8

In Vitro Leukocyte Adhesion in Endothelial Tissue Culture Models Under Flow

Scott Cooper, Melissa Dick, Alexander Emmott, Paul Jonak, Léonie Rouleau and Richard L. Leask
Department of Chemical Engineering, McGill University, Canada

1. Introduction

Atherosclerosis, an inflammatory disease which causes thickening and stiffening of arteries, is a major cause of death in the United States (Lloyd-Jones et al., 2010). These deaths occur because of vessel occlusion created by atherosclerotic plaques and thrombus shedding, leading to heart attack, ischemia, or stroke. Atherosclerosis is expected to be the leading cause of death worldwide within 10 years (Lloyd-Jones et al., 2010).
Inflammation plays a significant role in the initiation and progression of atherosclerosis. The cells that line the arteries, endothelial cells (ECs), mediate the inflammatory process. The forces created by blood flow affect the inflammatory response of ECs and the interaction with blood components. This chapter summarizes the background in our recent studies on the response of ECs to blood forces and the interaction of inflammatory cells.

2. Background

2.1 Pathogenesis of atherosclerosis

Vascular anatomy

Arteries have three tissue layers: the intima, media, and adventitia. The intima is lined with a monolayer of ECs in direct contact with blood. The ECs act as a protective membrane, allowing diffusion from the blood stream into the artery. ECs are capable of expressing specific genes in response to physical stresses which cause the vessel to remodel leading to the development of atherosclerosis. In addition, the intima can contain other cells (smooth muscle cells, fibroblasts and inflammatory cells), an extracellular matrix (ECM), and is only a few cell layers thick in healthy tissue. The internal elastic membrane, consisting of a layer of elastic connective tissues, separates the intima and media. The media layer is mainly comprised of smooth muscle cells (SMCs) and ECM. Although the media is involved in atherosclerosis, remodelling is less evident. The adventitia is relatively unaffected by atherosclerosis. It is separated from the media by the external elastic membrane comprised mainly of collagen, providing structural support yet allowing for artery expansion when required (Waller et al., 1992).
Over time, an atherosclerotic plaque grows by the accumulation of lipids, inflammatory cells, vascular cells and matrix material in the intima. It often produces a fibrous cap, over a necrotic lipid core, which can weather and rupture over time. The artery is able to

compensate for some intimal thickening by expanding outwards, instead of allowing for the plaque to impede blood flow. Eventually the vessel can no longer expand outwards, and negative remodelling can occur. Blood flow is therefore disturbed through the formation of a stenosis (Shah, 2006). This may lead to ischemia and angina pectoris (Libby, 2002).

Atherosclerosis can occur in any size of artery. However, clinical manifestations frequently occur in medium and large arteries when the EC layer is breached by erosion or disruption of the fibrous cap. Disruption may occur from a thinning of the fibrous cap as there is increased lipid accumulation, inflammatory cell recruitment and matrix metalloproteinase (MMP) expression, as well as the expression of cytokines inhibiting collagen synthesis. When the plaque is opened up to the blood stream, platelets cause blood coagulation and thrombus formation. There are two possible outcomes after thrombus formation. First, the thrombus may be broken down and reabsorbed. A second outcome is that the thrombus is disrupted by the blood flow and detached from the site of injury. This embolism may then travel through the vasculature to small arteries, where it causes ischemia and potential heart attack or stroke (Libby, 2002).

The role of inflammation

Over the past two decades, it has been recognized that inflammation plays a critical role in the development and progression of atherosclerosis (Libby, 2002). Indeed, the localization of plaques to regions of disturbed blood flow (curvature, bifurcations, and branches) has been linked to an inflammatory response of ECs due to hemodynamic forces (Libby, 2002; Shah, 2006). In these areas, ECs become inflamed, causing an influx of leukocytes (Shah, 2006). It has been found that nuclear factor κB (NF-κB), a transcription factor responsible for expressing genes involved in the inflammatory cascade, is activated at sites of disturbed blood flow (Van der Heiden et al., 2010).

Additionally, monocytes, part of the family of leukocytes, are attracted into the intima through the EC layer due to the existence of a chemical gradient. During inflammation, a chemokine called monocyte chemoattractant protein-1 (MCP-1) is expressed within the intima layer (Libby, 2002). MCP-1 is expressed constitutively, by both the EC layer, and the SMCs within the intima (Schwartz et al., 1991). The receptor for MCP-1 on the monocyte (the CCR2 receptor) is attracted to the MCP-1 within the intima, and monocytes migrate into the intima through diapedesis (Libby, 2002).

Also flowing in the blood stream are low-density lipoproteins (LDL), including cholesterol. LDLs are brought across the EC layer and into the intima. Reactive oxygen species within the intima, including $\cdot OH$ and $\cdot O_2$, oxidize the LDLs, turning them into oxidized low-density lipoproteins (Ox-LDL). These Ox-LDL molecules are also responsible for stimulating ECs and SMCs to secrete additional MCP-1 (Schwartz et al., 1991).

Once inside the intima, monocytes begin to express characteristics of macrophages, activated by the presence of macrophage colony stimulating factor (M-CSF) (Libby, 2002). M-CSF also activates Ox-LDL receptors on the macrophages, turning them into scavengers for Ox-LDL (Libby et al., 2002). Macrophages begin to take up Ox-LDL, filling themselves with lipids and transforming into foam cells (Ross, 1993; Shah, 2006). The accumulation of macrophage foam cells, as well as collagen, elastin, and proteoglycans, within the intimal layer is known as the fatty streak, and is an early indication of a complex atherosclerotic lesion (Ross, 1993; Schwartz et al., 1991). It has been shown that the progression of a foam cell to a more advanced lesion may be halted or reversed, possibly through a decrease in blood LDL levels (Schwartz et al., 1991).

In advanced lesions, macrophages are unable to take up any additional Ox-LDL, and these lipid molecules begin to accumulate within the intima instead (Schwartz et al., 1991). Ox-LDLs are toxic, and begin to injure and kill ECs, SMCs, and macrophages. When the macrophage is injured, its lipid contents are released into the intimal layer, forming a lipid core, also comprised of enzymes, cytokines, and growth factors that have accumulated within the intima (Schwartz et al., 1991; Shah, 2006).
In order to protect the body from the growing necrotic lipid core within the intima, a fibrous plaque is formed over the lesion. This prevents direct contact between the accumulation of cells within the intima and blood flow. The fibrous plaque is composed primarily of collagen, elastin, and proteoglycans that were found in the original fatty streak (Ross, 1999). T lymphocytes and macrophages release MMPs, which break down the extracellular matrix within the intima, allowing for these components to be used within the fibrous plaque (Libby, 2002).

2.2 Mechanisms in leukocyte-endothelium adhesion
2.2.1 Intercellular adhesion at the arterial surface
The initiation of atherosclerosis is hypothesized to start with endothelial injury, which triggers inflammatory pathways integral to the progression of the disease (Ross et al., 1977). Leukocytes, including neutrophils and monocytes, preferentially adhere to sites of inflammation. The events that take place during leukocyte recruitment are shown in the representative drawing, Figure 1.

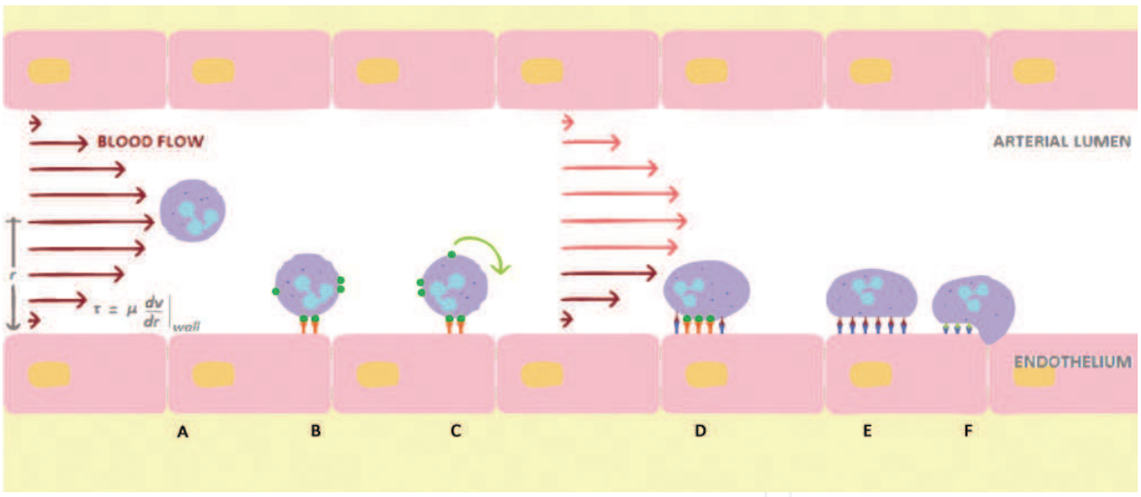


Fig. 1. Leukocyte recruitment to a site of endothelial injury. When injured, the endothelium expresses an increase in cell adhesion molecules (CAMs). (A) Leukocytes circulate within the blood stream. (B) Ligands on the leukocytes attach to selectins on the endothelium, effectively tethering the leukocyte. (C) The leukocyte begins to roll across the endothelium, reducing its velocity by forming and breaking selectin-ligand bonds. (D) Leukocytes begin to make bonds between CAMs and integrins. This firmly attaches the leukocyte to the endothelium. (E) Leukocytes migrate towards a cellular junction through CAM-integrin interactions. (F) CAM-CAM interactions allow the leukocyte to migrate through the endothelium by diapedesis.

Leukocyte tethering and rolling

Leukocytes circulating within the bloodstream must make their way to the site of injury, located on the endothelium. Although leukocytes flow in close contact with the endothelial layer, they do not stick until the inflamed endothelium starts to express adhesive molecules (Kelly et al., 2007). Tethering and rolling of leukocytes along the endothelial wall is due to a class of cell adhesion molecules (CAMs) known as selectins. There are three selectins involved: E-selectin, P-selectin, and L-selectin. Both E- and P-selectins are expressed on the endothelium; E-selectin is synthesized and expressed after endothelial stimulation, whereas P-selectin is expressed constitutively and stored, then quickly released upon stimulation (Kelly et al., 2007). L-selectin differs in that it is not expressed on the endothelium, but instead is constitutively expressed on the leukocyte surface. Both E- and P-selectin recognize carbohydrate ligands on the surface of leukocytes, while L-selectin recognizes a series of ligands expressed on the endothelium. When the selectins come in contact with their ligands they will bind, tethering the leukocyte to the endothelium (Miyasaka et al., 1997). Tethering facilitates leukocyte rolling along the EC surface. The velocity of the travelling leukocyte will be reduced as more selectin-ligand bonds form, allowing leukocyte adhesion (Kelly et al., 2007; Kubes & Kerfoot, 2001).

Leukocyte adhesion

Firm adhesion begins when integrins, another class of CAMs, are activated on the surface of leukocytes by chemoattractant cytokines, termed chemokines. Chemokines are secreted by circulating leukocytes and ECs (Kelly et al., 2007). One class of integrin responsible for neutrophil adhesion is the β_2-integrin. When activated by cytokines, certain β_2-integrins bind to intracellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2, respectively) on the EC surface, effectively adhering the leukocyte to the EC. Also activated by cytokines, $\alpha_4\beta_1$-integrin will bind with vascular cell adhesion molecule 1 (VCAM-1) (Kelly et al., 2007; Rao et al., 2007). The bound integrin-CAM complex results in firm adhesion of the leukocyte to the endothelial surface (Miyasaka et al., 1997). Although under transcriptional regulation, both adhesion molecules ICAM-1 and VCAM-1 have been shown to be upregulated at sites in the vasculature prone to developing atherosclerotic lesions (Iiyama et al., 1999).

Leukocyte migration

Once the leukocytes have firmly adhered to the endothelium, they may migrate from the lumen of the blood vessel into the subintimal space. Initially, leukocytes must make their way to the closest EC junction through a process termed locomotion. The movement of the leukocyte is made possible through interactions with leukocyte integrins and both ICAM-1 and -2 located on the endothelial surface (Schenkel et al., 2004). At the junction, the leukocytes will encounter another cell adhesion molecule, called platelet endothelial CAM 1 (PECAM-1). PECAM-1 is expressed both on the leukocyte and the endothelium. An interaction between the complementary PECAM-1 molecules allows leukocytes to migrate through the gap junction by diapedesis (Rao et al., 2007; Schenkel et al., 2004), a process also called transmigration.

2.2.2 Idealized arterial hemodynamics

Hemodynamics, the mechanics of blood flow, influence many of the physiological processes of the vascular system (Glagov et al., 1988). From an engineering perspective, blood flow through medium and small arteries (such as the right and left coronary arteries) is often

simplified by assuming steady laminar flow in a straight, rigid vessel (Ku, 1997; Nichols & O'Rourke, 1990). Additionally, blood is assumed to be a Newtonian fluid to simplify the flow dynamics to Hagen-Poiseuille flow (Nichols & O'Rourke, 1990). Such assumptions allow us to reduce the governing equations describing pressure-driven flow into a one-dimensional velocity profile in the form of Hagen-Poiseuille flow (Nichols & O'Rourke, 1990). For a cylindrical vessel model of arterial perfusion, the velocity profile as a function of a radial dimension is described by:

$$v(r) = 2\left(\frac{Q}{\pi R^2}\right)\left[1 - \left(\frac{r}{R}\right)^2\right] \quad (1)$$

where Q is the volumetric flow rate of blood and R is the hydraulic radius of the vessel.
Wall shear stress (WSS) is a tangential force per unit area of a fluid-wall interface that results from flow parallel to the vessel wall. For fluids with constant dynamic viscosity μ (Newtonian fluid), the WSS is the product of the viscosity and the shear rate γ, evaluated at the vessel wall:

$$\tau_w = \mu\gamma|_{r=R} \quad (2)$$

The wall shear rate of a fluid is the velocity gradient evaluated at the fluid-wall interface. For Hagen-Poiseuille flow through a cylindrical vessel (Equation 1), the wall shear rate is expressed as:

$$\gamma|_{r=R} = \left.\frac{dv(r)}{dr}\right|_{r=R} = \frac{4Q}{\pi R^3} \quad (3)$$

Combining Equations (2) and (3) produces an expression for WSS that is dependent on both the vessel geometry and the volumetric flow rate:

$$\tau_w = \mu\frac{4Q}{\pi R^3} \quad (4)$$

This equation is accepted as a reasonable model of the average WSS for arteries that are absent from serious geometric disturbances (Ku, 1997). Arterial WSS values range from 5 to 70 dyne/cm^2 with average WSS values of approximately 15 dyne/cm^2 being observed in coronary arteries (Glagov et al., 1988; Malek et al., 1999). Moderate levels of steady, laminar shear stress (> 10-15 dyne/cm^2) are believed to induce an atheroprotective EC phenotype while low shear stresses (< 4 dyne/cm^2) are believed to induce an atheroprone EC phenotype (Malek et al., 1999). An atheroprone EC phenotype describes one which facilitates the disease pathway marked by an increase in adhesion molecules and a decrease in vasodilators (as described in Sections 2.1 & 2.2) (Libby, 2002; Malek et al., 1999).
The fluid flow regime is determined by the dimensionless Reynolds number (Re), which represents the ratio of inertial to viscous forces. For flow in a cylindrical channel, the Reynolds Number is described by:

$$Re = \frac{\rho DU}{\mu} \quad (5)$$

where ρ is the fluid density, D is the hydraulic diameter, and U is the average fluid velocity. A Reynolds Number below 2300 indicates laminar flow that will behave predictably while a value above this threshold suggests the presence of flow disturbances. The average arterial conditions are within a laminar flow regime (Nichols & O'Rourke, 1990) and vary depending on the artery and metabolic demand (Myers et al., 2001; Nichols & O'Rourke, 1990).

Localized hemodynamics of leukocyte adhesion

The progression of leukocyte adhesion is strongly influenced by local hemodynamic forces. As the cell is passing along the wall, the torque imparted on the cell by the blood stream causes the cell to spin. As a result, the state of loose attachment with selectin-ligand bonds constantly forming and breaking has become known as cell rolling. The blood stream imposes not only torque but also shear stress on the slow moving cell. In turn, the membrane of the cell will try to distribute this stress by elongating in the direction of flow, allowing for increased binding with the vessel wall. Firrell and Lipowsky found that leukocytes rolling along rat arteriolar walls would elongate by around 140%, allowing their contact area to jump from approximately 14 μm² to 50 μm² (Firrell & Lipowsky, 1989).

Modelling of bond forces and leukocyte attachment is well documented in the literature (Cozens-Roberts et al., 1990; Evans et al., 2004; Lawrence et al., 1997; Tees & Goetz, 2003). For successful adhesion, a fine balance between the adhesive force and the hemodynamic force must be met. This adhesive force is dependent on several factors, including: the receptor density of both cells, the rate of reaction with respect to both bond formation and dissociation and the strength of the bonds and their response to strain. For instance, the bonds between E-, P-, and L-selectins and their respective ligands behave as catch-slip bonds (Lawrence et al., 1997; Marshall et al., 2003; Sarangapani et al., 2004). Though receptor-ligand bonds spontaneously dissociate (Tees & Goetz, 2003), slip bond behaviour describes an increasing probability of dissociation with increasing tensile force. Catch-slip bond behaviour, on the other hand, strengthens with increasing tensile force until some optimal force has been met. Furthermore, the rate at which this force is increased, known as the force gradient, also affects the strength of the selectin-ligand bonds (Evans et al., 2004). This is of particular interest to the study of stenotic arteries as plaque formation leads to distinct regions of varying force gradients.

2.3 Leukocyte adhesion in Parallel-Plate Flow Chambers (PPFCs)

The flow between two parallel plates has often been used to investigate the effects of blood flow on ECs and their interactions with blood components. Traditionally, parallel-plate flow chambers (PPFCs) have been used to provide an environment suited for tissue and suspension culture experiments under laminar flow. In classical PPFCs, fluid is driven through a channel formed by two narrowly separated plates in parallel. ECs are cultured on the bottom surface of the upper plate (often, a glass coverslip) while suspension cultures of leukocytes (neutrophils or monocytes) are prepared in the perfusion medium and their movement visualized within the chamber (Lawrence et al., 1987). This allows for regional or complete surface quantification of cells that are either adherent or, if observed in real-time, leukocytes that are undergoing rolling adhesion. A schematic of a PPFC is presented in Figure 2.

In a well defined PPFC, the WSS can be accurately characterised for steady, laminar flow of a Newtonian fluid as a function of a constant measurable volumetric flow rate Q:

$$\tau_w = \frac{6\mu Q}{wh^2} \tag{6}$$

where w is the width of the plate perpendicular to flow and h is the height of the interstitial gap between the two plates. For a constant volumetric flow rate, the velocity profile is parabolic and the WSS is uniform across the upper and lower plates save for the boundaries defined by the gasket (where the flow field approaches zero) and in the region of

developing flow at the inlet of the chamber (Bacabac et al., 2005; Lawrence et al., 1987). In practice, PPFCs are designed with a large *w/h* ratio allowing most of the flow field to be homogenous over the surface of the cells (Bacabac et al., 2005).

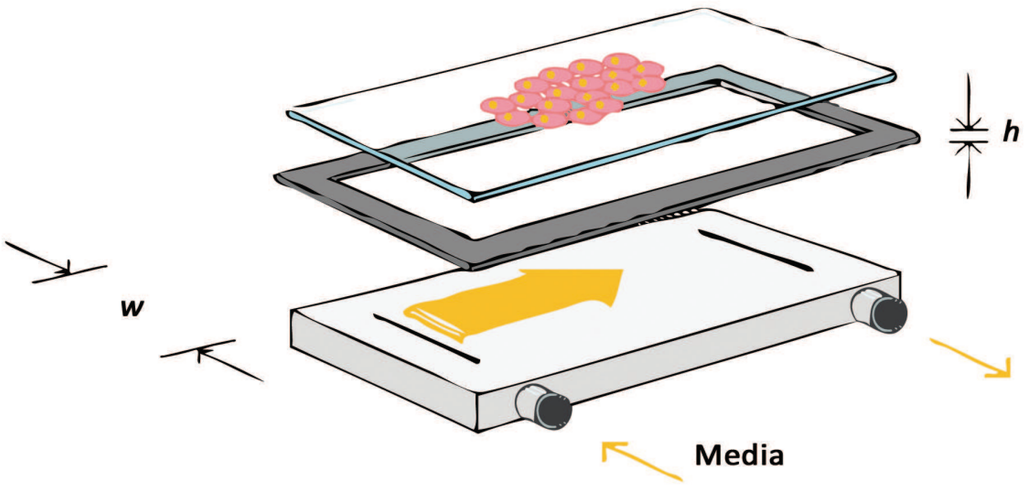


Fig. 2. Schematic of a Parallel-Plate Flow Chamber.

Studies of leukocyte adhesion using PPFCs have become the benchmark for revealing the role of shear in the adhesion pathway. Early results revealed a discreet shear dependence on both non-specific (Forrester & Lackie, 1984) and adhesion molecule-mediated adhesion (Alon et al., 1995; Finger et al., 1996; Lawrence et al., 1997). Further studies highlighted the role of endothelial dysfunction and inflammation as a precursor to adhesion when conditioned with flow (Alcaide et al., 2009; Sheikh et al., 2003; Sheikh et al., 2005). These findings complement the paradigm of leukocyte adhesion at sites of vascular inflammation; however, they do not address focal adhesion in non-uniform shear fields. This was considered by performing flow experiments using a step disturbance across the plate of the flow chamber (Burns & DePaola, 2005; Chen et al., 2006). The flow fields created by the step introduce regions of flow reversal and spatial WSS gradients to represent physiological hemodynamics (Burns & DePaola, 2005; Chen et al., 2006). Despite disturbed flow, leukocyte adhesion is increased in areas of high WSS gradients, with the highest incidence in re-attachment zones (Chen et al., 2006).

3. Asymmetric stenosis tissue culture model

3.1 Experimental methods

3.1.1 Asymmetric stenosis model design

Parallel-plate flow chambers are not ubiquitous when characterizing the role of WSS in endothelial dysfunction and leukocyte adhesion. Cone-plate viscometers (Shankaran & Neelamegham, 2001), animal models (Walpola et al., 1993, 1995) and three-dimensional (3D) tissue culture models (Hinds et al., 2001) have an increasing presence in the field.

A 3D model of an idealized coronary artery with an eccentric stenosis has been developed by our research group to reveal the effect of spatial WSS gradients on both endothelial inflammation and leukocyte adhesion (Rouleau et al., 2010a, 2010b). The eccentric stenosis

geometry with a 50% occlusion (i.e. 50% area reduction, orthogonal to flow) has been chosen to represent a clinically relevant atherosclerotic lesion (Brunette et al., 2008; Wexler et al., 1996). The model measures 10 cm in length with an internal diameter of 3.175 mm, Figure 3. Sylgard™ 184 silicone elastomer is cast and cured in PVC moulds to create semi-compliant structures that maintain their geometric integrity through the stages of sterilization and cell culture preparation.

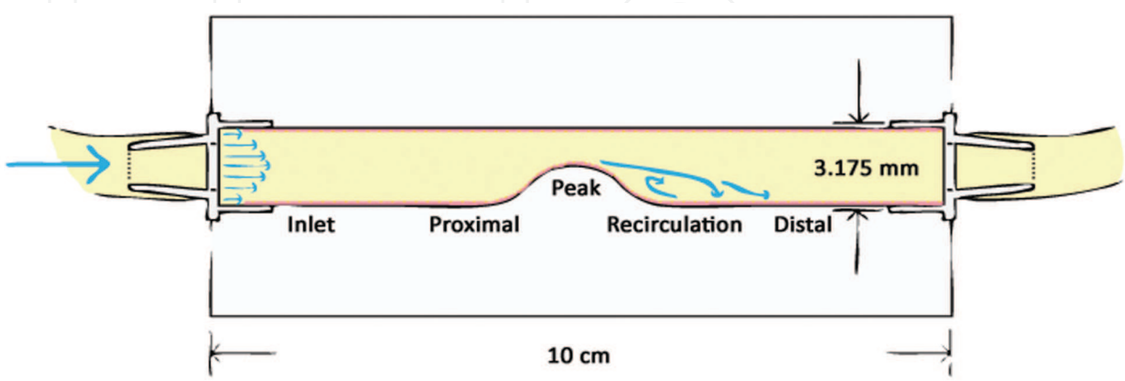


Fig. 3. Three-dimensional asymmetric stenosis model schematic with regional classifications defined using computational fluid dynamics and photochromic molecular flow visualization (Section 3.1.3).

3.1.2 Perfusion design

The perfusion flow loop consists of a media reservoir with tubing, flow dampeners, and an 8-roller peristaltic pump head with a programmable drive to produce steady or pulsatile laminar flow at the entrance of the models, Figure 4. ECs are cultured in the internal lumen of the model until they form a continuous, confluent monolayer. Inlet WSS values of 4.5, 9 and 18 dynes/cm^2 were chosen to represent moderate physiological shear in coronary arteries whereas inlet WSS values of 1.25 and 6.25 dynes/cm^2 were chosen to represent moderate to high shear for *in vitro* neutrophil adhesion. The WSS field was experimentally and numerically determined (Rouleau et al., 2010b).

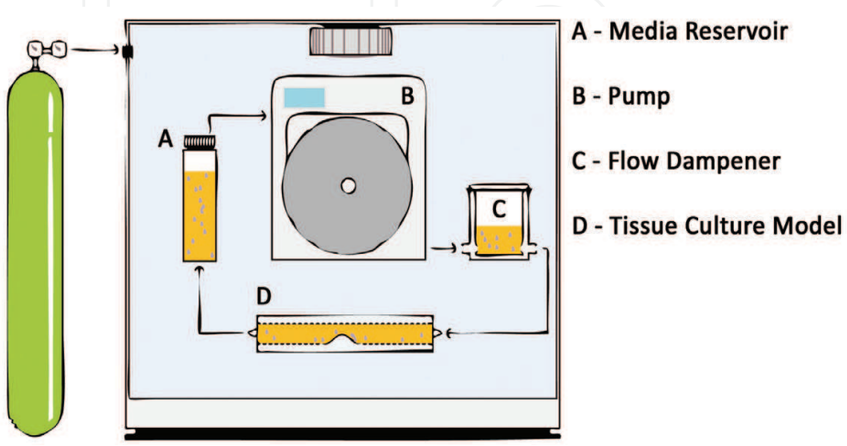


Fig. 4. Schematic of a steady-flow perfusion experiment with an asymmetric stenosis model.

3.1.3 Computational fluid dynamics and photochromic molecular flow visualization

Computational fluid dynamics (CFD) is a theoretical branch of research that relies on the power of modern computers to estimate fluid behaviour. The popularity of CFD lies in its ability to simulate physical experiments, thereby providing direction for further work or allowing for quick testing of key variables. With respect to cardiovascular flow studies, CFD is a numerical solution to a continuum of the Navier-Stokes (NS) equation. Although CFD is a powerful tool, it is only as accurate as the input data (e.g. geometry and mechanical properties). Defining the geometry and mechanics of healthy and diseased tissue is a constant endeavour in biomedical engineering (Choudhury et al., 2009; Tremblay et al., 2010). If the proper information is available, then CFD simulations are feasible, however experimental validation is still crucial. CFD has been performed for our stenosis model, yielding flow profiles at 6.25 dynes/cm^2 and 1.25 dynes/cm^2, respectively (Figures 5 & 6).

Fig. 5. Velocity profile, with an appreciable recirculation zone, in the asymmetric tissue culture model at 6.25 dynes/cm^2.

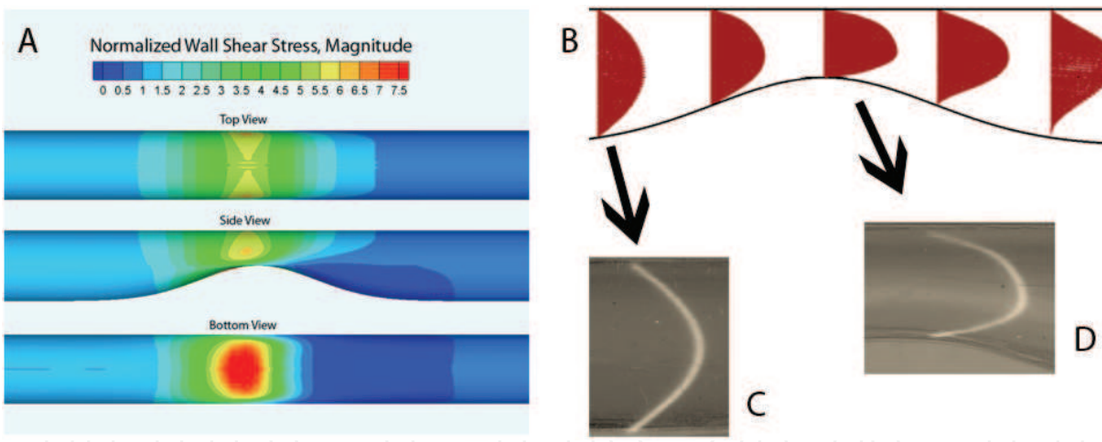


Fig. 6. Flow analysis of the asymmetric tissue culture model. (A) CFD normalized WSS contour plot. (B) Velocity profile at 1.25 dynes/cm^2. (C,D) PMFV velocity profiles at the inlet and peak, respectively.

We have used the photochromic molecular flow visualization (PMFV) technique to validate our CFD flow in the stenosis model (Ethier C.R. et al., 2000; Mahinpey et al., 2004). Using the flow profile at a given position, we are able to estimate the shear stress acting on the wall of a channel. Photochromic species reversibly change conformation when excited by a light source, such as a laser, resulting in an observable colour change. A PMFV setup includes a solution of photochromic dye, a laser, and a high-resolution camera. In practice, the photochromic dye solution is pumped through a micro-channel, the laser is triggered and the resulting pulse passes through the solution orthogonal to flow, activating any dye it contacts (Couch et al.,

1996; Park et al., 1999). At this moment, a narrow column of visible dye will appear within the solution. The solution is in motion, however, so a fraction of a second later, the excited dye will have displaced with flow. This displacement is recorded by a camera, providing a snapshot of the flow profile and subsequently, the shear stress on the opposing walls. Photochromic visualization results are presented in Figure 6 (c) & (d) to validate our CFD simulation.

3.1.4 Cellular analysis

EC morphology is often a good predictor of EC phenotype. Healthy ECs elongate in the direction of flow, whereas dysfunctional ECs may become randomly oriented and cobblestone in appearance (Dartsch & Betz, 1989). The shape index (SI) is a metric of EC morphology defined as (Nerem et al., 1981):

$$SI = \frac{4\pi \cdot Area}{Perimeter^2} \tag{7}$$

Generally, elongated cells have a lower SI than rounded cells. In concert with the SI, the angle of orientation evaluates the proportion of EC elongation relative to the direction of flow. ECs in regions of observed elongation will be narrowly distributed near 0° (i.e. in the axis of flow) while regions that appear cobblestoned will have a much wider distribution.

Protein and mRNA regulation of inflammatory markers and transcription factors defines the endothelial phenotype and relates to leukocyte adhesion and atherogenesis. Gene regulation is quantified for large cultures using Q-PCR, while the resultant protein expression is observed using Western Blotting. Regional inflammation around the stenosis can be observed using immunostaining and confocal microscopy for adhesion molecules and the translocation of inflammatory transcription factors to EC nuclei. Our analysis includes, but is not limited to, specific inflammatory markers and adhesion molecules, including: ICAM-1, VCAM-1, E-selectin and NF-κB.

3.2 Results and discussion

3.2.1 Endothelial cell morphology

The stenosis model was first used to investigate the morphological effects of shear gradients caused by the stenosis. A morphological response is one of the last measurable changes which occurs in the cascade of events following introduction of flow. As ECs experience a steady WSS, they tend to become elongated and aligned in the direction of flow, representing a healthy endothelium. When exposed to low shear magnitude or WSS spatial gradients, ECs tend to take on a more cobblestone and random morphology which is indicative of an unhealthy endothelium (Helmlinger et al., 1991; Levesque et al., 1986; Levesque & Nerem, 1985; Nerem et al., 1981; Nerem, 1993).

Perfusion experiments to evaluate EC response were run at wall shear stress values of 4.5, 9 and 18 dyne/cm^2 which corresponded to Reynold's numbers of 50, 100 and 200, respectively. It was found that at all times and inlet WSS values the shape indices in the inlet and outlet of the stenosis model were statistically similar to that of a straight model. These results demonstrate that these values can be a good reference point for morphological changes in the regions surrounding the stenosis. Furthermore, the longer the perfusion time, the more elongation was observed in the direction of flow.

Effect of wall shear stress gradients on endothelial cell morphology

The stenosis model allows the observation of the morphological response of the ECs to WSS gradients. Figure 7 shows the WSS patterns within the model as a function of position. A

positive shear gradient is found in the proximal region of the stenosis, reaching a peak WSS just upstream of the apex. A negative WSS gradient is observed in the recirculation zone of the stenosis, however, downstream of the flow reattachment point, laminar Hagen-Poiseuille flow resumes.

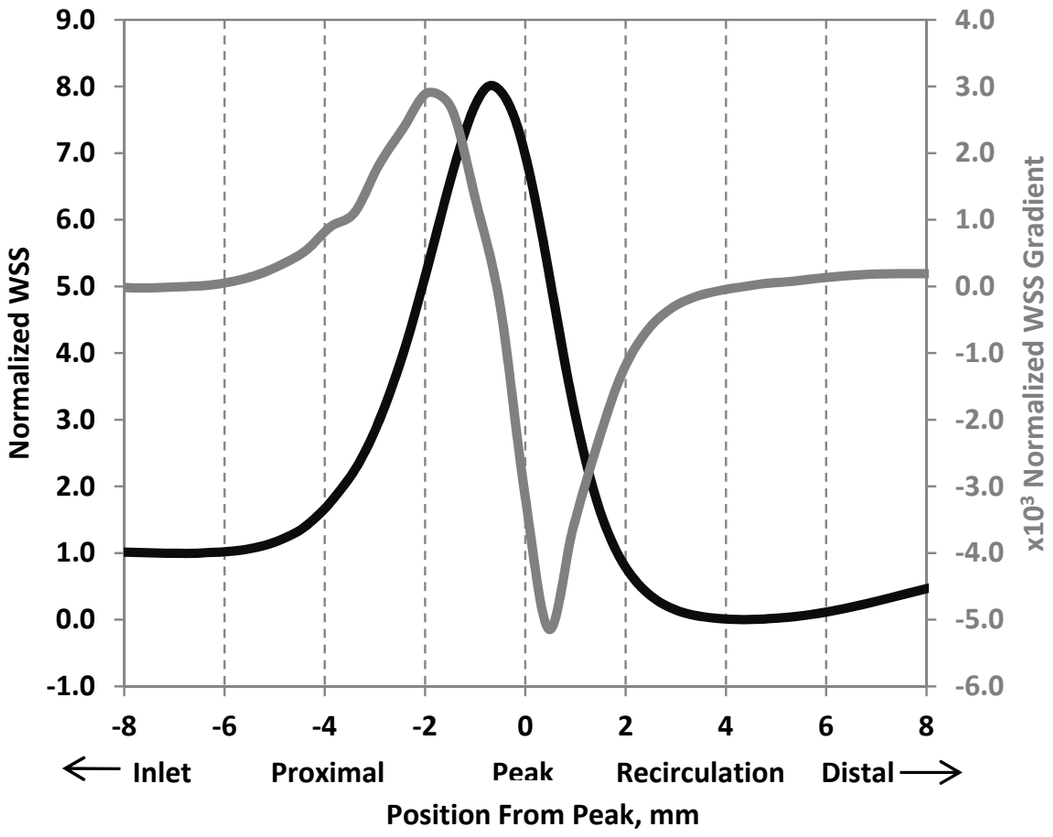


Fig. 7. CFD simulation of the WSS and WSS gradient Profile along the bottom central axis of the stenosis model (– Normalized WSS Magnitude; – Normalized WSS gradient).

It was found that after sufficient time had passed, morphological trends formed throughout the model. The inlet and outlet of the model showed similar shape indices as the straight tube controls, making them acceptable internal controls. These uniform internal controls can be compared to the other regions of the models. The deceleration, or recirculation zone (depending on the flow patterns, governed by the Re), showed the highest shape index, perhaps indicative of the most inflammatory response. The acceleration, or proximal zone, showed a slightly elevated shape index compared to the control regions, though this value was still statistically lower than that found in the deceleration zone. It was expected that the increased shear in this zone would result in more elongated ECs, however the results suggest that WSS gradients can have a more drastic effect on endothelium health than WSS magnitude alone.

It can be concluded that the deceleration zone could potentially present an inflamed endothelium and therefore one would predict to see an increase in regional expression of proteins linked to inflammation in that region. In turn, this should also lead to the largest amount of neutrophil adhesion in the recirculation zone.

3.2.2 Regional inflammation and adhesion molecule expression

WSS magnitude and duration was investigated to determine the effect these factors have on inflammatory response. Straight, cylindrical models were perfused under 4.5, 9 and 18 dyne/cm^2 inlet WSS conditions for various time periods (up to 24 hours). VCAM-1 and ICAM-1 mRNA expression decreased with increasing WSS magnitude and time, indicative of an atheroprotective phenotype. An increase in WSS magnitude resulted in a decrease in E-selectin mRNA expression; however, E-selectin expression increased from 0-12 hours, and sharply fell by 24 hours.

Inflammation was considered in the stenosis model by observing regional endothelial CAM expression using immunostaining and confocal microscopy. Perfusions with neutrophils were run at 1.25 dyne/cm^2, revealing an increase in CAM expression at the peak of the stenosis, Figure 8 (Rouleau et al., 2010a). Furthermore, perfusions were run at a higher inlet WSS of 6.25 dyne/cm^2, with no noticeable difference in regional CAM expression. These WSS values are consistent with the conditions used for neutrophil adhesion experiments.

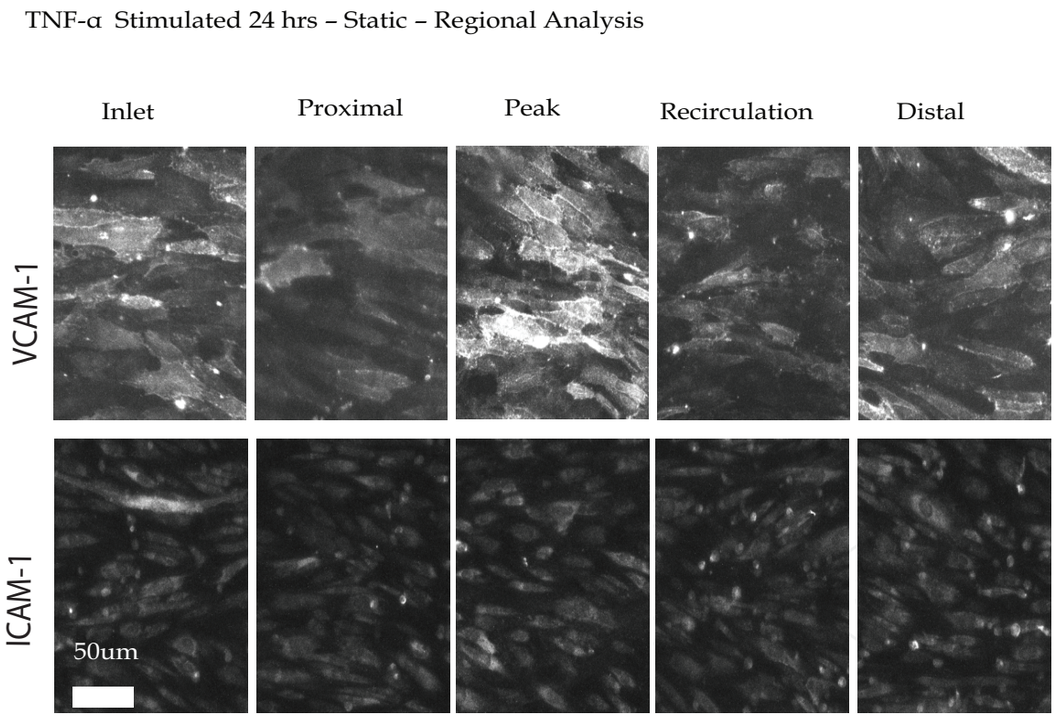


Fig. 8. Regional ICAM and VCAM expression. Copyright Springer, Annals of Biomedical Engineering, 38, 2010, pp. 2797, Neutrophil Adhesion on Endothelial Cells in a Novel Asymmetric Stenosis Model: Effect of Wall Shear Stress Gradients. Rouleau, L.; Copland, I; Tardif, J-C.; Mongrain, R. & Leask, R., Figure 6 with kind permission from Springer Science+Business Media B.V.

Using inlet WSS values of 4.5, 9 and 18 dyne/cm^2, more physiologically relevant hemodynamics were present. During these perfusions, ICAM-1, VCAM-1 and E-selectin levels were quantified and NF-κB translocation was observed to provide a robust picture of the regional inflammation around the stenosis. Similar to the experiments at 1.25 and 6.25 dyne/cm^2, there was an upregulation of ICAM-1 and VCAM-1 at the stenosis peak and in the proximal and recirculation zones. Similarly, E-selectin and NF-κB were also upregulated in these areas. It can then be concluded that there is a higher likelihood of increased neutrophil adhesion around the stenosis of the model.

3.2.3 Regional neutrophil adhesion

In perfusion experiments, it was shown that both WSS magnitude and perfusion time significantly affected the adhesion of a leukocyte cell line (NB4 cells). The trends showed that flow conditioned cells resulted in reduced adhesion of the NB4 cells for both the TNF-α stimulated and non-stimulated ECs. The two WSS magnitudes investigated, 1.25 dynes/cm^2 and 6.25 dynes/cm^2, resulted in a 3 fold and 15 fold decrease in adhesion, when compared to cells that were kept static prior to the adhesion experiments, respectively. Furthermore, experiments were run under even higher WSS conditions (12.5 dynes/cm^2) and it was found that very few cells were able to adhere to the ECs. This data demonstrates the influence of hemodynamic and attractive (ligand-receptor) forces acting on the neutrophils. The higher hemodynamic forces push the neutrophils off of the binding sites, overcoming the attractive forces which form during the adhesion of the neutrophils. For both the low (1.25 dynes/cm^2) and high (6.25 dynes/cm^2) shear stress conditions it was found that an increase in perfusion time from 1 to 6 hours resulted in an increase in adhesion, with a more noticeable increase occurring in non-stimulated ECs, potentially showing that at the shorter time point in TNF-α stimulated cells, a maximum *in vitro* adhesion is reached.

Regional neutrophil adhesion

The stenosis model presents a unique 3D environment which allowed for the investigation of the spatial differences in adhesion on and around a stenosis. Videos of the adhesion assays showed that there was a region of flow recirculation downstream of the stenosis. Immediately downstream of the separation point there was a distinct line of NB4 cell adhesion. It is postulated that this focal neutrophil adhesion was facilitated by low WSS and minimal fluid momentum caused by backflow in the recirculation zone.

For the rest of the analysis, the average adhesion was evaluated for each region of the model, Figure 9. TNF-α stimulation increased adhesion in all regions save for the stenosis peak. It was found that both the WSS magnitude and perfusion duration affected the incidence of adhesion. For example, it was found that at low inlet WSS (1.25 dyne/cm^2), ECs in the recirculation and distal regions showed a significant increase in adhesion from 1 to 6 hours.

It was found that in general, the recirculation zone tended to have the highest cell adhesion. It is hypothesized that the recirculation of NB4 cells results in a higher concentration of cells flowing along the endothelium (Rouleau et al., 2010a). Furthermore, the leukocytes have a lower momentum in the recirculation zone due to the decreased shear. This would allow for an increase in adhesion in this region. The endothelium in this location is also exposed to reduced WSS, leading to an increased inflammatory response.

The proximal and distal regions have lower incidence of adhesion than the recirculation region but more than at the stenosis peak. Interestingly, there seemed to be greater adhesion in the proximal region than the inlet of the model which may be due to a positive wall shear

gradient. As the fluid reaches the stenosis, the projected surface area decreases resulting in an increase in WSS. Hinds et al. found a similar result in their studies using monocytes (Hinds et al., 2001). Comparing these two results, it can be seen that there is increased adhesion of leukocytes to ECs in the presence of complex wall shear stress gradients.

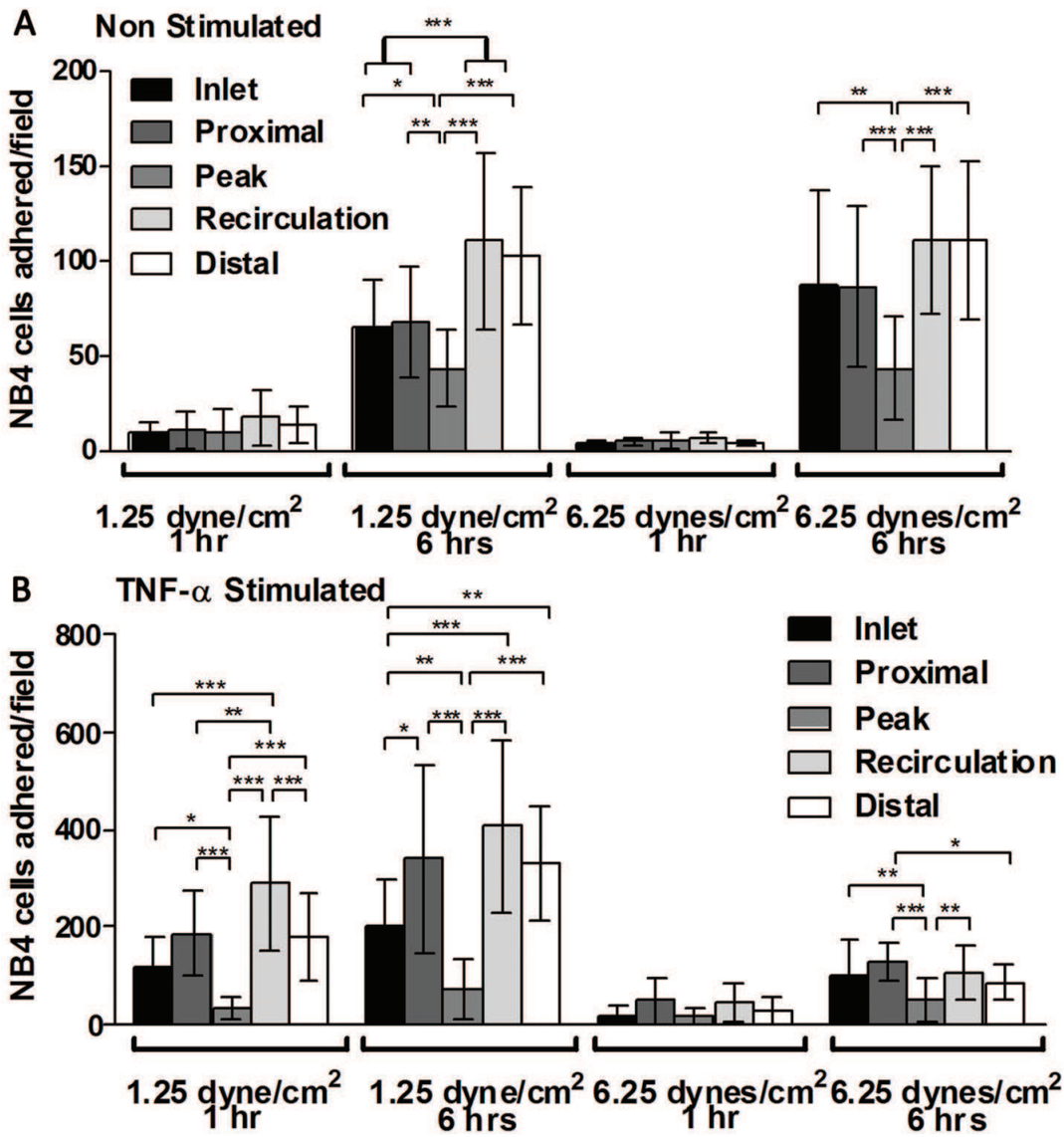


Fig. 9. Regional neutrophil (NB4) adhesion in the asymmetric tissue culture model. Copyright Springer, Annals of Biomedical Engineering, 38, 2010, pp. 2798, Neutrophil Adhesion on Endothelial Cells in a Novel Asymmetric Stenosis Model: Effect of Wall Shear Stress Gradients. Rouleau, L.; Copland, I; Tardif, J-C.; Mongrain, R. & Leask, R., Figure 7 (c) & (d) with kind permission from Springer Science+Business Media B.V.

In all instances, the stenosis peak had relatively low adhesion, which is a result of the high shear forces. By the peak of the stenosis, WSS values were appreciably larger than those found in the

inlet (Figure 7). These hemodynamic forces exceed the adhesive force needed for the neutrophils to adhere. Extending static adhesion past 1 hour resulted in little or no additional adhesion. This was likely due to a lack of adhesion sites for the NB4s to bind to. Ultimately, under any conditions, there will be a point where the endothelium becomes saturated with bound NB4s and simply cannot facilitate further adhesion, *in vitro*. Although this leaves a certain limitation on the results of the aforementioned experiments, it also demonstrates the high levels of focal adhesion which can occur as a result of endothelial inflammation.

4. Conclusions

Atherosclerosis is an inflammatory disease. *In vitro* studies of the interaction of inflammatory cells with the endothelium have advanced our understanding of the role of inflammation in atherosclerosis development and progression. Our novel three dimensional dynamic cell culture model of a coronary stenosis has shown the importance of spatial gradients in wall shear stress in EC response and leukocyte attachment. Leukocyte attachment is increased in the proximal and distal regions of the stenosis. The increased attachment occurs in regions where the ECs have an inflamed phenotype. The results suggest that the hemodynamics created by the stenosis geometry create an inflammatory response of the endothelial cells that promotes leukocyte attachment. These results help to explain disease stability in established coronary stenoses.

5. References

Lloyd-Jones, D.; Adams, R.; Brown, T.; Carnethon, M.; Dai, S.; De, S.; Ferguson, T.; Ford, E.; Furie, K.; Gillespie, C.; Go, A.; Greenlund, K.; Haase, N.; Hailpern, S.; Ho, P.; Howard, V.; Kissela, B.; Kittner, S.; Lackland, D.; Lisabeth, L.; Marelli, A.; McDermott, M.; Meigs, J.; Mozaffarian, D.; Mussolino, M.; Nichol, G.; Roger, V.; Rosamond, W.; Sacco, R.; Sorlie, P.; Roger, V.; Thom, T.; Wasserthiel-Smoller, S.; Wong, N. & Wylie-Rosett, J. (2010). Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation*, 121, 7, (February 2010), pp. (e46-e215)

Waller, B.; Orr, C.; Slack, J.; Pinkerton, C.; Van, T. & Peters, T. (1992). Anatomy, histology, and pathology of coronary arteries: a review relevant to new interventional and imaging techniques--Part I. *Clin Cardiol*, 15, 6, (June 1992), pp. (451-457)

Shah, P. (2006). Pathogenesis of Atherosclerosis, In: *Essential Cardiology,* C. Rosendorff, pp. (409-418), Humana Press, Retrieved from <http://dx.doi.org/10.1007/978-1-59259-918-9_22>

Libby, P. (2002). Inflammation in atherosclerosis. *Nature*, 420, 6917, (December 2002), pp. (868-874)

Van der Heiden, K.; Cuhlmann, S.; Luong, l.; Zakkar, M. & Evans, P. (2010). Role of nuclear factor kappaB in cardiovascular health and disease. *Clin Sci (Lond)*, 118, 10, (May 2010), pp. (593-605)

Schwartz, C.; Valente, A.; Sprague, E.; Kelley, J. & Nerem, R. (1991). The pathogenesis of atherosclerosis: an overview. *Clin Cardiol*, 14, 2 Suppl 1, (February 1991), pp. (I1-16)

Libby, P.; Ridker, P. & Maseri, A. (2002). Inflammation and Atherosclerosis. *Circulation*, 105, 9, (March 2002), pp. (1135-1143)

Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*, 362, 6423, (April 1993), pp. (801-809)

Ross, R. (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med*, 340, 2, (January 1999), pp. (115-126)

Ross, R.; Glomset, J. & Harker, L. (1977). Response to injury and atherogenesis. *Am J Pathol*, 86, 3, (March 1977), pp. (675-684)

Kelly, M.; Hwang, J. & Kubes, P. (2007). Modulating leukocyte recruitment in inflammation. *J Allergy Clin Immunol*, 120, 1, (2007), pp. (3-10)

Miyasaka, M.; Kawashima, H.; Korenaga, R. & Ando, J. (1997). Involvement of selectins in atherogenesis: a primary or secondary event? *Ann N Y Acad Sci*, 811, (April 1997), pp. (25-34)

Kubes, P. & Kerfoot, S. (2001). Leukocyte recruitment in the microcirculation: the rolling paradigm revisited. *News Physiol Sci*, 16, (April 2001), pp. (76-80)

Rao, R.; Yang, L.; Garcia-Cardena, G. & Luscinskas, F. (2007). Endothelial-dependent mechanisms of leukocyte recruitment to the vascular wall. *Circ Res*, 101, 3, (August 2007), pp. (234-247)

Iiyama, K.; Hajra, L.; Iiyama, M.; Li, H.; DiChiara, M.; Medoff, B. & Cybulsky, M. (1999). Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. *Circ Res*, 85, 2, (July 1999), pp. (199-207)

Schenkel, A.; Mamdouh, Z. & Muller, W. (2004). Locomotion of monocytes on endothelium is a critical step during extravasation. *Nat Immunol*, 5, 4, (April 2004), pp. (393-400)

Glagov, S.; Zarins, C.; Giddens, D. & Ku, D. (1988). Hemodynamics and atherosclerosis. Insights and perspectives gained from studies of human arteries. *Arch Pathol Lab Med*, 112, 10, (October 1988), pp. (1018-1031)

Nichols, W. & O'Rourke, M. (1990). McDonald's Blood Flow in Arteries; theoretical, experimental and clinical principles. Third Edition, (1990), pp. (1-456), Lea & Febiger, Philadelphia

Ku D. (1997). Blood flow in arteries. *Annual Review of Fluid Mechanics*, 29, 1, (1997), pp. (399-434)

Malek, A. M.; Alper, S. L. & Izumo, S. (1999). Hemodynamic shear stress and its role in atherosclerosis. *JAMA*, 282, 21, (December 1999), pp. (2035-2042)

Myers, J.; Moore, J.; Ojha, M.; Johnston, K. & Ethier, C. (2001). Factors influencing blood flow patterns in the human right coronary artery. *Ann Biomed Eng*, 29, 2, (February 2001), pp. (109-120)

Firrell, J. & Lipowsky, H. (1989). Leukocyte margination and deformation in mesenteric venules of rat. *Am J Physiol*, 256, 6 Pt 2, (June 1989), pp. (H1667-H1674)

Lawrence, M.; Kansas, G.; Kunkel, E. & Ley, K. (1997). Threshold levels of fluid shear promote leukocyte adhesion through selectins (CD62L,P,E). *J Cell Biol*, 136, 3, (February 1997), pp. (717-727)

Evans, E.; Leung, A.; Heinrich, V. & Zhu, C. (2004). Mechanical switching and coupling between two dissociation pathways in a P-selectin adhesion bond. *Proc Natl Acad Sci U S A*, 101, 31, (August 2004), pp. (11281-11286)

Cozens-Roberts, C.; Quinn, J. & Lauffenberger, D. (1990). Receptor-mediated adhesion phenomena. Model studies with the Radical-Flow Detachment Assay. *Biophys J*, 58, 1, (July 1990), pp. (107-125)

Tees, D. & Goetz, D. (2003). Leukocyte adhesion: an exquisite balance of hydrodynamic and molecular forces. *News Physiol Sci*, 18, (October 2003), pp. (186-190)

Marshall, B.; Long, M.; Piper, J.; Yago, T.; McEver, R. & Zhu, C. (2003). Direct observation of catch bonds involving cell-adhesion molecules. *Nature*, 423, 6936, (May 2003), pp. (190-193)

Sarangapani, K.; Yago, T.; Klopocki, A.; Lawrence, M.; Fieger, C.; Rosen, S.; McEver, R. & Zhu, C. (2004). Low force decelerates L-selectin dissociation from P-selectin

glycoprotein ligand-1 and endoglycan. *Journal of Biological Chemistry*, 279, 3, (January 2004), pp. (2291-2298)

Lawrence, M.; McIntire, L. & Eskin, S. (1987). Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion. *Blood*, 70, 5, (November 1987), pp. (1284-1290)

Bacabac, R.; Smit, T.; Cowin, S.; Van Loon, J.; Nieuwstadt, F.; Heethaar, R. & Klein-Nulend, J. (2005). Dynamic shear stress in parallel-plate flow chambers. *J Biomech*, 38, 1, (January 2005), pp. (159-167)

Forrester, J. & Lackie, J. (1984). Adhesion of neutrophil leucocytes under conditions of flow. *J Cell Sci*, 70, (August 1984), pp. (93-110)

Finger, E.; Puri, K.; Alon, R.; Lawrence, M.; von Andrian, U. & Springer, T. (1996). Adhesion through L-selectin requires a threshold hydrodynamic shear. *Nature*, 379, 6562, (January 1996), pp. (266-269)

Alon, R.; Hammer, D. & Springer, T. (1995). Lifetime of the P-selectin-carbohydrate bond and its response to tensile force in hydrodynamic flow. *Nature*, 374, 6522, (April 1995), pp. (539-542)

Sheikh, S.; Rahman, M.; Gale, Z.; Luu, N.; Stone, P.; Matharu, N.; Rainger, G. & Nash, G. (2005). Differing mechanisms of leukocyte recruitment and sensitivity to conditioning by shear stress for endothelial cells treated with tumour necrosis factor-alpha or interleukin-1beta. *Br J Pharmacol*, 145, 8, (August 2005), pp. (1052-1061)

Sheikh, S.; Rainger, G.; Gale, Z.; Rahman, M. & Nash, G. (2003). Exposure to fluid shear stress modulates the ability of endothelial cells to recruit neutrophils in response to tumor necrosis factor-alpha: a basis for local variations in vascular sensitivity to inflammation. *Blood*, 102, 8, (October 2003), pp. (2828-2834)

Alcaide, P.; Auerbach, S. & Luscinskas, F. (2009). Neutrophil Recruitment under Shear Flow: It's All about Endothelial Cell Rings and Gaps. *Microcirculation*, 16, 1, (2009), pp. (43-57)

Chen, C. N.; Chang, S.; Lee, P.; Chang, K.; Chen, L.; Usami, S.; Chien, S. & Chiu, J. (2006). Neutrophils, lymphocytes, and monocytes exhibit diverse behaviors in transendothelial and subendothelial migrations under coculture with smooth muscle cells in disturbed flow. *Blood*, 107, 5, (March 2006), pp. (1933-1942)

Burns, M. & DePaola, N. (2005). Flow-conditioned HUVECs support clustered leukocyte adhesion by coexpressing ICAM-1 and E-selectin. *AJP - Heart and Circulatory Physiology*, 288, 1, (January 2005), pp. (H194-H204)

Shankaran, H. & Neelamegham, S. (2001). Effect of secondary flow on biological experiments in the cone-plate viscometer: methods for estimating collision frequency, wall shear stress and inter-particle interactions in non-linear flow. *Biorheology*, 38, 4, (2001), pp. (275-304)

Walpola, P.; Gotlieb, A.; Cybulsky, M. & Langille, B. (1995). Expression of ICAM-1 and VCAM-1 and monocyte adherence in arteries exposed to altered shear stress. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 15, 1, (January 1995), pp. (2-10)

Walpola, P.; Gotlieb, A. & Langille, B. (1993). Monocyte adhesion and changes in endothelial cell number, morphology, and F-actin distribution elicited by low shear stress in vivo. *Am J Pathol*, 142, 5, (May 1993), pp. (1392-1400)

Hinds, M.; Park, Y.; Jones, S.; Giddens, D. & Alevriadou, B. (2001). Local hemodynamics affect monocytic cell adhesion to a three-dimensional flow model coated with E-selectin. *J Biomech*, 34, 1, (January 2001), pp. (95-103)

Rouleau, L.; Copland, I.; Tardif, J.; Mongrain, R. & Leask, R. (2010). Neutrophil adhesion on endothelial cells in a novel asymmetric stenosis model: effect of wall shear stress gradients. *Ann Biomed Eng*, 38, 9, (September 2010), pp. (2791-2804)

Rouleau, L.; Farcas, M.; Tardif, J.; Mongrain, R. & Leask, R. (2010). Endothelial cell morphologic response to asymmetric stenosis hemodynamics: effects of spatial wall shear stress gradients. *J Biomech Eng*, 132, 8, (August 2010), pp. (081013-

Brunette, J.; Mongrain, R.; Laurier, J.; Galaz, R. & Tardif, J. (2008). 3D flow study in a mildly stenotic coronary artery phantom using a whole volume PIV method. *Medical Engineering & Physics*, 30, 9, (November 2008), pp. (1193-1200)

Wexler, L.; Brundage, B.; Crouse, J.; Detrano, R.; Fuster, V.; Maddahi, J.; Rumberger, J.; Stanford, W.; White, R. & Taubert, K. (1996). Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. *Circulation*, 94, 5, (September 1996), pp. (1175-1192)

Rouleau, L.; Rossi, J. & Leask, R. (2010). Concentration and time effects of dextran exposure on endothelial cell viability, attachment, and inflammatory marker expression in vitro. *Ann Biomed Eng*, 38, 4, (April 2010), pp. (1451-1462)

Choudhury, N.; Bouchot, O.; Rouleau, L.; Tremblay, D.; Cartier, R.; Butany, J.; Mongrain, R. & Leask, R. (2009). Local mechanical and structural properties of healthy and diseased human ascending aorta tissue. *Cardiovasc Pathol*, 18, 2, (March 2009), pp. (83-91)

Tremblay, D.; Cartier, R.; Mongrain, R. & Leask, R. (2010). Regional dependency of the vascular smooth muscle cell contribution to the mechanical properties of the pig ascending aortic tissue. *J Biomech*, 43, 12, (August 2010), pp. (2448-2451)

Ethier C.; Prakash, S.; Steinman, D.; Leask, R.; Couch, G. & Ojha, M. (2000). Steady Flow Separation Patterns in a 45 Degree Junction. *J Fluid Mechanics*, 411, (2000), pp. (1-38)

Mahinpey, N.; Leask, R.; Ojha, M.; Johnston, K. & Trass, O. (2004). Experimental study on local mass transfer in a simplified bifurcation model: potential role in atherosclerosis. *Ann Biomed Eng*, 32, 11, (November 2004), pp. (1504-1518)

Couch G.; Johnston, K. & Ojha, M. (1996). Full-field flow visualization and velocity measurement with a photochromic grid method. *Meas Sci Technol*, 7, (1996), pp. (1238-1246)

Park.H.; Moore J.; Trass O. & Ojha, M. (1999). Laser photochromic velocimetry estimation of the vorticity and pressure field-two dimenstional flow in a curved vessel. *Experiments in Fluids*, 26, (1999), pp. (55-62)

Dartsch, P. & Betz, E. (1989). Response of cultured endothelial cells to mechanical stimulation. *Basic Res Cardiol*, 84, 3, (May 1989), pp. (268-281)

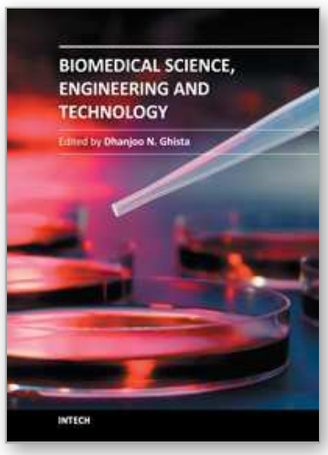
Nerem, R.; Levesque, M. & Cornhill, J. (1981). Vascular endothelial morphology as an indicator of the pattern of blood flow. *J Biomech Eng*, 103, 3, (August 1981), pp. (172-176)

Levesque, M. & Nerem, R. (1985). The elongation and orientation of cultured endothelial cells in response to shear stress. *J Biomech Eng*, 107, 4, (November 1985), pp. (341-347)

Levesque, M.; Liepsch, D.; Moravec, S. & Nerem, R. (1986). Correlation of endothelial cell shape and wall shear stress in a stenosed dog aorta. *Arteriosclerosis*, 6, 2, (March 1986), pp. (220-229)

Helmlinger, G.; Geiger, R.; Schreck, S. & Nerem, R. (1991). Effects of pulsatile flow on cultured vascular endothelial cell morphology. *J Biomech Eng*, 113, 2, (May 1991), pp. (123-131)

Nerem, R. (1993). Hemodynamics and the vascular endothelium. *J Biomech Eng*, 115, 4B, (November 1993), pp. (510-514)



Biomedical Science, Engineering and Technology

Edited by Prof. Dhanjoo N. Ghista

ISBN 978-953-307-471-9
Hard cover, 902 pages
Publisher InTech
Published online 20, January, 2012
Published in print edition January, 2012

This innovative book integrates the disciplines of biomedical science, biomedical engineering, biotechnology, physiological engineering, and hospital management technology. Herein, Biomedical science covers topics on disease pathways, models and treatment mechanisms, and the roles of red palm oil and phytomedicinal plants in reducing HIV and diabetes complications by enhancing antioxidant activity. Biomedical engineering coves topics of biomaterials (biodegradable polymers and magnetic nanomaterials), coronary stents, contact lenses, modelling of flows through tubes of varying cross-section, heart rate variability analysis of diabetic neuropathy, and EEG analysis in brain function assessment. Biotechnology covers the topics of hydrophobic interaction chromatography, protein scaffolds engineering, liposomes for construction of vaccines, induced pluripotent stem cells to fix genetic diseases by regenerative approaches, polymeric drug conjugates for improving the efficacy of anticancer drugs, and genetic modification of animals for agricultural use. Physiological engineering deals with mathematical modelling of physiological (cardiac, lung ventilation, glucose regulation) systems and formulation of indices for medical assessment (such as cardiac contractility, lung disease status, and diabetes risk). Finally, Hospital management science and technology involves the application of both biomedical engineering and industrial engineering for cost-effective operation of a hospital.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Scott Cooper, Melissa Dick, Alexander Emmott, Paul Jonak, Léonie Rouleau and Richard L. Leask (2012). In Vitro Leukocyte Adhesion in Endothelial Tissue Culture Models Under Flow, Biomedical Science, Engineering and Technology, Prof. Dhanjoo N. Ghista (Ed.), ISBN: 978-953-307-471-9, InTech, Available from: http://www.intechopen.com/books/biomedical-science-engineering-and-technology/in-vitro-leukocyte-adhesion-in-endothelial-tissue-culture-models-under-flow

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen

IntechOpen