CHAPTER 6

6 CONCLUSIONS AND FUTURE WORK

In conclusion, the research objectives outlined in Section 1.2 have been fulfilled. Novel contributions of this work include the conceptualization and development of a particle-based hemodynamic parameter intended to quantify the likelihood of critical blood particle deposition based on local discrete near-wall residence times and concentrations. Significant quantitative correlations have been established between the proposed near-wall residence time (NWRT) parameter and available *in vitro* deposition data sets for suspensions of monocytes and platelets in non-parallel axisymmetric domains. For applications in realistic three-dimensional geometries, composite models of blood particle deposition that incorporate surface reactivity as well as platelet activation within the NWRT framework have been established. Numerical results correlate with observed sites of intimal thickening in multiple vascular systems, i.e., the rabbit aorto-celiac junction, the human carotid artery bifurcation, and the femoropopliteal bypass distal anastomosis.

Calculation of the NWRT parameter in complex branching geometries was based on the effective evaluation of a number of point-force model terms that have previously not all been included in a model for bioparticle transport. Efficient multi-processor solution algorithms were necessary considering the complexity of the systems of interest in conjunction with the number of particles required for Eulerian style convergence. The contributions of an effective particle-based hemodynamic parameter, a comprehensive bioparticle point-force model, and applications to the evaluation of intimal thickening in different vascular geometries, are summarized below.

6.1 Discussion of Fundamental Contributions

Although the complex multifaceted mechanisms responsible for atherosclerotic lesion formation and general intimal thickening (IT) are not fully understood, a review of recent literature indicates that a variety of hemodynamic and/or particle interaction mechanisms are capable of providing an inciting stimulus at the cellular level (Helmke and Davies, 2002; Kleinstreuer et al., 2001; Liu, 1999). Furthermore, a number of studies stress the significance of critical blood particles, such as monocytes and platelets, in the initialization and progression of IT and thrombus formation (Davies, 1994; Ross, 1993; Schwartz et al., 1993; Sottiurai et al., 1999). Hemodynamic characteristics such as low and oscillatory wall shear stress (WSS) have been associated with endothelial expression of adhesive molecules and thrombogenic compounds (Gimbrone et al., 1997; Westmuckett et al., 2000). To simulate the adhesion process for individual monocytes, reaction kinetics theory has been used to model interactions between surface and cell bound molecules 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 approximation is often applied to capture locations of initial interaction in the absence of near-wall hydrodynamic forces. Alternatively, 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. However, 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.

Development of the NWRT Approach

Considering the limitations of the available deposition models, an alternative method was necessary to approximate the nano-scale molecular adhesion process of critical blood particles in geometrically large and complex systems with high particle counts. This work hypothesizes 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 interactions have been quantified by the near-wall residence time (NWRT) parameter. Regions of endothelial cell activation, e.g., up-regulation of adhesive and thrombogenic compounds, have been associated with low wall shear stress conditions.

In this study, particle hemodynamic simulations were based on an effective one-way coupled point-force representation of particle motion. By comparison to available experimental trajectories, it was shown that fluid-element pathlines may be used to simulate non-interacting blood particles removed from wall boundaries under dilute transient conditions. However, when particle-wall interactions are significant, an extended form of the particle trajectory equation is required which includes terms for Stokes drag, near-wall drag modifications, or 'lubrication' forces, pressure gradients, and near-wall particle lift. Still, additional physical and/or biochemical wall forces in the nano-meter range cannot be readily calculated; hence the near-wall residence time (NWRT) model indicating the probability of blood particle deposition was necessary. Lagrangian-style near-wall force terms, which are typically presented for simple two-dimensional shear flows, have been extended to complex three-dimensional flow domains (cf. Sect. 2.3.2). In order to effectively compute the large number of particle trajectories required to resolve regions of particle stasis, the proposed particle-tracking algorithm stores all transient velocity field solution data on a shared memory architecture (SGI Origin 2400) and computes particle trajectories using an adaptive parallel approach. Compared to commercially available particle tracking packages, the algorithm presented is capable of reducing computational time by an order of magnitude for typical transient one-way coupled blood particle simulations in complex flow domains.

The effectiveness of the NWRT parameter, as well as the necessity of the underlying point-force near-wall terms, has been assessed by comparisons to available *in vitro* deposition data in non-parallel axisymmetric geometries. Factors including the convective-diffusive transport of particles, finite particle size and inertia, as well as near-wall hydrodynamic interactions were found to significantly influence blood particle deposition. Of the models studied, the near-wall residence time (NWRT) approach was found to be a particularly effective indicator for the deposition of monocytes ($r^2 = 0.74$) and platelets ($r^2 = 0.57$) given that nano-scale physical and biochemical effects must be approximated in

computational simulations involving relatively large-scale geometries and complex flow fields. Still, surface reactivity (e.g., up-regulation of adhesive or thrombogenic molecules) remains a major determinate of where particles deposit and a major hurdle for effectively modeling adhesion in realistic systems.

Surface reactivity and particle activation are generally considered significant factors in blood particle attachment and have been directly related to hemodynamic conditions. Considering firm monocyte adhesions, regions of low WSS have been associated with endothelial cell expression of adhesive molecules such as VCAM-1 (Gimbrone et al., 1997) as well as a reduced likelihood of significant particle rolling and re-suspension (Zhu, 2000). A direct relationship has been identified between local endothelial 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. 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).

To encapsulate the effect of local shear stress on the biophysical processes of particle-wall interactions, composite NWRT models for monocytes and platelets have been defined. These models are based on the hypothesis 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. For monocytes, deposition is most probable in regions where the local near-wall residence time is high and wall shear stress is below a critical ‘threshold’ (Chang and Hammer, 1999), i.e., a WSS-limiter model. To assess mechanical platelet activation, a platelet stimulation history (PSH) function was defined based on shear stress exposure and used to scale local NWRT contributions. To quantify the potential for platelet adhesion in the presence of surface bound coagulate and anti-coagulate proteins, a WSS-based surface reactivity (SR) factor was defined and incorporated in the

composite NWRT model. The resulting composite models were tested in the rabbit aorto-celiac junction, the human carotid artery bifurcation, and the distal femoral anastomosis.

To elucidate the mechanisms by which particle-hemodynamics influence arterial lesion initialization and progression, the rabbit aorto-celiac junction was selected as an initial atherosclerotic model. Low WSS and high oscillatory shear index (OSI) parameters proved ineffective compared to localized *in vivo* results of monocyte accumulation and lesion initialization. The NWRT parameter, with a limiting WSS condition, identified the lateral flow divider as most susceptible to monocyte deposition, as observed *in vivo*. A representative quantitative correlation between monocyte deposition and NWRT occurrence was established ($r^2 = 0.77$ and $p < 10^{-4}$) on a highly focal basis for an averaged data set. 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.

As a second model of early arterial lesions, the human carotid artery bifurcation was selected to evaluate potential correlations between intimal thickening and particle-wall interactions, determined by the composite NWRT based models for monocytes and platelets. It was found that the composite NWRT models effectively identified generalized early IT features including: (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. 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, the results of this study indicate that particle-wall

interactions are also significant in establishing sites most susceptible to early lesion formation.

Regarding distal anastomotic intimal hyperplasia (DAIH), recent *in vivo* animal studies have illustrated a shift in maximum IH occurrence based on anastomotic geometry and the associated hemodynamic conditions (Keynton et al., 2001; Loth et al., 2002). For comparable realistic anastomotic configurations, 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.

Conclusions Regarding the NWRT Concept

Based on the comparison studies, conclusions can be drawn regarding the effectiveness of the NWRT parameter as well as the general significance of critical blood particles in the initialization and progression of early intimal thickening. In general, agreements with monocyte deposition data, sites of atherosclerotic lesion initialization, and DAIH occurrence suggest that the composite NWRT-based models are sufficiently detailed, yet computationally efficient as required for application in complex branching blood vessels. The reviewed literature (Chapter 1) indicates multiple potential pathways for the initialization and development of IT. For instance, endothelial cell production of growth factors, in the absence of particle-wall interactions, has been shown to increase at low and oscillatory shear in culture (Mondy et al., 1997; Pearson, 1994). However, results of the current study indicate that significant particle-wall interactions appear to be a necessary component for IT initialization and progression in all systems considered, whereas relations to other hemodynamic wall parameters such as low WSS and high OSI alone were not

significant. Nevertheless, composite particle-wall interaction models including shear stress based reactivity factors were necessary to establish significant correlations with observed sites of monocyte deposition and IT in realistic vascular geometries.

While WSS alone plays a significant role in vascular biology and arterial wall self-regulation, results of this study indicate that significant particle wall interactions in regions of activated or dysfunctional endothelial cells functions as a highly aggressive mitogenic pathway which may result in a majority of the IT initialization and progression observed. Moreover, composite NWRT comparisons indicate that monocyte deposition is potentially most significant with regard to atherosclerotic lesion initialization. However, intimal monocyte transport, LDL uptake, endothelial cell activation and dysfunction, and platelet attachment or interaction may all influence the arterial lesion pattern during and after initialization. In contrast, composite NWRT results imply that platelet interactions with the vascular surface are most significant with respect to DAIH formation. Indeed, this observation is consistent with the high smooth muscle cell content of hyperplasic developments (cf. Chapter 1). However, this remark is based on the assumptions underlying the composite NWRT parameters, including potentially incomplete surface reactivity and particle activation models, as well as limited DAIH observations. Therefore, the role of monocytes in the formation of DAIH, while potentially significant, remains largely undetermined.

6.2 Discussion of Applications

Conventional End-to-Side Anastomoses

Consistent with the research objectives, a particle hemodynamics analysis of the femoropopliteal bypass distal anastomosis has been conducted. Depending on the graft diameter selected and the initial graft-end cut made by the vascular surgeon, so-called ‘conventional anastomoses’ can vary significantly. In this study, the anastomotic configurations resulting from three common graft-end cut styles (straight, curved, and S-shaped) have been investigated including significant changes in the geometry of the recipient

artery. Considering the results of Chapter 3, most aggressive DAIH occurrence was expected in regions of significant platelet interaction with the vascular surface. Graft performance was assessed with respect to the potential for IH occurrence at locations critical to flow delivery. Of the configurations evaluated, straight and curved graft-end cuts with a graft-to-artery diameter ratio of 1.5:1 were found to significantly reduce the potential for critical IH development while maintaining a graft lumen sufficient to reduce the risk to early thrombotic occlusion. Nevertheless, the potential for significant IH occurrence via platelet and/or endothelial response pathways was evident in all conventional anastomoses considered, particularly along the critical suture-line region. Therefore, geometric modifications of the distal anastomosis in an effort to reduce hemodynamic wall parameter occurrence as well as to mitigate regions of significant particle-wall interactions is warranted. In addition to alterations of the junction region, the results of this study imply that upstream hemodynamics, such as inlet graft curvature, as well as arterial geometry resulting from the construction of the junction significantly influence the potential for IH and should be considered in anastomotic revisions.

Alternative End-to-Side Configurations

Regarding the poor clinical performance of conventional femoropopliteal bypass configurations, potential anastomotic geometries intended to reduce DAIH formations have been analyzed. While a number of theories have been proposed to explain the improved clinical performance of the Miller cuff configuration, results of this study indicate a redistributed occurrence of the composite NWRT parameter for platelets, compared to conventional anastomoses. As observed *in vivo* (Tyrrell and Wolfe, 1997), NWRT contours reveal that the construction of the cuff moves suspected sites of DAIH occurrence away from the artery and to the graft-to-vein junction. However, other studies suggest that the improved performance of the Miller geometry is a result of the biological properties of the vein cuff (Kissin et al., 2000).

Considering the virtually prototyped models, anatomic features consistent with venous anastomoses were found to reduce the particle-hemodynamic potential for DAIH formations in locations critical to flow delivery. Significant graft and arterial curvatures and

the application of an unexpanded anastomotic design redistributed and reduced WSS-based hemodynamic parameters and NWRT occurrence; however, the particle-hemodynamic potential for DAIH formation was not eliminated. Considering the proliferative nature of IH formations, it appears that eventual occlusion of the virtual prototyped configurations is expected.

In conclusion, the application of a multiple-pathway particle-hemodynamics model for IH in distal anastomotic designs indicates that occlusive formations are an inevitable consequence of the un-physiological distal end-to-side anastomosis, particularly for the case of proximal outflow. Nevertheless, surgical benefits of the end-to-side distal anastomosis, such as ease of construction and the ability to deliver proximal outflow, ensure its continued implementation until a better alternative is proven. As such, results of this study suggest the implementation of concave-up graft inlets, relatively unexpanded anastomotic configurations, and arterial curvatures which moderate flow redirection. Elimination of proximal outflow significantly reduced particle-wall interactions resulting in a preferred configuration, where applicable. Clinical testing will be necessary to determine if the unexpanded anastomotic design suggested can accommodate moderate IH formation in the immediate junction and lateral wall regions without significantly altering graft function. In contrast to the physiologically flawed end-to-side distal anastomosis, recent advancements in vascular devices, such as drug-impregnated stents, can be used to supplement end-to-end configurations, potentially resulting in a clinically superior junction.

6.3 Future Directions

Given the complexity of the systems considered, a number of assumptions have been implemented. Extensive validations have indicated that a majority of these assumptions are justifiable; however, further investigations as well as model extensions may better elucidate the roles of critical blood particles in vascular diseases. Assumptions warranting future study relate to:

- vascular geometries,
- fluid flow, and
- particle transport

as discussed below.

The assumption of rigid vascular walls is a potentially conservative approximation considering that compliant walls reduce disturbed flow characteristics. Moreover, Leuprecht et al. (2002) has recently indicated that wall compliance has a negligible effect on end-to-side hemodynamics. Nevertheless, minor wall motions may significantly influence particle interactions in the near-wall region. Furthermore, the inclusion of wall compliance is essential in addressing issues regarding compliance mismatch and intramural stress, which may have a significant impact on suture-line DAIH. Other geometric influences include out-of-plane curvature, which may have a significant effect on lumenal particle transport and WSS-based hemodynamic parameters. Considering the distal end-to-side anastomosis, the assumption of in-plane vascular geometries produces increased regions of particle stasis, particularly at the lateral wall location. Therefore, a particle-hemodynamics investigation of out-of-plane curvature is expected to reveal a reduced potential of IH along the lateral walls, which may be more consistent with *in vivo* results. Considering the near-wall region, geometric modifications due to lesion growth and particle attachment may considerable alter local hemodynamics. These effects should be accounted for in future simulations, possibly with the inclusion of a porous media model to approximate thrombus formations.

Variables that influence the hemodynamic flow field will most likely influence particle-hemodynamic indications for IT. Increased flow-rate division, 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. Furthermore, the Quemada

model was found to result in viscosity values significantly greater than the Newtonian limit, throughout a majority of the anastomotic junction. The resulting increase in lateral momentum transport equalized input velocity profiles and reduced recirculation, particularly in the Miller cuff configuration. Comparisons of these results with *in vitro* studies implementing Newtonian solutions and dilute particle suspensions will most likely reveal considerable discrepancies.

Considering particle transport, the assumptions of spherical particles and smooth walls eliminate the possibility for contact in Stokes flow. Blood particle deposition *in vivo* is largely a result of particle asymmetry, e.g., activated platelets form spiny spheroids with pseudopod extensions, and surface non-uniformity, e.g., microvilli extensions. Direct numerical simulations of platelet and monocyte interactions with the vascular surface might allow for improved near-wall drag and lift approximations. Other factors related to blood particle localization, such as rolling and intimal monocyte transport, also warrant further investigations. For example, particle rolling is a likely mechanism by which monocytes might collect in the stenotic throat of the Hinds et al. (2001) configuration. Nevertheless, NWRT agreements with monocyte and platelet deposition data as well as sites of lesion initialization are very reasonable.

Regarding blood particle transport due to dispersion, a number of open questions remain. The current assumption of Gaussian dispersion is only valid for a constant concentration shear field. In reality, the persistent dispersion of blood particles is determined by local velocity gradients, red blood cell concentrations, and red blood cell motion including deformations. Therefore, improvements of the implemented dispersion estimate requires knowledge of the local hematorcrit, which is complicated by the flexible nature of red blood cells. While the applied dispersional effect is incrementally small, it may play a significant role in the near-wall environment.

Considering the composite NWRT models, a number of components are in need of further refinement. Limiting wall shear stress values have been 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. For instance, 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 relatively high WSS-gradients in the junction regions, 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.

Similarly, the surface reactivity factor (SR) was intended to approximate differences in platelet affinity associated with various vessel wall components. However, reaction rates for platelet adhesion to *in vivo* PTFE versus endothelium are currently not available, such that a value of unity has been implemented. In refining the 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. While the PSH is intended to capture mechanical platelet activation, the significant influences of chemical agonists have been ignored. Future studies should model the transport and local concentration of agonist species, as derived from sites of vascular injury and thrombosis, and include their effects in the simulation of activation.

To further refine the simulation of particle-to-wall interactions, it is suggested that Adhesive Dynamics style micron-scale analysis be conducted within regions of significant near-wall stasis and/or concentrations. For example, 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. However, the enhancement of current particle-wall interaction models, as well as composite hemodynamic wall parameters, is largely depended upon the availability of targeted experimental data sets. Quantitative data regarding the

histopathological development of intimal thickening 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.