CHAPTER 3

3 A NEAR-WALL RESIDENCE TIME MODEL WITH CORRELATIONS TO PARTICLE DEPOSTION AND INTIMAL THICKENING

3.1 Introduction

The relative significance of factors contributing to the complex multi-faceted processes of vascular diseases is not fully known. However, the interaction of critical blood particles, such as monocytes and platelets, with the vascular surface provides a necessary stimulus for intimal thickening initialization and progression, as well as a means for thrombus formation and potential vessel occlusion (Ross, 1993; Schwartz et al., 1993; Davies, 1994). Blood particle *adhesion* is an initial step in a cascade of events by which monocytes and platelets may contribute to the formation of stenotic developments and/or mural thrombi.

For blood particle adhesion to occur, hemodynamic forces must first transport the cell to within a critical distance of the vascular surface. 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/re-suspension and possible migration into the wall. The initial adhesion of platelets to a surface involves glycoprotein complexes exposed on the surface membrane of activated platelets; various vessel wall collagens and possibly other structures; and adhesive proteins, such as vWF, fibronectin, and fibrinogen, which are found in the plasma, the platelet granules, and the vessel wall (Colman et al., 1994). Monocyte deposition and transmigration also involves cell and surface bound molecules, such as selectins, immunoglobulins, integrins and their corresponding ligands (Springer, 1995).

Despite the complex biophysical processes involved in blood particle adhesion, a number of researchers have established correlations between particle attachment and local wall shear stress conditions. A direct increase in platelet adhesion and accumulation with shear rate has been observed *in vitro* and *en vivo* for platelets depositing on subendothelium and collagen-coated surfaces (Alevriadou et al., 1993; Badimon et al., 1986; Sakariassen et al., 1988; Sixma and de Groot, 1994; Turitto and Baumgartner, 1975; Wootton and Ku,

1999). The effect of shear rate on platelet deposition is due, in part, to (a) enhanced diffusion of platelets in whole blood resulting from fluctuations in local fluid velocities due to red blood cell rotation, deformation and migration induced by fluid shear (Zydney and Colton, 1988); and (b) enhanced platelet reactivity (or activation) partially due to shear stress exposure (Hellums, 1994). Alternatively, production of anti-thrombogenic compounds by an intact endothelial layer has been directly related to WSS. With respect to monocytes, regions of low wall shear stress generally allow for prolonged vessel wall contact, thereby facilitating adhesive interactions (Henry and Chen, 1993). Moreover, low wall shear stress has been associated with endothelial cell expression of adhesive molecules such as VCAM-1 (Gimbrone et al., 1997).

Mathematical models for blood particle adhesion that are more accurate than the wall shear stress correlations, typically consider the convection and diffusion of platelets or monocytes within a system. Due to an extremely low Stokes number, platelets are often represented as a solute in a multicomponent mixture (Friedman et al., 1970; Turitto et al., 1980; Basmadjian et al., 1990; Wootton et al., 2001). Shear dependent parameters, determined from experimental studies, are used to account for platelet diffusion and the rate of platelet adhesion. These models ignore the discrete nature of hydrodynamic interaction forces that arise in the near-wall region and, due to the assumed constants, typically perform poorly in complex flows. The attachment of individual platelets to a vascular surface has been rarely modeled (Fogelson and Peskin, 1988).

The transient motion of individual blood particles, particularly monocytes, has been simulated to visualize fluid flow characteristics, including stasis and sites of potential particle deposition (Ehrlich and Friedman, 1977; Buchanan et al., 2000). To simulate the adhesion process for monocytes, reaction kinetics has been used to model interactions between single receptors and ligands, often on a stochastic basis (Zhu, 2000). Due to computational requirements, such adhesive dynamics models are presently not applicable to large-scale geometries with significant numbers of particles. In such cases, a simple particle-surface contact model is often applied to capture locations of initial interaction in the absence of near-wall hydrodynamic forces (Hyun et al., 2001).

For geometrically large and complex systems with high particle counts, an alternative methodology is needed to approximate the nano-scale molecular adhesion process in order to effectively capture the *probability* for critical blood particle deposition. It has been widely proposed that for monocytes and platelets sufficiently close to a reactive surface, adhesion probability and local residence time are positively correlated (Richardson, 1973; Karino and Goldsmith, 1979a & b; Henry and Chen, 1993; Hellums, 1994; Ross et al., 1998). Other researchers have shown that adhesion is not only dependent on contact time (Rinker et al., 2001, Swift et al. 1998); however, residence time appears to be the common thread for general blood particle deposition.

It is hypothesized that blood particle deposition is most likely in regions of near-wall particle stasis and/or elevated concentrations, coincident with regions of activated or dysfunctional endothelial cells. Regions of enhanced particle-wall interaction may be quantified by the near-wall residence time (NWRT) parameter. Endothelial cell activation and dysfunction, e.g., up-regulation of adhesive molecules, generation of mitogenic factors associated with smooth muscle cell proliferation, and exposure of subendothelial collagen, have been correlated with wall shear stress based hemodynamic parameters. Aggravating hemodynamic parameters include low wall shear stress (WSS), large variations in WSS direction over time, as well as large spatial and temporal gradients of the wall shear stress vector field in terms of magnitude and direction.

In this chapter, the effectiveness of the NWRT concept is first established by comparison to *in vitro* monocyte and platelet deposition results on surfaces without an endothelial coating. Composite NWRT-based parameters are then formulated for the deposition of monocytes and platelets, which take into account factors such as particle dispersion, the local WSS environment, and the particle shear stress exposure history. These shear stress based components are intended to model phenomena such as endothelial cell activation and dysfunction, monocyte rolling and resuspension, and platelet activation. Implementing the rabbit aorto-celiac junction and the human carotid artery bifurcation, for which some particle deposition and intimal thickening localization data is available, the proposed NWRT-based models are evaluated. Finally, results of the NWRT-based models

are compared to recent *in vivo* observations of intimal hyperplasia in animal models of the distal femoral anastomosis.

3.2 Comparison of Particle Deposition Models for Non-Parallel Flow Domains

3.2.1 Overview

This study investigates the effectiveness of various approximations for blood particle attachment and deposition that are applicable to complex flow fields with high particle counts. It is expected that deposition models such as the wall shear stress correlations, the multi-component approach, and the surface-contact approximation may be insufficient to capture likely sites of particle adhesion in flow environments with stagnation, recirculation, and reattachment. Hence, the NWRT method for predicting the likelihood of blood particle attachment is evaluated in the context of non-parallel flow environments. Given the need to efficiently identify sites of likely monocyte and platelet deposition in complex hemodynamic flow systems (e.g., the carotid artery bifurcation or end-to-side anastomoses) the performance of potential deposition models is assessed by comparison to available 2-D axisymmetric experimental results.

3.2.2 Systems

To evaluate the performance of the potential models with respect to platelet deposition, an axially-symmetric stagnation point flow cell was selected, which has been widely implemented. Recently, Affeld et al. (1995) used this *in vitro* style model to quantify the deposition of pre-activated platelets ($d_p \approx 3$ μm, $\tau_p = 1.53 \times 10^{-6}$ s, $\rho/\rho_p \approx 0.964$) onto a glass surface over times ranging from 160 to 180 s. Platelet rich plasma (PRP: $\rho = 1.03$ g/cm^3; $\mu = 0.014$ dyne s/cm^2) was used as the working fluid such that the shear induced dispersional effects of red blood cell collisions would not complicate the results. The flow apparatus of Affeld et al. (1995) consisted of a pair of axisymmetric disks 0.04 cm apart with a circular inlet pipe of diameter d = 0.067 cm fixed at the center of the upper disk (Fig.

3.2.1a). The PRP entered with a fully developed profile at a steady rate of 5.72×10^{-3} g/s, which resulted in an inlet Reynolds number of $Re_d = 9.5$. It was assumed that the flow chamber is completely filled with plasma such that an air-space does not arise at the entrance. Due to the axisymmetric design, the mean velocity decreases with radial distance (Fig. 3.2.1b).

A tubular geometry consisting of a "stenosis" and a sudden expansion was selected to evaluate models for monocyte deposition. Hinds et al. (2001) studied the adhesion of preactivated dilute U937 cells ($d_p = 20$ μm, $\tau_p = 2.96 \times 10^{-5}$ s, $\rho/\rho_p \approx 0.939$) in this style system, coated with E-selectin. Properties of the suspension include $\rho = 1.00$ g/cm^3, $\mu = 0.008$ dyne s/cm^2, and a mean concentration of 5×10^5 cells/ml. The inlet diameter was 0.635 cm and the stenosis diameter ratio was 0.62 (Fig. 3.2.2a). The transient input pulse was characterized by a mean Reynolds number of 107, a Reynolds number range of $Re_{max} = 132$ to $Re_{min} = 64$, and a Womersley parameter of Wo = 3. Simulated velocity vectors and contours of magnitude at the minimum flow rate illustrate the significant vortical flow features within this system (Fig. 3.2.2b). Due to the conditions of both *in vitro* flow systems, platelet and monocyte deposition models will be assessed under the assumptions of pre-activated blood particles, reactive surfaces, and in the absence of red blood cell induced particle dispersion.

3.2.3 Results

Platelet Adhesion in a Stagnation Flow Geometry

The normalized platelet density across the circular stagnation plate per unit area, as measured by Affeld et al. (1995), was found to qualitatively match simulated wall shear stress magnitudes through $r/R \approx 0.6$ (Fig. 3.2.3). For all data points reported through $r/R = 1.5$, a moderate quantitative correlation resulted, i.e., $r^2 = 0.25$ and $p = 3.4 \times 10^{-4}$ (JMP 4 statistical software, SAS Institute, Cary, NC). Considering that red blood cells were absent and that platelets were pre-activated, this result might imply an increased surface reaction rate with higher shear stress; however, the thrombogenic surface of interest was uncoated glass.

The multicomponent mixture model was found to be an effective approach for simulating particle concentration away from reactive walls. Briefly, assuming platelets to be a dilute chemical species (Stokes number St = $\rho_p d_p^2 U/(18\mu L)$ << 1 and mass concentration $c = m_p/m$ << 1) the multicomponent model for platelet transport by convection and diffusion assuming a constant mixture density may be written

$$\frac{\partial c}{\partial t} + (\vec{u} \cdot \nabla)c - \nabla \cdot (D \nabla c) = 0 \qquad (3.2.1a)$$

where c is the ratio of local particle to mixture mass. In the absence of red blood cells, platelet diffusion is governed by Brownian motion and can be represented using the Stokes-Einstein equation, resulting in an effective diffusion coefficient $D = 1.25 \times 10^{-9}$ cm^2/s for a platelet radius of $a_p = 1.5$ μm. The inlet boundary condition for the multicomponent mixture model was a uniform platelet concentration, c_∞, such that the local mass-flux profile matches that of the inlet velocity. Multicomponent deposition flux models implement a surface reaction rate, k_t, derived from experimental studies of initial platelet deposition (Turitto et al., 1980). It is generally assumed that platelet attachment occurs due to diffusion only. The boundary condition for a reactive surface is formulated by equating mass diffusion at the wall with the adhesive flux

$$D \left. \frac{\partial c}{\partial n} \right|_{wall} = k_t c_{wall} \qquad (3.2.1b)$$

where n is the surface normal coordinate. Hence, impact driven deposition, which may occur due to finite particle inertia and size in stagnation or recirculating type flows is not accounted for by the typical deposition flux model for platelet transport (Turitto and Baumgartner, 1975). Therefore, this model results in a maximum wall-flux at the site of minimum measured deposition, i.e., the stagnation point location (Fig. 3.2.4). While the surface reaction rate was assumed to be $k_t = 3.5 \times 10^{-6}$ cm/s, modifications of this constant did not alter the overall characteristics of the result. Apparently, ignoring particle deposition driven by finite particle size may be inappropriate for stagnation type flow.

Assuming platelets to be spherical elements with a mean diameter of 3 μm that are transported by convection *without* near-wall hydrodynamic forces, and which deposit at the site of initial particle contact results in a significant correlation (Fig. 3.2.5; $r^2 = 0.35$ and $p <$

10^{-4}). For this simulation, initialization of 10,000 cells grouped near the centerline was required to effectively resolve the assumed Gaussian distribution of particle diameter (Corash et al., 1977) and resulted in a convergent solution. Interestingly, this efficient approach captures the site of maximum measured platelet deposition.

Including near-wall forces and calculating the NWRT, based on 10,000 particles with s = 2, resulted in a strong and statistically significant correlation (Fig. 3.2.6; $r^2 = 0.57$ and $p < 10^{-4}$). Qualitatively, this model performs well through one inlet pipe radius. Thereafter, the NWRT model indicates a correct trend of diminished deposition; however, the rates of reduction differ (Fig. 3.2.6a). The primary mechanisms by which the NWRT-values decrease beyond $r/R = 1$ were observed to be off-wall velocity components and near-wall particle lift. In this radially symmetric geometry, the mean flow velocity decreases linearly with distance r, such that the NWRT might be expected to increase past $r/R = 1$. However, it was observed in the near-wall region that viscous drag due to the presence of the wall increased the velocity difference between the particle and the surrounding fluid, i.e., the slip-velocity. In turn, the increased slip-velocity enhanced lift away from the wall in this high-shear environment, resulting in reduced NWRT-values for $r/R > 1$. However, the lift relation used here is for rigid spheres. It is widely observed that flexible and non-spherical cells, such as activated platelets, are influenced by much higher degrees of near-wall lift (Goldsmith and Turitto, 1986). Moreover, flow separation due to a sharp inlet might increase plasma off-wall velocity components past $r/R = 1$ resulting in reduced downstream particle-wall interactions.

Monocyte Deposition in a Stenotic Geometry

The cell deposition results of Hinds et al. (2001) are shown in Fig. 3.2.7a for 2.5 hours of model perfusion. Error bars indicate variance in the angular coordinate at a particular axial location. Interestingly, overall particle deposition shows a significant and direct correlation ($r^2 = 0.66$ and $p < 10^{-4}$) with the time-averaged wall shear stress magnitude (Fig. 3.2.7). Low shear stress has been shown to promote monocyte adhesion and to play a role in endothelial activation and expression of monocyte adhesive molecules. However, for this low shear stress environment, regions of lowest shear do not correlate with regions of highest deposition concentration *under transient flow conditions*. Therefore, other factors may play

predominant roles in the likelihood for particle attachment including the convection and possible diffusion of blood particles as well as the resulting near-wall particle concentration and residence times.

Sites of particle contact in the absence of near-wall forces indicate reasonable agreement with deposition data in the stenosis and expansion regions; however, no mechanism is available to account for deposition in the stenotic throat for this dilute flow system (Fig. 3.2.8a). The minimum extent of the recirculation zone (or reattachment point) was found to be approximately 1.04*d*, which nearly coincides with a maximum of particle deposition in the expansion region. To produce the expected local minimum in particle deposition at this location, near-wall forces should be included in the simulation of blood particle motion.

Convergent NWRT results based on 40,000 initial trajectories with a five-particle-diameter lift-off distance, i.e., $h = 5d_p$, indicate a strong and significant correlation (Fig. 3.2.9; $r^2 = 0.74$ and $p < 10^{-4}$). While a correct local minimum is identified at $x/d = 1.05$, the NWRT was not capable of capturing the peak in deposition midway through the throat (Fig. 3.2.9a). Hinds et al. (2001) speculated that a transient recirculation zone might be responsible for this peak in particle attachment. However, simulations using a highly refined mesh did not identify vortical flow patterns in the throat. The reason for this peak in deposition may be particle rolling from the $x/d < -1$ region, which is not included in the particle trajectory equation and hence in the NWRT model.

3.2.4 Discussion

The adhesion process by which platelets and monocytes attach to a vascular or synthetic surface is not fully understood. Furthermore, vascular systems of interest often consist of large flow domains with significant numbers of particles. For such systems, the nano-scale process of adhesion cannot be resolved due to computational limitations. Hence, the effectiveness of *models* for both platelet and monocyte attachment has been investigated in axisymmetric flow systems with components of flow stagnation, recirculation, and reattachment.

The results of this study are consistent with the overwhelming evidence in the literature that predicts platelet deposition to increase directly with wall shear stress; however, this

effect is widely attributed to red blood cell induced dispersion and mechanical platelet activation. Considering that the system of Affeld et al. (1995) lacked red blood cells and implemented pre-activated platelets, the direct correlation observed here indicates that surface reactivity rate may increase with shear stress, as suggested by Weiss (1995). However, shear stress alone does not account for the convection of platelets that is, by all accounts, important in diffusion limited deposition occurring in complex flow domains (Turritto et al., 1980). For monocytes, increased shear stress may result in greater monocyte deformations and/or more frequent penetration of microvilli through surface barriers (Rinker et al., 2001). Still, wall shear stress models alone do not address the availability of near-wall particles.

The salient feature of the multicomponent mixture approximation is that platelet concentration is modeled as a chemical species and is transported in the flow by convection and diffusion. In relatively parallel and low shear flow, this model correctly assumes that platelet deposition is due to diffusion. This approach also typically implements a constant surface reaction rate which accounts for activation and platelet binding affinity. However, as indicated by Wootton et al. (2001), the use of a constant surface reaction rate in regions of flow recirculation and reattachment results in poor model performance. Similarly and consistent with the results here, David et al. (2001) found that the multicomponent model was inappropriate for stagnation style flow when a constant reaction rate was implemented. The present study suggests that the multicomponent mixture approach is inappropriate for non-parallel flow systems because it ignores the hydrodynamic interaction of discrete particles with a boundary and neglects the potential for inertial driven impaction arising from finite particle size. While wall shear stress dependent surface reactivity, as implemented by David et al. (2001), may play a role in platelet deposition, the influence of finite particle size should not be overlooked in modeling a nano-scale process such as adhesion.

Due to ever-present computational constraints, approaches which model platelets and monocytes as discrete elements are limited to a relatively small number of representative particles. However, these models performed well with respect to identifying sites of likely particle adhesion in most instances observed. The assumption that particles adhere at initial surface contact provided an approximation that performed moderately well in all instances

except for the stenotic throat region, which was comprised of an un-physiological abrupt angle and tubular section. By comparison, the surface contact model has performed well in the application of Buchanan et al. (2002) where sites of likely particle deposition were identified in a realistic vascular configuration. Nevertheless, the inclusion of hemodynamic particle-surface interactions and the NWRT model resulted in improved quantitative correlations for particle adhesion, despite the assumption of spherical elements.

In a certain respect, the NWRT captures the deposition efficiency as discussed by Karino and Goldsmith (1979a & b). For example, a local area interacting with a large number of fast moving particles and an area interacting with a small number of slow moving particles may result in similar deposition counts. On a local volumetric basis, the NWRT takes into account the number of particles that are present as well as the time available for particle interaction with the local surface in calculating the probability for particle deposition. Furthermore, particles that are closer to the wall are given increased weight via the wall proximity scale factor.

The NWRT was devised, in part, to account for unknown variables such as exact vessel wall structure and nano-scale surface forces and biological reactions. For the case of platelets depositing on glass, the surface geometry can be resolved almost exactly. However, activated platelets form spiny spheroids with pseudopod extensions. These extensions can be up to several platelet diameters in length. Therefore, without a direct numerical simulation of the exact platelet shape and motion the fine scale details of platelet-wall interactions are masked. To avoid the exact simulation of platelet wall interactions, the NWRT concept allows for the simulation of platelet attachment on a probabilistic basis. That is, the closer a particle is to a wall and the longer it remains there, the higher the probability for deposition. A platelet that is two diameters away from the wall may have a pseudopod contacting the wall and, therefore, makes a scaled contribution to the local NWRT-value.

The scale factor s was set to unity for monocytes and factor of two for the simulation of platelet deposition. These factors were selected to match experimental results; however, an argument can be made that platelets adhere much faster than monocytes and are much more likely to firmly attach at initial contact. Hence, proximity between the surface and the cell becomes increasingly important compared to the time available for interaction. The

dependence of the model on the proximity factor can be increased by raising the exponent of the scale factor. The result is that the particle must be closer to the wall to register a significant NWRT-value.

Primary limitations of the implemented discrete element approach include the omission of particle rolling and the assumption that the near-wall flow field is unaffected by particle deposition and possible aggregation. Particle rolling is a likely mechanism by which monocytes might collect in the stenotic throat of the Hinds et al. (2001) configuration. In the stagnation system, a developing thrombus would provide a likely mechanism for lifting subsequent particle trajectories away from the surface possibly resulting in the observed void of deposited platelets in the downstream portion. Furthermore, initial particle conditions were found to significantly influence the discrete element models. In each case, it was assumed that inlet conditions were sufficient for fully developed velocity and particle concentration profiles to develop. The effect of red blood cell induced dispersion can most likely be simulated by the introduction of a shear dependent diffusion term (Zydney and Colton, 1988). Unfortunately, monocyte dispersion is currently not well understood for shear rates beyond 20 s^{-1}.

Of the models studied, the most effective with respect to the adhesion of platelets and monocytes in non-parallel flow domains accounted for finite particle size and particle inertia. In particular, the NWRT approach was found to be useful as well as necessary given that 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 fully be included in simulations involving relatively large-scale geometries. Still, surface reactivity (e.g., up-regulation of adhesive molecules or exposure of subendothelial collagen) remains a major determinate of where particles deposit and a major hurdle for effectively modeling adhesion in realistic systems.

3.3 An Extended NWRT-Based Model for Cell-Wall Interactions

The *in vitro* experimental results implemented in the previous section for the validation of the NWRT parameter differ from realistic conditions with respect to several key aspects. For instance, the experimental model of Hinds et al. (2001) was set in a low shear environment, e.g., $\tau_{max} \approx 1.4$ dyne/cm^2, and the walls were uniformly reactive. Similarly, the perfusion system of Affeld et al. (1995) implemented pre-activated platelets and an artificial thrombogenic surface. Furthermore, in both *in vitro* experiments and subsequent NWRT simulations, red blood cells were absent such that collisional dispersion was not introduced.

To address the above issues, models for blood particle deposition in realistic large-scale branching blood vessels that incorporate surface reactivity as well as platelet activation within the NWRT framework are proposed, as outlined in Fig. 3.3.1. Clearly, monocytes and platelets are characterized by different momentum response times and adhere via different biochemical mechanisms. Hence, separate models are required in an effort to accurately predict near-wall blood particle motion and to approximate the potential for blood particle attachment. Components of these models are discussed below. The models are then tested in the rabbit aorto-celiac junction and the human carotid artery bifurcation. Finally, results of the NWRT-based models are compared to recent *in vivo* observations in animal models of the distal femoral anastomosis.

3.3.1 A NWRT-Based Model for Monocyte Adhesion

The dispersional effect of red blood cells on monocyte trajectories in a variable shear field is simulated using a discrete perturbation method. Assuming blood particle dispersion to be a Gaussian process, i.e., as it would occur in a constant concentration shear field, the mean lateral displacement is given by

$$\langle \Delta x_i \rangle = \sqrt{2 D_p \, \Delta t} \tag{3.3.1}$$

where Δt is a time-step much greater than the particle momentum response time, $\tau_p = \rho_p d_p^2 / 18\eta$. Discrete particle motions have been simulated by interpreting the standard deviation of a Gaussian probability function to be the square of the above relation. A random

walk method is then employed where displacements are generated and superimposed on the local particle motion, i.e., a Monte Carlo type approach. For monocytes, a constant dispersion coefficient for a shear rate of 20 s^{-1} has been selected due to a lack of a better estimate (Goldsmith and Karino, 1977), i.e.

$$D_p = 1.5 \times 10^{-7}\ cm^2 / s \qquad (3.3.2)$$

Unfortunately, estimates for monocyte dispersion variability were not available in higher shear environments (cf. Appendix B). While the applied dispersional influence of red blood cell mixing is incrementally small, it may play a significant role in the near-wall environment.

Surface reactivity is generally considered a significant component of blood particle attachment. With regard to monocytes, conditions such as secondary flow, low WSS, and high WSS have been associated with endothelial cell expression of adhesive molecules such as VCAM-1 (Gimbrone et al., 1997; Malinauskas et al., 1998; Truskey et al., 1999). Considering firm monocyte attachment, regions of low WSS indicate enhanced surface reactivity as well as a reduced likelihood of significant particle rolling and re-suspension. Therefore, monocyte deposition is suspected in regions where local near-wall residence time is high and wall shear stress is below a critical 'threshold' (Chang and Hammer, 1999). To encapsulate both of these factors in a composite model, contributions to the local NWRT-value will only be accepted in regions where the time-averaged WSS is below a critical value, i.e., a WSS-limiter model (Fig. 3.3.1). In the absence of a better estimate, the limiting value of WSS will be selected as the magnitude of the time-averaged wall shear stress for the artery of interest.

3.3.2 A NWRT-Based Model for Platelet-Wall Interactions

A direct correlation between platelet adhesion and shear rate has been observed *in vitro* for deposition on subendothelium (e.g., Turitto and Baumgartner, 1975) and collagen-coated surfaces (e.g., Sixma and de Groot, 1994). This relation has been found to hold at shear rates that are very high by *in vivo* standards, e.g., 20,000 s^{-1}. It is suspected that the rate of platelet adhesion and aggregation to collagen and subendothelial tissue increases in regions of elevated shear due to (a) increased platelet dispersion arising from enhanced red

blood cell mixing; and (b) shear stress modulated platelet activation (Wootton and Ku, 1999). Regarding an intact vascular surface, endothelial cells regulate platelet adhesion by the production of thrombogenic and anti-thrombogenic compounds (Pearson, 1994). Hence, platelet dispersion and activation, as well as surface reactivity, have been addressed in a model for platelet-wall interactions.

Effective Dispersion

As discussed in Appendix B, the solute diffusivity expression of Aarts et al. (1986), which is based on deposition studies, has been selected to quantify platelet dispersion

$$D_p \approx 1.05\times10^{-9}\,\dot{\gamma}^{0.297+1.29\bar{h}-0.90\bar{h}^2} \tag{3.3.3}$$

where $\dot{\gamma}$ is the local strain rate and $\bar{h}$ is the mean hematocrit, assumed to be 40% (D_p is in cm^2/s).

Platelet Activation

Hellums (1994) demonstrated *in vitro* that the steady threshold shear stress required for activation, τ_{active}, decreases asymptotically with time, *t*, as (cf. Fig. 1.6.1)

$$\tau_{active}(t) = \frac{c}{\sqrt{t}} \tag{3.3.4}$$

where *c* is a constant. To establish platelet activation, Boreda et al. (1995) defined *c* in the above relation to be the platelet stimulation function, *PSF*, and fit the data compiled by Hellums (1994) to the expression

$$PSF \approx \tau\sqrt{t} \tag{3.3.5}$$

The requirement for mechanical activation, in the absence of a chemical agonist, is then PSF $\geq$ 1000.

Because the above relations are based on *in vitro* observations, application to realistic systems may not be appropriate. As discussed in Section 1.6.1, the threshold curve for platelet activation shifts dramatically to lower exposure times and lower stress levels in response to platelet agonists, thrombogenic surfaces, and in the presence of red blood cells (Goldsmith et al., 1994). Furthermore, it has been found that mechanical platelet activation

is also a function of shear stress exposure rate (Holme et al., 1997) such that the above relations for platelet activation may not be directly applicable to time-varying shear fields. Nevertheless, Boreda (1995) approximated platelet activation within a stenotic geometry by applying Eq. (3.3.5) in the form

$$PSF = \tau(\Delta t)^{0.452} \tag{3.3.6}$$

where Δt represents the observation time-step. The resulting PSF-value is largely dependent on the observation time-step size selected such that this relation is inappropriate. Furthermore, a cumulative *PSF* cannot be calculated due to the power-law format. For example, a constant exposure shear stress of $\tau = 5$ dyne/cm^2 sampled in 0.25 and 0.5 second intervals for a total period of 1 second results in cumulative *PSF*-values of 10.7 and 7.3, respectively. An appropriate relation for platelet activation should be independent of sample time-step size for a constant shear-exposure period.

Alternatively, Eq. (3.3.5) might be applied to a variable shear environment by considering the time-averaged local shear stress exposure, $\bar{\tau}$, and the total platelet residence time, *t*, i.e.,

$$PSF \approx \bar{\tau}\sqrt{t} \tag{3.3.7}$$

While mathematically sound, the correct period of time that τ should be averaged over is not known. Platelets are capable of activation in less than 0.1 s (Richardson, 1973 and 1981), and, yet may require greater than 300 seconds (Hellums, 1994) of low shear stress exposure for a significant response to occur. Selecting a large time-scale for the computation of $\bar{\tau}$ does not properly account for significant but brief shear stress changes in a transient shear field. Similarly, a small temporal region of interest ignores the cumulative shear stress exposure.

In this study, it has been assumed that shear stress exposure sensitizes platelets to many chemical agonists (Goldsmith et al., 1994) and that a spectrum of platelet activation may occur (Hellums, 1994). Mechanical factors that affect platelet activation include the level of shear stress a platelet is subjected to and the exposure time (Hellums, 1994), as well as the rate of shear stress application (Holme et al., 1997). Considering only the former two factors in the context of a time-dependent shear field, a platelet stimulation history (PSH) may be expressed as

$$PSH = \frac{1}{\tau_{mean} \cdot T} \int_{t_{in}}^{t} \tau(t) \cdot dt \qquad (3.3.8)$$

where t_{in} and t are the initial and current times, and $\tau(t)$ is the local shear stress exposure for an individual platelet. The mean wall shear stress (τ_{mean}) of the vessel and the input waveform time period (T) are included to non-dimensionalize the PSH-parameter. This representation captures the cumulative effects of shear stress exposure as well as temporally local conditions without the artificial residence-time bias associated with temporal averaging. For numerical applications, it will be assumed that all particles have an initial *PSH* value of zero. Particles will be initialized on the same plane such that cumulative PSH-values will be consistent within a single geometry. Moreover, particles will be initialized in similar geometries at equal distances from regions of interest such that comparisons will be possible. As indicated in Fig. 3.3.1, contributions to the local NWRT-parameter will be scaled by the *PSH* value of the particle of interest. In this manner, an approximate measure of mechanically induced platelet activation has been included in formulating the NWRT-based model for platelet-wall interactions.

Surface Reactivity Considerations

As discussed in Section 1.4, activated platelets can release chemotactic and mitogenic growth factors such as platelet derived growth factor (PDGF) and vascular constrictive agents such as thromboxane A2 (TxA_2). These compounds contribute to generalized intimal thickening (IT) by promoting smooth muscle cell migration and proliferation (Liu, 1999). Permanent adhesion of platelets to the vascular surface may not be necessary for significant promotion of IT to occur. Alternatively, mural thrombi resulting from significant platelet adhesion and aggregation may evolve into active atherosclerotic plaque-like lesions (Leu et al., 1988; Sloop, 1999), particularly on synthetic surfaces (Sloope et al., 2002).

Where present, endothelial cells are capable of synthesizing and expressing pro-coagulate and anti-coagulate proteins, which regulate the adhesion of platelets to the vascular surface. Endothelial cell derived anti-thrombogenic compounds include prostacyclin (PGI_2), nitric oxide (NO), and tissue factor pathway inhibitor (TFPI) (Pearson, 1994). In addition to preventing platelet adhesion, NO is a vaso-relaxant, which strongly inhibits SMC

proliferation. Endothelial cells produce TFPI to counteract thrombogenic TF production, which occurs in areas of activation (Grabowski et al., 2001). A direct relationship has been identified between local endothelial cell WSS exposure and the expression of anti-thrombogenic compounds such as PGI_2, NO, and TFPI (Grabowski et al., 1985; Harrison et al., 1996; Westmuckett et al., 2000; Grabowski et al., 2001). *Therefore, the potential for platelet adhesion to an intact endothelial lining is mitigated in regions of high WSS and promoted in regions of low WSS*.

Synthetic surfaces such as PTFE, which often lack an endothelial coating, generally absorb thrombogenic plasma proteins such as fibrinogen and von Willebrand factor very rapidly (Colman, 1993). Adhesion of a platelet monolayer and limited aggregation then ensue. Watase et al. (1992) implemented PTFE conduit in an animal model and observed consumption of the early thrombus formations by leukocytes and macrophages. Fibroblasts[1] were then found to occur, followed by growth of endothelial-like cells, appearance of SMCs, and proliferation of fibroblasts producing collagen fibrils. However, arterial shear stress levels were found to interrupt the development of a neointima. Sottiurai et al. (1983) observed the structure of late-stage neointima in PTFE femoral bypass conduits excised from humans. It was reported that an inorganized layer of fibrin, erythrocytes, leukocytes, and thrombi formed a heterogeneous pseudo-intima. A developed neointima containing fibroblasts occurred only adjacent to the anastomosis.

It is concluded that the luminal surface of a PTFE vascular conduit in the arterial system is primarily covered in fibrin, collagen, and subendothelial tissue prior to significant IT development. In contrast to an intact endothelium, platelet adhesion to collagen and subendothelial tissue has been shown to correlate directly with WSS (Wootton and Ku, 1999). As discussed (Section 1.5.1), this direct relationship is predominantly due to shear dependent platelet dispersion and platelet activation. However, shear dependent surface reactivity has also been proposed for platelet adhesion to subendothelial tissue (Weiss, 1995) and other non-endothelial cells. For instance, thrombogenic TF expression by monolayers of human fibroblasts has been shown to increase directly with shear stress (Grabowski et al., 1993).

[1] Fibroblasts are undifferentiated cells in connective tissue that give rise to cells that are the precursors of bone, *collagen*, and other connective tissues. Fibroblasts are able to produce extracellular matrix components including *collagen*, elastin and *glycoproteins*.

Surface Reactivity Model

Due to endothelial cell production of anti-thrombogenic compounds, platelet interactions with the vascular surface are mitigated in regions of high wall shear stress and promoted in regions of low WSS. It is hypothesized that regions of low and high WSS are relatively proportional to the mean WSS of the vessel of interests. A surface reactivity (*SR*) factor to quantify the local significance of platelet interactions with an intact endothelium and based on the potential for adhesion in the presence of coagulate and anti-coagulate proteins, may then be written as:

$$SR = \frac{\tau_{mean}}{\tau} \qquad (3.3.9a)$$

Considering the heterogeneous luminal surface of an arterial PTFE conduit, it is assumed that the expected increase in platelet adhesion with shear stress is due exclusively to enhanced dispersion and mechanical platelet activation. These factors are simulated in the model by the inclusion of an effective dispersion coefficient and the PSH. Assuming the heterogeneous PTFE surface to be uniformly reactive results in a constant surface reactivity condition, i.e.,

$$SR = constant \qquad (3.3.9b)$$

It is expected that platelet adhesion to the heterogeneous PTFE lumen surface will be significantly more likely than to an intact, but possibly activated, endothelial layer, as could be indicated by a constant SR > 1. However, reaction rates for platelet adhesion to *in vivo* PTFE versus endothelium are currently not available, and most likely change significantly over time, such that the graft surface reaction constant will initially be assigned

$$SR = 1.0 \qquad (3.3.9c)$$

As indicated in Fig. 3.3.1, the surface reactivity factor is implemented to scale the local value of the NWRT-parameter in an effort to account for endothelial expression of thrombogenic and anti-thrombogenic compounds. Where necessary, *SR*-factors can also be used to approximate differences in platelet affinity associated with various vessel wall components. The composite NWRT-based model for platelets accounts for red blood cell induced particle dispersion and a spectrum of mechanical activation on an individual platelet basis. This model is intended to capture the likelihood of significant platelet-wall

interactions where platelet derived growth factors may signal intimal hyperplasia development and/or mural thrombi may rapidly develop into active atherosclerotic plaque-like formations. The latter mechanism of plaque generation is a likely scenario on an *in vivo* PTFE surface where the initial neointima is poorly organized.

3.4 Identifying Sites Susceptible to Early Lesion Growth in the Rabbit Aorto-Celiac

3.4.1 Overview

To elucidate the mechanisms by which particle-hemodynamics influence arterial lesion initialization and progression, the rabbit aorto-celiac junction has been selected as an atherosclerotic model. For this geometry, detailed quantitative results are available regarding sub-endothelial macrophage accumulation (Malinauskas et al., 1995), LDL permeability (Barakat et al, 1997; Malinauskas, 1993), and lesion initialization (Barnes et al, 1999; Ivey et al., 1995; Zeindler et al, 1989). The hemodynamic characteristics of the rabbit aorto-celiac junction are also well established. Barakat *et al.* (1997) investigated the fluid mechanics of the rabbit abdominal aorta under steady conditions using an *in vitro* flow model. Malinauskas *et al.* (1998) visualized steady and transient flow patterns in the rabbit aorto-celiac junction and suggested a predominant role for secondary velocity in the localization of intimal monocytes and the initialization of arterial lesions. Buchanan *et al.* (1999) and Buchanan (2000) simulated steady and transient flows within models of the rabbit aorto-celiac and abdominal aorta and calculated WSS-based hemodynamic wall parameters, as well as fluid element pathlines, particle trajectories, and potential particle deposition sites based on a particle contact model.

3.4.2 System

The geometry used in this study is based on measurements of rabbit aortic casts made by Malinauskas (1993). Buchanan (2000) transformed these measurements into a geometric surface model (Fig. 3.4.1) and a validated computational mesh, which resulted in grid independent velocity and shear stress fields. The average angle between the celiac and distal aorta reported by Malinauskas (1993) was 88^{o} with a 4^{o} out-of-plane incline, which has been reproduced in the model. The proximal and distal aorta diameters are 0.48 cm and 0.457 cm, and the celiac diameter is 0.276 cm. The transient input pulse shown in Fig. 3.4.1 is based on the rabbit thoracic flow rate waveforms of Avolio *et al*. (1976) with a systolic peak Reynolds number of 860, a Reynolds number of -90 in the diastolic trough and a secondary peak Reynolds number of 145. The mean Reynolds number for the pulse is 224 and the

Womersley parameter is 5.32. A constant flow rate division ratio of 70:30 was assumed for the distal artery and celiac branch outlets, respectively.

3.4.3 Results

An overview of the transient vortical flow characteristics occurring in the aorto-celiac junction can be visualized using slices of the velocity field as well as particle trajectories released randomly over an initial pulse, as show in Figs. 3.4.2a & b. Due to the extremely small dispersion constant applied, collisional effects are not visible in the particle trajectories. Further details of the transient hemodynamic patterns within this system have been described at length based on both *in vitro* (Malinauskas et al., 1998) and computational (Buchanan et al., 1999; Buchanan, 2000) results. The corresponding hemodynamic wall parameters including the new NWRT-based model are now presented and compared to *in vivo* monocyte deposition and lesion formation data.

WSS-Based Hemodynamic Parameters

Wall shear stress based hemodynamic wall parameters, which are intended to capture the aggravating effects of the flow field on the endothelium including potential adhesive molecule expression, are presented in Fig. 3.4.3. For other flow systems, such as the carotid artery bifurcation, regions of low WSS in conjunction with regions of elevated oscillatory flow, as indicated by the OSI, have been identified with intimal thickening (Ku et al., 1985). Low time-averaged WSS and high OSI are observed on the dorsal aortic wall directly opposite the celiac branch with a critical contour extending part way up the lateral wall toward the distal quadrant of the flow divider. Interestingly, a majority of the flow divider, including the distal region, is characterized by high WSS and low OSI. Strong spatial changes in mean WSS-vector magnitude and direction, as indicated by the WSSG and WSSAG, respectively, are observed on the distal portion of the flow divider. Reduced contours of both the WSSG and WSSAG extend around the junction in a narrow band.

NWRT-Based Models

Results of the NWRT model for the rabbit aorto-celiac geometry, which indicate the probability for particle deposition based on local residence time and concentration, are given in Fig. 3.4.4. Without a limiting shear stress condition for the deposition of monocytes, the entire distal portion of the flow divider displays elevated NWRT contours. As discussed, the likelihood for monocyte deposition is assumed to drop dramatically for regions of locally elevated mean wall shear stress. For all WSS-limiter values considered, composite NWRT contours imply significant monocyte deposition on the lateral flow divider wall (Fig. 3.4.4). Allowing for higher threshold values of WSS results in more elevated NWRT contours extending distally from the lateral flow divider region. It appears that elevated WSS may be a mechanism which reduces monocyte deposition along the distal most portion of the flow divider allowing for a peak in NWRT along the lateral flow divider wall.

Monocyte deposition and lesion formation data are often presented in a flattened transverse *en face* view, as with the upper panels of Fig. 3.4.5. In this representation, data is presented as a flattened surface viewed from above in the context of Fig. 3.4.1. A composite rabbit aorto-celiac monocyte deposition pattern compiled from Malinauskas *et al*. (1995) and lesion formation in hypercholesterolemic young rabbits from Barnes & Weinberg (1999) have been selected for qualitative comparisons to NWRT results. The reported monocyte percentages (Fig. 3.4.5a) are averaged values based on data (Malinauskas et al., 1995 & 1998) taken from the four animals that were used to construct the computational aorto-celiac geometry (Malinauskas, 1993). The gridlines in Fig. 3.4.5a form the 0.5 mm $\times$ 0.5 mm sites sampled by Malinauskas *et al.* (1995). Figures 3.4.5c-f indicate corresponding results of the NWRT monocyte models, i.e., transverse *en face* views of Fig. 3.4.4. The WSS-limiter model (Fig. 3.4.5d-f) indicates a pattern that is very similar to the averaged monocyte deposition data in the upper half of Fig. 3.4.5a, with a NWRT peak on the lateral flow divider wall and near zero values in the distal flow divider region. Of the WSS-limiter values considered, it appears that the higher condition, τ_{limit} = 19. dyne/cm^2 (Fig. 3.4.5d), best captures the continually elevated monocyte deposition contours that extend downstream along the lateral wall. In the lower half of Fig. 3.4.5a, a similar pattern is observed; however, the deposition magnitude is considerably less, most likely due to significant out-of-plane

celiac and aorta curvature as well as helical flow introduced by the aortic arch. Regarding lesion growth, sites of initialization are well captured with peaks in the NWRT contours, particularly for τ_{limit} = 19. dyne/cm^2 (Fig. 3.4.5d). It is expected that the full 'Anitschkow pattern', evident in Fig. 3.4.5b, is a result of continued lesion formation and/or the product of other atherogenic entities.

A significant quantitative correlation was established between the monocyte deposition data presented in the upper half of Fig. 3.4.5a and the comparable NWRT result including a WSS-limiter condition of τ_{limit} = 19. dyne/cm^2 (Fig. 3.4.6a; $r^2 = 0.77$ and $p < 10^{-4}$). In the absence of the WSS-limiter, a much weaker correlation resulted (Fig. 3.4.6b; $r^2 = 0.25$ and $p = 0.12$). To establish these correlations, available data was extracted from Fig. 3.4.5a and compared to consistently localized (0.5 mm $\times$ 0.5 mm) NWRT results. In this manner, artifacts introduced by area averaging were considerably minimized.

Similar to the NWRT parameter, locations of initial particle-wall contact in the absence of near-wall forces have been used to identify sites of potential monocyte adhesion. For the calculation of a deposition fraction parameter, χ, sites of surface contact were area-averaged on a local basis and nondimensionalized by the number of occurences.[5] Simulated particles were removed from the flow field upon initial surface contact. A linear comparison of surface contact and NWRT results, computed on a local 0.5 $\times$ 0.5 mm basis and including a WSS-limiter condition of τ_{limit} = 19. dyne/cm^2, indicates a direct relationship (Fig. 3.4.7; $r^2 = 0.25$ and $p < 0.05$) between the two particle-based parameters. In contrast to the surface contact model, the NWRT parameter provides more detailed information regarding particle-wall interactions such that a stronger localized correlation with observed sites of monocyte deposition was identified.

3.4.4 Discussion

Although the complex multifaceted mechanisms responsible for atherosclerotic lesion formation and general intimal thickening are not well understood, it is now widely accepted that abnormal hemodynamic influences play a significant role. Hemodynamic factors such as relatively large gradients in wall shear stress vector magnitude and direction have been

associated with endothelial cell up-regulation of adhesive molecules, secretion of mitogenic and thrombolytic agents, replication, orientation, and shape (Kleinstreuer et al., 2001). It has been established that the accumulation of monocytes and macrophages within the arterial wall is one of the earliest events in lesion formation (Ross, 1993), with a high spatial correlation in rabbits (Malinauskas et al., 1995). Mitogenic factors released by adherent monocytes and macrophages contribute to smooth muscle cell migration and proliferation resulting in general intimal thickening (Ross, 1993). Malinauskas *et al*. (1998) suggested that 'secondary velocities' serve as a generalized mechanism for transporting monocytes and other atherosclerotic entities to the vascular surface, controlling local residence time, and influencing the expression of local adhesive molecules. Attached or circulating platelets may also play a major role in lesion growth by stimulating smooth muscle cell migration and proliferation (Davies, 1994). Hence, complex hemodynamics, often labeled 'disturbed flow', may influence the atherogenic process by stimulating or injuring the endothelium, transporting critical blood elements to the vascular surface, and regulating the time available for physical and biological interactions.

Regarding early lesion formation in rabbits fed a high cholesterol diet, Zeindler *et al.* (1989) reported that early lesion development occurs initially lateral to the junction orifice and then continues to grow downstream. Other researchers have also reported similar results (Barnes et al., 1999; Ivey et al., 1995). Complementing these observations, Malinauskas *et al.* (1995) showed that monocytes and macrophages are preferentially located within the arterial intima about the lateral flow divider walls in the rabbit model. Furthermore, early lesion development has not typically been observed on the dorsal wall of the artery opposite the junction orifice. The earliest stages of lateral lesion growth typically occurred just distal to the tip of the crescent-shaped fold that forms the flow divider and progresses distally (Zeindler et al., 1989). However, the flow divider itself is often spared lesion formation (Zeindler et al., 1989).

For an in-plane aorto-celiac geometry similar to the model studied here, Buchanan *et al.* (1999) established significant positive correlations between the WSSG and intimal monocyte data on a segmental-averaged basis. Furthermore, Buchanan (2000) estimated a similar segmental-averaged correlation for the WSSAG hemodynamic wall parameter.

Individually, the high OSI and low WSS regions did not correlate with intimal macrophages, LDL permeable sites, and lesion growth data.

The segmental-averaged correlations of Buchanan *et al.* (1999) and Buchanan (2000) are based on large areas, e.g., approximately 1 cm wide cross-sections, such that detailed spatial relationships between local hemodynamic wall parameters and atherosclerotic entities are masked. For example, the distal region of the divider is generally devoid of intimal macrophages in the initial stages of lesion formation; however, WSSG and WSSAG contours express maximum values at this location. In reference to this observation, Buchanan *et al.* (1999) acknowledged that regions of highest LDL permeability and sites most susceptible to monocyte deposition are associated with elevated WSSG and WSSAG; however, the relationship is not exclusive. That is, peak values of the hemodynamic wall parameters and regions of highest atherosclerotic entity occurrence do not necessarily coincide on a local basis. Moreover, peak hemodynamic wall parameters may occur in regions devoid of the precursors for lesion formation and lesion development.

Observations of lesion formation *in vivo* report that the site of lesion origin is just distal to the tip of the crescent fold that forms the flow divider, i.e., the distal portion of the flow divider lateral wall (Zeindler et al., 1989). This region is characterized by low WSS and moderately high OSI (Fig. 3.4.3). However, the dorsal aorta opposite the celiac is a region of minimum WSS and maximum OSI, yet no atherosclerotic entities are reported in this area. Clearly, for the rabbit aorto-celiac geometry, regions of low WSS and high OSI alone cannot be used to identify areas susceptible to monocyte deposition and subsequent lesion formation. Indeed, the determination of near-wall particle interaction and the potential for particle deposition requires simulating the convection and diffusion of individual blood particles as well as modeling surface and particle reactivity.

The NWRT model with a WSS-limiter is intended to capture regions of elevated near-wall monocyte concentration and residence time in conjunction with surface reactivity. Assuming a critical value of wall shear stress, above which monocytes cannot deposit, the composite NWRT-WSS model was found to indicate monocyte deposition and lesion initialization in local agreement with *in vivo* data.

In this study, quantitative correlations between NWRT results and observed monocyte deposition locations were established on a highly focal basis. Considering that the sample area size was 0.5 mm $\times$ 0.5 mm, very close agreement is required to generate a significant correlation. Nevertheless, a statistically significant relationship was established between the NWRT-WSS model and selected deposition data from Malinauskas *et al.* (1995). Due to variability among *in vivo* cases as well as geometric and numerical assumptions, the reported correlation is intended to only corroborate the observed qualitative results for a specific dataset comparison.

Techniques that calculate the formation and breakage of molecular bonds require precise knowledge of the distance between biologically active elements, e.g., surface bound receptors and monocyte ligands (Chang and Hammer, 1999). Specifically, difficulty arises in modeling only the time during which the cell is in transient contact with the surface, and not including the time when the cell is too far from the surface for bond formation to be possible (Zhu, 2000). The size of the binding pocket for monocyte adhesion is on the order of 10 nm, which is an order of magnitude less than the vessel surface roughness height. To accurately implement an adhesive dynamics model, a mesh capable of resolving the vessel surface roughness height would be required. Clearly, this is not presently feasible for the simulation of blood particles in complex three-dimensional vessel geometries.

The NWRT concept simplifies the complex biophysical process of particle deposition by modeling local particle residence time in the absence of an active endothelium. Sites of highest near-wall particle concentration and residence time, in conjunction with local wall shear stress below a critical value, are hypothesized to be where both deposition and monocyte transmigration are most likely. The assumptions of spherical particles and smooth walls eliminate the possibility for particle-to-wall contact in Stokes flow. Hence, particle rolling does not occur in this numerical model. Obviously, no attempt has been made to model monocyte movement within the intima. Nevertheless, agreement with monocyte deposition data and the reported site of lesion initialization is very reasonable. Intimal monocyte transport, LDL uptake, endothelial cell activation and dysfunction, as well as platelet attachment may all influence the arterial lesion pattern during and after initialization.

The limiting wall shear stress values were selected to generally approximate the effects of adhesive molecule expression, monocyte rolling, and monocyte lift-off in the NWRT-based model. Numerical estimates for this parameter were based on time-averaged WSS-means of the junction vessels, in the absence of definite experimental results. Implementing the celiac mean (τ_{limit} = 19. dyne/cm^2) as the WSS-limiter condition resulted in the strongest agreement with averaged deposition data. Due to large WSS-gradients in the junction region, it was found that results of the composite model were relatively insensitive to the WSS-limiter condition selected. Still, the inclusion of this parameter was necessary to reflect the importance of low-wall-shear-stress in the onset of atherogenesis, and hence to establish a significant quantitative correlation for intimal monocyte deposition as well as qualitative agreement with the site of reported lesion initialization. For example, the NWRT model with a WSS-limiter indicated that the distal center of the flow divider would be devoid of lesion formation, as observed *in vivo*.

In addition to the significant lateral and distal lesion formations observed for hypercholesterolemic young rabbits (Fig. 3.4.5b), Barnes and Weinberg (1999) reported significant lesion formation proximal to the celiac orifice of old rabbits. It is expected that this shift in lesion formation pattern is due to altered geometric, hemodynamic, and wall tissue conditions, which may influence particle-wall interactions and LDL permeability throughout the aorta. A potential increase in curvature of the aorta may significantly influence hemodynamic interactions along the ventral wall. Buchanan (2000) indicated that anatomic conditions upstream of the celiac induce significant helical flows that increase critical blood particle interactions with the vascular surface. A gradual loss of compliance within the aortic arch could alter helical flow patterns that continue down the aorta potentially increasing monocyte adhesion proximal to the celiac. Furthermore, permeability of the arterial wall as well as transmural flow may change over time, potentially resulting in variations in lesion occurrence. Hence, aortic model features such as proximal anatomic character, wall compliance, and transmural flow are most likely required in order to appropriately simulate changes in lesion formation associated with age. Furthermore, increased flow rate division ratios, input pulse severity, and elevated mean Reynolds number

are all expected to significantly increase secondary flow features, which directly relate to the transport of blood particles.

Considering the near-wall environment, minor wall motion may significantly influence particle-wall interactions. Furthermore, lesion growth and particle attachment may alter near-wall hemodynamics. For instance, King and Hammer (2001) implemented an Adhesive Dynamics approach in conjunction with micron-scale experiments to show that inter-particle hydrodynamics significantly influence particle rolling characteristics as well as the near-wall flow field. Using the NWRT concept to identify regions of significant particle wall interaction, as a result of luminal transport, in conjunction with promising Adhesive Dynamics techniques may provide a feasible approach to more accurately evaluate sites of likely monocyte deposition as well as lesion formation within computationally large and complex vessel configurations.

In conclusion, a model for the likelihood of monocyte deposition based on hemodynamic transport, near-wall particle concentration, and residence time has been devised and applied to the rabbit aorto-celiac system. The convection and diffusion of monocytes in a NWRT-formulation was found to identify specific sites of lesion initialization, whereas a low WSS and high OSI condition proved indiscriminate. The agreements with monocyte deposition measurements and sites of lesion initialization suggest that the NWRT-based model with a WSS-limiter condition is sufficiently detailed, yet simple enough for application in complex branching blood vessels such as the rabbit aorto-celiac junction. Quantitative data regarding lesion growth history as well as refined correlations for adhesive molecule expression, endothelial cell activation, monocyte wall interaction, red blood cell induced dispersion, and platelet activation will allow future studies to better relate hemodynamic characteristics to sites of potential lesion formations.

3.5 Identifying Sites of Intimal Thickening in the Human Carotid Artery Bifurcation

3.5.1 Overview

The carotid artery bifurcation (CAB) was selected as a test scenario to evaluate a qualitative correlation between the NWRT-based models and intimal thickening. Zarins et al. (1983) established typical, i.e., statistically averaged, intimal thickening patterns in human specimens without symptoms of significant vascular disease, i.e., early lesion formation. Early intimal thickening (IT) was reported to be most significant along the outer wall of the internal branch, particularly in the sinus region, cf. Fig. 3.5.1. Ku et al. (1985) made laser Doppler velocimetry measurements of pulsatile flow in a model of the human carotid bifurcation. By comparison to the measurements of Zarins et al. (1983), it was determined that IT correlated with the reciprocal of the mean shear stress ($r^2 = 0.67$, $p < 0.001$) and the OSI ($r^2 = 0.67$, $p < 0.001$). These correlations were based on data reported at selected axial cross-sections including a common carotid artery (CCA) far proximal region, three internal carotid artery (ICA) locations, and one distal external carotid artery (ECA) position. Intimal thickening data was not available for the CCA just proximal to the flow divider and the outer ECA near the flow divider such that comparisons to WSS and OSI were not made in these regions. Other studies of the carotid luminal surface indicate that early lesions tend to develop on the flow divider side of the CCA (i.e., side-wall near the origin of the flow divider) and opposite the flow divider in the sinus region of the ICA (Grottum et al., 1983).

Masawa (1994a) observed that early IT followed a helical pattern beginning in the CCA on the outer wall opposite the sinus region. The extent of maximum intimal thickening (MIT) then shifted to the side wall of the CCA in the region of the flow divider, as depicted by the red circumferential bands in Fig. 3.5.1. (It is noted that the red bands indicate only the circumferential extend of maximum intimal thickening, and do not represent luminal encroachment.) Continuing along a helical path, MIT was observed in a narrow region of the carotid sinus just distal to the flow divider. MIT then occupied an increasing region approximately centered along the outer wall of the internal branch. Little IT was observed in the far distal ICA. For corresponding cross-sectional regions, Masawa et al. (1994b) reported the magnitude of IT as represented by stenotic area reduction percentage (cf. Fig. 3.5.1). The

most significant area reduction due to IT occurred in the ICA sinus just proximal to the flow divider (22.3 %). IT along the outer wall of the CCA is most likely due to significant *in vivo* curvature in this region, as observed by Masawa et al., (1994a).

A considerable amount of variation in CAB geometries and subsequent lesion initialization has been observed *in vivo* (Zarins et al., 1983; Masawa et al., 1994a & b). Hence, only qualitative comparisons between IT and hemodynamic conditions are possible for the representative CAB model implemented. Considering the above observations of early IT in the CAB, significant general and recurrent features include: (1) IT along the CCA side wall near the proximal origin of the flow divider; (2) A highly focal region of maximum IT in the proximal sinus along the outer wall; (3) a distally oriented expansion of IT in the region of the sinus; and (4) relatively little or no IT in the far distal ICA. IT in the proximal CCA is expected to be a function of in-plane and out-of-plane vessel curvature.

Hemodynamic wall parameters which correlate with early IT observations are of interest. Regions of low WSS and high OSI have been associated with endothelial and smooth muscle cell expression of a variety of growth factors which may stimulate IT initialization (Liu, 1999; Pearson, 1994) supporting the correlations established by Ku et al. (1985). Significant interactions of critical blood particles, such as monocytes and platelets, with the vascular surface have also been implicated with the initialization and progression of atherosclerosis (Ross, 1993; Schwartz et al., 1993; Davies, 1994). While local shear stress and stress oscillations may affect surface interactions with blood particles, the transport of circulating cells is established solely by luminal convection and diffusion. It is hypothesized that regions of early IT in the CAB may be best identified by considering regions of enhanced particle-wall interaction, including elevated near-wall concentrations and/or stasis, coinciding with regions of activated or dysfunctional endothelial cells. The composite NWRT models are intended to encapsulate these factors in a format that may be effectively applied in complex vascular geometries. To evaluate the proposed hypothesis regarding early intimal thickening, the composite NWRT models will be evaluated, along with other WSS-based hemodynamic parameters, in a representative CAB model (Milner et al., 1998; Hyun, 1998) by qualitative comparison to the four generalized early IT factors described.

3.5.2 System

For comparison to *in vivo* IT data, the realistic CAB geometry of Hyun (1998), which is based on the geometry measurements of Motomiya and Karino (1984), Archie (1997) and Milner et al. (1998), has been selected, cf. Fig. 3.5.1. The branch angle is approximately 40^{o} in the region of the flow divider, and gradually decreases such that the far distal branches are parallel. The common carotid artery (CCA) diameter is 8 mm and the maximum internal and external diameters are 8.6 and 5.6 mm, respectively. The input flow waveform, which originated from Milner et al. (1998) cf. Fig. 3.5.1, and characterized by minimum, maximum and mean Reynolds numbers of 50, 1200, and 321, respectively. The input waveform Womersley number is Wo = 5.52. A similar outlet waveform was applied far downstream of the ICA, and a constant pressure outlet was assumed for the far distal ECA.

3.5.3 Results

WSS-Based Hemodynamic Parameters

The sinus and CCA side-wall are regions of low WSS and high OSI (Fig. 3.5.2a & b). The CCA outer wall opposite the sinus (ventral side) and just proximal to the flow divider is also a region of nearly equivalent low WSS and high OSI, yet little IT is expected in this region compared with the sinus. Apparently, low WSS and possibly high OSI may be necessary for significant IT to occur; however, the relationship is not exclusive. Hence, factors such as critical blood particle localization as determined by convection and diffusion also play a significant role in IT formation. Furthermore, regions of low WSS and high OSI do not expand progressively along the outer sinus wall as observed with the generalized IT factors.

Considering variations in mean WSS magnitude and direction (Figs. 3.5.2c & d), it is apparent that the WSSG is inappropriate for identification of early IT in the CAB. The WSSAG correctly indicates the sinus and CCA sidewall in the proximal region of the flow divider. However, the ventral CCA and outer ECA show potential IT similar to that found in the sinus. While significant deviations in the WSS-vector direction may stimulate IT to some

degree, this factor is apparently not dominate and/or becomes significant only in the presence of other components.

NWRT-Based Models

Convergent profiles of the NWRT parameter, based on 200,000 monocyte trajectories, indicate a limited but significant region of expected IT along the CCA side wall in the proximal region of the flow divider (Fig. 3.5.3a). Consistent with IT observations of Masawa (1994a & b), the NWRT-contours then follow a helical pattern such that a focal maximum is observed along the proximal outer sinus wall (cf. Fig. 3.5.3a view of the sinus). Consistent with the generalized IT observations, contours of the NWRT parameter expand along the outer sinus wall and then contract, such that a limited occurrence is observed in the distal ICA. A significant region of particle-wall interaction is indicated by the NWRT parameter very near the flow divider; however, little or no IT is observed along the remaining inner walls. A helical pattern similar to that found in the ICA is also observed in the ECA, but with limited interaction along the outer-wall.

Monocyte trajectories elucidate the source of the helical NWRT pattern which may provide an explanation for early lesion formation. As the CCA sidewall narrows in the vicinity of the flow divider, particles are convected to within a critical distance of the vascular surface. These and other entrained particles then spiral around the luminal axis within the near-wall region interacting with the ICA side-walls and finally the distal sinus region. A portion of this near-wall layer of particles also enters the recirculation zone created within the proximal sinus region. These recirculating particles most likely account for the narrow band of maximum early IT observed *in vivo* and the corresponding NWRT contours (Fig. 3.5.3a, view of the sinus). A break in the elevated NWRT contour along the outer sinus wall is attributed to the mean reattachment length, which on average separates proximally recirculating particles and those spiraling down the ICA branch. A significant number of particles also interact with the apex of the flow divider, due to inertial driven impaction.

A relatively high WSS-value of τ_{limit} = 14 dyne/cm^2 resulted in NWRT-contours similar to those observed without a WSS-limiter condition (Fig. 3.5.3b). With the WSS-limiter condition, significant NWRT contours are more confined to the immediate region of

the flow divider, and NWRT-values are eliminated in the distal ICA region. Hence, high WSS may be one mechanism by which monocyte interaction with the vascular surface is limited near the flow divider and in the distal ICA.

Considering platelets in the absence of PSH and SR conditions, NWRT contours are similar to results for monocytes as well as the generalized IT features (Fig. 3.5.4a). It is expected that the mean reattachment location is less evident due to the shear rate dependent dispersion approximation implemented for platelets. Including both the PSH and SR factors amplified NWRT contours along the CCA side wall and in the sinus region (Fig. 3.5.4b). Furthermore, NWRT contours at the flow divider apex and in the ECA are dramatically reduced (Fig. 3.5.4b). Comparison to WSS contours indicates that the observed composite NWRT modifications are largely due to the PSH, which is intended to capture the degree of mechanical platelet activation. For example, platelets interacting with the flow divider apex have a relatively low PSH, such that the local composite NWRT-value is reduced (cf. Fig. 3.3.1).

3.5.4 Discussion

The significant role of critical blood particles such as monocytes and platelets in the initial stages of atherogenesis is well established (Ross, 1993; Schwartz et al., 1993; Davies, 1994). In this study, it was found that composite models for particle-wall interaction which include particle and surface reactivity conditions effectively identify early IT features in the CAB. Other hemodynamic wall parameters, such as regions of low WSS and high OSI also indicate sites of early IT as reported by Ku et al. (1985). However, regions of significant low WSS and high OSI were also observed to occur where relatively little or no IT is expected, e.g., the outside ECA wall near the flow divider. Moreover, the data of Masawa et al. (1994a & b) results in a more composite description of early IT implicating the CCA side wall and suggesting a helical IT pattern, the latter of which is not indicated by WSS parameters alone. *While WSS plays a significant role in vascular biology and arterial wall self-regulation, particle-wall interactions are also significant in establishing sites most susceptible to early lesion formation.*

The NWRT parameter, which encapsulates regions of near-wall particle stasis and/or elevated concentrations, was found to effectively capture significant regions of particle-wall interaction. In a composite model for blood particle deposition, local shear stress exposure was assumed to influence EC production of adhesive and thrombogenic compounds and to affect platelet activation. Regions of elevated particle-wall interaction, as captured by the NWRT parameter, coinciding with endothelial cell and/or platelet reactivity were found to qualitatively correlate with observed sites of early lesion growth. Moreover, among the hemodynamic wall parameters tested, the composite NWRT-based models best indicated general IT features reported by multiple *in vivo* studies for the CAB.

3.6 Identifying Sites of Intimal Hyperplasia and Possible Thrombosis Formation in Distal Femoral Anastomoses

3.6.1 Overview

Due to a predominate role in late bypass graft failure, localization and biological content of distal anastomotic intimal hyperplasia (DAIH) are of significant interest. In a study of late-stage PTFE femoral bypass grafts excised from humans, DAIH was predominately found at the toe and heel of the graft, and on the floor of the artery (Sottiurai et al., 1983). A heterogeneous pseudo-intima that lacked an endothelial cover was observed on a majority the PTFE surface. Other *in vivo* studies of DAIH characteristics have largely implemented animal models based on an assumed similarity with humans in the intimal thickening (IT) process.

Sottiurai et al. (1989) qualitatively characterized the location and biological content of DAIH in canine PTFE iliofemoral grafts. As observed in humans, DAIH was reported to localize in the heel and toe of the graft and on the floor of the host artery; however, quantitative measurements and graft geometry details are not provided. Bassiouny et al. (1992) constructed canine model iliofemoral bypass grafts of saphenous vein and PTFE. Biologic content of DAIH and qualitative localization were considered after eight weeks. Transparent silicone cast models were also constructed for flow visualization experiments. Bassiouny et al. (1992) observed that suture line intimal thickening was greater in PTFE anastomoses than in vein anastomoses while arterial floor IH was consistent among both groups. It was postulated that suture line DAIH formation was due to surgical injury and compliance mismatch, while DAIH at other locations arose from hemodynamic mechanisms. Bassiouny et al. (1992) visualized hemodynamic flow patterns in cast models and found a qualitative correlation between intimal thickening and sites of observed low WSS as well as long near-wall particle residence times.

In a review of *in vivo* DAIH observations from excised human and animal grafts, Sottiurai (1999) reports frequent observations of blood-borne elements (such as thrombocytes and leukocytes) interacting with an intact arterial wall endothelium. Transmission electron micrograph analyses indicate blood particle adherence to endothelial gap junctions as well as

transmigration in regions of DAIH formation. These observations provide evidence that monocytes and platelets may interact with the vascular surface and stimulate a smooth muscle cell response without apparent endothelial disruption (Sottiurai, 1999). Furthermore, Sottiurai (1999) suggests that suture line injury and compliance mismatch enhance the pathogenesis of DAIH, whereas particle-wall interactions and the local shear stress environment are the underlying stimuli.

Several recent studies have implemented canine models to evaluate possible correlations between WSS-based hemodynamic parameters and sites of DAIH (Keynton et al., 2001; Li and Rittgers, 2001; Loth et al., 2002). Specifically, Keynton et al. (2001) constructed PTFE carotid bypass grafts in six dogs and made *in vivo* wall shear rate (WSR) estimates along the toe and floor immediately following implantation. The distal anastomoses were characterized by a 30^o angle, no proximal outflow, and a graft-to-artery diameter ratio (DR) of either 1.0 or 1.5. While *limited IH occurred along the toe region* and was different between DR groups, the *most significant IH occurred along the artery floor* and was consistent among DR groups. Considering the toe and floor only, DAIH was found to weakly correlate with exponential functions of the inverse of the mean WSS ($r^2 = 0.23$) and the OSI ($r^2 = 0.36$). Interestingly, Keynton et al. (2001) observed that a vast majority of DAIH occurs in regions where the mean wall shear rate is below a critical value and that IH often remains absent over the full range of wall shear rates.

Loth et al., (2002) reported the distribution of DAIH in seven canine iliofemoral PTFE grafts after 12 weeks of implantation. Anastomotic configurations were characterized by a low graft angle (approximately 10^o), a graft-to-artery diameter ratio of 1.6, and a proximal-to-distal outlet flow division of 20:80. DAIH was found to be *most significant along the graft hood and the suture line at the toe and heel*, but was *absent along the arterial floor*. DAIH was found to strongly correlate with the inverse of the WSS along the hood; however, regions of equally low WSS were observed at the floor (where no DAIH occurred) such that an overall relationship was relatively weak ($r^2 = 0.276$).

A weak and inconsistent relationship between DAIH and low WSS as observed by Keynton et al. (2001) and Loth et al. (2002) is evidence that other factors may act in conjunction with WSS in the development of DAIH. Surgical injury, compliance mismatch,

and intramural strain may potentially all act synergistically with WSS in the development of DAIH. Due to convective and diffusive transport, localized regions of elevated particle-wall interactions have also been identified in non-uniform vascular configurations. Moreover, these regions do not necessarily coincide with regions of low WSS and/or high OSI.

While local WSS conditions affect endothelial cell interactions with critical blood particles, it is hypothesized that the near-wall localization of platelets by convective and diffusive transport plays a significant role in IH development. Therefore, regions of IH initialization in the distal femoral anastomotic geometry might be best identified by considering regions of enhanced platelet-wall interaction, including elevated near-wall concentrations and/or stasis, coinciding with regions of down-regulated endothelial cell expression of anti-thrombogenic compounds. It is assumed that local WSS exposure controls both endothelial cell reactivity and platelet activation as described in the composite NWRT model for platelets (cf. Sect. 3.3). The proposed hypothesis along with the performance of WSS-based hemodynamic parameters are to be evaluated based on qualitative comparisons to recent observation of system specific DAIH occurrence.

3.6.2 Systems

Three geometries where constructed, as described in Sect. 2.3.1, for comparison with system specific DAIH observations. Case A is characterized by a relatively high graft-angle (approximately 30^{o}) and no proximal outflow (Fig. 3.6.1). Case B is geometrically similar to Case A, but allows 20% of the inlet mass flow to exit proximally. Case C is characterized by a low graft-angle (approximately 10^{o}) and a proximal-to-distal outlet flow division ratio of 20:80 (Fig. 3.6.1). All configurations assume a 6 mm graft inlet diameter, a graft-to-artery diameter ratio of DR = 1.5, and a standard human femoral input pulse (Fig. 3.6.1).

3.6.3 Results

WSS-Based Hemodynamic Parameters

For Case A, the mid-floor region as well as the heel and distal toe are sites of low WSS and high OSI (Fig. 3.6.2). However, the toe region in the vicinity of the suture line is an area of high WSS and low OSI. Changes in mean WSS magnitude and direction, as quantified by the WSSG and WSSAG, are most pronounced along the suture line with elevated contours occurring at the toe and heel (Fig. 3.6.2). Significant contours of the WSSG and WSSAG also encompass the sinus region and extend to the artery floor.

Considering Case B, regions of low WSS and high OSI are again observed in the mid-floor and distal toe regions (Fig. 3.6.3), as with Case A. However, proximal outflow increases WSS magnitude and reduced oscillations in the proximal artery. Furthermore, proximal outflow mildly reduced WSSG values in the toe region, whereas WSSAG contours remain largely consistent with those observed for Case A.

Case C exhibits low WSS and high OSI along the arterial floor, while the graft hood and toe regions are largely characterized by elevated WSS and low OSI (Fig. 3.6.4). WSSG contours are generally consistent among the graft hood, sinus, and floor regions, with a maximum occurring at the toe in the vicinity of the suture line. As illustrated by the WSSAG field, WSS-vectors change direction most severely along the suture line.

NWRT-Based Model

Convergent profiles of the NWRT parameter, based on 400,000 platelet trajectories, indicate significant particle-wall interactions at the toe, sinus and floor of Case A (Fig. 3.6.5a). The composite NWRT model, which includes conditions for platelet activation and surface reactivity, indicates critical contours in the junction area with particular emphasis in the vicinity of the toe as well as an elevated region that emanates from the heel and enters the sinus (Fig. 3.6.5b). Consistent with the 4 mm artery and applied inlet conditions, a value of WSS_{mean} = 14 dyne/cm^2 was used to compute PSH and SR factors. Furthermore, the inclusion of the PSH and surface reactivity conditions results in highly significant NWRT contours in the mid-floor region. Comparison to WSS values (Fig. 3.6.2) for Case A

indicates that the elevated composite NWRT contours observed along the arterial floor are due primarily to the SR factor in the presence of significant particle-wall interactions.

In comparison to Case A, the proximal outflow of Case B results in elevated particle-wall interactions in the sinus region and moderately reduced NWRT occurrence along the artery floor (Fig. 3.6.5). Including PSH and SR factors for Case B diminishes NWRT-values in the sinus region and magnifies values near the toe. While the SR factor is consistent between Cases A and B, a lack of platelet-wall interaction is responsible for the reduced composite NWRT contours along the artery floor observed with the latter configuration (Figs. 3.6.5b & d).

Due to secondary velocities instigated by the significantly curved graft-hood of Case C, a large amount of platelet-wall interaction is observed in this region. Elevated NWRT contours are also evident along the suture line at the toe and heel as well as in the enlarged arterial sinus. Interestingly, very little particle-wall interaction is observed along the arterial floor. Inclusion of PSH and SR factors magnifies NWRT contours along the graft hood and suture line at the toe and heel. Due to a lack of particle-wall interaction, composite NWRT contours remain largely absent within the low shear stress environment of the arterial floor.

3.6.4 Discussion

Wall-shear-stress and particle-residence-time based hemodynamic wall parameters have been evaluated for three common distal anastomotic configurations. As evident in the present results and observed by a number of researchers (Keynton et al., 1991; Li and Rittgers, 2001; Longest and Kleinstreuer, 2000; White et al., 1993, etc.) factors such as graft-to-artery angle, anastomotic shape, and outlet flow-division ratio largely influence mean WSS fields, variations in WSS magnitude and direction, and regions of localized particle-wall interactions. Other system factors influencing hemodynamic interactions with the vascular surface include inlet flow waveform, diameter ratio, and vessel dimensions in the anastomotic region (Ethier et al., 1998; Kleinstreuer et al., 1996; Loth et al., 1997; Lei et al., 1997, Moore et al., 1999; etc.). Similarly, variations in anastomotic system configurations have been associated with differences in DAIH localization and progression (Keynton et al., 2001; Loth et al., 2002). Potentially, mechanical factors most closely related to initial DAIH may be identified by comparisons of intimal thickening (IT) occurrence and simulated hemodynamic parameters within similar anastomotic systems.

A number of hemodynamic and/or blood element interactions with the vascular surface have been directly associated with smooth muscle cell (SMC) migration, proliferation, and synthesis of excess extra-cellular matrix, which characterize IH. For example, endothelial secretion of platelet derived growth factor (PDGF), which is a potent stimulant of SMC migration and proliferation, has been associated with reduced shear stress in baboon prosthetic vascular grafts (Mondy et al., 1997). Endothelial cell production of other growth factors, such as endothelin, has been shown to increase at low and oscillatory shear in culture (Pearson, 1994; Sharefkin et al., 1991). Furthermore, endothelial expression of a smooth muscle cell *inhibitor*, nitric oxide (NO), has been shown to *decrease* in regions of low mean shear (Harrison et al., 1996). Changes in endothelial cell shape, potentially indicating elevated permeability and thrombogenicity, have been associated with gradients of the time-averaged WSS magnitude and direction (Helmke and Davies, 2002; Kleinstreuer et al., 2001). With regard to critical blood particle interactions with the vascular surface, it has been well documented that adherent monocytes may penetrate the endothelium 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; Sottiurrai et al., 1999). Hemodynamic characteristics such as secondary flow, low WSS, and high WSSG have also been associated with endothelial cell expression of adhesive molecules such as VCAM-1 (Gimbrone et al., 1997; Malinauskas et al., 1998; Truskey et al., 1999). Similarly, activated platelets can release chemotactic and mitogenic growth factors such as platelet derived growth factor (PDGF) and vascular constrictive agents such as thromboxane A2 (TxA_2) (Liu, 1999). Permanent adhesion of platelets to the vascular surface may not be necessary for significant promotion of IT to occur (Savage et al., 1996). Alternatively, mural thrombi resulting from significant platelet adhesion and aggregation may evolve into active atherosclerotic plaque-like lesions (Leu et al., 1988; Sloop, 1999), particularly on synthetic surfaces (Sloope et al., 2002). A direct relationship has been identified between local endothelial cell WSS exposure and the expression of anti-thrombogenic compounds such as PGI2, NO, and tissue factor pathway inhibitor (TFPI) (Grabowski et al., 1985; Harrison et al., 1996; Westmuckett et al., 2000; Grabowski et al., 2001). Hence, a variety of hemodynamic and/or particle interaction mechanisms are fundamentally capable of providing the necessary cellular level stimulus required for IH to occur. However, the relative importance of these factors in the development of DAIH is largely unknown.

Several recent studies have implemented canine models to evaluate localization of DAIH as well as possible correlations with WSS-based hemodynamic parameters (Keynton et al., 2001; Li and Rittgers, 2001; Loth et al., 2002). Relatively weak and limited correlations with the inverse of WSS and with OSI have been estimated and attributed to elevated endothelial production of endothelin and reduced production of NO (Keynton et al., 2001, Loth et al., 2002). Including factors such as distance from the suture line and vascular conduit type resulted in a significantly stronger correlation (Loth et al., 2002); however, these variables do not necessarily indicate mechanisms responsible for DAIH at the cellular level. For an anastomotic configuration similar to Case A, Keynton et al. (2001) observed IH at the immediate toe junction and more severely along the artery floor. DAIH at other locations was not reported. For a low-angle anastomosis similar to Case C, Loth et al. (2002) reported

predominate IH along the graft hood and suture line, particularly at the toe and heel, with relatively little occurrence near the arterial floor.

In comparison, WSS-based hemodynamic parameters for Case A (Fig. 3.6.2) are generally inconsistent with the DAIH observations of Keynton et al. (2001). The mid-floor region exhibits low WSS and high OSI (where maximum IH is expected); however, maximum WSS and very low OSI are observed at the toe very near the suture line (where secondary formation of IH is reported). Changes in WSS vector magnitude and direction are elevated along the floor, but exhibit maximums along the suture line particularly in the immediate toe region. Similarly for Case C (Fig. 3.6.3), WSS and OSI results contrast the DAIH observations of Loth et al. (2002). The graft hood (where maximum IH is expected) is a region of relatively high WSS and low OSI, whereas the arterial floor (where little IH is expected) is a region of relatively low WSS and high OSI. WSSG and WSSAG contours also fail to properly identify the correct local proportion of DAIH formation for Case C.

While regions of expected IH formation may exhibit elevated WSS-based hemodynamic parameters, the resulting correlations are not qualitatively significant. That is, peak values of the hemodynamic parameters and regions of highest DAIH do not necessarily correlate on a local basis throughout the system. As suggested by Loth et al. (2002), this discrepancy may be due to a variable cellular response of different SMC phenotypes, which comprise IH in the artery and on the graft surface. Furthermore, other factors possibly interacting with the WSS-based parameters, or interaction of the WSS-based parameters (beyond a low WSS and high OSI condition), may produce a more significant cellular response with respect to early DAIH development.

The results of the current study indicate that sites of low WSS and/or high OSI do not necessarily correlate with region of elevated particle-wall interaction, as quantified by elevated near-wall particle concentrations and/or stasis (Figs. 3.6.2-3.6.4 vs. 3.6.5). For instance, composite NWRT contours indicate that little particle-wall interaction is expected in the distal toe region of Case A and along the floor of Case C, which are characterized by relatively low WSS and high OSI contours. Alternatively, significant particle-wall interactions are predicted along the upper graft surfaces of Cases A and C, which exhibit relatively high WSS and low OSI in comparison to the arterial floor.

Consistent with the results of Keynton et al. (2001), the composite NWRT results for Case A implicate the mid-floor region as a site of expected DAIH (Fig. 3.6.5b). Furthermore, due to an elevated near-wall particle concentration, NWRT contours indicate local maximum IH occurrence in the toe region very near the suture line, which is also in agreement with *in vivo* observations. The study of Keynton et al. (2001) does not provide DAIH observations in the region of the heel and sinus such that comparisons in these areas have not been evaluated.

Composite NWRT contours are also generally consistent with the results of Loth et al. (2002). Specifically, NWRT-contours predict significant IH along the graft hood and the suture line at the toe and heel (Fig. 3.6.5f). Due to an observed lack of particle-wall interaction along the arterial floor (Fig. 3.6.5e), little IH is expected in this region, despite low WSS occurrence. These particle-wall interaction features are largely evident without platelet activation and surface reactivity factors (Fig. 3.6.5e). However, inclusion of the SR factor was largely responsible for magnifying NWRT contours along the floor of Case A (Fig. 3.6.5a & b), while the PSH variable significantly increased NWRT contours along the graft hood of Case C (Fig. 3.6.5e & f).

The study of Loth et al. (2002) implies that maximum DAIH occurs along the graft hood. The significant extent of NWRT occurrence within the sinus region of Case C may be inconsistent with this observation (Fig. 3.6.5f). However, a refined geometric comparison indicates that the sinus region observed by Loth et al. (2002) in canine iliofemoral bypass configurations is significantly smaller than implemented for Case C. Moreover, increasing the arbitrarily selected surface reactivity factor of the graft to a value greater than unity will magnify composite NWRT values relative to those found on the arterial surface. A graft surface reactivity factor SR > 1 may be justified considering that platelets more readily interact with heterogeneous pseudo-intima in comparison to the endothelial layer covering early hyperplasic lesions found in the artery. However in refining a SR factor, the relative responses of the primary cell types comprising the various forms of graft and artery IH must be considered, as well as the highly increased and transient thrombogenicity of the suture line.

The shift in maximum DAIH occurrence from the floor in Case A to the graft hood with Case B is expected to be primarily a factor of either the inclusion of proximal outflow,

as suggested by Li and Rittgers (2001), or the anastomotic geometry. Composite NWRT results for Case B indicate that proximal outflow does, in fact, reduce platelet recirculation in the vertical plane thereby moderately reducing significant particle-wall interactions along the mid-floor region (Figs. 3.6.5 a & c). Moreover, proximal outflow increases WSS along the arterial floor such that a reduced SR factor results for Case B (Figs. 3.6.2 & 3.6.3). However, comparison of NWRT results indicates that the primary mechanism for the shift in maximum DAIH occurrence might be due to the anastomotic geometry. The low graft angle and significant hood curvature of Case C induce elevated secondary velocities which significantly increase particle-wall interactions along the outer graft surface and result in a large portion of the flow exiting near the toe opposite the arterial floor.

In addition to platelet interactions with the vascular surface, monocytes may also play a significant role in DAIH development. It was found that NWRT-values based on idealized spherical monocytes ($\tau_p = 3.35\times10^{-6}$, $\rho/\rho_p = 0.964$) were largely similar to NWRT contours for platelets. Assuming monocyte adhesive molecule VCAM-1 expression by endothelial cells to be inversely proportional to the normalized local WSS resulted in composite NWRT contours similar to those observed for platelets in Case A. The absence of a particle activation condition for monocytes reduced composite NWRT values along the graft hood of Case C, compared to platelet model results.

While the computational geometries implemented are similar to the anastomotic configurations of Keynton et al. (2001) and Loth et al. (2002), system variations are present. The sinus region and raised arterial floor evident in both computational models, while observed in some cases, may not be consistent with the canine anastomotic systems. Minor wall motion, lesion growth, and particle attachment may significantly alter near-wall hemodynamics *in vivo*. Furthermore, the DAIH results of Keynton et al. (2001) are based on a carotid artery bypass model whereas Loth et al. (2002) implemented an iliofemoral configuration. Flow waveform differences may contribute to the observed variations in DAIH occurrence as speculated by Ethier et al. (1998) and Kleinstreuer et al. (1996). However, the identified flow features responsible for the particle-wall interactions that underlying the shift in DAIH with geometric design and outflow condition are expected to remain largely unchanged.

Vascular injury (Bassiouny et al., 1992) and compliance mismatch (Abbott et al., 1987) have also been associated with DAIH development, particularly along the suture line (Hofer et al., 1996; Loth et al., 2002). Sottiurai (1999) suggests that suture line injury and compliance mismatch enhance the pathogenesis of DAIH, whereas particle-wall interactions and the local shear stress environment are the underlying cellular level stimuli. The suture line region is initially extremely thrombogenic in comparison to the graft and artery surfaces. Platelet interactions with the suture line could be incorporated within the composite NWRT model by the inclusion of a locally large surface reactivity factor. An alterative scenario is that endothelial cells which migrate and develop over the graft-to-artery juncture are highly activated by large variations in WSS vector magnitude and direction (as evident by WSSG and WSSAG contours, Figs. 3.6.2-3.6.4) such that excessive SMC progression ensues. Clearly, further detailed histopathological studies are necessary to determine which of these, or other, scenarios is largely responsible for the high degree of suture line IH observed *in vivo*.

With respect to graft and artery compliance, it is reported that elevated intramural stress, often elicited by increased mismatch, is associated with suture line IH (Leuprecht et al., 2002; Thubrikar et al., 1995). An alternative scenario is that graft and artery compliance affect the static anastomotic geometry and/or wall motion, particularly in the region of the suture line. Hence, compliance mismatch also provides a geometric potential for increased WSS-based hemodynamic parameter occurrence as well as particle-wall interactions.

In conclusion, the composite NWRT model for platelet-wall interactions, which includes mechanical factors for both surface reactivity and platelet activation, was found to effectively capture significant regions of reported DAIH occurrence for multiple anastomotic configurations. Local shear stress exposure was assumed to influence endothelial cell production of thrombogenic compounds and to affect platelet activation. Comparisons of other WSS-based hemodynamic wall parameters with reported DAIH observations resulted in inconsistent qualitative correlations when considering multiple locations within a single configuration. However, large variations in WSS vector magnitude and direction, as encapsulated by the WSSG and WSSAG parameters, were consistently observed along the suture line in all configurations studied. While WSS plays a significant role in vascular

biology and arterial wall self-regulation, platelet interaction with the vascular surface also appears to be a highly significant mechanism in the development of DAIH at the cellular level. Quantitative data regarding the histopathological development of DAIH as well as refined correlations for adhesive molecule expression, endothelial cell activation and expression of thrombogenic compounds, platelet-wall interaction, red blood cell induced dispersion, and platelet activation will allow future comparison studies to better relate hemodynamic characteristics to sites of potential lesion formations.