Biomechanical characterization of the remodeling of atherosclerotic arteries and plaque rupture

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Declaration of originality

I hereby declare that the work presented in this thesis has not been submitted for a higher degree to any other university or institution. To the best of my knowledge this submission contains no material previously published or written by another person, except where due reference is stated otherwise. Any contribution made to the research by others is explicitly acknowledged.

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Declaration of contributions

All of the work presented in this thesis was solely performed by Alireza Rezvani Sharif except for the contributions that are specified below:

I. The collection of human coronary arteries and abdominal aortas was performed by Prof. Davood Kazemi-Saleh and Dr. Zahra Pourjafar.

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Publications

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Abstract

Introduction. Myocardial infarction is one of the leading causes of death in the world, resulting mostly from the sudden rupture of atherosclerotic plaques. Atherosclerotic plaques often form in specific regions within the arterial tree characterized by complex blood flow patterns. The progression of initial plaques depends on arterial wall remodeling defined as any persistent changes within the composition and size of the artery allowing adaptation to new circumstance. Plaques that are prone to rupture may often be clinically silent until the time of rupture; hence, the detection of vulnerable plaques is of great importance. It has been hypothesized that mechanical fatigue caused by pulsatile blood pressure is the main mechanism underlying atherosclerotic plaque rupture. This thesis aimed to characterise the remodeling and rupture of atherosclerotic plaques from a mechanical perspective.

Methods. For the characterization of remodeling of atherosclerotic arteries, the alteration of composition and geometry of atherosclerotic arteries were examined separately. To analyse the modification of mechanical properties of atherosclerotic lesion components with plaque development, 20 human abdominal aortas, and 20 human coronary arteries were extracted at autopsy from subjects who died mostly due to post-accident complications. The force-spectroscopy mode of the atomic force microscopy (AFM) and histological examination were used to determine the elastic moduli of specified locations within samples. To investigate the leading causes of expansive remodeling of atherosclerotic arteries, as well as its consequences, many idealised models mimicking different stages of plaque development were designed. Using fluid-solid interaction analysis, the distribution of mechanical stresses among different models was estimated and the results were compared. For the mechanical characterization of plaque rupture, the geometry of atherosclerotic coronary plaques was reconstructed from histological images. Pulsatile blood pressure was considered as the external load and stress distribution within each model was estimated using finite element method. The process of mechanical fatigue failure within atherosclerotic plaques was simulated based on fracture mechanics roles. Then, the effect of plaque morphology, mean and pulse blood pressure and lipid pool stiffness on the number of fatigue cycles required for the fracture of atherosclerotic plaques was investigated.

Results. The outcomes of the AFM test on the atherosclerotic abdominal aorta and coronary arteries indicated the high variability of Young's modulus at different locations of plaque. Fibrous cap showed a lower stiffness than the fibrous tissue beneath the lipid pool. Calcification zones and lipid pools were the stiffest and softest components of atherosclerotic lesions

respectively. With atherosclerotic plaque development, reduction of elastin lamellae stiffness, as well as stiffening of inter-lamellar zones, were detected in the medial layer of the diseased portion of the abdominal aortic wall. Moreover, the increase of media stiffness due to the build-up of fibrosis tissue and reduction of the elastic modulus of internal elastic lamina was observed in coronary arteries. Significant differences were observed between the stiffness of the medial layer in diseased parts and free-plaque segments in incomplete plaques. The results of computational modeling on the remodeling of atherosclerotic arteries showed that in atherosclerotic plaques with expansive remodeling, the level of endothelial shear stress, as well as the level wall circumferential stress in the diseased-free wall of the artery, remain approximately in the physiological range. However, higher levels of stress are induced at the shoulder and cap of plaques with expansive remodeling compared to atherosclerotic plaques which do not exhibit remodeling. With the numerical simulation of fatigue failure of atherosclerotic plaques, it was found that the required time for the plaque rupture decreased with increase of mean and pulse pressure and with reduction of lipid pool stiffness.

Conclusion. Development of atherosclerotic plaques leads to alteration of micromechanical properties of the arterial wall in both elastic and muscular arteries. Findings suggest that most atherosclerotic arteries exhibit expansive remodeling to preserve the normal level of mechanical stresses sensed by endothelial and smooth muscles cells. The increase of pulse and mean blood pressure, intensification of stiffness mismatch between plaque components, as well as the expansive remodeling of atherosclerotic arteries, can increase the risk of plaque rupture.

Key words: Atherosclerosis, Remodeling, Mechanical stresses, Numerical simulation, Atomic force microscopy

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List of Abbreviations

AFM	Atomic force microscope
AHA	American Heart Association
DWA	Diseased wall angle
ECM	Extracellular Matrix
EEL	External Elastic Lamina
ER	Expansive remodeling
ET-1	Endothelin-1
FCT	Fibrous cap thickness
FGF	Fibroblast growth factor
HS	Heparin sulfates
ICAM-1	Intercellular adhesion molecule–1
IEL	Internal Elastic Lamina
IL-1	Interleukin-1
L	Lipid pool
LDL	Low-density lipoprotein
MMP	Matrix metalloproteinase
NO	Nitric oxide
NR	No remodeling
NL	No lipid pool
PCT	Plaque cap thickness
PDGF	Platelet-derived growth factor
RI	Remodeling index
SPA	Stress phase angle

SMC	Smooth muscle Cell
SSTO	Shear stress temporal oscillations
TAWSS	Time average wall shear stress
TGF-β	Transforming growth factor beta
2D	Two-dimensional
3D	Three-dimensional
VCAM-1	Vascular cell adhesion molecule–1
WSS	Wall shear stress
WSSAD	Wall shear stress angle deviation
WSSTG	Wall shear stress temporal gradient
WTS	Wall tensile stress

Chapter 1

Introduction

1.1 The circulatory system

The human circulatory system is distributed all over the body and consists of the heart, arteries, veins and the microcirculatory system. The main functions of the circulatory system are the carrying of blood throughout the body, transfer of oxygen and nutrients to the cells and the removal of waste products of cellular metabolism. The systemic circulation starts when the left ventricle of the heart pumps oxygenated blood to the aorta through the aortic valve. The aorta extends upward to form an arch then it continues downward and after passing through the diaphragm it extends down to the abdomen where it splits into common iliac arteries. Different branches of the aorta spread oxygenated blood through the body. For instance, the coronary arteries provide blood supply to the heart muscle itself. The arteries branch into smaller blood vessels called arterioles. Arterioles subdivide further into the smallest vessels called capillaries which are the vessels that reach different types of cells in the human body. Capillaries play the key role in the maximum transmission of ingredients carried within the blood to the different organs throughout their extremely thin wall which allow oxygen and nutrients diffuse to cells. Venules are very small blood vessels which remove the deoxygenated blood and unwanted chemicals from the cells and conduct them into the veins which are responsible for transporting blood toward the right arteriole of the heart. The deoxygenated blood flows through the tricuspid valve into the right ventricle where it is pumped toward the lungs to receive oxygen. The oxygenated blood returns to the left atrium of the heart through the pulmonary veins and pass through the mitral valve to the left ventricle to complete the circulatory system (1, 2).

1.2 Blood vessels

1.2.1 Arterial wall structure

The healthy arterial wall consists of three layers of intima, media, and adventitia. The adventitia, the outermost layer of the artery, principally consists of a dense network of collagen fibers (type I), elastin, fibroblasts, fibrocytes, and nerves. This layer acts as a protective sheath, provides structures for the underneath layers and preserves the vessel from overstretching and rupture at higher pressures (3).

The medial layer, the thickest layer of the artery, mainly consists of smooth muscle cells (SMCs), collagen fibers (types I and III) and elastin. Internal elastic lamina (IEL) and external elastic lamina (EEL) are thin layers of elastin fibers which separate the medial layer from intima and adventitia respectively. SMCs within the medial layer are responsible for the synthesis of all extracellular matrix (ECM) constituents. Considering the arrangement of the SMCs in the medial layer, arteries can be divided into elastic and muscular. Elastic arteries have relatively large diameters and can be found close to the heart such as the aorta, common carotid arteries, common iliac arteries, and the main pulmonary artery. The medial layer of elastic arteries has a composite-like lamellar structure which is made by the repetition of two sets of sublayers: elastin sheets which are organized in rings around the lumen and interlamellar layers containing collagen bundles, SMCs, and proteoglycans. Elastin fibers take up the force at the beginning of loading and play the key role in the elastic behaviour of the elastic arteries in response to the variation in blood pressure over the cardiac cycle. By increasing the blood pressure, collagen fibers which are stiffer engage as load-bearing fibers to support the integrity of the artery tissue (4, 5). Muscular arteries such as femoral, celiac and coronary arteries deliver blood to the specific organs of the body. The medial layer of muscular arteries contains several layers of SMC, embedded in a loose connective tissue matrix. The high amount of SMCs in muscular arteries enables them to actively constrict and relax. The modification from elastic arteries to muscular arteries take place gradually and the corresponding artery segments lose their lamellar structure progressively as occurs in carotid arteries after the bifurcation (6).

The intima, the innermost layer of the blood vessel consists of a single layer of endothelial cells, called endothelium, laying on a thin basal membrane (basal lamina) which consists mainly of collagen type IV. There is also a subendothelial layer including some collagenous bundles, elastic fibrils, and SMCs (3, 7). Endothelial cells tend to be elongated in the direction of the blood flow and act as a semipermeable membrane for nutrients and chemical signals. In healthy arteries, the intima is very thin and it does not have a significant contribution to the mechanical behaviour of the arterial wall (3).

1.2.2 Mechanical stresses and the arterial wall

The haemodynamic conditions inside the blood vessels create two major mechanical stresses: wall shear stress and wall circumferential stress.

Wall Shear stress

Wall shear stress (WSS) is the tangential stress derived from the friction of the blood flow on the endothelial surface of the arterial wall. WSS is proportional to the product of the blood viscosity and the spatial gradient of blood velocity (shear rate) at the arterial wall as follows:

$$\tau=\mu\times\frac{dv}{dy} \quad (1\text{-}1)$$

in which, τ is wall shear stress, μ is blood viscosity, dv represents changes in flow velocity and dy stands for changes in the unit of radial distance from the wall (8). The pulsatile nature of blood flow, as well as the particular shape of blood vessels, result in very complex blood flow pattern within the arteries. For instance, the blood velocity is slower along the inside wall of the curvature compared to the outer wall of curvature. Also, in the vessel bifurcations, blood flow near the flow divider is greater than near the outer wall. As a consequence, the wall shear stress pattern varies not only along the artery length but also around the artery circumference. The wall shear stress pattern also alters continuously with the cardiac cycle due to the pulsatile changes in the rate and direction of blood flow (9).

Wall tensile stress

Wall tensile stress (WTS) is caused by pulsatile blood flow which produces strain on the vessel wall in a direction perpendicular to the endoluminal surface. Arterial wall tension is defined as the force per unit length of the vessel. According to the Laplace's law, arterial wall tension is proportional to the blood pressure and the vessel radius as follows:

$$T = P \times r \quad (1\text{-}2)$$

where T is arterial wall tension, P stands for blood (transmural) pressure and r is the radius of the artery. WTS is defined as a tension per unit of arterial wall thickness (10). If the artery is considered as a thin and homogenous cylinder, WTS can be calculated as below:

$$\sigma_\theta=\frac{P\times r}{t} \quad (1\text{-}3)$$

In this equation, σ_θ is WTS, P represents internal pressure, r and t respectively stand for the radius of the artery and the arterial wall thickness at P. This equation is derived by assuming the arterial wall as a thin and homogenous cylinder in which the stress is relatively constant through the wall thickness (10, 11). However, when the ratio of vessel radius to the vessel thickness is not large enough, the artery is like a thick cylinder and the stress variation from

the inner wall to the outer wall can be significant. In this case, the circumferential stress can be calculated based on Lame's equation as follows:

$$\sigma_\theta = \frac{P \times r_i^2}{r_o^2 - r_i^2}\left(1+\frac{r_o^2}{r^2}\right) \quad (1\text{-}4)$$

in which σ_θ is WTS, P represents internal pressure, r and t are respectively the radius of the artery and the arterial wall thickness. Based on this equation, the circumferential stress can be calculated at the inner surface ($r = r_i$) and the outer surface of the arterial wall ($r = r_o$). The results show that the circumferential stress is maximum at the inner surface of the arterial wall and minimum at the outer surface of the arterial wall (12).

1.2.3 Mechanical characteristics of the arterial wall

Arteries are not rigid pipes and have remarkable mechanical characteristics which are essential for their optimal function.

Incompressibility

Experimental measurement of the arterial wall compressibility shows that the volume of the artery under the physiologic range of pressure is preserved. Consequently, arterial tissue can be considered incompressible. This feature can be related to the high percentage of water content in the arterial wall (13).

Residual stresses

One of the salient features of the arterial wall is the existence of residual stresses in the unloaded vessel. This characteristic of the arterial wall was reported firstly by Fung and Vaishnav who showed that the arterial ring opens when it is cut radially (14, 15). This observation showed that due to the existence of residual stresses in the arterial wall even after depressurizing, the luminal part of the artery is under compression, while the external part is under tension (16). Considering the fact that the circumferential stress level at the inner surface of the arterial wall is higher than the outer surface, it has been proposed that residual stresses homogenize the stress distribution within the arterial wall in vivo (17).

It has been hypothesized that the residual stresses are created by the different growth of the inner part of the arterial wall with respect to the outer wall (18). Further studies showed that even the lamellar unit in the aorta is subject to residual stresses at the microscopic level (19, 20). The most popular technique for the quantification of the residual stresses in blood vessels

is based on opening angle measurement. In this method, a ring of the blood vessel is radially cut and the opened angle of the resulting circular section is measured and the equivalent residual stresses are calculated (16, 21).

Anisotropy

Anisotropy is one of the fundamental characteristics of the artery tissue, characterized by different mechanical behavior in the circumferential and axial directions. Animal and human experiments indicated that both the media and adventitia layer of arteries represents anisotropic behavior (22, 23).

Viscoelasticity

Like most soft tissues, arteries have a certain degree of viscoelasticity (24, 25). The viscoelastic behavior of the arterial wall can be characterized through different ways such as stress relaxation in response to constant deformations, creep, frequency-dependency of strength and the hysteresis they display under cyclic loads (25, 26).

Non-linear stress-strain relationship

Arteries display a highly nonlinear stress-strain response during both loading and unloading. The stiffness of arteries progressively increases with elevating applied loads. This feature can be related to the recruitment of collagen fibers in the higher pressures (22, 26).

1.2.4 Arterial wall remodeling

Arterial wall remodeling defined as any persistent changes within the composition or size of the artery allowing adaptation to new circumstances (27, 28). The processes involved in the arterial remodeling include regulation of vascular cell migration, mitosis and apoptosis rates, as well as control of matrix synthesis, deposition, and degradation (29). In normal arteries, remodeling is a homeostatic response to long-term changes in hemodynamic conditions and involves production of mediators which affect the structure and function of the artery (30). The main mediators are matrix-producing cells, primarily SMCs and matrix degrading proteases, predominantly matrix metalloproteinases (MMPs) (8). MMPs are endopeptidases produced by SMCs and macrophages and their activity is required for the breakdown of the ECM and reorganisation of the artery (31)

There are hypotheses that mechanical stresses can regulate arterial wall remodeling through stimulating MMPs expression and activation, as well as evoking migration, differentiation, and proliferation of SMCs (27, 32). It is believed that arterial wall remodeling occurs in a way that the shear stress sensed by endothelial cells and the circumferential stress induced in the arterial wall remains constant. Moreover, it has been shown that the arrangement of collagen and elastin fibers in the blood vessel may be affected by the induced stresses (33).

1.3 Cardiovascular diseases and atherosclerosis

Cardiovascular diseases including hypertension, coronary heart disease, heart failure, and stroke are the main cause of morbidity and mortality in the world (34). Cardiovascular diseases are mainly caused by atherosclerosis (35), a common disorder of the arteries, characterized by the accumulation of cells, lipid, connective tissue, calcium, and cellular waste products inside the intimal layer of the arterial wall. Atherosclerosis appears most often in specific sites within the circulatory system such as abdominal aorta, coronary carotid, iliac, femoral, and cerebral arteries (36). Risk factors for atherosclerosis include elevated cholesterol, cigarette smoking, diabetes mellitus, family history, hypertension, and obesity (37).

The atherosclerotic process starts early in life and advances throughout adulthood. The evolution and progression of early plaques depend on arterial wall remodeling. Plaques can gradually narrow arterial lumen size, resulting in the reduction of blood supply to the downstream arteries. They may also trigger the signs of ischemia during the periods of increased oxygen demand as in exercise. Although the atherosclerotic plaque development was thought to always lead to gradual luminal narrowing, it has been shown that most coronary arteries enlarge in response to atherosclerotic plaque development in a way that the luminal area is preserved. The expansion of atherosclerotic arteries has been observed until the atherosclerotic lesion occupies 40% of the internal elastic lamina area. The maintenance of luminal area in atherosclerotic arteries may prevent signs of ischemia in patients. The concept of arterial wall enlargement in response to local atherosclerotic plaque formation is known as the expansive (outward) remodeling of atherosclerotic arteries (38).

As the disease develops, atherosclerotic plaques may cause clinical syndromes in two different ways: plaque rupture and plaque erosion. Plaque rupture most often occurs at the fibrous cap of lipid-rich plaques which acts as a protective shield for the underlying lipid pool. The rupture of atherosclerotic plaques, can expose highly thrombogenic materials to the

circulation and eventually induces thrombus formation inside the lumen leading to the complete blockage of the artery (39). Plaque erosion is characterized by the absence of or disrupted endothelium and may cause thrombus formation on the lumen surface. Compared to plaque rupture, plaque erosion is a weaker thrombogenic stimulus and frequently observed in high stenotic arteries (40, 41). Plaque disruption has been reported to be more common than plaque erosion by a ratio on the order of 3 to 1 (42) and it is the leading cause of more than 60% of strokes and 70% of myocardial infarctions (43).

1.3.1 Determination of plaque vulnerability

The precise mechanisms of plaque rupture still remain unclear. Identifying the vulnerable plaques is a key factor in planning preventive measures (44). Measurement of the luminal stenosis by angiography is a conventional method to evaluate the severity of atherosclerotic plaques. However, it has been shown that the determination of luminal stenosis cannot be adequate for demonstrating the vulnerability of atherosclerotic plaques and most of the ruptures occur in the lesions with low-grade stenosis degree. Statistics show that more than 80% of plaque rupture occurs in atherosclerotic lesions with luminal stenosis of less than 70% (43). Hence, for the evaluation of plaque instability, other parameters such as plaque morphology and composition should be considered in addition to luminal stenosis (45).

At present, the contribution of some plaque features such as large lipid core, thin fibrous cap, calcification, high content of inflammatory cells, accumulation of macrophages, intra-plaque haemorrhage, ECM degradation, and expansive remodeling have been identified as risk factors for plaque vulnerability (44, 46, 47). Considering the morphological and structural features of atherosclerotic plaques, they can be classified into two groups of stable and vulnerable plaques. Stables plaques are characterized by a thick fibrous cap, small lipid core and the abundance of SMCs. Whereas, vulnerable plaques are distinguished by a thin fibrous cap, large lipid pool, reduced fibrous cap SMCs, increased SMCs apoptosis and abundance of inflammatory cells such as macrophages (41, 44, 48).

1.3.2 Mechanical stress analysis

From a biomechanical point of view, it is believed that WSS and WTS play an important role in the formation, progression, and rupture of atherosclerotic plaques (49). For instance, it

has been hypothesized that mechanical fatigue caused by pulsatile blood pressure is the main mechanism underlying atherosclerotic plaque rupture and the mechanical stress distribution within atherosclerotic plaques can be used for the assessment of plaque vulnerability (50, 51). Great effort has been made to calculate mechanical stresses within the atherosclerotic plaques and link them to the atherosclerosis disease. Finite element method (FEM) and computational fluid dynamics (CFD) have been employed respectively for the structural analysis and simulation of blood flow within atherosclerotic arteries (52, 53). Moreover, by considering the continuous interaction between blood flow and arterial wall structure, fluid-structure interaction (FSI) modeling has been used for more accurate determination of mechanical stresses distribution within atherosclerotic arteries. In FSI modeling the effect of arterial wall deformation and subsequent geometry adaptation on the blood flow pattern is considered through an iterative process (54, 55).

To reconstruct the geometry of atherosclerotic plaque, histological examination as a gold standard for the determination of plaque composition (56), as well as different medical imaging modalities such as magnetic resonance imaging (MRI) (54, 57), computed tomography (CT) (58), micro-computed tomography (μCT) (59), intravascular ultrasound (IVUS) (60) and optical coherence tomography (OCT) (61) have been employed.

In addition to geometric features, the accuracy of stress analysis depends on the mechanical properties of the arterial wall and plaque tissue. Richardson et al proposed that the lack of sufficient data on the stiffness of arterial wall and atherosclerotic plaque components is possibly the most uncertain aspect in the study of plaque rupture (62). Different techniques such as tensile test (23, 63, 64), inflation test (65), and indentation test (66-69) have been used for the mechanical characterization of atherosclerotic arteries. However, there are still few data on the mechanical properties of different layers of arteries and plaque components.

1.4 Organization of the thesis

The main objective of this project is to analyze the remodeling and rupture of atherosclerotic plaques from a mechanical perspective.

Chapter 2 provides a literature review on the role of mechanical stresses on the formation, progression, remodeling, and rupture of atherosclerotic plaques. Moreover, this chapter discusses different techniques used for mechanical characterization of atherosclerotic arteries.

Chapter 3 presents precise data relevant to the mechanical characterization of the human atherosclerotic abdominal aorta with atherosclerosis disease progression regarding the detailed structure of the plaque and arterial lamellar structure. In human abdominal aorta as an elastic artery, the stiffness of elastin lamellae and inter-lamellar zones, as well as different plaque constituents and how they change during disease progression, are reported using AFM indentation.

Chapter 4 investigates the alteration of mechanical properties of the arterial wall and atherosclerotic plaque components in human coronary arteries with atherosclerotic plaque development. In human coronary artery as a muscular artery, the stiffness of the medial layer, internal elastic lamina, and external elastic lamina, as well as different plaque components, and their change during atherosclerotic plaque development are reported using AFM indentation test.

Chapter 5 describes the effect of arterial wall remodeling, fibrous cap thickness, and disease wall angle on the mechanical stresses distribution within the arterial wall and atherosclerotic plaque. For this purpose, many idealized models mimicking different stages of atherosclerotic plaque development are designed and the level of wall circumferential stress and wall shear stress among different models are compared.

Chapter 6 examines the influential parameters on rupture of atherosclerotic plaques. Patient-specific coronary plaque geometries are generated from histological images and used for computational stress analysis. Fatigue analysis and crack propagation modeling are performed to investigate the effect of different geometries, pulse and mean pressure, as well as lipid pool stiffness, on the fatigue life of atherosclerotic plaques.

Chapter 7 represents the conclusion, limitations and future directions of this thesis.

Chapter 2

Literature Review

2.1 Formation and progression of atherosclerotic plaques

Although the precise mechanism of plaque initiation is not clearly understood, it is well known that the abnormal function of endothelial cells is the key factor in the initiation and progression of atherosclerotic plaques (70). It is believed that the accumulation of low-density lipoprotein (LDL) in the subendothelial space is the main culprit in the formation of atherosclerotic plaque (71). LDLs may be modified by oxidation and aggregation and act as chronic stimulators for the adaptive immune response (72). In this time, endothelial cells become activated from the injury and begin to secrete chemokines in the luminal surface and express adhesion factors such as intercellular adhesion molecule–1 (ICAM-1), vascular cell adhesion molecule–1 (VCAM-1) and selections. These adhesion molecules bind integrins with receptors on monocytes and T-cells from the circulation and facilitate their migration and adhesion into the intima and stimulate their differentiation into macrophages and dendritic cells (40). The macrophages and dendritic cells which have membrane-bound lipid droplets in their cytoplasm and ingest oxidized LDL are called foam cells (71). The next step in the atherosclerotic process is fatty streak formation which is primarily composed of foam cells and can be considered as infancy lesions (46). Gradually, isolated small lipid pools appear beneath the layers of foam cells and develop into confluent necrotic cores through the invasion of macrophages. This process is accelerated by the apoptosis and necrosis of macrophages and SMCs in the plaque region (40).

The continued inflammation will cause the further progression of the atherosclerotic lesion. Growth factors such as platelet-derived growth factors (PDGFs), thrombin, fibroblast growth factor (FGFs), interleukin-1(IL-1) and endothelin-1(ET-1) as well as inhibitors such as heparin sulfates (HS), nitric oxide (NO) and transforming growth factor beta (TGF)-β produced by macrophage foam cells and endothelial cells stimulate SMCs phenotype switching from contractile to synthetic state (48, 73, 74). Contractile to synthetic phenotypic modulation in SMCs links with the capability of SMCs to become proliferated and migrate from the medial layer to the intima. Synthetic SMCs can synthesize collagen fibers up to 25 times more than contractile SMCs (75). The continuous synthesis and organisation of the ECM in the atherosclerotic lesions by SMCs, leads to the formation of a thick and strong fibrous cap which covers the thrombogenic lipid pool. Macrophages and SMCs in the plaque region also secrete enzymes, such as MMPs for degrading elastin and collagen fibers to facilitate the migration of macrophages and SMCs towards the atherosclerotic lesion (71). In advanced atherosclerotic

lesions, ECM and necrotic core materials may act as a nidus for calcium deposits. Calcification can increase over time, leading to the formation of a large calcified region with the plaque (40).

2.1.1 Mechanical stresses and atherosclerotic plaque development

It is well known that atherosclerotic plaques often form in regions characterized by complex blood flow patterns such as the outer wall of bifurcations, and the inner wall of curvatures (76, 77). Different hemodynamic parameters at the plaque region have been proposed to be involved in plaque formation and progression. It has been shown that low and oscillatory wall shear stress affect the biochemistry and permeability of endothelial cells, leading to the atherosclerotic plaque formation (78, 79). Previous studies have shown that the distribution of early atheroma in the blood vessels is coincident with regions with low shear stress; while, plaque development is inhibited or retarded in regions with relatively high wall shear stress (70, 80). Moreover, it has been revealed that plaque progression often occurs in the downstream part of the plaque due to the induction of blood flow recirculation in this region (81, 82). Taylor et al noted that regions with low time average wall shear stress (TAWSS) and high shear stress temporal oscillations (SSTO) are predisposed to the development of plaque (83). Other hemodynamic factors such as wall shear stress angle deviation (WSSAD) and wall shear stress temporal gradient (WSSTG) have been introduced as important parameters in atherogenesis (84, 85).

Some investigators sought a relationship between WTS and susceptible regions for atherosclerotic plaque formation. Thubrikar et al conducted an animal study and showed that by the reduction of intramural stresses, atherosclerotic plaque development in rabbit carotid arteries is inhibited (86). They also quantified WTS in bovine coronary arteries and suggested that the stress concentration at the arterial branch can injure the artery and make this region prone to atherosclerosis (87). In another study, this group showed that the morphology of endothelial cells at arterial branch sites is different from unbranched reigns due to the induction of higher level of circumferential stress at this region. They showed that with the reduction of stress level, the morphology of branch endothelial cells modifies and becomes similar to that of other regions (88). Finally, they concluded that high WTS caused by blood pressure is the main factor responsible for atherogenesis (89). Kaazempur-Morfrad et al performed stress analysis and histological examination on the human carotid bifurcation and found that early inflammation and atherogenesis often occurs at the regions with highest variations in cyclic

strain (90). This group also showed that the hemodynamic parameters influencing atherogenesis such as WSS, OSI, and cyclic strain may be dissimilar among different subjects (91).

Several studies took into account the simultaneous effect of WSS and WTS on atherogenesis. Based on MRI- based models of healthy arteries, it has been proposed that atherosclerotic plaque formation and development often occur in certain regions with low wall shear stress and high tensile stress (92). Tarbell's group defined stress phase angle (SPA) as the phase angle between WTS and WSS and found that regions that are predominantly susceptible to atherosclerotic plaque development such as the outer wall of the carotid sinus can be characterized by large negative values of SPA (93). They also showed that large negative SPA has a pathologic effect on the NO production by endothelial cells which is a critical component of normal vascular tone and has anti-inflammatory properties (94).

Using different technical approaches, several investigators examined the effect of mechanical stresses on the protein expression by endothelial cells and SMCs *in vitro* and attempted to link their findings to the formation and progression of atherosclerosis disease. It has been demonstrated that low shear stress attenuates NO production by endothelial cells, stimulates uptake and synthesis of LDL, prompts inflammation, and induces oxidative stress (8). Moreover, low shear stress can stimulate SMCs proliferation and migration from media to intima through PDGF-β released by endothelial cells (95). Mechanical strain can also play a critical role in the phenotype switching of the SMCs. The imposition of the physiological level of mechanical stretch that is required to maintain the contractile phenotype of SMCs and the absence of strain leads to the development of proliferative phenotype of SMCs (96, 97).

Mechanical stresses can also influence the distribution of different cell types in atherosclerotic plaques. It has been proposed that due to the existence of low shear stress in the downstream part of the plaques, a higher number of SMCs migrate into this region, contributing to the plaque progression at the distal part of the plaque. Moreover, it has been suggested that the accumulation of a higher number of macrophages at the upstream areas of the plaque can be related to the induction of high shear stress in this region (98). FSI models have been used to investigate the correlation between hemodynamic parameters and histological markers of atherogenesis in the carotid bifurcation. The results revealed that the WSSTG and WSS negatively correlate with macrophage accumulation and lipid deposition and positively with the content of collagen fibers and SMCs. Moreover, a positive relationship between OSI with

macrophage accumulation, lipid deposition and the reduction of the amount of collagen fibers and SMCs was found (99).

2.1.2 Classification of atherosclerotic plaque

Based on morphological features of atherosclerotic plaques in the process of disease progression, a classification scheme has been proposed by the American Heart Association (AHA) representing the sequence of plaque development. In this classification, atherosclerotic plaques are divided into eight groups and labelled by Roman numbers. Early atherosclerotic plaques (type I lesions) are identified by the accumulation of lipoproteins, the increase of intimal macrophages, and the formation of dispersed foam cells. Type II lesions include fatty streaks containing layers of macrophage foam cells and lipid-laden SMCs and droplets of extracellular lipid. In preatheroma or intermediate lesions (type III lesions), pools of extracellular lipid are present between the SMCs and form the main component of the thickened intima. Type IV lesions, known as atheroma, are characterized by the presence of a lipid pool made by free cholesterol esters and surrounded by foam cells. Type V lesions or fibro-atheroma are distinguished by the excessive high amounts of SMCs and fibrous tissue. They usually consist of one or more lipid cores of unequal sizes surrounded by thick layers of fibrous tissue and might contain organized thrombi. Type VI lesions, also called ruptured lesions, are identified by large lipid core (>40% by area), thin fibrous cap (<65 μm) and extensive inflammation at plaque shoulders. These lesions may also contain a surface defect, intra-plaque haemorrhage, and thrombus. Advanced plaques might progress into calcified lesions (type VII) or fibrotic lesions (type VIII). In calcified lesions, large calcifications are within the intimal layer in addition to fibrous tissue and in some cases lipid deposits. Whereas, fibrotic lesions are characterized by the presence of high amount of fibrous connective tissue and lack of lipid core (100, 101).

Based on the morphological features of atherosclerotic lesions such as the fibrous cap thickness, the association between extracellular lipid accumulation and the fibrous cap formation, and the existence of thrombosis, an alternative, and simpler classification, was later defined by Virmani et al. In this category, atherosclerotic plaques were classified into seven groups. Intimal thickening is distinguished with the accumulation of SMCs in the intimal layer in the absence of lipid droplets and macrophage foam cells. Intimal xanthoma or fatty streak is characterized by the focal accumulation of foam cells without a necrotic core or fibrous cap.

Plaques with pathological intimal thickening are characterized by the existence of SMCs in the proteoglycan-rich matrix with areas of extracellular lipid accumulation without necrosis. Fibrous cap atheroma is defined by a well-formed necrotic core with an overlying fibrous cap. Thin fibrous cap atheroma is specified with a thin fibrous cap infiltrated by macrophages and lymphocytes with rare SMCs and an underlying necrotic core which may contain intraplaque haemorrhage. Calcified nodule refers to the lesions with fibrous cap disruption and thrombi associated with eruptive nodular calcification. Finally, fibrocalcific plaques or collagen-rich plaques are described with thick fibrous cap, large areas of calcification and a small necrotic core with few inflammatory cells (45).

2.2 Arterial wall remodeling

2.2.1 Arterial wall adaptation to mechanical stresses

It is well known that arteries remodel their geometry, structure and mechanical properties in response to alterations of blood flow and blood pressure to restore the physiological level of WSS and WTS sensed by endothelial cells and SMCS respectively (11, 27, 29).

Arterial wall response to the alteration of blood pressure

Animal and human studies have been performed to examine the effect of hypertension on the arterial wall. It has been shown that continued hypertension (high WTS) is associated with the thickening and stiffening of the arterial wall by modifying collagen fiber synthesis by SMCs (11). The upregulation of TGF-β_1 production in response to high WTS is a mechanism that may explain the increase of ECM production and deposition in hypertensive arteries (102).

Wolinsky and his colleagues conducted a series of studies to elucidate the effect of hypertension on the aortic wall. First, they introduced the lamellar structure of the arterial wall including elastin lamellae and interlamellar zone (103). Then, they showed that the ratio of lamellar unit numbers to aortic diameter is approximately constant for different adult mammalians except for the human abdominal aorta (104). Moreover, they indicated that in different species, the average tension per lamellar unit of an aortic media is the same (5). They also investigated the effect of short term and long term hypertension on the rat thoracic aorta. Results showed that after 2.5 months of hypertension, the diameter of the aorta in hypertensive rats was significantly larger than controls. However, the number of medial lamellar units in

hypertensive arteries was equal to those of normotensive arteries, leading to the increasing tension per lamellar unit in hypertensive animals (105). After long-term (16 months) hypertension, no significant difference was observed between the diameter of hypertensive arteries and normotensive arteries. Instead, arterial wall hypertrophy (the increase of wall thickness to the lumen diameter ratio) was observed in hypertensive arteries. It was suggested that with the progressive increase of arterial wall thickness in response to hypertension, wall tension restores to its normal level (106).

Matsumoto et al studied the effect of hypertension on the stress distribution through the wall thickness in the rat thoracic aorta. They found that with the thickening and stiffening of the aortic wall in response to hypertension, arterial wall stress is kept constant at the physiological level. They also indicated that morphological changes within the hypertensive arteries occur much earlier than composition changes. Moreover, the hypertensive rat aortic wall restores its normal level of elastic properties in 16 weeks (107).

Rachev et al proposed a theoretical model to examine the remodeling of hypertensive arteries. In this model, it was hypothesized that the artery remodels its zero-stress configuration in such a way that the stress and strain distribution in the hypertensive artery remain in the physiological level. The model predicted the thickening and increase of the inner diameter of the hypertensive arteries which is consistent with experimental data (108). In further studies, the response of the human aortic lamellar structure to high blood pressure was investigated and it was found that the thickening of aortic wall is required to maintain the circumferential stress profile across the medial layer of the hypertensive aorta (109).

Boutouyrie et al investigated the effect of pulse pressure and mean pressure on the arterial wall remodeling. They found that carotid pulse pressure can be considered as a strong independent determinant of the carotid artery enlargement and wall thickening, whereas the level of mean blood pressure does not influence arterial wall remodeling. Based on these observations, they suggested that the cyclic stretch can play a critical role in the remodeling of elastic arteries (110).

Arterial wall response to the alteration of blood flow

Modification of arterial wall dimensions has been observed with the alternations of shear stress. Outward remodeling in response to increased flow (high WSS) and inward remodeling due to the reduction of blood flow (low WSS) have been observed (29, 111). It has been shown

that vascular remodeling in response to flow changes depends on the presence of endothelium and tends to restore physiological levels of WSS (112). The adaptive process includes modifications of luminal diameter as well as relatively small changes in arterial wall thickness relied on the rearrangement of ECM. The disruption of IEL within the arteries exposed to high blood flow have been observed. The existence of gaps within the IEL facilitates the increase of luminal area in response to high blood flow (113).

Langille and his colleagues investigated the effect of reduced carotid blood flow in young and mature rabbits. Using ipsilateral external carotid artery ligation, they reduced common carotid blood flow by 60% and demonstrated that this flow drop results in a significant decrease of elastin mass and reduction of arterial wall diameter in immature rabbits. However, adult rabbits showed only decreased internal diameter after flow reduction and no significant changes in vessel mass or wall constituents were observed. It was concluded that there is a shear stress threshold for adult arteries to adapt morphologically (114).

2.2.2 Remodeling of atherosclerotic arteries

The concept of expansive remodeling (positive or outward remodeling) of the arterial wall in response to the formation of atherosclerotic plaque was introduced by Glagov et al in 1987. They studied histological sections of left main coronary arteries and found that most of the atherosclerotic plaques with stenosis less than 40%, expand in a way that the luminal area is preserved. They showed that for stenosis greater than 40% , the atherosclerotic artery loses its capacity to become enlarged and the lumen starts to narrow. When the ability of arteries to preserve lumen size, in response to growing atherosclerotic plaque fails to occur the result is stenosis, leading to blood flow reduction to vital organs.

In 1995, Pasterkamp et al introduced the concept of constrictive remodeling of atherosclerotic plaques. They observed the reduction of IEL area in atherosclerotic femoral arteries with respect to the reference site and found that the development of atherosclerotic lesions may be accompanied by local shrinkage of the artery. Based on this observation, they introduced constrictive remodeling (negative or inward remodeling) of atherosclerotic arteries in which the arterial wall paradoxically exhibits shrinkage in response to atherosclerotic plaque formation leading to the exacerbation of the luminal narrowing (115). Consequently, they proposed that in addition to plaque accumulation, the type of arterial wall remodeling is a key determinant of luminal stenosis in atherosclerotic arteries (31, 116). Moreover, due to the high

luminal restriction within atherosclerotic arteries with constrictive remodeling, they are readily detectable angiographically (117).

In further studies based on IVUS images, the EEM of coronary atherosclerotic arteries was examined and it was shown that the EEM area at the lesion site can be larger, equal or even smaller than the reference site that contains the least amount of plaque. Remodeling index (RI) defined as the ratio of EEM area at the lesion site to the EEM area at the reference site has been used to describe dynamic changes of the EEM area over time and determine the type of remodeling within atherosclerotic arteries. Based on this definition, remodeling of atherosclerotic arteries was categorized into three different groups of negative remodeling ($RI<0.95$), intermediate remodeling (no remodeling) ($0.95<RI<1.05$) and positive remodeling ($RI>1.05$) (118).

Feldman et al proposed another description for the characterization of remodeling of atherosclerotic arteries. They defined the remodeling index (RI) as the slope of the relation between EEM area and plaque area. Based on this definition, they categorised the remodeling of atherosclerotic arteries into three different groups of constrictive remodeling ($RI<0.75$), compensatory expansive remodeling ($0.75<RI<1.25$) and excessive expansive remodeling ($RI>1.25$). They found that 60% of minimally diseased coronary arteries exhibit compensatory expansive remodeling, 21% shows excessive expansive remodeling, whereas 19% represents constrictive remodelling (119).

Atherosclerotic plaques which undergo expansive remodeling are more likely to be eccentric and usually composed of large lipid burden surrounded by thin and inflated fibrous caps (118, 120). Conversely, atherosclerotic plaques with constrictive remodeling are often concentric and associated with higher grade of stenosis and may disrupt the blood supply to the downstream tissue. They are often stable fibrotic plaques with small lipid pool and thick fibrous cap and usually contain a large amount of calcium (118, 121). Reliable evidence shows that atherosclerotic arteries with expansive remodeling compared with lesions with constrictive remodeling have higher macrophages count, inflammatory cells and subsequent protease activities (117, 122). Considering these histological markers, it can be proposed that expansive remodeling of atherosclerotic arteries acts like a double-edged sword; while it prevents luminal stenosis and subsequent ischemia symptoms, it is associated with unstable angina and may increase the risk of plaque rupture (32, 122, 123). Moreover, due to low luminal stenosis, the clinical identification of atherosclerotic plaques with expansive remodeling is often more complicated than those with constrictive remodeling (124).

It has been shown that the balance between ECM degradation by MMPs and ECM production by SMC is the major determinant of the type of arterial wall remodeling (125). For instance, when fibro-proliferative processes dominate inflammation and subsequent matrix breakdown, the atherosclerotic wall undergoes constrictive remodeling (126). Macrophages play an essential role in the expansive remodeling of atherosclerotic lesions. Degradation of the matrix scaffold by macrophage MMPs may weaken the arterial wall, leading to the increase of arterial wall diameter compared to the neighbouring, non-involved areas. The increase of inflammation activities may also cause thinning and eroding of the fibrous cap, result in weakening and destabilization of atherosclerotic plaques (127, 128).

It has been suggested that the inability of blood vessels to remodel properly is an important clinical issue (28). A correlation has been suggested between the lack of compensatory expansive remodeling and the history of coronary heart disease (129). The major parameter controlling the expansive remodeling of atherosclerotic arteries is endothelial shear stress. The dysfunction of endothelial cells can play a key role in the development of early plaques into constrictive ones. Constrictive remodeling is the most significant component of restenosis after balloon angioplasty, which is controlled by wall shear stress and wall tensile stress (130). Moreover, it has been suggested that the pattern of arterial wall remodeling before intervention can predict the possibility of diffuse in-stent restenosis (131).

The existence of atherosclerotic plaque leads to a complex connection between the mechanical stresses and arterial wall remodeling. Although in normal arteries, low shear stress stimulates an adaptive response of the arterial wall leading to constrictive remodeling and, consequently, an increase in endothelial shear stress to physiologic levels, in atherosclerotic arteries low shear stress leads to the development of focal plaque, inflammation of the wall beneath the plaque and production of enzymes that shift the ECM balance toward degradation. Therefore, low shear stress can promote arterial expansion and accommodation of the enlarging plaque (8, 132, 133). By using intracoronary ultrasound, biplane coronary angiography, Stone et al conducted a follow-up study to examine the effect of shear stress on the remodeling of atherosclerotic coronary arteries. Their results showed that atherosclerotic plaque development and outward remodeling often occur in the regions of low shear stress (134). On the other hand, the study of Samady et al based on radiofrequency IVUS images showed that that coronary segments with low shear stress develop plaque with constrictive remodeling and expansive remodeling occurs in the regions of high shear stress (135).

Animal models can be beneficial for the better understanding of multifaceted diseases such as atherosclerosis. For instance, the Yucatan micropig model has been used to investigate the correlation between de novo atherosclerosis with the direction and extent of remodeling induced by angioplasty (136). Apolipoprotein E-deficient ($apoE^{-/-}$) mouse model has been widely used for studying the mechanisms of atherosclerotic plaque development (137, 138). It has been observed that the aortic root of $apoE^{-/-}$ mouse shows expansive remodeling in response to plaque formation (139). However, some features of human atherosclerotic plaques such as plaque rupture and the subsequent thrombosis processes as well as constrictive remodeling are not observed in $apoE^{-/-}$ mice (140, 141). Another issue related to the apoE genetically modified mice models is the accelerated mode of disease progression over months; whereas, the disease progresses over several decades in humans. Therefore, it may be reasonable that some characteristics of atherosclerotic lesions in animal models are completely different from the process that occurs in humans (40).

2.3 Atherosclerotic plaque imaging modalities

Advances in atherosclerosis imaging technology have provided a range of diagnostic tools for the characterization of atherosclerotic plaques and the possibility of realistic 3D plaque geometry reconstruction with a high resolution. Each method has its own advantages and disadvantages for imaging atherosclerosis and the accurate segmentation and reconstruction of plaque components is one of the main challenges in the development of medical imaging based computational models. In this section, some of the image techniques used for the assessment of atherosclerotic plaques are discussed.

2.3.1 Computed tomography angiography (CTA)

Computed tomography operates by using an X-ray generator that rotates around the object and allows better visualization behind dense structures. CTA is a computed tomography method used to visualize arteries. In this technique, the patient receives an intravenous injection of contrast and then the artery is scanned using a high-speed CT scanner. The spatial resolution of CTA can be considered suitable and it is able to quantify the vessel diameter, determine the degree of luminal stenosis and assess the amount of lipid, fibrous tissue and calcification within the plaques. The main disadvantage of this technique is that the patients receive radiation and

it can limit the temporal resolution of CTA. Moreover, the existence of dense structures such as bone can cause artifacts within the images (58).

2.3.2 MRI

MRI evaluates the biophysical response of tissues placed in a strong static magnetic field. MRI does not involve X-rays or ionizing radiation and has the greatest potential for the non-invasive and comprehensive assessment of atherosclerotic plaques compared to other imaging techniques. By using different sequences, MRI can provide insights, not only on luminal stenosis and the atherosclerotic lesion but also on different plaque components, such as the lipid pool, intra-plaque haemorrhage, calcifications, and fibrous tissue. It can be also used for the measurement of the actual blood flow as well as the quantification of fibrous cap thickness and inflammation. Therefore, the popularity of MRI for the characterization of atherosclerotic plaques has been increased. However, considering the resolution of MRI, it is only suitable for the study of large arteries, such as the carotid arteries. Due to the small dimension and the continuous motion of coronary arteries during data acquisition, it is difficult to characterize atherosclerotic plaques within coronary arteries with MRI (142).

2.3.3 Optical coherence tomography (OCT)

OCT uses low-coherence interferometry and typically near-infrared light to generate cross-sectional images of biological samples. The use of relatively long wavelength light allows it to penetrate into the scattering medium. In ex vivo experiments, OCT has been shown to identify plaque morphology with high sensitivity. Considering the high spatial resolution of OCT, it is the most accurate technique for the identification of plaque rupture, plaque erosion, thrombus formation and quantification of macrophage content in the fibrous cap. However, since OCT uses light to create the image, it has limited tissue penetration and the quality of the OCT images is affected by blood (61).

2.3.4 Intravascular ultrasound (IVUS)

IVUS is one of the first methods used for the assessment of plaque morphology and it is an excellent tool for assessing the remodeling of atherosclerotic arteries. IVUS provides real-time

cross-sectional images of the artery perpendicular to the long axis of the catheter. IVUS can also detect some of the important features of atherosclerotic plaques such as the presence of thrombi, plaque eccentricity, plaque length, luminal stenosis and the existence of calcification. However, IVUS cannot determine the detailed structure and composition of the plaque with sufficient robustness and accuracy. Additionally, IVUS is an invasive technique and it is not suitable for screening purposes. These technical limitations restrict IVUS from becoming a clinically useful tool in the determination of plaque vulnerability (143).

2.3.5 Ultrasound

Ultrasound is a non-invasive and inexpensive technique, which use the sound waves to provide information on the flow dynamic and luminal stenosis in both 2D and 3D. It has a high temporal and spatial resolution depending on its frequency; however, it cannot penetrate dense structures like bone and dense calcifications. Therefore, the arterial tissue behind these structures cannot be detected and ultrasound technique can only be used to image superficial vessels, such as the carotid arteries and femoral arteries. Recent advances in semi-automatic image analysis facilitate the global measures of the diseased artery such as the luminal stenosis with ultrasound. However, since this method is operator dependence and has limited capability in the determination of plaque composition, it is less popular than other techniques for the characterization of different plaque components (142).

2.4 Mechanical characterization of atherosclerotic arteries

Obtaining accurate data regarding the mechanical properties of healthy and diseased arterial tissue is essential for realistic stress prediction. A significant amount of research has been done on healthy arterial tissue (144, 145). For instance, a recent study reported the biaxial mechanical properties of some of the large and medium-sized arteries such as human thoracic and abdominal aorta, subclavian, common carotid, renal and common iliac arteries (146). Another group has evaluated the layer-specific mechanical properties of human healthy coronary arteries and aorta (145). For the determination of mechanical behavior of elastin lamellae and inter-lamellar zones within the human thoracic aorta, a microstructural model based on the lamellar structure of aortic wall has been suggested. (147).

Despite the efforts made, mechanical characterization of the atherosclerotic arteries is still challenging due to the heterogeneity and anisotropic mechanical behaviour of the plaque tissue (148). Different *in vitro* techniques such as tensile test, unconfined compression test, and indentation test have been suggested for mechanical characterization of atherosclerotic arteries (148-150). The material properties of atherosclerotic arteries have also been estimated using *in vivo* techniques such as elastography and pulse wave imaging (151, 152). However, these *in vivo* methods cannot determine the local stiffness of the arterial wall and atherosclerotic plaque.

2.4.1 Tensile properties of atherosclerotic arteries

Several studies evaluated the mechanical properties of atherosclerotic arteries using tensile experiments (150). In 1993, Lendon and his colleagues designed a tensometer to measure the stress-strain relationship of the cap and intimal tissue of atherosclerotic plaques obtained from human aorta. Their experiments showed significant differences in the mechanical properties of non-ulcerated and ulcerated atherosclerotic plaques (153). Loree et al studied the stress-strain behaviour of human aortic intimal plaques. They classified the samples into three groups of cellular, hypocellular and calcified lesions and found that the degree of cellularity and calcification do not have a significant effect on the static circumferential tangential modulus of the atherosclerotic plaque. It was also shown that in the physiologic range of loading, cellular and hypocellular plaques display highly anisotropic and nonlinear behaviour (154).

Some papers reported the global stiffness of atherosclerotic arteries using tensile test. Maher et al obtained atherosclerotic carotid samples from endarterectomy surgery and classified them into three different groups of calcified, mixed or echolucent. The results of radial compressive test and circumferential tensile test showed that calcified plaques are more than twice as stiff as the echolucent samples. The inhomogeneous nature and variations in the mechanical properties of plaques taken from different anatomical sites (internal, external and common carotid arteries) were highlighted (155). In another study, the ultimate tensile stress and strain of human carotid plaques was reported based on the uniaxial tensile testing (156). It has shown that atherosclerotic coronary arteries are generally stiffer than the healthy ones (157).

Due to the difficulty of separation of plaque tissue constituents, few investigators have measured the tensile properties of plaque components. In 2004, Holzapfel et al measured anisotropic mechanical properties of different plaque components within human iliac arteries using uniaxial tensile testing. Their results highlighted the heterogeneity and nonlinear mechanical behaviour of atherosclerotic plaques components. They also determined the

ultimate tensile stresses/ stretches of plaque components and showed that the lowest fracture stress is related to the fibrous cap in the circumferential direction. Based on their results, the adventitia and nondiseased media represent the highest and lowest mechanical strength respectively (23). Another group performed uniaxial tensile testing on the carotid plaques obtained by endarterectomy and determined hyperelastic properties of different types of atherosclerotic tissue including media, lipid, fibrous cap and intraplaque haemorrhage (63).

2.4.2 Compressive properties of atherosclerotic arteries

The compressive mechanical properties of atherosclerotic arteries have been measured using different techniques such as unconfined compression and indentation tests.

Unconfined compression test

In the unconfined compression test, the biological tissue is mounted between two metal plates. The top plate is often fixed and attached to a load measuring device, while the bottom plate can go up and down with a desirable speed. In this test, the induced displacement or force can be considered as loading conditions. Material stiffness and frequency-dependent dynamic characteristics of the tissue respectively can be determined by using static loading condition and cyclic loading condition at different frequencies (149).

Lee et al employed dynamic mechanical testing to determine the uniaxial unconfined compressive stiffness of cellular, hypocellular and calcified fibrous caps, obtained from atherosclerotic human abdominal aortas. It was found that calcified and hypocellular fibrous caps are respectively 4-5 times and 1-2 times stiffer than cellular fibrous caps. They also found that the stiffness of plaque fibrous cap intensified with the increase of the frequency of loadings (158). In another study, an experiment was designed to examine the effect of different lipid compositions on the dynamic shear modulus of the lipid pools using a torsion rheometer. Results showed that the lipid pool stiffness depends on the concentration of cholesterol monohydrate crystals (159). By performing uniaxial compressive tests, the history-dependent nonlinear and inelastic mechanical behaviour of human atherosclerotic plaques under finite deformation have been highlighted (160).

Indentation test

Indentation test is a suitable method for measuring local material properties of relatively small and inhomogeneous material. There are a variety of instruments used to indent samples

which are capable of applying a wide range of loads (161). In the micro-indentation test, an indenter applies physiologically relevant strains to the biological tissue to perform local measurements. The initial contact point between the indenter and the sample is detected and the applied force and the indentation depth are continuously reordered. As a consequence, a force-depth curve is achieved for each indented location. Due to the nonlinearity of the contact condition, complex mechanical strain and stress fields induced around the indentation location and tissue stiffness cannot be calculated directly from the force-depth curves. Consequently, numerical simulation (inverse finite element method) is used to extract the mechanical properties of the tissue from the force-depth data (149, 162).

Barrett et al performed indentation test on the carotid atherosclerotic plaque samples and fit the measured indentation response to finite element modeling to estimate the shear modulus of human carotid atherothrombotic tissue. The inferred shear modulus was found to be in the range 7–100 kPa, with a median value of 11 kPa (163). Chai et al combined micro-indentation test with confocal laser scanning microscope imaging to determine the compressive elastic modulus of different atherosclerotic lesion constituents in the axial direction. The confocal microscope was used to visualize the collagen fiber structure and analyze its effect on the mechanical properties of the tissue. They generated a finite element model to simulate the indentation test and assumed isotropic neo-Hookean behavior for the plaque tissue. It was found that collagen-rich locations are stiffer than collagen-poor locations. Furthermore, they indicated that the axial compressive mechanical behavior of atherosclerotic plaques are comparable at the different locations within the plaques such the middle of the fibrous cap, the shoulder regions, and remaining plaque tissue (68).

Considering the high heterogeneity of atherosclerotic plaques, even micro-indentation might be inadequate to identify local mechanical properties of atherosclerotic plaque components. To overcome this issue, nano-indentation can be employed (149, 164). Nano-indentation have been widely used for the determination of local elastic modulus of biological tissue such as microstructural components of the bone (165), lung tissue (166), and vascular tissues (67, 167, 168).

Ebenstein et al used nano-indentation to measure the mechanical properties of different components of human atherosclerotic plaque components including blood clots, fibrous tissue, and calcification. They demonstrated that the stiffness of plaque tissue increases with the accumulation of mineral content (67). Akhtar et al utilised nanoindentation for measuring micromechanical properties of adult ferret descending aorta and vena cava. They found that

the increase of elastic modulus within samples is correlated with elastic fiber density (167). In another study performed by Hemmasizadeh and his colleagues, mechanical properties of the porcine aortic wall in the radial direction were measured using a custom-made nano-indentation technique. A quasi-linear viscoelastic model was applied to the results and it was found that the outer half of the aortic wall is stiffer and shows less relaxation compared to the inner half of the artery (168).

Atomic force microscope (AFM) is a high-resolution type of scanning probe microscope which is generally used to scan the surface of materials. The force-spectroscopy mode of AFM is a practical and popular method to apply nano-indentation and perform very local force-indentation measurements, to obtain the mechanical characteristics of cells and biological tissues (169, 170). The AFM consists of a cantilever with a tapered probe on the order of nanometers which is typically made from silicon or silicon nitride. When the probe tip interacts with the specimen, the cantilever deforms like a spring in response to forces between the tip and the sample. This deflection is measured by sensing the position of a laser beam that reflects off the cantilever arm (171, 172).

The application of AFM-based nanoindentation for the determination of the elastic and viscoelastic response of the intimal surface of healthy human femoral arteries was described by Lundkvist and his colleagues (24). In this study, an elastic modulus of 34.3 kPa was reported for the upstretched, intimal vessel wall. Peloquin et al used AFM indentation to measure the mechanical properties of subendothelium in bovine carotid arteries. They used scraping for eliminating the endothelial cells from the subendothelial matrix. Then, they performed AFM test on both untreated and scraped bovine carotid arteries and fitted the data to the Hertz model. They found that the elastic modulus of endothelium and subendothelium is quite similar (173).

Two different groups performed AFM test to characterize mechanical properties of aortic plaques within $ApoE^{-/-}$ mouse (66, 69). They did histological examinations to identify different components of atherosclerotic plaque including cellular fibrotic areas, hypo-cellular fibrous cap, and lipid-rich regions. Then, AFM test was performed on the cross-sections of atherosclerotic arteries and several sites within each cross-section corresponding to different plaque constituents were probed. They applied the Hertz model to the experimental data and calculated the elastic modulus of desirable locations. Tracqui et al reported the average elastic modulus of cellular fibrotic regions and hypocellular fibrous caps as 10.4 ± 5.7 kPa and 59.4 ± 47.4 kPa respectively. The elastic modulus of the lipid pool was determined as 5.5 ± 3.5 kPa (66). Hayenga et al reported the median values of 1.5 kPa and 18.7 kPa respectively as the

elastic modulus of lipid-laden plaques and unloaded healthy aortic wall. They also extracted two mechanically separate populations with median values of 9.8 and 76.7 kPa, based on the calculated elastic modulus within different locations of the arterial wall and proposed that the higher value is related to the presence of elastin fibers in the aortic wall (69). In another study, the effect of aging on the elastic and viscoelastic properties of elastin lamellae and inter-lamellar regions of the medial layer of sheep aorta were investigated using frequency modulated AFM technique. It was found that the elastic modulus, as well as the storage and loss modulus of both elastin lamellae and inter-lamellar zones, increase with aging (174).

2.5 Rupture of atherosclerotic plaques

Since the late 1980s and with the development of computational modeling, biomechanical factors have been introduced as important triggers in plaque rupture. Preliminary studies were carried out to associate extreme mechanical stress situations with the site of intimal tears. They found a correlation between the location of plaque rupture with regions of high circumferential stress concentration and locations of foam cells accumulation (175, 176). In 1992, using two-dimensional (2D) idealized models of atherosclerotic plaque, Loree et al examined the effect of fibrous cap thickness and luminal stenosis on the circumferential stress distribution within the atherosclerotic plaque. They indicated that mechanical stress concentrations frequently occur in the shoulder of fibrous cap regions, where atherosclerotic plaques are more likely to rupture. Furthermore, they found that with the increase of luminal stenosis, as well as the increase of fibrous cap thickness, the level of circumferential stress within the atherosclerotic plaque decrease (177). In another study, using three-dimensional (3D) axisymmetric idealized models, the effect of lipid core size, fibrous cap thickness and calcification on the stress distribution within atherosclerotic plaques was investigated. It was found that thin fibrous cap, large lipid pool and the existence of calcification results in the intensification of stress level at the plaque cap (178). Further studies based on realistic models of carotid plaques confirmed that the stress level on the plaque cap significantly increases with the decrease of fibrous cap thickness and the increase of lipid core volume (179, 180).

Cheng et al reconstructed the cross-sectional geometry of atherosclerotic plaques based on histological images of coronary arteries and performed FEM analysis to estimate the mechanical stress distribution within the plaques. Comparing the results of stress analysis with the histological images, confirmed that plaque rupture often occurs in the locations of high

stress concentration. They proposed that due to local variation in the mechanical properties of atherosclerotic plaques, the location of stress concentration is not always matched with plaque rupture site. They also introduced a critical value of 300kPa for the disruption of coronary plaque, according to the stress analysis on ruptured and stable atherosclerotic lesions (56). In further studies, FSI analysis was performed to predict the location of plaque rupture based on pre-rupture medical images. The results were compared with the locations of carotid atheroma rupture observed in vivo and it was found that plaque rupture most often occurs in the region of high first principal stress (181).

The sensitivity of mechanical stress distribution within atherosclerotic arteries to the various parameters have been studied in detail. The accurate reconstruction of plaque geometry is of vital importance. Histological examination has been introduced as the gold standard for determining plaque geometry and composition (61). For accurate prediction of the stress field from *in-vivo* plaque geometries, the zero-stress state of the atherosclerotic plaque should be determined first. Different shrinkage procedures have been proposed for reconstructing the zero-stress state of carotid atherosclerotic models from *in-vivo* MRI data (182). Williamson et al analyzed the sensitivity of circumferential stress distribution to the alteration of mechanical properties of atherosclerotic plaque component. Their results showed that uncertainty in the material properties of the arterial wall and atherosclerotic plaque generates relatively small errors in the prediction of wall stresses (183). In another study, the effect of intima stiffness and plaque morphology on the stress pattern of atherosclerotic plaques were investigated simultaneously. The results showed that in models with stiff and intermediate media, the cap thickness is the main determinant of plaque vulnerability; while in soft intima models, the thickness and angle of the lipid pool had a bigger impression on the peak cap stress(184).

It has been shown that different computational strategies such as 2D structure only models, 3D structure-only/fluid-only models, one-way FSI models and fully coupled FSI models, can influence on the accuracy of the estimation of mechanical stress distribution. Comparing the results of FSI models with structure only models revealed that there could be a large difference in the maximum wall shear stress and maximum principal stress estimated by different methods (185). Despite the fact that fully coupled FSI analysis is the most accurate method for the determination of mechanical stress field, in some cases other computational techniques can be used to reduce the computational cost (55). It has been also shown that boundary conditions and more specifically the degree of freedom of artery movement can also influence the stress

distribution in coronary arteries (186). Although the effect of residual stresses is often ignored in computational modeling, it has been shown that residual stresses can influence the stress distribution within atherosclerotic arteries (21).

The effect of calcification on plaque stability is controversial. Some investigators suggested that calcification does not have a significant effect on the plaque vulnerability (47), while others believed that the existence of calcification should be considered for the risk assessment of atherosclerotic plaques (187-189). It has been suggested that calcium buried deep in plaques could hardly increase plaque vulnerability, and may even stabilize the plaque from rupture. However, the existence of calcification within the fibrous cap close to the lumen could increase the stress concentration due to compliance mismatch, leading to the increase of the probability of plaque rupture (188). By constructing an idealized 3D plaque model, Hoshino et al showed that the effect of calcification on the plaque vulnerability depends on the relative position and orientation of the calcium deposit in the plaque region. By bearing load, the calcium deposit could decrease the stress level in some areas, while it could increase the peak stress at other regions by introducing a great dissimilarity in the plaque stiffness (189). It has been hypothesized that cellular-level microcalcifications within thin fibrous caps can contribute to plaque rupture by inducing local stress concentration and the following interfacial debonding (190). The results of FSI modeling confirmed that local stress concentrations derived from embedded calcification spots within the fibrous cap region may be the underlying mechanism that increases the risk of plaque rupture (191).

In 2005, Ohayon et al proposed that 3D structural analysis combined with IVUS imaging can be used for the in-vivo prediction of plaque rupture during balloon angioplasty (60). In another study, by reconstructing FEM models from *in vivo* high-resolution MRI, it was showed that the stress level in ruptured plaques is much higher than unruptured plaques. It was also indicated that the presence of a moderate carotid stenosis with a thin fibrous cap can present a high risk for plaque rupture (192). Using MRI-based FSI models it has also been proposed that determination of longitudinal fibrous cap stresses may be beneficial for determination of plaque vulnerability (193).

Tang et al developed a 3D FSI model from high-resolution MR images of a human atherosclerotic carotid artery to estimate stress distribution conditions, which may be related to plaque rupture (54). Their results showed that mechanical stress distribution within atherosclerotic plaques is affected by the fibrous cap thickness, lipid pool size, material properties of plaque components and calcification (54, 194). In 2005, this group introduced a

stress-based index to assess atherosclerotic plaque vulnerability. Close agreement between the proposed vulnerability index and histological analysis was observed in assessment of plaque instability (195). This group also showed that localized critical stress conditions had a much better correlation with plaque vulnerability, compared to global maximum stress values (196). In further studies, they suggested that both low WSS and low WTS contribute in atherosclerotic plaque progression (197).

2.5.1 Fatigue failure hypothesis

For the first time, Bank et al hypothesized that mechanical fatigue caused by pulsatile blood pressure is the underlying cause of atherosclerotic plaque rupture (50). Mechanical fatigue is introduced by the weakening of materials exposed to a cyclic load with a magnitude lower than the ultimate strength of the material. Mechanical fatigue leads to the formation of microscopic cracks at locations of high stress concentration. When the crack reaches a critical size, it propagates progressively in the perpendicular direction. Fatigue failure is a catastrophic event and often happens abruptly and without warning at stress levels much lower than those required with a single maximal load. Based on fracture mechanics theories, with the increase of loading cycles, the final rupture occurs at lower levels of stress (198).

Reliable evidence confirmed the role of mechanical fatigue in the rupture of atherosclerotic plaques. With a normal heart rate, cardiovascular tissues are exposed to more than 35 million stress cycles per year derived from the blood pressure pulse wave (199). Rupture of atherosclerotic plaques occurs at locations of stress concentration (56). Furthermore, animal and human studies have shown that plaque rupture occurs at stress levels much lower than the ultimate stress which plaque tissue can tolerate (200, 201). Using cyclic fatigue test, cracks in porcine coronary arteries have been detected (202).

Based on this hypothesis, Versluis et al analyzed the effect of anatomy, tissue stiffness and blood pressure on the fatigue failure of atherosclerotic plaques. Many 2D idealized models were designed by changing the lumen shape and fibrous cap thickness. Stress distribution was estimated in these models based on FEM. It was assumed that the initial crack forms at the location of stress concentration. The fatigue crack growth process was modeled according to the evolving stress distribution within the plaque and Paris law, a well-known relation in fracture mechanics. They found that the location of initial crack formation depends on the lumen shape, fibrous cap thickness, and plaque component's stiffness. It was shown that blood

pressure and lipid pool stiffness can affect the rate of crack propagation (199). In a similar study based on 2D idealized models, the effect of formation of the initial crack at any possible location around the lumen was investigated. Atherosclerotic plaque lesion was divided into three regions of mid-cap zone, shoulder zone, and backside zone. Then, the most critical location for crack initiation (where the fatigue life reached its minimum) in each region was determined. Results showed that cracks which initiate at the location of maximum stress concentration are not necessarily the most dangerous since the path which different cracks should traverse to reach the lipid pool is not the same. They also showed that with the reduction of fibrous cap thickness, as well as the increase of lipid pool size, the plaque fatigue life is reduced significantly (203). The simulation of plaque rupture has been also performed in realistic models of carotid plaques reconstructed from MR images. In this study, it was shown that atherosclerotic plaques without ulceration have a longer fatigue life compared to those with fibrous cap defect. Moreover, it was shown that the rate of crack propagation increases exponentially with the increase of crack length (204).

2.6 Summary

It is well known that mechanical stresses play a critical role in the initiation, development, and rupture of atherosclerotic plaques. During atherosclerosis disease, the arterial wall experiences remodeling, which is governed by the force-sensing cells and determined by the re-organization of structural components of the arterial wall at the micrometer length scale. Therefore, there is a need to develop reliable methods for the assessment of the stiffness of arterial wall and atherosclerotic plaque components at the micron length scale According to the author's knowledge, no comprehensive work was dedicated to examining the alteration of mechanical properties and geometrical features of atherosclerotic plaques with atherosclerotic plaques development. Moreover, little work has been carried out to study the effect of mechanical fatigue on the rupture of atherosclerotic arteries. Based on the arguments above, one of the main objectives of this thesis is the description of how the composition and the geometry of atherosclerotic plaques change during the atherosclerosis disease progression. In particular, this thesis aims to contribute to the characterization of mechanical properties of the arterial wall and atherosclerotic plaque components in both elastic and muscular arteries by means of AFM test. Moreover, with the efforts in numerical simulation of plaque stress, more insights will be gained regarding the remodeling of atherosclerotic arteries. Finally, the

mechanical fatigue analysis on the realistic models of atherosclerotic plaques derived from histological analysis of human coronary arteries will provide a biomechanical framework for the assessment of plaque vulnerability in patient-specific models.

Chapter 3

Mechanical Characterization of the Lamellar Structure of Human Abdominal Aorta in the Development of Atherosclerosis: An Atomic Force Microscopy Study

Chapter outline

It is well known that arteries remodel their geometry and composition to adapt to new circumstances. However, the effect of atherosclerosis disease on the alteration of the stiffness of arterial wall has not been studied in detail. This Chapter presents precise data relevant to the mechanical properties of atherosclerotic plaque components and the lamellar structure of human atherosclerotic abdominal aorta with atherosclerosis disease progression. Experiments were carried out on twenty human abdominal aortas obtained from individuals who died due to post-accident complications. As the abdominal aorta is an elastic artery, the focus was on the alteration of the stiffness of elastin lamellae and inter-lamellar zones during atherosclerosis disease progression. The force-spectroscopy mode of the atomic force microscopy (AFM) was employed to apply nano-indentation for the quantification of micromechanical properties of different constituents of atherosclerotic arteries. The results showed the softening of elastin lamellae and stiffening of inter-lamellar zones within the abdominal aortic wall with atherosclerosis disease progression. Findings of this chapter may shed light on the remodeling of elastic arteries during atherosclerotic plaque development.

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Chapter 4

Progressive changes of elastic moduli of arterial wall and atherosclerotic plaque components during plaque development in human coronary arteries

Chapter outline

Following Chapter 3, this chapter investigates the alteration of mechanical properties of the arterial wall and atherosclerotic plaque components within human coronary arteries during atherosclerosis disease progression. In contrast to the abdominal aorta, the coronary artery is a muscular artery and contains dense layers of SMCs within its medial layer. IEL and EEL which are made by elastin fibers, separate the medial layer from intima and adventitia respectively. In this chapter, the stiffness of the medial layer, IEL, EEL, and different plaque components are reported for healthy, mildly diseased and advanced atherosclerotic coronary arteries.

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Chapter 5

Arterial wall remodeling in the development of atherosclerotic plaques: mechanical stress analysis

Chapter outline

In previous chapters, the alteration of the arterial wall stiffness during atherosclerotic plaque development were investigated in both elastic and muscular arteries. In this chapter, the focus was shifted on the effect of geometrical features of remodeling of atherosclerotic arteries. Considering different types of arterial wall remodeling introduced by previous studies, many idealised models were designed to mimic different stages of atherosclerotic plaque development. The distribution of wall circumferential stress and wall shear stress were estimated for different models using FSI analysis. Then, the effect of arterial wall remodeling, plaque cap thickness, existence of lipid pool, and disease wall angle on the mechanical stress level were investigated. Results showed that in atherosclerotic arteries with compensatory expansive remodeling, the physiological levels of wall circumferential stress within the disease-free wall and wall shear stress preserve. However, they are more vulnerable to rupture due to the induction of stress concertation within the atherosclerotic plaque.

This chapter under the title of "Arterial wall remodeling in the development of atherosclerotic plaques: mechanical stress analysis" has been submitted in the Journal of Biomechanical Engineering.

Abstract

Purpose: The evolution and progression of atherosclerotic plaques depend on arterial wall remodeling. Although atherosclerotic coronary arteries generally undergo compensatory expansive remodeling, some do not exhibit arterial wall remodeling at all or even experience constrictive remodeling. The present study aimed to examine the effect of arterial wall remodeling on the mechanical stresses field within the arterial wall and atherosclerotic plaque.

Methods: Various idealized models of atherosclerotic plaques were designed to include alterations of plaque cap thickness, lipid pool, remodeling mode, and angle of the disease wall to mimic different stages of atherosclerotic plaque development. Fluid-solid interaction analysis was performed to estimate endothelial shear stress and wall circumferential stress in the models.

Results: In atherosclerotic arteries with compensatory expansive remodeling, high level of circumferential stress was induced in the shoulder and middle of plaque fibrous cap; while, the level of circumferential stress in the disease-free wall, as well as endothelial shear stress, remained almost equal to that of the healthy artery during plaque development. In the absence of expansive remodeling, non-physiological ranges of circumferential stress in the disease-free wall and high level of endothelial shear stress were observed. Although the existence of lipid pool increased the stress level within the atherosclerotic plaque, it did not affect the stress distribution within the arterial wall significantly.

Conclusions: The compensatory expansive remodeling of the arterial wall is crucial to preserve the physiological range of mechanical stresses during plaque development. However, atherosclerotic plaques with compensatory expansive remodeling are more vulnerable to rupture due to induction of higher level of stress in the atherosclerotic plaque while atherosclerotic plaques without remodeling are more prone to plaque erosion due to high shear stress.

Keywords: Atherosclerosis, Remodeling, Wall Circumferential Stress, Wall Shear Stress, Smooth Muscle Cell, Endothelial Cell

5.1 Introduction

Myocardial infarction is one of the leading causes of human mortality mainly through the disruption of atherosclerotic plaques. Plaque disruption results in the formation of blood clots and complete blockage of the artery. Plaques that are prone to rupture may often be clinically silent until the time of rupture, hence identifying vulnerable plaque is of great importance (41). Although, the percentage of luminal stenosis is considered as the main criterion for the detection of vulnerable atherosclerotic plaques, the highest frequency of plaque rupture does not necessarily occur in the presence of a high degree of luminal stenosis (204). Various parameters such as a thin fibrous cap, a large lipid pool, and the presence of calcification zones contribute to atherosclerotic plaque vulnerability (44).

From a biomechanical point of view, circumferential stress caused by pulsatile luminal pressure and wall shear stress due to pulsatile blood flow play important roles in the formation, progression, and rupture of atherosclerotic plaques (51, 56), through influence on the proliferation, survival/apoptosis, and migration of endothelial cells and smooth muscle cells (SMCs) (95, 229). It is hypothesized that atherosclerotic plaques are formed in the regions with low and oscillatory shear stress (8), and high circumferential stress (89) such as the lateral walls of bifurcations and the inner wall of vessel curvature. Moreover, it has been suggested that the existence of a negative phase angle between circumferential stress and wall shear stress increases the possibility of plaque formation (232).

The fracture of atherosclerotic plaques is mainly due to plaque fatigue failure caused by pulsatile circumferential stress (56, 199). Plaque fracture exposes thrombogenic materials to the circulating blood and eventually induces thrombus formation in the lumen which can block arteries and cause ischemic events. Moreover, high shear stress can lead to plaque erosion and formation of superficial thrombosis (43). Plaque fracture has been reported to be more common than plaque erosion by a ratio of 3 to 1 (42).

Arterial wall remodeling is defined by the persistent changes in composition and size of arteries allowing adaptation to new circumstances. The mechanisms of remodeling are crucial to maintain arterial function and reduce the possibility of pathologic conditions. Considering the effect of mechanical stresses on the migration, differentiation, and proliferation of endothelial and smooth muscle cells, it has been suggested that cells within the walls are responsible for arterial remodeling through local protein synthesis, deposition, and in some cases degradation (8). It is well known that in hypertensive arteries, the increase of wall tensile

stress lead to overproduction of extracellular matrix (ECM) by SMCs results in the thickening and stiffening of the arterial wall in a way that the wall tension remains roughly unchanged compared to normotensive arteries (11, 107). Moreover, with the alteration of blood flow and the consequent changes in the shear stress level of endothelium, the diameter of the artery changes to restore the endothelial shear stress to its physiological range (112).

The evolution and progression of early plaques depend on vascular remodeling which should be considered separately from atherosclerosis disease development. In fact, while plaque formation is a clinical condition, the arterial wall remodels itself to adapt to new circumstances. Glagov et al. found that in early atherosclerotic plaques, the luminal area remains almost constant due to the compensatory enlargement of the arterial wall until the stenosis exceeds 40%, and then the area decreases, resulting in disturbed flow and cardiovascular complications (38). Further studies showed that although about 60% of atherosclerotic arteries undergo compensatory expansive or positive remodeling, almost 20% of vessels experience constrictive or negative remodeling in a way that the arterial wall shrinks at the lesion site, exacerbating rather than compensating luminal stenosis. It has been reported that about 20% of atherosclerotic arteries experience excessive expansive remodeling in which the arterial lumen enlarges during atherosclerotic plaque development (119).

The importance of arterial wall remodeling within atherosclerotic arteries is emphasized by the observation that luminal stenosis correlates more closely with the type (outward or inward) and the magnitude of remodeling rather than the plaque size (32). Previous studies have shown that although atherosclerotic plaques with constrictive wall remodeling may disrupt the blood supply to the downstream tissue, they are often stable fibrotic plaques with small lipid pools and thick fibrous caps which can clinically be readily identified (45, 233). On the other hand, atherosclerotic plaques which undergo expansive remodeling are usually composed of large lipid cores surrounded by thin and inflated fibrous caps and are more prone to rupture (8, 233).

With finite element analysis of two-dimensional idealized models, Ohayon et al. introduced the parameter of remodeling index and suggested that the combination of fibrous cap thickness, necrotic core thickness, and remodeling index should be considered for the determination of plaque instability (234). Similarly, using idealized plain strain geometries, Cilla et al compared the level of maximal principal stress in coronary arteries with positive remodeling and negative remodeling (235). However, as far as the author is aware, there is no work carried out on the effect of arterial wall remodeling on the circumferential stress within different parts of the arterial wall and atherosclerotic plaque. In the current study, the level of wall circumferential

stress at different locations of the arterial wall and atherosclerotic plaques were compared between models with compensatory expansive remodeling and without remodeling.

5.2 Methods

To mimic different stages of atherosclerotic plaque development and examine effects of atherosclerotic plaque remodeling on the mechanical stress field within arterial wall and atherosclerotic plaque, several 3D idealized models of atherosclerotic plaque were designed with varying plaque cap thickness (PCT), diseased wall angle (DWA) and lipid pool (191). It was assumed that initial atherosclerotic plaques are eccentric and plaque growth occurs with increasing the thickness of plaque cap or increasing the diseased wall angle. Models were divided into two groups: atherosclerotic arteries with compensatory expansive remodeling and atherosclerotic arteries without. Considering the high variety of atherosclerotic plaques, using idealised models of plaque facilitates performing sensitivity analysis. To determine shear stress and circumferential stress, fluid-structure interactions (FSI) method, as the most accurate techniques for the estimation of stress distribution within atherosclerotic arteries was utilized (55).

5.2.1 Geometrical model

Figure 5-1a and 5-1b represent the cross-section of the idealised models. The diameter of the lumen in the healthy portions of the model of coronary artery was assumed to be to be 3.4 mm. The thickness of the arterial wall was assumed constant and equal to 0.3mm (234). In all idealized models, the length of atherosclerotic plaque was set to be 10 mm, and the lengths of proximal and distal sections were set at 20 mm to avoid potential boundary condition effect. As a geometrical constraint, the lumen and artery borders were considered to be circular. Four different values of 0.5, 1, 1.5 and 2mm were chosen as the thickness of the plaque cap (Figure 5-1a). The parameter of the diseased wall angle was introduced to describe incomplete atherosclerotic plaques (Figure 5-1b) and was elevated from 120º to 360º to mimic the progression of atherosclerotic plaques along the circumference. In complete eccentric plaques, due to the interconnection of plaque shoulders, a secondary fibrous cap was created. The thickness of the secondary fibrous cap was assumed to be 0.25 of the initial plaque cap (0.125, 0.25, 0.375 and 0.5mm for different cases). In each model, the effect of the existence of lipid

pool was investigated. The angle and thickness of lipid pool were considered respectively half of the diseased wall angle and half of plaque cap thickness.

In models of atherosclerotic arteries without remodeling, it was assumed that the outer diameter of the atherosclerotic artery did not change during lesion growth and remained constant. Consequently, the lumen area decreased continuously with the increase of the plaque cap thickness or diseased wall angle. Figure 5-1c shows a typical model of the atherosclerotic artery without remodeling. Models with compensatory expansive remodeling were designed based on the definition of Glagov et al (38). In the initial stage, the lumen was assumed similar to that of the healthy artery, the thickness of the plaque cap was considered to be 0.5 mm, and for different diseased wall angles, initial atherosclerotic plaques were created by the expansion of artery. With the gradual increase of plaque cap thickness, it was assumed that atherosclerotic arteries expand in a way that the luminal area remained constant until the percentage of plaque burden reached 40%. The percentage of plaque burden in each model was calculated based on equation 5-1. After this, the expansive remodeling ceased as the dimensions of the arterial wall were considered constant, and by the further progression of the disease, the luminal area decreased gradually by means of the increase of plaque cap thickness. Figure 5-1d represents a typical model of atherosclerotic arteries with compensatory expansive remodeling.

$$\text{Percentage of plaque burden } = 100 * \frac{Plaque_{area}}{Plaque_{area} + Lumen_{area}} \tag{5-1}$$

Considering four different plaque cap thicknesses, four different diseased wall angles, two types of arterial wall remodeling, and the existence or lack of lipid pool, 64 distinct idealized plaque topologies were designed for the computational study. Table 5-1 represents the luminal stenosis of various models by considering the type of remodeling, the angle of the disease-free wall and the thickness of the plaque cap.

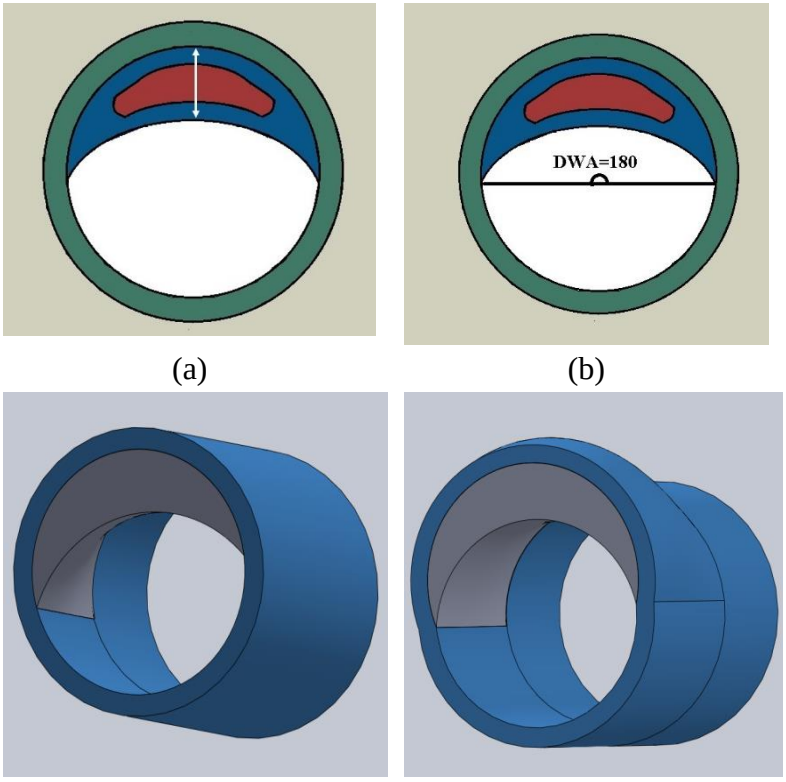


Figure 5-1 The characteristics of idealized models of atherosclerotic coronary arteries designed for numerical simulation. a) The plaque cap thickness in the cross-section of the model. b) The diseased wall angle (DWA) in the cross-section of the model. c) A typical model with no remodeling, lack of lipid pool, DWA=180, and PCT=1mm. d) A typical model with expansive remodeling, lack of lipid pool, DWA=180, and PCT=1mm.

Table 5-1 The percentage of luminal stenosis for different idealized models based on their disease wall angle (DWA) and plaque cap thickness (PCT).

		PCT=0.5 mm	PCT=1mm	PCT=1.5 mm	PCT=2 mm
DWA=120º	Expansive remodeling	0	0	0	0
	No remodeling	12%	21%	31%	43%
DWA=180º	Expansive remodeling	0	0	0	11%
	No remodeling	17%	32%	45%	57%
DWA=240º	Expansive remodeling	0	0	6%	27%
	No remodeling	25%	40%	58%	73%
DWA=360º	Expansive remodeling	0%	4%	24%	41%
	No remodeling	33%	52%	79%	92%

5.2.2 Numerical Simulation

To estimate mechanical stress fields within atherosclerotic plaque models, fully coupled FSI analysis was performed by coupling ANSYS CFX and ANSYS Mechanical modules. Hexahedral elements were generated to fit the shape of the arterial wall, atherosclerotic plaque, and fluid domain. Mesh density sensitivity analyses were performed on both fluid and solid domains by increasing the number of elements in the mesh until the difference in peak stress was <2% of the previous mesh. For a typical model with expansive remodeling, diseased wall angle of 180 and fibrous cap thickness of 1mm, the vessel consisted of 405234 elements and the fluid domain consisted of 195634 elements. The inner surface of the arterial wall and the corresponding fluid boundary were defined as the fluid-structure interface. A no-slip condition between solid and fluid domains was applied. As the convergence criteria, the residuals were limited to less than 1e-6. The level of shear stress and circumferential stress as main contributors in the progression and rupture of atherosclerotic plaques were reported for different models.

Fluid domain

Blood was assumed to be Newtonian and incompressible with a density of 1050 Kg/m^3 and dynamic viscosity of 0.0035 Pa.s. The incompressible Navier–Stokes equations were used as the governing equations:

$$\frac{\partial u_i}{\partial x_i} = 0 \tag{5-2}$$

$$\frac{\partial u_i}{\partial t} + u_j \frac{\partial u_i}{\partial x_j} = \frac{1}{\rho_b} \frac{\partial p}{\partial x_i} + \frac{1}{\rho_b} \frac{\partial \tau_{ij}}{\partial x_j} \tag{5-3}$$

where x_i and x_j are the coordinates, u_i represents the velocity vector, p stands for the blood pressure, ρ_b is the blood density and τ_{ij} is the fluid stress tensor (186). The finite-volume method was implemented using ANSYS CFX to solve the governing equations. A typical physiological coronary flow waveform with a parabolic velocity profile was considered at the inlet and stress-free boundary condition was imposed at the outlet of the models (54, 191). To avoid the negative effect from boundary condition at the entrance of the models, an additional tube of the same inlet diameter and following a direction perpendicular to the inlet cross-section was merged with the original vessel. To apply stress-free boundary condition at the inlet, the normal derivative of the velocity components was set to zero.

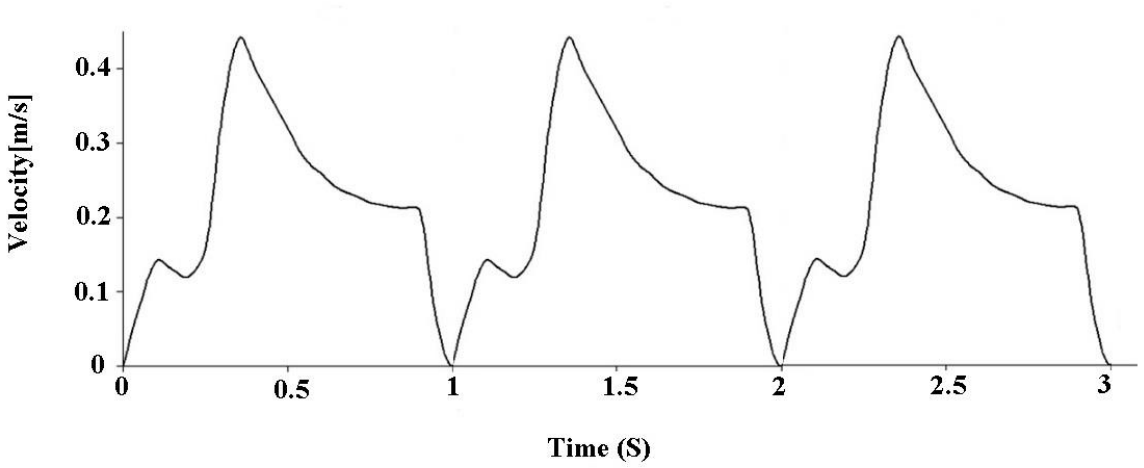


Figure 5-2 Inlet velocity waveform for the models of atherosclerotic coronary artery

Solid domain

Both arterial wall and atherosclerotic plaque were assumed to be hyperelastic, isotropic, incompressible and homogeneous. The 3D nonlinear modified Mooney–Rivlin model was used to describe the material properties of the vessel wall and atherosclerotic plaque (236). The strain energy function of the used model was as follows:

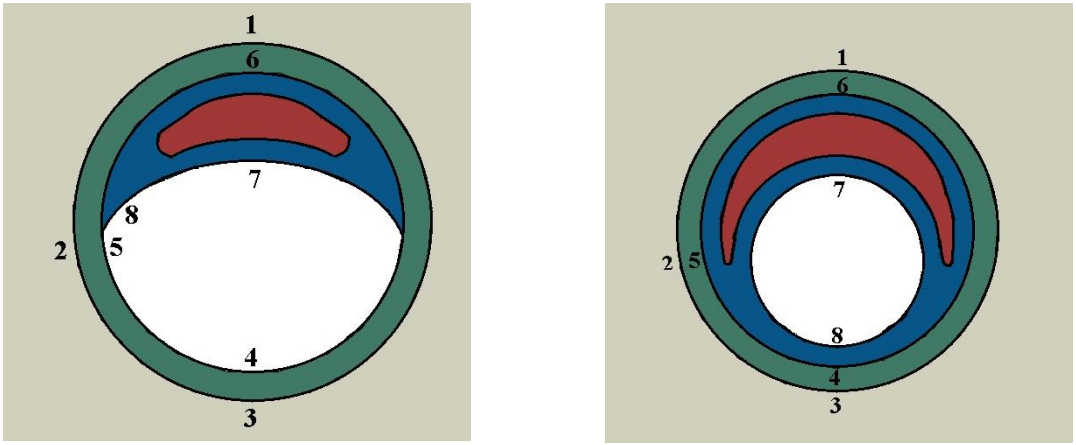
$$W=C_1\ (I_1-3)+D_1\ [\exp\ (D_2\ (I_1-3))-1] \quad (5\text{-}4)$$

where I_1 and I_2 are the first and second strain invariants. The parameters C_1, D_1 and D_2 are material constants. According to the existing literature, following values were chosen for the coronary arterial wall as: c_1 = 36.8 kPa, D_1 = 14.4 kPa, D_2=2; atherosclerotic plaque: c_1 = 73.6 kPa, D_1 = 28.8 kPa, D_2 = 2.5 and lipid pool: c_1 = 2 kPa, D_1 = 2 kPa, D_2 = 1.5 (204).

To apply proper boundary conditions and allow radial expansion and rotational movement of the artery, the models were embedded in a cube of soft elastic material with a very low Young's Modulus of 1 kPa (221). This soft elastic material was considered to simulate the connective tissues around the coronary arteries. To prevent rigid body motion, all boundaries of the surrounded tissue were fully constrained (193); however, the artery and surrounding tissue were allowed to deform. The entire structure was meshed using hexahedron elements and finite element method (FEM) was applied for the estimation of the circumferential stress field.

Stress analysis

To compare the level of circumferential stresses in pathologically meaningful parts of models, eight different locations were defined (Figure 5-3a): 1- The outer part of media layer behind the atherosclerotic plaque; 2- The outer part of media layer behind the plaque shoulder; 3- The outer part of media layer in disease-free wall; 4- The inner part of media layer in disease-free wall; 5- The inner part of media layer close to plaque shoulder; 6- The inner part of media layer behind the atherosclerotic plaque; 7- The centre of atherosclerotic plaque close to the lumen; 8- The shoulder of atherosclerotic plaque. As shown in Figure 5-3b, due to lack of diseased wall in complete atherosclerotic plaques, the intended locations for reporting circumferential stresses were changed slightly. Since the shear stress level was almost constant in each cross-section of the artery, the maximum value of shear stress was reported for each model.



(a) (b)

Figure 5-3 Specified regions within arterial wall and atherosclerotic plaque for determination of circumferential stress. a) An incomplete atherosclerotic plaque with DWA=180, b) A complete atherosclerotic plaque with DWA=360

5.3 Results

Figure 5 represents the Von Mises stress distribution in the cross section of models of atherosclerotic plaque by considering the type of arterial wall remodeling and the existence or lack of lipid pool. The stresses were reported by averaging over a 3D surface. As can be seen, although the stress concentration in the model with compensatory expansive remodeling occurred at the shoulder of atherosclerotic plaque, the stress concentration in the model without remodeling appeared in the arterial wall close to the plaque shoulder. Moreover, higher levels of stress observed in the atherosclerotic plaques with lipid pool compared to those which do not have a lipid pool.

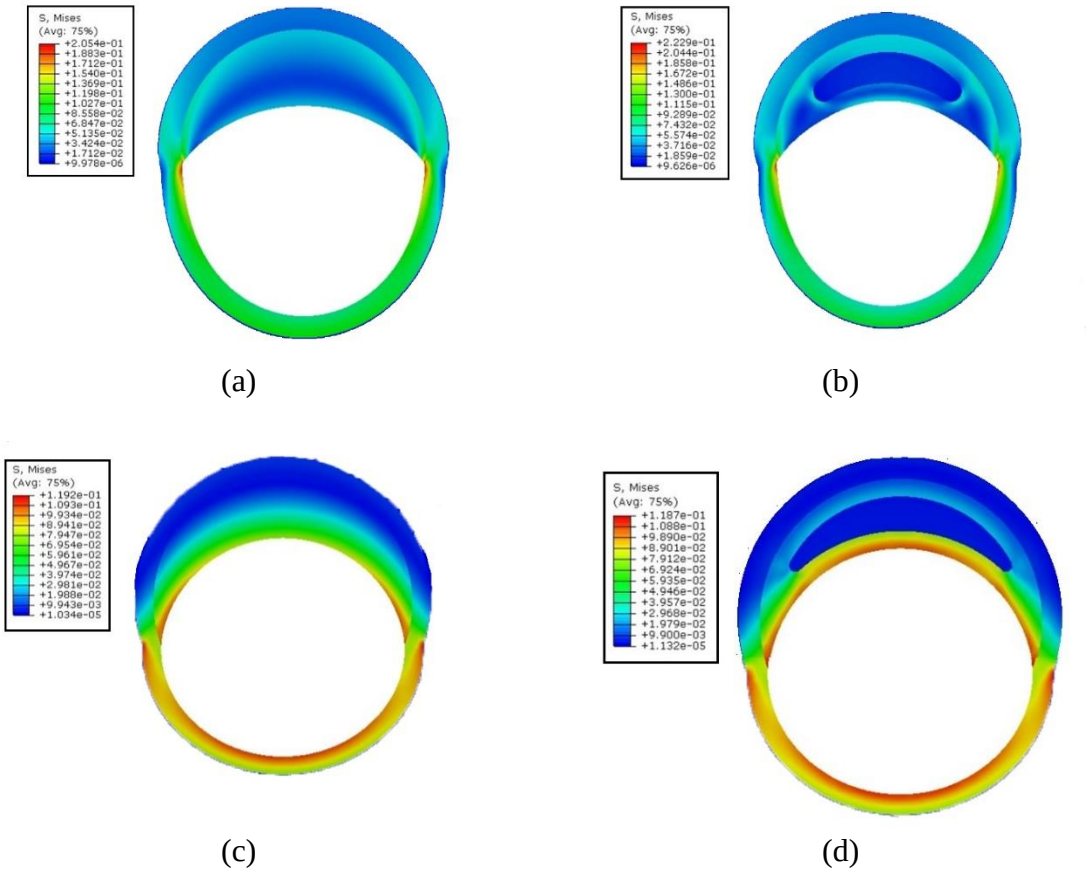


Figure 5-4 The pattern of Von Mises stress (MPa) in the idealized models of atherosclerotic coronary plaques with DWA=180 and PCT=1mm. a) Model without remodeling and lack of lipid pool. b) Model without remodeling and the existence of lipid pool. c) Model with expansive remodeling and lack of lipid pool. d) Model with expansive remodeling and the existence of lipid lipid.

Figures 5-5, 5-6, 5-7 and 5-8 compare the level of wall circumferential stress at specified locations of the arterial wall and atherosclerotic plaque among healthy arteries, atherosclerotic arteries without remodeling and atherosclerotic arteries with compensatory expansive remodeling with the existence or lack of lipid pool. Figure 5-5, 5-6 and 5-7 represent the circumferential stress level at different locations of incomplete atherosclerotic plaques with diseased wall angles of 120, 180 and 240 respectively, while Figure 5-8 shows the circumferential stress level at defined zones of complete atherosclerotic plaques.

As shown in Figures 5-5, 5-6 and 5-7, the media layer behind the atherosclerotic plaque (Location 1 and 6) experienced lower levels of circumferential stresses compared to the healthy artery regardless of the thickness of plaque cap, the type of remodeling, the existence of lipid

pool and diseased wall angle. Similarly, the level of circumferential stress within all areas of the medial layer of complete atherosclerotic arteries was significantly lower than that of a healthy artery (Figure 5-8).

The circumferential stress levels at the media layer of all incomplete plaques with compensatory expansive remodeling, close to the atherosclerotic plaque shoulder (Location 2 and 5) were almost equal to the stress level of healthy arteries. The thickness of the plaque cap, the existence of lipid pool, as well as diseased wall angle, did not have a considerable effect on the stress level at these sites. On the other hand, the stress level at Locations 2 and 5 of models without arterial wall remodeling did not overlap with that of the healthy artery. The induction of low-stress level (about 60% of physiological value) at the outward of the medial layer and high circumferential stress (almost 1.5 of physiological value) at the inward of media layer, lead to high circumferential stress gradient within the medial layer of atherosclerotic plaque without remodeling.

The circumferential stress levels in the disease-free wall section (Location 3 and 4) of incomplete atherosclerotic plaques with compensatory expansive remodeling were almost equal to the healthy artery regardless of diseased wall angle, the existence of lipid pool and thickness of the plaque cap. Similarly, the circumferential stress level in the disease-free wall of atherosclerotic arteries without remodeling was almost equal to that of the healthy artery when the diseased wall angle was equal to 120°. However, with the growth of the diseased wall angle, the stress level at the disease-free wall of models without remodeling reduced significantly compared to the healthy artery. For instance, for the atherosclerotic plaques without remodeling with the diseased wall angle of 240° and plaque cap of 2mm (Figure 5-7), the stress level at location 3 and 4 were about 70% of that of the healthy artery (Figure 5-7).

In all models, the circumferential stress level in the middle of plaque cap (Location 7) and shoulder of plaque (Location 8) were greater in models with compensatory expansive remodeling compared to models without remodeling. Moreover, the existence of a lipid pool led to the increase of stress level in these regions. However, the stress level difference between models with compensatory remodeling and without remodeling decreased with plaque development in the circumferential direction. With increasing the plaque cap thickness as well as growing of diseased wall angle, the level of circumferential stress in the middle of the plaque cap and plaque shoulder decreased considerably for all models. Hence, the development of atherosclerotic lesions without lipid pool led to the reduction of the possibility of plaque rupture.

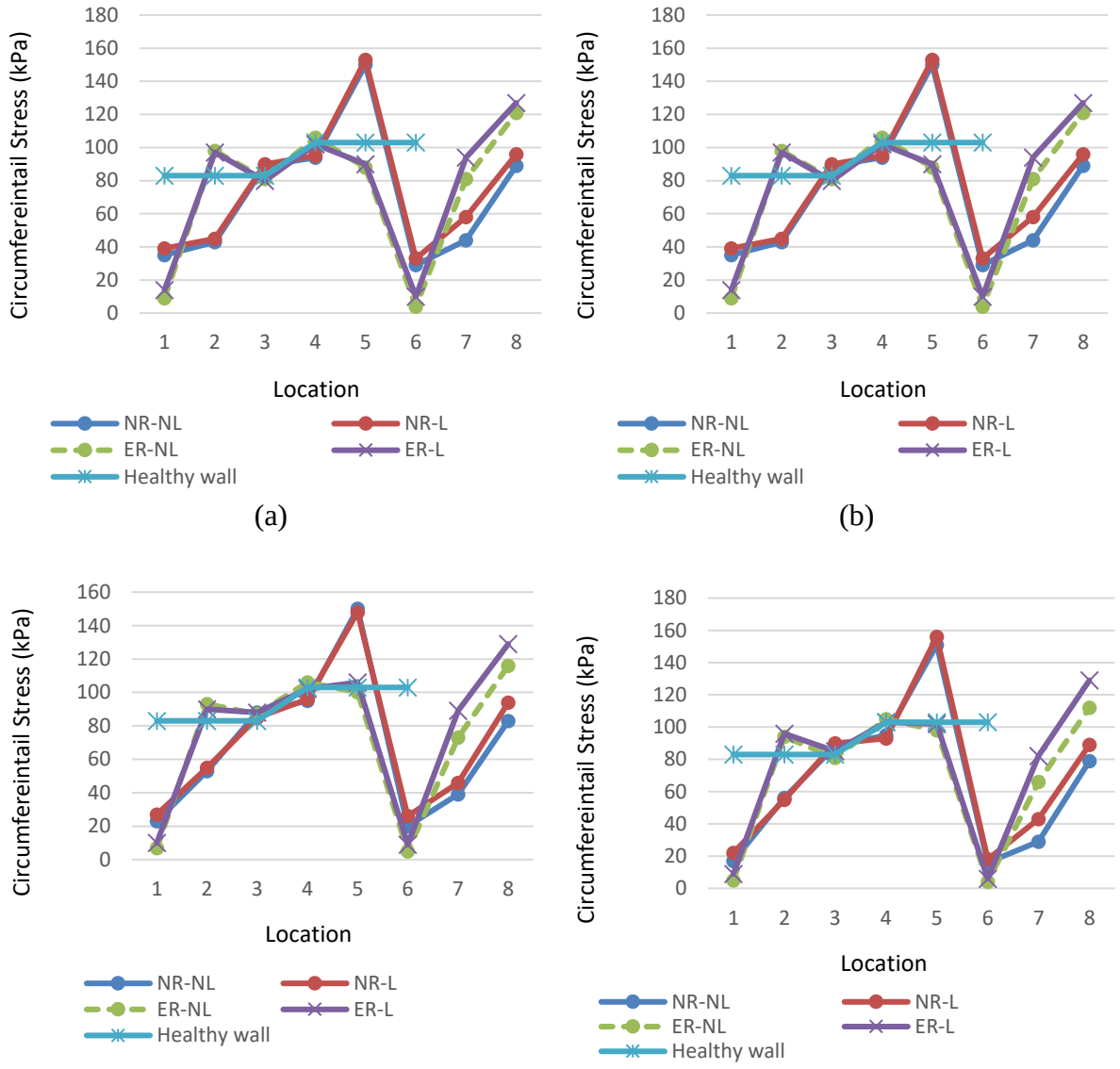


Figure 5-5 Circumferential stress levels in specified locations of the arterial wall and atherosclerotic plaque in idealized models of atherosclerotic plaque with different plaque cap thickness values and DWA=120, comparing with a healthy artery. a) PCT=0.5 mm, b) PCT=1 mm; c) PCT=1.5 mm, d) PCT=2 mm. The results related to plaques with no remodeling and no lipid pool (NR-NL), plaques with no remodeling which contain lipid pool (NR-L), plaques with expansive remodeling and no lipid pool (ER-NL), and plaques with expansive remodeling which contain lipid pool (ER-L) are reported.

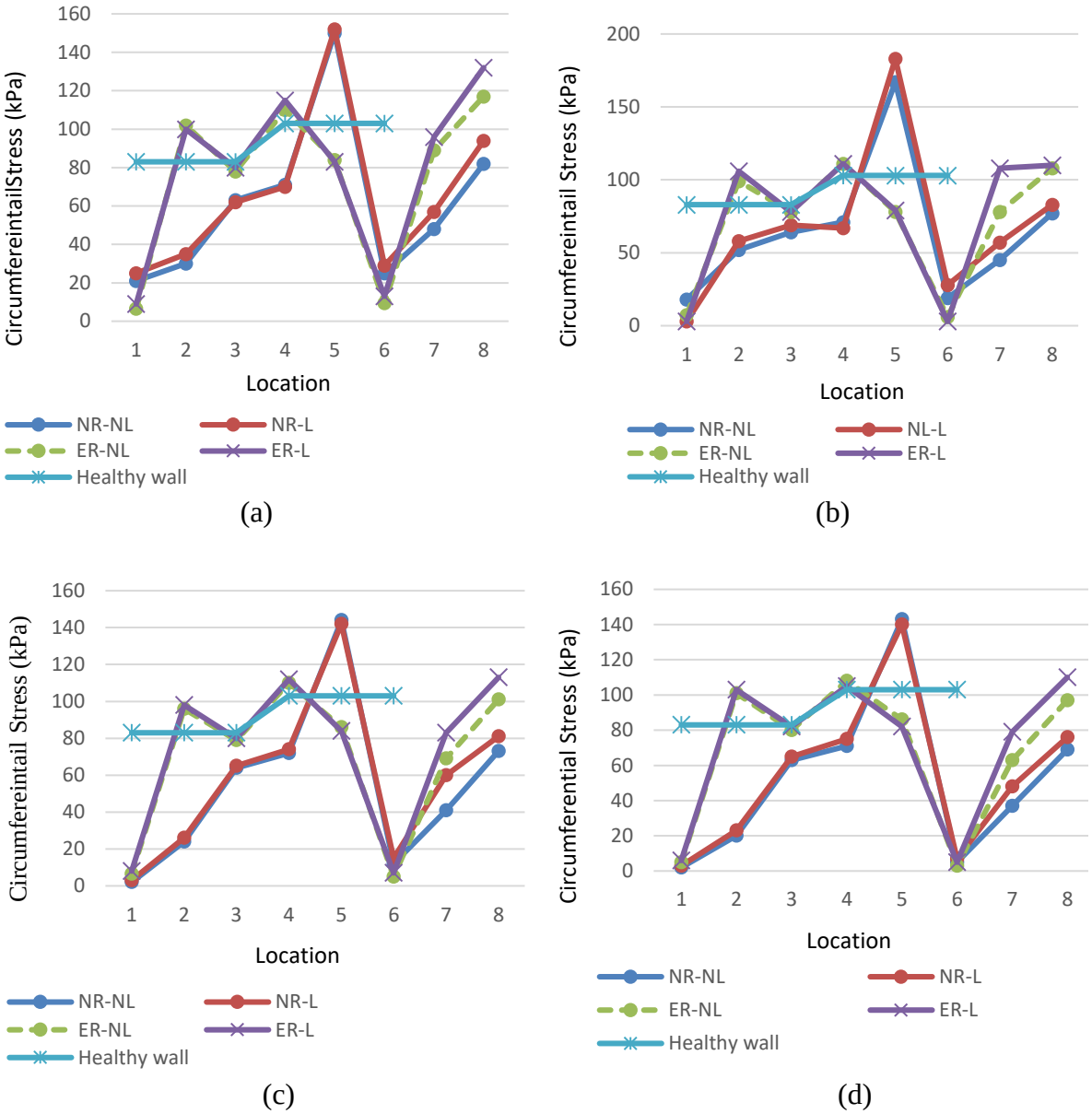


Figure 5-6 Circumferential stress in specified locations with idealized models of atherosclerotic plaque with DWA=180, comparing with the healthy artery. a) PCT=0.5 mm, b) PCT=1 mm; c) PCT=1.5 mm, d) PCT=2 mm.

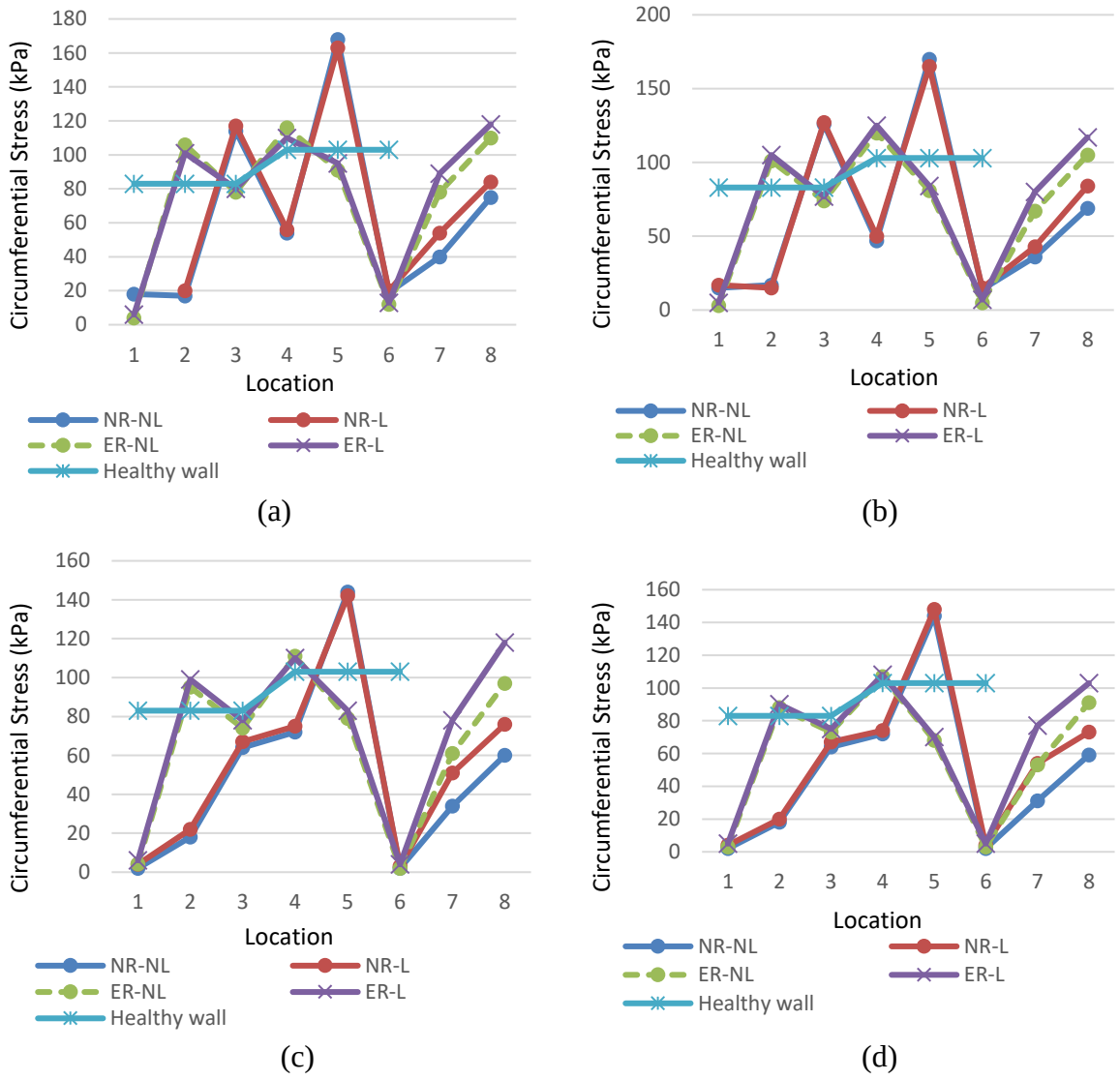


Figure 5-7 Circumferential stress in different locations of idealized models with DWA=240, comparing with healthy artery. a) PCT=0.5 mm, b) PCT=1 mm; c) PCT=1.5 mm, d) PCT=2 mm.

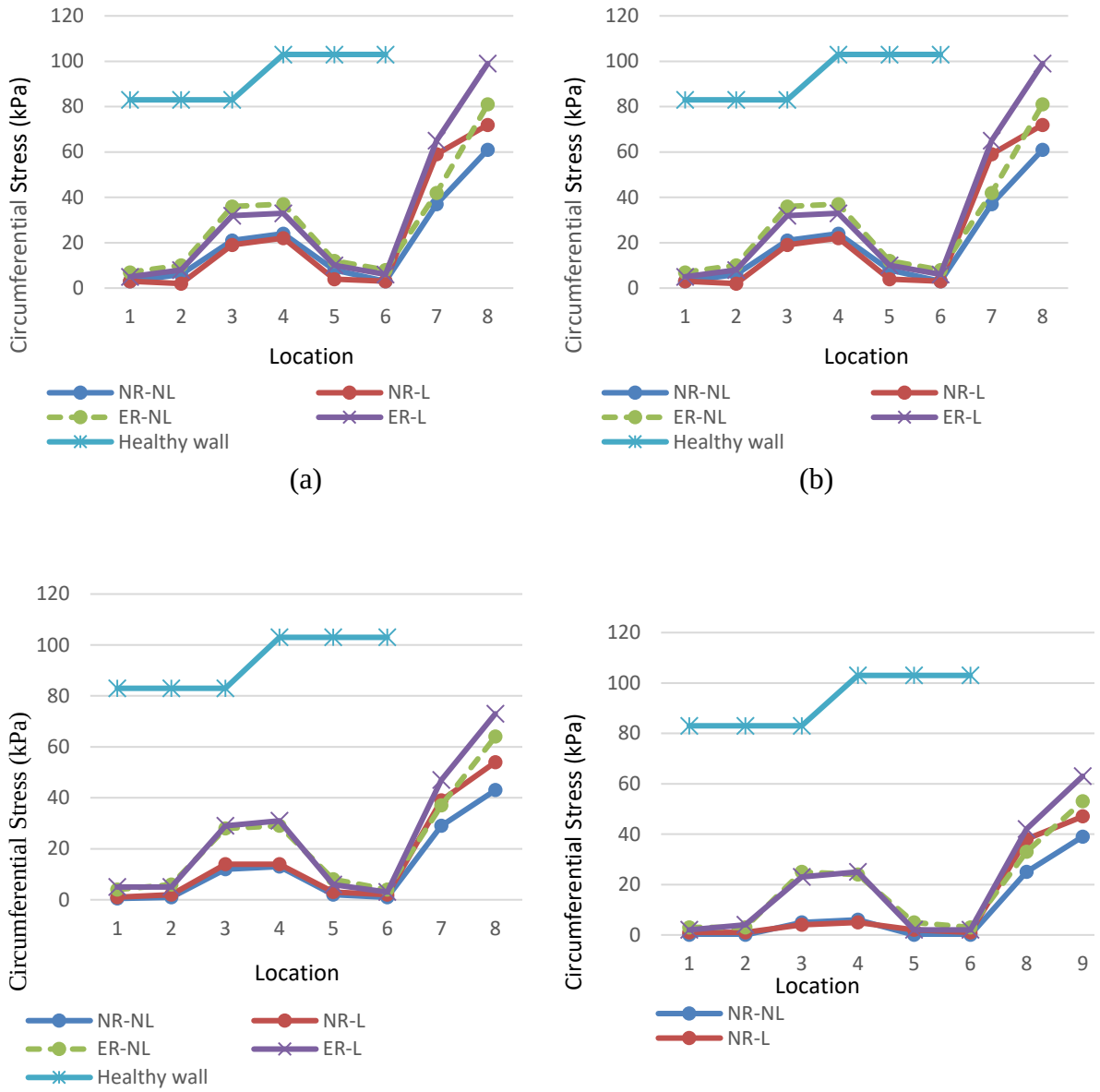


Figure 5-8 Circumferential stress in different locations of idealized models with DWA=360, comparing with the healthy artery. a) PCT=0.5 mm, b) PCT=1 mm; c) PCT=1.5 mm, d) PCT=2 mm.

Figure 5-9 shows the shear stress pattern in the region of stenosis in four different models with various plaque cap thickness, disease wall angle and type of remodeling. The existence of lipid pool did not affect the distribution of shear stress within the models significantly (results not shown). In the model with compensatory expansive remodeling, disease wall angle of 180° and plaque cap thickness of 1mm (Figure 5-7a), the shear stress remained constant and equal to the healthy artery due to the expansion of the arterial wall and the preservation of the luminal area. However, in the model without remodeling with same plaque cap thickness and diseased wall angle (Figure 5-7b), due to the reduction of luminal area, the shear stress increased up to 1.25 times greater than that of the healthy artery in the region of stenosis and reduced to less than 40% of the healthy artery in the downstream of atherosclerotic plaque. Figure 5-8c and 5-8d represent the shear stress distribution in the models with compensatory expansive remodeling and without remodeling respectively, with disease wall angle of 360° and plaque cap thickness of 2mm. As can be seen, although arterial wall expansion cannot prevent luminal stenosis in this case (Figure 5-8c), the level of shear stress in the model with expansive remodeling is much lower (less than 2%) than that of the model without remodeling (Figure 5-8d). Moreover, in the model without remodeling, vortex and secondary flow (negative shear stress) were observed in the downstream of atherosclerotic plaque.

Figure 5-10 compares the maximum shear stress levels in different models regarding the type of remodeling, the thickness of plaque cap and diseased wall angle. The existence of lipid pool did not affect the shear stress level within the models significantly. As an overall trend, the alteration of shear stress was significantly greater in atherosclerotic plaques without remodeling compared to the atherosclerotic plaques with compensatory expansive remodeling. In models with compensatory expansive remodeling, the level of shear stress remained constant until the degree of stenosis reached 40%, such as all models with disease wall of 120º. Then, when the arterial wall expansion ceased, the shear stress level increased gradually and reached to more than double of physiological range in complete atherosclerotic plaque with the cap thickness of 2mm. For all models with compensatory expansive remodeling, the pressure drop due to stenosis was less than 2%. However, in atherosclerotic plaques without remodeling, the rate of rising in shear stress, as well as the pressure drop due to stenosis, was more rapid especially when the luminal stenosis was more than 70%.

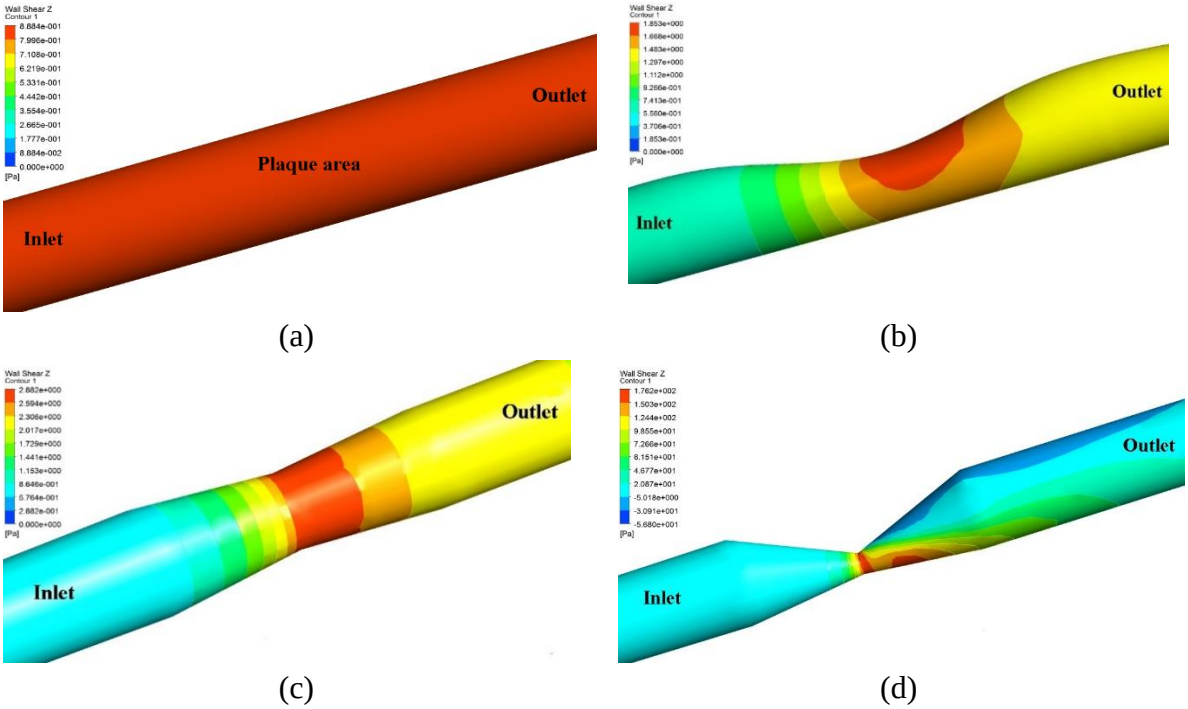


Figure 5-9 The shear stress distribution (Pa) in the idealized models of atherosclerotic coronary plaques. a) Model with compensatory expansive remodeling, DWA=180 and PCT=1mm. b) Model without remodeling with DWA=180 and PCT=1mm. c) Model with compensatory expansive remodeling, DWA=360 and PCT=2mm. d) Model without remodeling with DWA=360 and PCT=2mm.

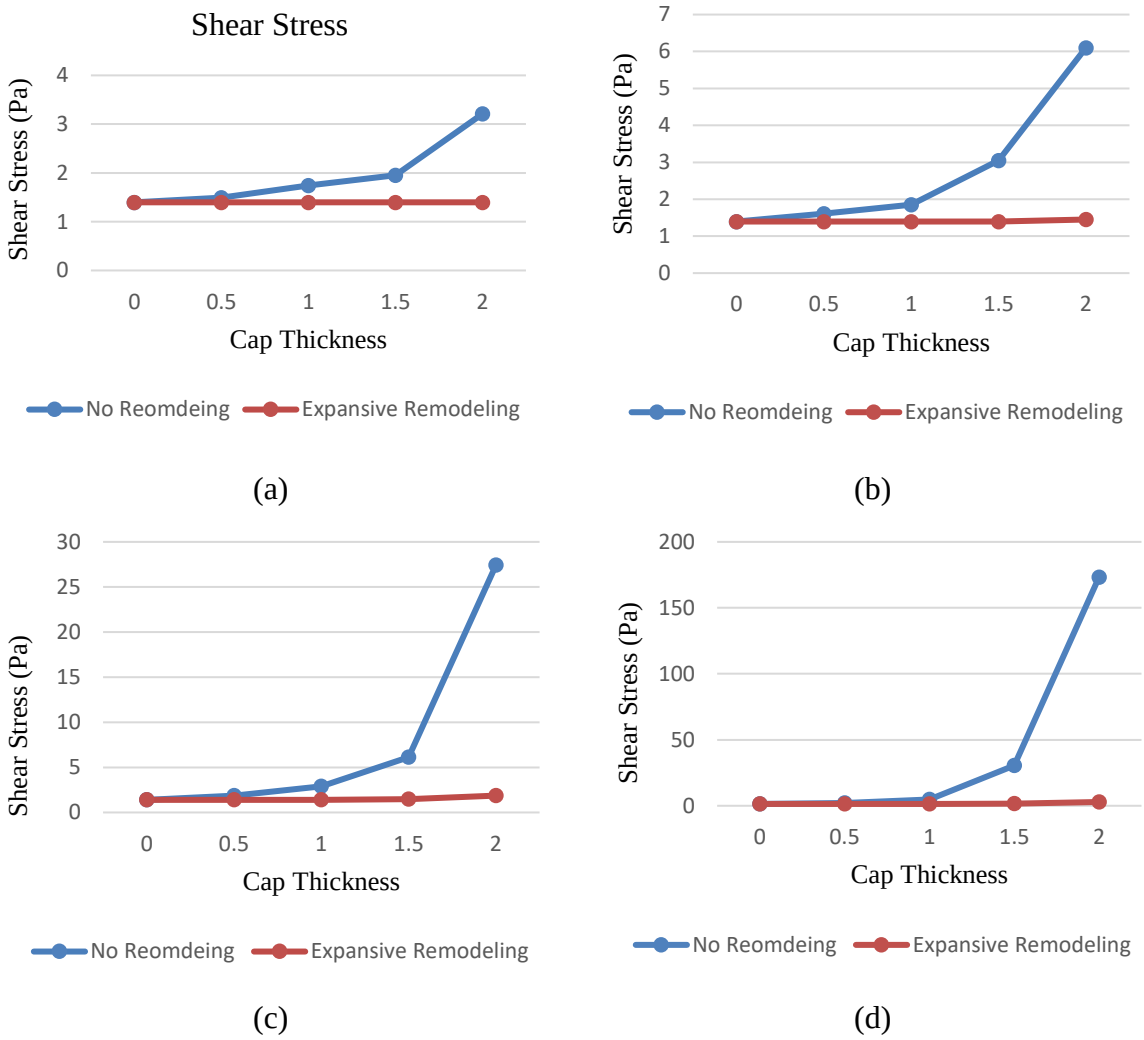


Figure 5-10 Shear stress (Pa) in different models with different disease-free wall angle and different remodeling type. a) DWA=120, b) DWA=180, c) DWA=240, d) DWA=360.

5.4 Discussion

It is well known that the biological ranges of shear stress and circumferential stress play key roles in regulating the biological function of endothelial cells and SMCs, extracellular matrix (ECM) synthesis, and the out of biological ranges are associated with clinical conditions and eventually rupture of atherosclerotic plaques (40, 51). Hence, computational stress analysis can assist in examining the atherosclerotic plaque vulnerability and obtaining a better insight into the role of biomechanical factors during plaque progression. In the present study, we used idealized models to examine the alternation of mechanical stresses within the arterial wall and atherosclerotic plaques during plaque development. By formation and progression of

atherosclerotic plaques, significant alterations in the circumferential stress induced in different parts of the arterial wall were found. These alternations might influence the structure and function of the arterial wall primarily by inducing deformation on the ECM in which SMCs are embedded (8). Results indicate that not only the stress values changed dramatically, but also such changes caused different stress gradients within the plaque body and arterial wall both in diseased and disease-free parts. SMCs switch between the contractile and synthetic phenotypic states in response to the alternation of applied tension. The transition of SMCs from the contractile to the synthetic state facilitates the pathological conditions of SMCs (75, 237). Synthetic SMCs migrate and proliferate more readily and can synthesize collagen fibers, about 25 times more than that of contractile SMCs (75, 125). It is well known that the physiological range of circumferential stress on the arterial wall maintains medial SMCs in their contractile state and both low and pathological applied tensions cause phenotype switching of SMCs from contractile to synthetic proliferative(96).

Our results indicate that due to thickening caused by plaque formation, the reduction of circumferential stress level in the media layer of the arterial wall behind atherosclerotic plaques is inescapable, regardless of the type of arterial wall remodeling, disease wall angle, existence of lipid pool, and plaque cap thickness. This reduction was more pronounced in atherosclerotic plaques with expansive remodeling and became more significant with the increase in thickness of the plaque cap. Such a phenomenon might justify plaque growth through cap thickening. The reduction of the applied circumferential stress on the arterial wall can cause the phenotype switching of SMCs from contractile to synthetic proliferative (97). This process triggers migration of proliferated SMCs from media to intima layer and synthesis of high amount ECM components especially collagen fibers leading to neointima development (74). With the increase of disease wall angle, more sections of the arterial wall undergo low circumferential stress and in complete atherosclerotic plaques, the applied circumferential stress on all portions of the arterial wall is lower than in healthy arteries. The reduction in circumferential stress can cause wall atrophy (10) and also affects the expression of matrix metalloproteinase (MMPs) by SMCs. MMPs are the major proteases responsible for ECM degradation. The disruption of collagen networks by MMPs is required for the expansive remodeling of the arterial wall. Previous studies showed that cyclic strain increases the expression and activity of MMPs (238). Hence, it can be hypothesized that the diseased wall of atherosclerotic arteries, in which the strain level is very low, does not exhibit expansive remodeling. Consequently, if the full

circumference of atherosclerotic plaque is occupied by atherosclerotic plaque the expansive remodeling of atherosclerotic arteries would stop (122, 126).

Our results revealed that in atherosclerotic arteries which experience compensatory expansive remodeling, the level of circumferential stress at the disease-free wall is approximately equal to that of healthy arteries, however by the lack of expansive remodeling the circumferential stress at the disease-free wall does not remain in the physiological range. In the absence of expansive remodeling, a marked circumferential stress concentration was observed at the inner part of the disease-free wall near the plaque shoulder. The induction of excessive amount of circumferential stress on the endothelial cells can lead to the endothelial injury and reduction of nitric oxide (NO) expression by endothelial cells (229). NO has anti-atherogenic properties and the reduction of its expression promotes plaque progression (229). SMCs also might be affected by the existence of high circumferential stresses within the medial layer of atherosclerotic arteries. Pathological tension not only increases ECM synthesis by SMCs (96, 97) but also facilitates the migration of SMCs into the intima through the increase of MMPs activity (229). Hence, by considering all the processes mentioned above, it can be proposed that atherosclerotic plaques without expansive remodeling are more likely to develop into the circumference and become complete. This suggestion is supported by clinical data showing that atherosclerotic plaques without remodeling are often complete, concentric and stenotic (8, 122).

The effect of alternation of shear stress level is most commonly associated with the function of endothelial cells (97, 132, 133). It was found that with the compensatory expansion of the arterial wall up to %40 stenosis, the physiological shear stress stays still. However, the lack of compensatory expansive remodeling in atherosclerotic arteries accelerated luminal area reduction and intensified shear stress level in the region of stenosis which may cause macrophage accumulation (98) and may promote the possibility of plaque erosion (43). Moreover, the lack of compensatory expansive remodeling intensified the drop of shear stress and vortex formation in distal parts of the atherosclerotic plaque with the likelihood of endothelial injury and axial plaque progression in this region (8, 126). Consequently, very high shear stress gradient from the stenosis site to the distal part of atherosclerotic plaque was observed in atherosclerotic arteries without expansive remodeling. Our findings suggest that plaque erosion, as well as plaque progression in distal parts of stenosis, occurs more likely in atherosclerotic plaques with the high degree of luminal stenosis, predominately formed in the absence of expansive remodeling.

It was found that for preserving the constant blood flow rate in atherosclerotic arteries without remodeling, the required pressure gradient increased considerably, especially for the luminal stenosis of more than 70%. Hence, the upstream blood pressure should increase, resulting in elevation of blood pressure in the parallel non-stenotic arteries and eventually systemic hypertension. Considering the systemic limitation of the heart and vasculature, the physiological blood flow rate cannot be preserved in high stenotic arteries which can lead to blood supply shortage in downstream organs (239).

Our finding revealed that the level of circumferential stress at the middle and shoulder of atherosclerotic plaques with compensatory expansive remodeling was significantly higher than atherosclerotic plaques without remodeling. Moreover, the stress level at the middle and shoulder of plaque cap was significantly higher in the plaque containing lipid pool. The repetitive induction of high circumferential stresses in atherosclerotic plaques may result in the formation of small cracks and mechanical fatigue failure of plaques (199, 221). With the increase of plaque cap thickness as well as plaque development into the circumference, the reduction of circumferential stress level within atherosclerotic plaque was observed. Hence, it can be concluded that incomplete atherosclerotic plaques which undergo compensatory expansive remodeling are the most vulnerable plaques especially in the early stages of development. Our results are consistent with clinical data and previous studies based on numerical modeling (234, 235) and may explain why plaque rupture often occurs with only modest luminal stenosis.

Limitations

Although the progressive stiffness changes of the arterial wall and atherosclerotic plaque components during plaque development has been reported (205), these alterations cannot affect the mechanical stresses distribution within atherosclerotic arteries significantly(183). Consequently, in the current study it was assumed that the mechanical properties of the arterial wall and atherosclerotic plaque components remain constant during atherosclerosis progression. The results were achieved based on idealized models of atherosclerotic coronary arteries in the absence of calcification. The effect of microcalcifications on the plaque vulnerability have been widely investigated in the previous studies (190, 191). Considering the negligible effect of non-Newtonian behaviour of blood on the mechanical stress fields, blood was considered Newtonian. Although the range of reported stresses were in agreement with the

previous studies (191), further investigations are required to validate our conclusions using patient-specific plaque data.

5.5 Conclusion

The compensatory expansive remodeling of the arterial wall during atherosclerotic plaque development is crucial to preserve the physiological range of wall shear stress and wall circumferential stress. Moreover, the compensatory expansion of atherosclerotic plaques prevents the reduction of blood supply to the vital organs and the possibility of plaque erosion as well as postponing plaque progression downstream to atherosclerotic plaques. However, atherosclerotic plaques which undergo compensatory expansive remodeling are more vulnerable to rupture due to the induction of higher level of circumferential stress within atherosclerotic plaques. These findings may provide new insight into the role of arterial wall remodeling on the mechanical stress field within atherosclerotic arteries.

Chapter 6

Stress analysis of fracture of atherosclerotic plaques: Crack propagation modeling

Chapter outline

After the characterization of the remodeling of atherosclerotic arteries, this chapter aims to study the disruption of atherosclerotic plaques from a biomechanical point of view. We hypothesized that the rupture of atherosclerotic plaques occurs due to the mechanical fatigue caused by pulsatile blood pressure. Based on this hypothesis, we simulate the process of crack formation and progression in the realistic geometry of atherosclerotic plaques derived from histological examination of atherosclerotic coronary arteries. Then, based on fracture mechanic theories, the number of cycles required for plaque rupture (fatigue life) was calculated for different models and used for assessing the plaque vulnerability. Finally, the effect of different parameters such as pulse and mean pressure as well as lipid pool lipid pool stiffness, on the fatigue life of atherosclerotic plaques was investigated.

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Chapter 7

Conclusions, limitations and future directions

7.1 Discussion

Rupture of atherosclerotic plaques and the subsequent thrombus is one of the leading causes of morbidity and mortality over the world. From the biomechanical point of view, the non-physiological levels of mechanical stresses within atherosclerotic plaques can contribute to the formation, progression, and rupture of atherosclerotic plaque. Over the last few decades, numerical simulation has been extensively used for the mechanical stress analysis within the atherosclerotic arteries. In this project, several aspects regarding the biomechanical characterization of remodeling and rupture of atherosclerotic plaque were tackled and discussed.

In Chapter 3 and 4, the alteration of the mechanical properties of the arterial wall and atherosclerotic plaque during atherosclerotic plaque development were investigated in the abdominal aorta and coronary arteries respectively. In these Chapters, histological analysis was employed to highlight the different structure of the medial layer of the abdominal aorta and coronary arteries. By comparing the results presented in Chapter 3 and 4, it was revealed that the elastic modulus of IEL and EEL within coronary arteries are comparable with the elastic modulus of elastin lamellae in the abdominal aorta. The reduction of stiffness of IEL in coronary arteries and elastin lamellae in the aortic wall with plaque development were observed. Moreover, it was shown that the range of elastic modulus of the inter-lamellar zones within the abdominal aorta and the medial layer in coronary arteries are the same and the stiffness of both of inter-lamellar zones within the abdominal aortic wall and the medial layer in coronary artery increase with the growth of atherosclerotic plaque. The mechanical properties of different components of plaques such as lipid pool, calcification, and fibrous tissue were almost similar in the two samples, suggesting that the composition of atherosclerotic plaques formed in the coronary arteries and abdominal aorta is almost similar.

In Chapter 5, the effect of remodeling of atherosclerotic plaques on the mechanical stress distribution was investigated using FSI simulation. The simulations were carried out on the idealized models of atherosclerotic coronary arteries and the sensitivity analysis was performed on the desired parameters such as the mode of remodeling, lipid pool size, disease wall angle and fibrous cap thickness. The results revealed that in atherosclerotic plaques with expansive remodeling, the level of shear stress and circumferential stress sensed by endothelial cells and SMCs respectively remain in the physiological range. Therefore, the preservation of the physiological range of mechanical stress within the atherosclerotic arteries can be considered

as a trigger for the expansive remodeling of atherosclerotic plaques. Furthermore, it was observed that higher level of circumferential stress is present in the atherosclerotic plaques with expansive remodeling compared to atherosclerotic plaques without remodeling. This finding is in agreement with the clinical data showing that atherosclerotic plaques with expansive remodeling are more vulnerable to rupture. The focus of Chapter 6 was on the rupture of atherosclerotic plaques. It was hypothesized that mechanical fatigue caused by pulsatile blood pressure is the main mechanism underlying atherosclerotic plaque rupture. Histological examination as the gold standard for the determination of plaque composition was used for the reconstruction of plaque geometry. Based on fracture mechanics theories, the number of fatigue cycles required for plaque rupture was calculated and introduced as an index for the assessment of plaque vulnerability.

Overall, this thesis provides a biomechanical framework to study the remodeling and rupture of atherosclerotic plaques and establish a framework for developing clinical strategies to decrease the risk of myocardial infarction. Different parameters such as the expansive remodeling of atherosclerotic plaques and the existence of a large lipid pool, thin fibrous cap and calcification were introduced as the risk factor for plaque rupture. It was shown that the reduction of heart rate as well as pulse and mean pressure can increase the fatigue life of atherosclerotic plaques. Further analysis revealed that the pulse pressure has greater influence than the mean pressure on the rate of crack propagation in atherosclerotic plaques and its reduction can prolong the required time for plaque rupture independently. Moreover, it was found that with the increase of lipid pool stiffness the required time for plaque rupture increase. The presented results may elucidate the mechanisms of remodeling of atherosclerotic arteries (alterations of both composition and geometric features), as well as plaque rupture from a biomechanical point of view and may be used for determination of the vulnerability of atherosclerotic lesions.

7.2 Limitations

(i) Rupture of atherosclerotic plaques is a very complex process involving biochemical, biological and biomechanical factors. For the comprehensive assessment of plaque vulnerability, all of these factors should be considered. However, the focus of this thesis

was on the biomechanics of atherosclerotic plaques and the influence of biological and biochemical factors on the remodeling and rupture of atherosclerotic plaques was beyond the scope of this thesis.

(ii) In Chapter 3 and 4, the mechanical properties of the arterial wall and atherosclerotic plaque were reported for the three groups of healthy artery, mildly diseased artery, and advanced atherosclerotic artery. For the significance and clinical utility of the work, the results needed to be strengthened by a larger group of subjects.

(iii) It was desirable to use the patient specific material properties in the simulation and combine the morphological changes of atherosclerotic arteries with the alteration of mechanical properties of the arterial wall during plaque development. However, as mentioned in Chapter 3 and 4, the elastic modulus determined by the AFM test represents the local and compressive stiffness of arterial wall and atherosclerotic plaque and cannot describe the mechanical behaviour of the arterial tissue in the physiological range of blood pressure. Considering the difference between the local and bulk material properties, the results of AFM test did not use for the estimation of mechanical stress in Chapter 5 and 6.

(iv) To examine the effect of arterial wall remodeling on the mechanical stress distribution, the idealized models of atherosclerotic plaques were employed. Considering the high variability of atherosclerotic plaques, the use of idealized model facilitated the sensitivity analysis. Moreover, since the mode of remodeling is determined by comparing the external elastic lamina area at the plaque region with the adjacent non-diseased artery, the reconstructed models based on histological examination could not provide information regarding the remodeling of atherosclerotic arteries. However, for the verification and validation of all computational models, patient-specific studies are needed.

(v) The simulation of plaque rupture was performed based on 2D models due to the complexity of the simulation of crack propagation in 3D models. However, 2D models may overestimate the stress levels within the atherosclerotic plaques and performing 3D stress analysis can improve the accuracy of the analysis.

(vi) In the simulations, the material properties of atherosclerotic arteries were assumed constant and the viscoelastic and anisotropic behavior of arterial wall were not considered. Moreover, the effect of residual stresses, as well as the density and architecture of collagen fibers, on the stress distribution within atherosclerotic arteries were ignored. The blood flow was considered laminar and Newtonian and due to the lack of information, patient-specific boundary condition was not employed.

7.3 Conclusions

To summarize, the major findings of this thesis are listed below:

(i) Atherosclerotic plaque development results in the stiffening of interlamellar zones and softening of elastin lamellae in diseased portions of the abdominal aortic wall.

(ii) With atherosclerotic plaque development in coronary arteries, the stiffness of the medial layer in diseased parts of the artery increases, whereas the stiffness of the IEL decreases.

(iii) In advanced human atherosclerotic coronary arteries, the diseased wall is significantly stiffer than the plaque-free segment.

(iv) Calcification zone and lipid pool are respectively the stiffest and softest components of the atherosclerotic plaque.

(v) The stiffness and inhomogeneity of the atherosclerotic intima increase with the atherosclerosis disease progression.

(vi) The compensatory expansive remodeling of the arterial wall during atherosclerotic plaque development is crucial to maintaining the physiological range of wall circumferential stress in the disease-free wall sections of incomplete plaques.

(vii) The expansive remodeling of atherosclerotic plaques reduces the possibility of plaque erosion, as well as plaque development in the downstream region of plaque, by maintaining the physiological range of endothelial shear stress.

(viii) Atherosclerotic plaques which undergo compensatory expansive remodeling are more vulnerable to rupture due to the induction of higher level of circumferential stress within atherosclerotic plaques.

(ix) The initial crack often forms at the shoulder of atherosclerotic plaques and its location is influenced by the lumen shape, plaque eccentricity, the size and position of lipid pool and calcification zones, fibrous cap thickness, and stiffness of plaque components.

(x) With crack formation and propagation in the atherosclerotic plaque, the stress field within the plaque changes considerably which can lead to the initiation of secondary cracks.

(xi) The lipid pool stiffness affects the location of crack formation and the rate and direction of crack propagation within the atherosclerotic plaque.

(xii) The increase of both mean and pulse pressure reduces the number of cycles required for plaque rupture. However, pulse pressure has a greater influence than the mean pressure on the rate of crack propagation.

7.4 Future Directions

7.4.1 Non-invasive determination of material properties

In Chapter 3 and 4 the mechanical properties of atherosclerotic arteries with the AFM indentation test on the human samples extracted at autopsy. It is desirable to develop methods such as elastography and pulse wave imaging to estimate the mechanical properties of atherosclerotic arteries *in vivo*.

7.4.2 Patient-specific studies

It is desirable to conduct a longitudinal study to detect the remodeling of atherosclerotic arteries at multiple time points. In this way, the progression and rupture of atherosclerotic plaques might be predictable based on the distribution of mechanical stresses within the atherosclerotic plaque. Moreover, using patient-specific boundary conditions can improve the accuracy of the results.

7.4.3 Simulation of blood coagulation after plaque rupture

It has shown that the mechanical stress not only play a critical role in the rupture of atherosclerotic plaques, but also it can regulate blood clot formation(252). With the combination of biochemical and biomechanical analysis, the effect of shear rate on the thrombus formation after plaque rupture can be investigated.

7.4.4 Cellular mechanics

The abnormal function of endothelial cells and SMCs play a critical role in the process of atherosclerotic plaque formation, progression and rupture. The surface of endothelial cells is equipped with various mechanoreceptors which are capable to respond to mechanical stimuli. Studying the effect of different levels and types of mechanical stimuli on the endothelial and SMCs can be beneficial for better understanding the mechanisms involved in atherosclerosis disease. A pilot study to investigate the effect of shear stress on the expression of ICAM-1 by endothelial cells is detailed in Appendix 1.

Appendix1

A pilot study to examine the effect of shear stress on the expression of I-CAM 1 by endothelial cells

Summary

It is well known that the protein expression by endothelial cells may be affected by hemodynamic stimuli. In this study, the effect of shear stress on the expression of intercellular adhesion molecule-1 (ICAM-1) by endothelial cells was examined. Human brain microvascular endothelial cells (HBMEC) were subjected to a physiologically relevant range of laminar shear stresses (10-25 dyn/cm2) for two hours. Analysis of the fluorescent images revealed an increase of ICAM-1 expression by endothelial cells with the elevation of shear stress level. It was concluded that the expression of ICAM-1 by endothelial cells is regulated by shear stress level. This selective regulation of adhesion molecule expression in endothelial cells suggests that mechanical stresses may contribute in the process of protein expression by endothelial cells and thus can play a critical role in the development of atherosclerosis disease.

A1.1 Introduction

One of the earliest detectable cellular responses in the formation of atherosclerotic plaques is the adherence of leukocyte especially monocytes and lymphocytes (T cells and B cells) to the endothelial cells. The leukocytes migrate across the endothelial cell's barrier and accumulate in the subendothelial space, where some of the monocytes ingest lipid and become foam cells. The recruitment of leukocytes persists so long as the condition of hypercholesterolemia continues in the subject. During that time, the vascular endothelium participates in recruitment of leukocytes by expression of specific leukocyte adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). It has been hypothesized that VCAM-1 and ICAM-1 are regulated by blood flow and plasma cholesterol levels and play a key role in the formation and progression of atherosclerotic lesions. Of particular interest, ICAM-1 is a glycoprotein and a member of the immunoglobulin superfamily of adhesion molecules, which is continuously expressed at low levels in the normal endothelium. ICAM-1 mediate arrest, firm adhesion and focal recruitment of lymphocytes, monocytes, and neutrophils in developing lesions of atherosclerosis.

It is well known that atherosclerotic plaques often form at specific locations within the arterial tree such as the branch points, the outer wall of bifurcation, and the inner wall of curved arteries. These locations are characterized by the existence of disturbed blood flow pattern. This remarkable connection between hemodynamics and atherosclerosis has motivated various studies to perform in vitro studies for better understanding of the role of hemodynamic forces

such as laminar shear stress in the pathogenesis of atherosclerosis (253). In this study, we examined the short-term effect of physiologically relevant levels of laminar shear stress on the expression of I-CAM 1 by human brain microvascular endothelial cells (HBMEC).

A1.2 Methods

A1.2.1 Cell culture

Human brain microvascular endothelial cells-SV40(HBMEC-SV40) were maintained in M199 media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C. Passages 7-9 were used for all experiment. The culture condition was optimized prior to the experiment. Cells were stained with trypan blue and counted using the Countess cell counter. Then, cells were seeded on Collagen I coated Streamer™ culture slips with Teflon border at different seeding densities (Figure Appx.1). Cells were transferred into a cell culture incubator at 37°C maintained with 5% CO2 for three overnights to ensure their firm adherence to the culture slips. Several photomicrographs were taken from the cells under a phase contrast microscope to examine cell growth, adherence, and spread. Finally, different seeding densities were compared and the initial density of 2×10^5 was chosen for the shear test.

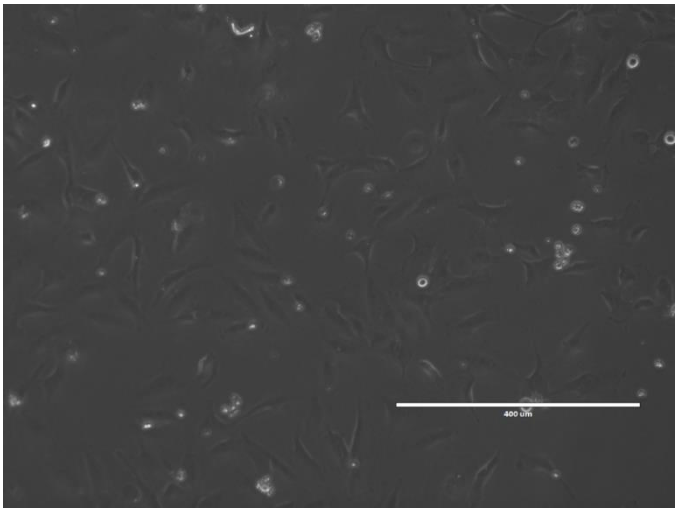
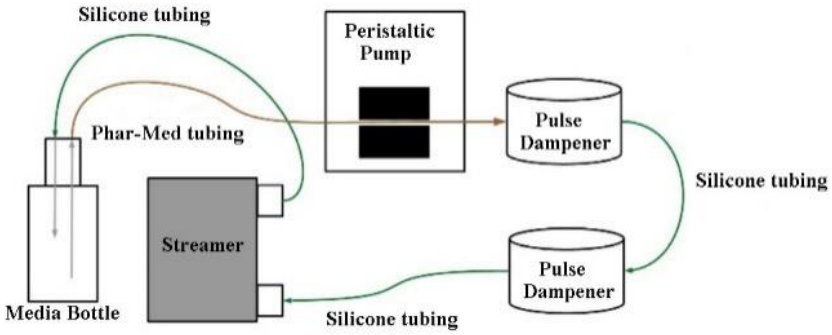


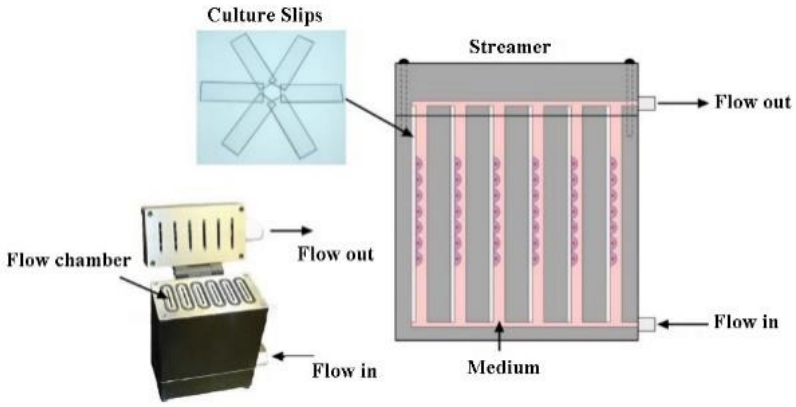
Figure A1.1 The optimization of cell culture on the culture slips.

A1.2.2 Shear stress test

The streamer system made by Flexcell International Corporation was utilized to apply laminar shear stress to endothelial cells. As can be seen in Figure Appx.2a, this system consists of a media bottle as a medium collection reservoir, a peristaltic pump to drive the medium through the system, two pulse dampener to prepare steady flow by reducing flow pulsation caused by the peristaltic pump and a streamer. Streamer includes six chambers designed to hold culture slips and induce the desired level of shear stress to the endothelial cells (Figure Appx.2b). When the streamer system was assembled, it was autoclaved using dry sterilization setting. Then, the prepared slides were inserted in the slide slots of the streamer under a tissue culture hood and the streamer system placed into the incubator (Figure Appx.2c). The level of shear stress and the time of shear exposure (two hours) were imported to the system and the experiment was conducted.



(a)



(b)

(c)

Figure A1.2 Shear stress machine made by Flexcell international corporation. (a) Different components of the machine including media bottle, silicone tubing, peristaltic pump, two pulse dampener, and streamer (from user manual of streamer system with permission). (b) The schematic diagram of the streamer, in which endothelial cells are cultured on the culture slips and exposed to the shear stress (from user manual of streamer system with permission). (c) The assembled shear stress machine which is placed into the incubator.

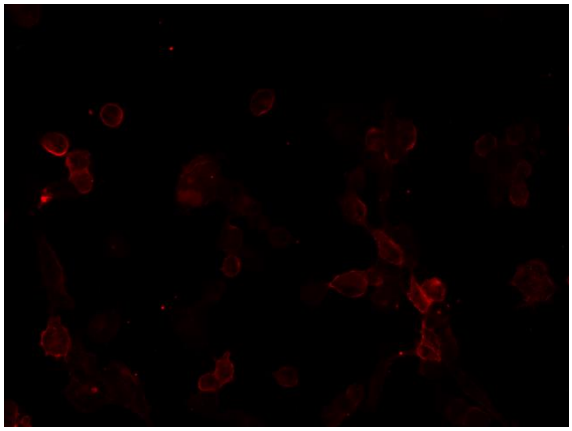
A1.2.3 Immunofluorescent staining with flow cytometric analysis

Followed by the shear stress experiment, slides were removed from the streamer and placed on the petri dishes. Cells were washed three times each for 5 minutes with 1 x PBS and then fixed at room temperature with 2% phosphate-buffered paraformaldehyde (PFA) prepared in 1 x PBS for 15 minutes. Again, cells were washed three times each for 5 minutes with 1 x PBS to discard any residual PFA solution. The slides were dried under the fume hood and stored at -80°C until the permeabilisation step. The cells were permeabilised with 50% ethanol for 10 minutes at room temperature. Cells were rinsed with 1 x PBS, once for 5 minutes and blocked with the blocking buffer (containing 1% bovine serum albumin (BSA) and 0.3% Triton X-100 in 1 x PBS) at room temperature. After one hour, the blocking solution was discarded and the primary antibody which was diluted (1:250) with antibody dilution buffer (1% BSA in PBS) was applied. Cells were left for overnight incubation at 4°C and then washed three times each for 5 minutes with 1x PBS. The secondary antibody conjugated with a fluorochrome diluted in blocking solution buffer (1: 500) and applied to the cells for two hours at room temperature in the dark. The secondary antibody solution was discarded and cells were washed three times with 1 x PBS, each for5 minutes followed by mounting with mounting media (DAKO aqueous mounting media) and coverslip. Images were taken by fluorescence microscope using an appropriate excitation wavelength.

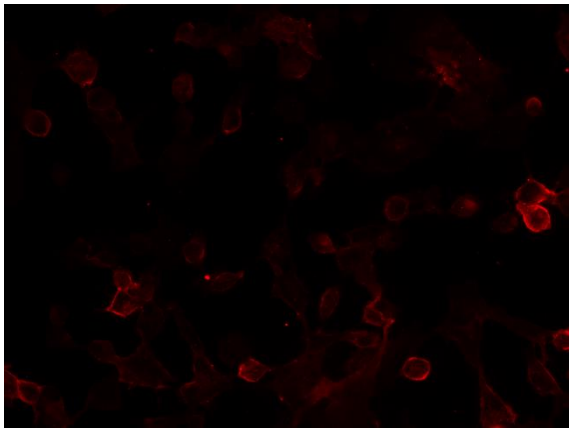
A1.3 Results

As can be seen in Figure Appx3, the induction of shear stress to the endothelial cells can increase the expression of ICAM-1 by endothelial cells in a force-dependent manner. The expression of ICAM-1 exposed to the different levels of shear stress was higher than the static condition. With the rise of shear stress level, the increase of ICAM-1 expression was observed. No significant alteration in the orientation of endothelial cells after two hours of inducing different levels of shear stress was observed. Considering the contribution of shear stress in the expression of ICM-1, it can be considered as a stimulus in the inflammation and atherosclerosis.

(a)



(b)



(c)

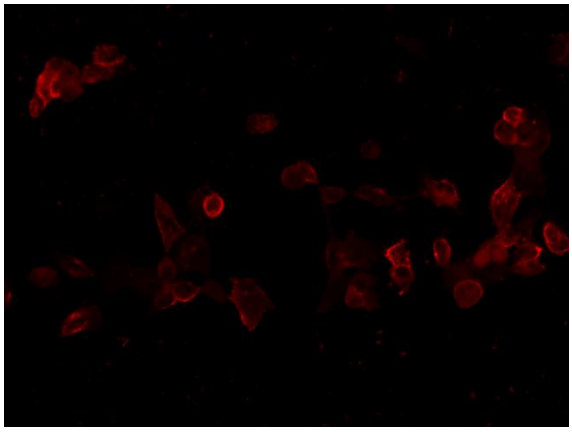


Figure A1.3 Immunocytochemistry of ICAM-1 surface expression by HBMEC exposed to the different level of shear stress. (a) HBMEC maintained under no flow condition (control). (b) HBMEC exposed to the shear stress of 10 $\mathrm{dyn/\,cm^2}$.(c) HBMEC exposed to the shear stress of 20 $\mathrm{dyn/\,cm^2}$.

A1.4 Discussion

In this study, the short-term effect of shear stress on the expression of ICAM-1 expression was investigated. Different levels of laminar shear stress were exposed to the endothelial cells and the expression of ICAM-1 was compared among them qualitatively using immunofluorescent staining. The results confirm the role of shear stress in the formation of intimal hyperplasia and atherosclerosis. However, the presented study has some limitations. First of all, the pulsatile behaviour of shear stress in the cardiovascular system which was neglected in this study can affect protein expression by endothelial cells (78). Moreover, in the vascular system, endothelial cells are exposed to shear stress and tensile stretch simultaneously and it is challenging to determine the response of endothelial cells when both stimuli are applied. Further studies can be performed to study the long-term and simultaneous effect of applying both pulsatile shear stress and tensile stress on the expression of different biomarkers involved in atherosclerosis disease (93, 229).

Appendix2

Full patient information

Table A2-1 Patient/specimen details of abdominal aorta samples

	Male/Female	Age	Luminal stenosis	Lesion type	Primary disease
1	Male	39	-	Healthy	-
2	Male	26	-	Healthy	-
3	Male	37	-	Healthy	-
4	Female	32	-	Healthy	-
5	Male	39	-	Healthy	-
6	Male	35	-	Healthy	-
7	Male	40	27%	III	Hypertension
8	Female	47	36%	IV	-
9	Male	42	19%	II	-
10	Male	42	41%	IV	-
11	Male	37	35%	III	-
12	Male	43	31%	III	-
13	Female	37	28%	III	Hypertension
14	Male	36	81%	IV	-
15	Male	38	70%	V	-
16	Male	50	79%	VI	Diabetic
17	Male	37	78%	VI	Diabetic
18	Female	44	65%	V	-
19	Male	48	68%	V	Hypertension
20	Female	41	73%	V	-

Table A2-2 Patient/specimen details of coronary artery samples

	Male/Female	Age	Luminal stenosis	Lesion type	Primary disease
1	Male	34	-	Healthy	-
2	Male	39	-	Healthy	-
3	Male	40	-	Healthy	-
4	Male	35	-	Healthy	-
5	Male	42	-	Healthy	-
6	Male	34	-	Healthy	-
7	Male	43	35%	III	Hypertension
8	Male	38	28%	III	-
9	Male	50	36%	IV	Diabetic
10	Male	38	18%	II	-
11	Male	44	22%	III	-
12	Male	39	43%	IV	-
13	Male	51	59%	V	Hypertension
14	Male	49	76%	VI	-
15	Male	52	68%	V	-
16	Male	37	65%	V	Hypertension
17	Male	51	85%	VI	Hypertension
18	Male	50	82%	VI	Diabetic
19	Male	38	79%	V	-
20	Male	45	63%	V	-

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