1998) extended the drift-flux concept for platelet transport to non-uniform geometries by postulating that radial dispersion is also caused by extensional shear. This effect was included in the framework of a drift-flux model as a platelet length scale multiplied by the first scalar invariant of the rate of deformation tensor (a measure of shear rate).

*More Advanced Constitutive Relations*

Phillips et al. (1991) modified and applied concepts suggested by Leighton and Acrivos (1987a) to develop a diffusion equation that describes the evolution of a particle concentration profiles in shear flow. This model is applicable for rigid spherical particles of volume fraction $0.0 < \alpha_p < 0.68$ and large particle Peclet numbers, i.e.,

$$Pe = \frac{a^2 \dot{\gamma}}{D_s} >> 1 \qquad (1.7.11)$$

The resulting constitutive relation is second-order and nonlinear with respect to the particle volume fraction, and can be solved in conjunction with the equations of motion.

To model particle interaction, Phillips et al. (1991) derived semi-empirical expressions for lateral particle flux via the two mechanisms discussed by Leighton and Acrivos (1987a). One of these Eulerian flux terms accounts for migration of particles from a region of high particle-to-particle collision rate to a region of lower interaction. The other flux term is due to the fact that an interaction between two particles will be affected by the spatially varying viscosity (caused by the existence of gradients in the particle concentration). These flux terms are proportionalities and require correction via empirically derived constants. Phillips et al. (1992) went on to determine these constants for rigid spheres in Couette and Poiseuille flows. Phillips et al. (1992) suggest that their model is only applicable in one-direction shear flows, however, the details in the model derivation should be consistent with more complex environments.

Hofer and Perktold (1997) directly applied the Phillips et al. (1992) model to flow through a stenosis using a variable viscosity particle flux model. In their model, the local particle volume fraction is computed via the Phillips et al. (1992) constitutive relation and is then used to compute the local fluid viscosity and density. The stenotic geometry used represents an extension of this constitutive equation to a multi-dimensioned shear environment. The resulting particle concentration profiles are qualitatively reasonable; however, empirical data was not available for validation. Hofer and Perktold (1997) acknowledge that the hard sphere model is not compatible with blood flow in which deformation and aggregation of the red blood cells are essential rheological factors. Furthermore, this collision model breaks down very near the wall and is not capable of accounting for near-wall hydrodynamic effects, e.g., wall induced lift forces.