The role of biomechanical forces in the natural history of coronary atherosclerosis

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ABSTRACT

Atherosclerosis remains a significant cause of morbidity and mortality worldwide, such that a thorough understanding of the underlying pathophysiological mechanisms is crucial if novel therapeutic strategies are to emerge. Although atherosclerosis is a systemic inflammatory disease, coronary atherosclerotic plaques are not uniformly distributed in the vascular tree. Experimental and clinical data highlight that biomechanical forces, including wall shear stress (WSS) and plaque structural stress (PSS), play a crucial role in the natural history of coronary atherosclerosis. Endothelial cell function is heavily influenced by changes in WSS and longitudinal animal and human studies have shown that coronary regions with low WSS undergo increased plaque growth. Local alterations in WSS may also promote transformation of stable to unstable plaque subtypes. Plaque rupture is determined by the balance between PSS and material strength, with plaque composition having a profound effect on PSS. Prospective clinical studies are now required to ascertain whether integrating mechanical parameters with medical imaging can improve our ability to identify patients at highest risk of rapid disease progression or sudden cardiac events.

KEY POINTS

- Atherosclerotic plaques are not uniformly distributed throughout the coronary tree, implying that local mechanical factors may determine plaque development and/or growth.
- Blood flow and particularly wall shear stress heavily influence endothelial function via diverse mechanisms.
- Biomechanical forces promote adverse changes in plaque composition, producing a 'high-risk' plaque phenotype that is more prone to rupture and sudden cardiac events.
- Plaque structural stress is determined by plaque composition, with plaque rupture occurring when plaque structural stress exceeds plaque strength.
- Prospective, observational studies suggest that integrating biomechanical parameters may improve our ability to identify patients at highest risk of rapid disease progression or plaque rupture.

INTRODUCTION

Ischaemic heart disease remains a leading cause of mortality and morbidity throughout the world. The development of atherosclerotic plaques within coronary arteries may give rise to a number of adverse clinical events, including sudden cardiac death and myocardial infarction (MI). Although the incidence of MI continues to decrease in developed countries as a consequence of lifestyle improvements, risk factor modification and therapeutic advances[1], the number of coronary events in developing and newly industrialised countries is rising[2]. Thus, a thorough understanding of the pathological mechanisms underlying atherosclerotic plaque initiation, development and failure is critically important if new diagnostic and therapeutic strategies are to successfully emerge.

Atherosclerosis predominantly affects the intimal layer of the arterial vessel wall and is characterised by deposition of extracellular lipids and inward migration of pro-inflammatory bone marrow-derived cells and proliferation and migration of local smooth muscle cells[3]. The initial pathological cellular changes associated with atherosclerosis can develop in early childhood, but overt macroscopic atherosclerotic plaques are rarely observed before the second or third decade of life[4]. Although atherosclerosis is a systemic disease that may affect several arterial territories at once, plaques are focally distributed within the coronary tree and individual lesions are often observed at different stages of development. Indeed, plaque evolution does not occur in a linear manner, with some plaques transforming into advanced lesions with large lipid necrotic cores and thin overlying fibrous caps (a 'thin-cap atheroma' (TCFA)), while others regress and become less likely to result in clinical events[5, 6]. While the physical substrate for plaque rupture has been defined from post-mortem studies, prediction of rupture prior to the event is a significant diagnostic challenge, with medical imaging based on plaque anatomy providing only limited ability to predict future cardiovascular events[7-9].

The suggestion that local environmental factors influence plaque development and behaviour means that novel modalities that can measure these factors may complement plaque imaging and improve our ability to predict future events.

The non-uniformity in atherosclerosis distribution within an individual and difficulty in predicting plaque behaviour from imaging alone may be explained, in part, through study of the biomechanical forces acting within the artery. For example, mechanical forces play an important role in cellular physiology[10], resulting in pro-atherogenic changes within the endothelium that ultimately influence the macroscopic and microscopic architecture of the plaque, which in turn regulates plaque development. These changes may also weaken the plaque, resulting in plaque failure[11]. We describe and review those biomechanical forces that act within coronary arteries and explore their mechanisms and role in the natural history of coronary atherosclerosis, both in animal models and human studies. Calculation and integration of biomechanical parameters may improve our ability to identify arterial regions at risk of atherosclerosis, allowing better identification of patients at the highest risk of clinical events.

BIOMECHANICAL FORCES

Coronary arteries are continually subjected to biomechanical forces during each cardiac cycle, due to the pulsatile pressure of blood passing from the aorta through the coronary tree. These complex mechanical forces act in circumferential, radial and axial directions, deforming individual cells, atherosclerotic tissue and the artery wall to create stresses. In biomechanics, a stress is defined as the force acting on a surface, divided by the area of that surface. Although exact measurement of coronary stresses *in vivo* is challenging, they can be estimated through advanced computational techniques. In the context of cardiovascular

research, the two important stresses that have been hypothesised to affect the natural history of atherosclerosis are wall (or endothelial) shear stress (WSS) and plaque structural stress (PSS) (**Table 1**).

Wall shear stress

WSS is the parallel frictional force exerted by blood flow on the endoluminal surface of the arterial wall. Magnitude of WSS is expressed using a variety of interchangeable units (e.g. 1 Pa = 1 N/m^2 = 10 $dynes/cm^2$), with values being influenced by changes in luminal geometry, blood flow velocity and plasma viscosity. WSS is dependent on anatomical location, with the highest values often observed in the distal vessel or in arterial side-branches[12]. WSS is also very sensitive to pre-existing arterial geometry, being increased at the outer curvature and reduced at the inner curvature of the vessel wall[13, 14]. If blood flow rate is constant, a stenotic atherosclerotic plaque will significantly increase WSS at the site of maximal stenosis, as blood becomes accelerated through a smaller, cross-sectional luminal area. In contrast, disturbed blood flow, defined as a pattern of flow that is non-uniform and irregular resulting in eddies and changes to flow direction[15], can be induced by arterial bifurcations, vessel curvature and typically observed downstream of a stenotic plaque[16] (**Figure 1**).

WSS can be estimated *in vivo* through computational fluid dynamics (CFD) simulations, a numerical method that mathematically approximates the flow fields of liquids within a structure. Several factors first need to be defined to perform CFD analysis, including 1) an accurate 3D geometry of the coronary arteries created from imaging, 2) coronary physiological parameters, such as coronary blood flow velocity and pressure, forming the loading conditions for the simulation and, 3) numerical solutions for the physical laws governing the motion and behaviour of blood **(Figure 2)**. Although these basic elements are

required for WSS calculations, there remains ongoing debate and research regarding the optimal appropriate methodology[17-19]. For example, recent data supports the use of 'true' anatomical coronary models, with blood flow also being modelled through side branches[20]. Nevertheless, this lack of a standardised approach for computational modelling has introduced heterogeneity in the metrics used to quantify and describe WSS in atherosclerosis, leading to some challenges in data interpretation between studies[21].

Plaque structural stress

PSS is the stress located within the body of an atherosclerotic plaque or the arterial wall as a consequence of vessel expansion and stretch induced by arterial pressure. However, PSS is also determined partially by plaque composition/morphology and the material properties of tissues. Synonymous terms include 'tensile stress', 'structural stress' or even 'biomechanical stress'. PSS is typically around 10^3-10^5 times greater than WSS in humans and often expressed in kilopascals (kPa)[22]. PSS correlates positively with vessel luminal area in a vessel of uniform wall thickness and pressure, such that higher PSS values are expected in the proximal segments of coronary arteries. In contrast, lower PSS values are expected at stenoses and with increasing vessel wall thickness PSS[23]. However, changes in composition and architecture of the plaque and artery wall can alter PSS significantly[24]. For example, PSS is markedly affected by the juxtaposition of tissues of different mechanical properties. Increased PSS levels may be a principal mechanism through which plaques rupture, resulting in thrombosis and sudden ischemic clinical events[25]. Localised high PSS concentrations may also affect cellular function, modifying the structural integrity of the plaque[26] **(Figure 1)**.

Estimation of PSS can be performed using structural mechanical techniques, including finite element analysis (FEA); a numerical method that mathematically models the effect of a force

being applied to a structure. FEA allows an approximation of the solution by first subdividing the structure being studied into many (often >10,000) smaller elements. The FEA solution first requires a model of the coronary artery, which is typically generated from a high-resolution imaging modality (e.g. intravascular ultrasound). The model must accurately depict both atherosclerotic lesion composition and architecture, as FEA simulations are sensitive to subtle changes in plaque or luminal geometry. Coronary arterial pressure and the material properties of each atherosclerotic plaque component and arterial wall are required to simulate lesion deformation. However, data on the material properties of atherosclerotic tissue are minimal and there is likely a variation between different patients and even plaque subtypes[27], which has potential to affect overall PSS calculations[28]. At present, the majority of studies use the material properties derived from *ex vivo* tensile testing of plaque samples[29-31], although research using patient-specific material properties is ongoing. Once complete, the final solution can allow estimation of either maximum principal stress and/or von Mises stresses, two differing engineering measures that can be used to predict whether any specific material is prone to structural failure[32](**Figure 3**).

Alternatively, PSS can be calculated through fluid-structure interaction (FSI) simulations, an approach that seeks to integrate both the fluid and structural mechanical forces into a defined solution[33]. Although FSI allows quantification of both WSS and PSS within an individual artery, the engineering processes involved are increasingly complex. The increased analytical demand of FSI modelling limits its current application either to idealised models or small clinical studies. Furthermore, it is unclear whether the small incremental gain in accuracy achieved by FSI simulations is clinically meaningful, when compared with computationally analyses that are less time consuming[34].

BIOMECHANICAL FORCES INVOLVED IN PLAQUE INITIATION

Atherosclerotic plaques are not uniformly distributed throughout the coronary tree[35, 36]. Biomechanical forces influence the initiation of atherosclerosis, with plaques developing predominantly near to side branches and/or bends in arteries. Blood flow at these sites is disturbed due to the uneven luminal geometry, leading to alterations in both the magnitude and direction of WSS. Arterial regions exposed to low time-averaged WSS or to patterns of WSS that change direction (oscillatory or tangential) appear prone to atherosclerosis, whereas regions exposed to WSS with higher magnitude and uniform direction are protected[16, 21, 37]. However, there may be a ceiling at which WSS loses its atheroprotective effects, with studies showing that supraphysiological WSS may also modify the endothelial response to promote plaque initiation[38].

A causal relationship between WSS and initiation of atherosclerosis has been established by studies where arterial flow is altered in experimental animals either by an extravascular cuff[39, 40], partial ligation, or tandem ligations of the carotid artery[41]. These studies revealed that although low WSS *per se* is not sufficient to induce atherosclerosis, it induces focal endothelial cell (EC) dysfunction and inflammation, which primes arterial regions for subsequent atherosclerosis initiation in response to hypercholesterolaemia. In addition, as magnitude of WSS varies with lumen remodelling, ECs overlying plaques are exposed to much higher levels compared to healthy endothelium[42]. Intriguingly, plaque rupture localises preferentially to the upstream portion of the plaque that is exposed to the highest levels of WSS[43]. The causal role of WSS in atherosclerosis is reinforced by studies showing that higher time-averaged WSS is associated with atheroprotection in healthy arteries, whereas supraphysiological levels of WSS correlate with EC injury and may be associated with

plaque instability. One possible explanation for these observations is that physiological and supraphysiological levels of WSS may have differing effects on EC function (see below).

Endothelial cell behaviour is affected by changes in blood flow

EC at atheroprone sites exposed to disturbed flow exhibit highly heterogenous and often contradictory behaviour[16, 44] **(Figure 1)**. For example, disturbed flow induces EC apoptosis via activation of JNK[45], p53[46], PKCζ[47] and via the unfolded protein response[48], and increases EC proliferation, which is also increased at atheroprone compared to atheroprotected sites[45, 49]. In contrast, disturbed flow has also been reported to *reduce* EC proliferation via down-regulation of the microRNA species miR-126-5p[50] and through the induction of irreversible cell cycle arrest (senescence) via p53-p21 signalling[51]. These studies suggest that atheroprone regions contain proliferating ECs as well as subpopulations in which the proliferative reserve has been exhausted, and that additional sub-populations undergo apoptosis. The processes that control differential cell fate decisions in ECs exposed to disturbed flow and the dynamic interactions between these different EC phenotypes should be studied further.

Mechanotransduction modulates plaque inflammatory signalling

The initiation of atherosclerosis involves the recruitment of circulating leukocytes to the vessel wall. This highly co-ordinated process, termed the leukocyte adhesion cascade, involves capture of leukocytes by activated ECs and subsequent rolling and transmigration into underlying vascular tissues. Monocyte migration is promoted by several proinflammatory mediators including cytokines (e.g. TNFα, IL-1) and oxidized lipoproteins. WSS has profound effects on inflammatory activation of ECs **(Figure 1)**, such that higher uniform WSS reduces subsequent induction of VCAM-1 and E-selectin expression in ECs in culture[52, 53] and adhesion of monocytes[54, 55]. In contrast, low oscillatory WSS promotes

monocyte adhesion by enhancing expression of inflammatory adhesion molecules, including JAM-A[54, 56, 57]. Consistent with these *in vitro* observations, *en face* staining of arteries demonstrates that atheroprone regions exposed to low WSS are more inflamed than sites exposed to high WSS[58, 59].

Flow regulates EC inflammatory activation by altering activity of a number of inflammatory pathways including NF-κB and MAP kinase signalling **(Figure 4)**. In unstimulated cells, NF-κB is held in the cytoplasm by its inhibitor IκB. Inflammatory mediators induce phosphorylation and degradation of IκB, leading to nuclear translocation and activation of NF-κB. The induction of inflammatory genes by NF-κB is enhanced in EC exposed to low oscillatory WSS compared to higher uniform WSS[58]. The mechanism relies on increased expression of RelA NF-κB subunits, whose transcription is increased by low WSS via a JNK-ATF2 pathway[59]. Although NF-κB expression is increased by low WSS it remains cytoplasmic and inactive in the majority of cells. Thus, increased NF-κB expression primes EC for enhanced activation in response to a second inflammatory stimulus, such as hypercholesterolaemia[58, 59]. Disturbed flow also promotes inflammation by suppressing the expression of MiR10a, a microRNA that negatively regulates canonical NF-κB signalling[60]. In contrast, high uniform WSS reduces NF-κB activity, which is mediated through several mechanisms including induction of KLF2, which inhibits NF-κB transcription by sequestering the co-activator CBP/p300[61], and activation of endothelial nitric oxide synthase (eNOS), which generates NO to suppress IκB kinase, a positive regulator of NF-κB[62]. High uniform flow also alters the function of NF-κB by enhancing NF-κB-dependent cytoprotective and anti-inflammatory responses and simultaneously inhibiting NF-κB-dependent inflammation[63].

WSS also regulates the activity of the pro-inflammatory MAP kinases JNK and p38 and downstream AP-1 family transcription factors. The mechanisms that link WSS with MAP kinases involve KLF2, which is induced by high WSS and subsequently inhibits phosphorylation and nuclear localization of AP-1 family members c-Jun and ATF2 [64-66]. High WSS also activates the transcription factor Nrf2[67-69] which function together with MAP kinase phosphatase-1 to reduce inflammation by dephosphorylating p38 and JNK[69, 70]. The inhibitory effect of high WSS on inflammatory MAP kinases is also mediated via downregulation of thioredoxin interacting protein (TXNIP) leading to inhibition of apoptosis signal-regulating kinase 1, a kinase that acts upstream of p38 and JNK [71].

Although higher or physiological WSS reduces inflammation, supraphysiological WSS or very high WSS can have the opposite effects. This was demonstrated using a murine model of arteriovenous fistula that generates enhanced WSS in the carotid artery[72]. Increased WSS induced activation of NF-κB and the expression of matrix metalloproteinases involved in inflammation and vascular remodelling. *In vitro* studies demonstrated that supraphysiological WSS (7.5 Pa) enhanced phosphorylation of p38, c-Jun and ATF2 compared to physiological WSS (1.5 Pa)[38], suggesting that supraphysiological WSS may contribute to plaque instability by triggering inflammation and/or EC erosion.

BIOMECHANICAL FORCES INVOLVED IN PLAQUE GROWTH

Plaque growth and arterial remodelling

There is increasing literature in both animals and humans demonstrating that WSS can affect both plaque growth and transformation into high-risk plaques. For example, atherosclerotic plaques responsible for MI are more frequent within proximal vessels or around bifurcations[73, 74]. Low WSS regions are present within proximal vessels and on the

contralateral wall to arterial side-branch divisions[12]. Animal studies using a partial arterial ligation model showed that co-localisation of low and oscillatory WSS acted synergistically to promote atherogenesis[75], whereas low WSS was associated with plaque burden and positive remodelling[76]. Similarly, the spatial and temporal relationship between low WSS and plaque progression was illustrated through longitudinal animal studies. Here, arterial segments with low WSS at baseline exhibited greater subsequent plaque progression compared with moderate/high WSS regions[77]. Furthermore, low WSS was frequently associated with excessive expansive remodelling, a form of compensatory remodelling where both lumen and vessel dimensions increase. These changes to the arterial structure act to decrease WSS within these segments, further driving rapid plaque progression[78]. This was in contrast to regions with negative (or constrictive) remodelling, where WSS was increased and plaque growth appeared almost attenuated.

The relationship between baseline WSS and plaque progression has also been found in humans (**Table 2**). The potential for *in vivo* WSS calculations was first demonstrated using a combination of coronary angiography and intravascular ultrasound (IVUS) to reconstruct luminal geometry[79], before being confirmed in a longitudinal study of twelve patients who had one coronary artery with a <50% obstruction[80]. Investigators observed significant increases in plaque thickness associated with regions of low WSS, which was again coupled with expansive remodelling, mirroring the results observed in animal models. However, in humans there is greater uncertainty over the relationship between WSS and vascular remodelling, as both constrictive remodelling and eccentric plaque development have also been observed[81, 82]. Other studies have reported contradictory findings and observed that high WSS regions were more likely to result in excessive expansive remodelling[83]. Notably, plaque growth was predicted better when WSS calculations were blended with established

imaging features known to predict disease progression[84]. The translational potential of baseline WSS calculations was tested in the PREDICTION study, which used coronary angiography and intravascular ultrasound (IVUS) to reconstruct 1,341 arteries from 506 patients undergoing percutaneous coronary intervention following an acute coronary syndrome[85]. Increases in plaque area were independently predicted by plaque burden, as quantified by IVUS, with decreasing luminal area (analogous to constrictive remodelling) predicted by low WSS. Additionally, both increased plaque burden (≥58%) and low WSS (<0.98 Pa) were associated with further luminal obstruction necessitating repeat coronary intervention. Further longitudinal studies are now warranted to assess whether integration of WSS calculations with anatomical imaging can better predict future clinical events. This may be increasingly possible through the use of improvements in non-invasive coronary imaging, allowing the construction of 3D arterial geometry with relative ease[86-88].

Transformation into high-risk plaques

Coronary plaques responsible for around two-thirds of acute coronary events, including sudden cardiac death and MI, are lipid-rich and frequently have a thin overlying fibrous cap[89, 90]. A hypothesised precursor lesion has been proposed, the thin-cap fibroatheroma (TCFA), which shares several of these characteristics and may also display additional structural and compositional features that act to induce instability (**Box 1**)[91]. However, post-mortem and clinical studies have shown that TCFA are not distributed evenly throughout the coronary arteries[35, 92], while longitudinal clinical studies have shown that stable plaques may evolve and transform into TCFA with the passage of time[5, 93]. Local alterations in WSS have been suggested as one mechanism that may be involved in this dynamic process.

Animal and *ex vivo* studies provide the majority of data linking alterations in WSS to changes in plaque composition. Regions of low WSS correlate with increasing lipid accumulation within the arterial wall[94, 95], possibly through inducing conformational changes in ECs that act to increase permeability to low-density lipoproteins[96, 97] or though up regulation of genes encoding the LDL receptor[98, 99]. This crucial pro-atherogenic effect of low WSS itself appears to be affected by total serum cholesterol levels, with low WSS and high cholesterol acting synergistically to promote increased plaque growth, lipid accumulation and leukocyte infiltration[100]. Smooth muscle cell migration and apoptosis may also be affected by changes to WSS[101, 102], resulting in regions of persistently low WSS having reduced collagen content[94] and being associated with fibrous cap thinning[103]. WSS has also been linked with increased plaque inflammatory activity and increased expression of MMPs[104], which act to reduce plaque strength through degradation of extracellular matrix proteins[105]. Finally, longitudinal studies in porcine models have shown that WSS changes dynamically over time, with TCFA developing more frequently in coronary regions exposed to low WSS throughout their evolution[103, 106].

Assessing the relationship in humans between WSS and coronary plaque composition has proven challenging, as non-invasive imaging modalities currently struggle to characterise plaque morphology in sufficient detail for subtle changes in composition to be studied[107-109]. Thus, the majority of human data on WSS have been obtained through use of invasive imaging modalities, including virtual-histology (VH)-IVUS[110] or optical coherence tomography (OCT)[111]. In a study of twenty patients low WSS regions were found to develop greater plaque and necrotic core progression over 6 months when compared with intermediate WSS regions[83]. Indeed, a negative relationship between WSS and lipid accumulation has been found to exist, with a 17% increase in necrotic core for every 1 Pa decrease in WSS[112].

A spatial relationship between low WSS regions and necrotic core was observed in early plaques (plaque burden <40%) imaged by VH-IVUS[113], while increases in necrotic core percentage occured at the ostium of the left anterior descending artery, an anatomical site typically affected by low WSS values[114]. WSS has also been calculated in patients presenting with an acute coronary syndrome imaged by OCT, with low WSS regions having a higher prevalence of macrophages, lipid-rich plaques and OCT-defined TCFA[115]. Furthermore, fibrous cap thickness was reduced in low WSS segments, with these coronary regions showing more superficial calcification.

BIOMECHANICAL FORCES INVOLVED IN PLAQUE FAILURE

The structural failure of the majority of atherosclerotic plaques is due to rupture of the fibrous cap[116], while repeated cycles of rupture and repair are thought to drive rapid plaque progression and growth[117]. Post-mortem studies have shown that ruptured plaques exhibit specific compositional features, including a large necrotic lipid core, macrophage infiltration and microcalcification, and an overlying fibrous cap with less collagen and fewer smooth muscle cells than stable lesions. Significant attempts have been made to identify plaques with unstable features *in vivo*, yet prospective studies have shown that few plaques labelled as 'high-risk' for rupture actually result in adverse clinical events[7-9]. Thus, rupture is not solely dependent on plaque morphology, and other local factors are likely involved. In recent years, there has been a resurgence of interest in the role of biomechanical forces in plaque rupture, as a consequence of improvements both in the imaging of coronary atherosclerosis and in computational power.

Experimental data

Increased PSS has long been hypothesised as a fundamental mechanism that precipitates rupture of a vulnerable plaque[25], as alterations to either plaque architecture or composition significantly affect PSS values. In idealised models of coronary plaques, PSS is increased with increased necrotic core thickness/size, during early stages of positive remodelling, when luminal curvature is increased (as would be expected at the plaque shoulder) and when fibrous cap thickness is reduced[24, 118, 119]. 3D idealised plaque models have confirmed these findings, where a reduction in fibrous cap thickness by 50% results in a 30% increase in PSS[120]. The thickness of the fibrous cap may be the principal determinant of PSS, as PSS values appear more sensitive to changes in fibrous cap thickness than necrotic core volume[121]. For example, PSS can exceed 300kPa if fibrous cap thickness is <60μm, a value whereby the cap is prone to failure[122].

Another key determinant of PSS is the location, extent and size of individual calcium deposits within the plaque. For example, superficial calcification around or within the fibrous cap increases PSS by almost 50%[123]. In addition, whereas larger plates of calcification (generally those >1mm in size) may be a plaque stabilising feature by FEA models[124], small foci of calcium significantly increase PSS[125]. In a study using idealised 3D models, maximal PSS values were not located on the calcium itself, but instead occurred just upstream[125], suggesting that calcium may focus high stress concentrations on tissues that may not have the mechanical strength to withstand these high stress values. Finally, microscopic, cellular-level microcalcifications (~5-10μm diameter) have been shown to be common and accumulate within either apoptotic smooth muscle cells or macrophages located in the fibrous cap[126]. When these minute particles of calcium were clustered together along the tensile axis of the cap, they could increase local PSS by a factor of >5[127]. This pattern was found to occur very

infrequently however, which may explain why so few TCFA progress to rupture in clinical studies. Intriguingly, recent research has shown that microcalcification may become be identified through use of novel imaging techniques, including ^{18}F-sodium fluoride positron emission tomography[128-130].

Clinical data to support role of PSS in rupture

Initial clinical studies examining the role of PSS in plaque rupture used histological plaque specimens as the geometrical basis for simulations. Here, PSS was found to be significantly increased in ruptured plaques responsible for sudden cardiac death when compared with stable lesions[131]. PSS levels in all ruptured plaques were found to exceed 300kPa, and this PSS value was tentatively suggested as a predetermined 'threshold' for rupture. However, rupture is not always found to occur at peak PSS location, implying that regions of relatively high, rather than absolute peak stress, may be of equal importance. Indeed, high PSS regions were found to accurately predict plaque rupture location in 82% of lesions studied[132]. PSS may also directly alter plaque composition towards a more unstable subtype. For example, consistent with idealised experimental models, PSS increased as the necrotic core increased, with significant increases in PSS observed near the luminal surface of the plaque[133]. High PSS levels have been correlated with both increased MMP expression[134] and macrophage accumulation[135], features known to weaken plaque mechanical strength. The regulation of vascular smooth muscle cells (VSMC) is also thought to be partly dependent on the degree of deformation (or stretch) within the artery wall[136], with increased stretch promoting VSMC phenotypic switch from a contractile to a synthetic phenotype[137]. Inhibition of VSMC activity and stretch-induced VSMC apoptosis may also lead to reduced collagen content within the lesion, which again may weaken plaque structural integrity[138]. Lastly, high local stress concentrations were found around intra-plaque neovascularisation that displayed early signs

of haemorrhage, suggesting that high PSS levels may trigger for neovessel rupture and intra-plaque haemorrhage, with consequent potential for rapid plaque growth[139].

Although histological analysis represents a useful tool to examine the composition and structure of atherosclerotic plaques, tissue processing can induce geometrical distortions that may affect the reliability of computational simulations[140]. Through improvement in medical imaging, it is increasingly possible to visualise coronary plaque morphology *in vivo*. Although most clinical studies of PSS in humans has been conducted in the carotid artery[141-143], a few studies have reported PSS calculations within the coronary tree using intravascular ultrasound (IVUS). Again, changes in plaque composition were found to modulate PSS, with increasing necrotic core elevating PSS, while increasing luminal stenosis attenuated values[124]. However, the relationship between calcification and PSS is more complex. While large plates of calcification appear to act as a 'stress-shield', tempering the effects of high PSS on the plaque, small calcific deposits may paradoxically increase PSS significantly, especially when they are in close proximity to one another[127]. IVUS was also used to create 3D models of the arterial wall in patients undergoing coronary intervention, with plaque rupture induced by balloon angioplasty[144]. The rupture site in this study co-localised with PSS location, demonstrating the feasibility of predicting rupture location. Finally, PSS calculated from 53 patients that underwent VH-IVUS before stenting demonstrated that stress values were highly heterogeneous throughout plaques, but increased values were observed in high-risk plaque regions in patients presenting with an acute coronary syndrome and within plaques classified as TCFA[145].

Alternatives mechanisms for rupture

Observational studies have demonstrated that plaque rupture most frequently occurs at a proximal location (upstream) to the site of maximal stenosis[146], a region where WSS is higher owing to increasing acceleration of blood through the luminal narrowing. This has led to the concept that increased WSS may promote plaque rupture[147, 148]. In support of this, experimental studies have shown that increased WSS may induce erosion of the plaque surface[149] and promote platelet accumulation and thrombogenicity[150, 151], two factors that increase the risk of intraluminal thrombosis. Indeed, plaque erosion is considered the aetiological mechanism underlying around one-third of MI, particularly in younger patients, smokers and women[152-154]. Clinical studies too have shown that high WSS correlates with plaque rupture location, although imaging was typically performed after rupture had occurred[155]. Finally, regions of high WSS and large plaque deformation have previously been found to co-localise[156], meaning it may be difficult to disentangle whether it is increased WSS or PSS that drives rupture.

Although we have focussed particularly on WSS and PSS, material fatigue is another potential mechanism promoting plaque rupture[157]. Fatigue is the accumulation of microscopic levels of damage as a response to repeated loading. Although fatigue is widely accepted as a mechanism of structural failure in non-organic materials, biological tissues are thought to respond differently due to their inherent capability to heal or change their structure as a consequence of damage. Nevertheless, experimental models have suggested a role for fatigue in plaque rupture[158], while a reduction in smooth muscle cells in the fibrous cap may inhibit the repair process, leading to accelerated rapid crack propagation and structural failure[159, 160]. Further studies are now required to assess the response of atherosclerotic tissue to multiple repeated loading cycles that may precipitate material fatigue

CONCLUSIONS

Biomechanical forces play a key role in the development of coronary atherosclerotic plaques through their effect on endothelial cell function. Mechanical stimuli also promote changes in plaque composition and plaque growth, with rupture governed by the dynamic interaction between plaque material strength and structural stress. Prospective clinical studies are now warranted to assess whether the integration of mechanical and medical imaging parameters improves our ability to identify those patients at highest risk of rapid disease progression or adverse clinical events.

REVIEW CRITERIA

Searches were performed on PubMed, MEDLINE and Central databases for full-text manuscripts published between 1990 and May 2015. The following search terms were used: "atherosclerosis", "coronary artery disease", "mechanotransduction", "shear stress", "endothelial stress", "wall stress", "tensile stress", "structural stress" and "plaque rupture". There were no limitations applied to language.

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CONTIBUTIONS

AJB, ZT and PC researched, wrote and critically edited the manuscript. JHG, HS and MRB reviewed, edited and added substantially to the scientific content presented.

DISCLOSURES

The authors have no competing interests to declare.

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Adam Brown is currently a British Heart Foundation Clinical Lecturer in Cardiology at the University of Cambridge and an Honorary Interventional Fellow at Papworth Hospital. He completed his undergraduate studies at the University of Cambridge and his early clinical training at both Addenbrooke's and Papworth Hospitals. His recently completed PhD focused on the use of intracoronary techniques, non-invasive imaging and mechanical modelling to identify and risk-stratify vulnerable coronary plaques.

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Professor Paul C. Evans

Paul Evans is Professor of Cardiovascular Science at the University of Sheffield. His research is focussed on the effects of shear stress on the physiology of vascular endothelial cells. These studies are relevant to vascular injury and atherosclerosis which develop predominantly at branches and bends in the arterial tree that are exposed to disturbed patterns

of blood flow. The cross-disciplinary nature of his work involves interactions with engineers, physicists and mathematicians as well as molecular and cellular biologists and vascular physiologists.

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Jonathan Gillard is Professor of Neuroradiology at the University of Cambridge, an Honorary Consultant Neuroradiologist at Addenbrooke's Hospital and a Fellow of Christ's College, Cambridge. He undertook his undergraduate training in Guy's Hospital London, graduating in 1988, where he also gained a first class BSc in Radiological Sciences focusing on the use of transcranial ultrasound. His basic radiological training was undertaken in Cambridge; his specialist neuroradiological training was undertaken at Johns Hopkins Hospital and University in Baltimore. His 1997 MD was based on spectroscopic imaging in acute stroke. Professor Gillard now leads active research groups using MR methodologies in atheroma imaging, cardiovascular imaging and neuro-oncology He has published over 210 peer reviewed articles and has a longstanding interest in medical education, at an undergraduate and postgraduate level. He is also interested in management and received his MBA from the Cambridge Judge Business School in 2010.

Professor Habib Samady

Habib Samady is Professor of Medicine, Emory University School of Medicine, and Director, Cardiac Catheterization Laboratory, at Emory University Hospital. He is a physiologically-oriented cardiologist and clinical investigator trained in general cardiology, nuclear cardiology and interventional cardiology at Yale University School of Medicine. In addition to patient care, Dr. Samady maintains an intensive research program: 1) hemodynamic mechanisms associated with progression of human coronary atherosclerosis; 2) evaluation of

pharmaceutical agents for treatment of coronary microvascular disease, endothelial dysfunction, coronary vasospasm and non-obstructive atherosclerosis, and 3) biomechanics of permanent and bioresorbable coronary vascular devices. He is Deputy Editor of JACC Cardiovascular Interventions and extensively published in the fields of intravascular imaging, coronary physiology, atherosclerosis assessment and stent biomechanics.

Professor Martin R. Bennett

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TABLE LEGENDS

Table 1

Descriptions of methodologies used and the biological impact of wall shear and plaque structural stresses

Table 2

Human prospective studies examining the relationship between baseline wall shear stress measures and changes to coronary plaque morphology

FIGURE LEGENDS

Figure 1 Biomechanical forces in atherosclerosis

Alterations in both wall shear stress (WSS) and plaque structural stress (PSS) play important roles in coronary plaque development, progression and plaque rupture. Alterations in blood flow induced by geometrical changes to the arterial lumen result in low WSS regions, which acts to promote expression of inflammatory adhesion molecules and modifies endothelial cell turnover. In established lesions, subtle changes in plaque composition and/or architecture increase PSS, making the plaque more vulnerable to rupture.

EC, endothelial cell; JNK, c-Jun N-terminal kinases; p53, tumour protein p53; PSS, plaque structural stress; VCAM-1, vascular cell adhesion molecule-1; WSS, wall shear stress

Figure 2. Wall shear stress calculations

Invasive catheter angiography of a right coronary artery (RCA) at baseline showing an atherosclerotic coronary plaque (**A**). Repeat angiography for stable angina performed at 4 years (**B**), showing a region of plaque progression with increase in luminal narrowing (arrow). Volume-rendered computerised tomography coronary angiogram of the RCA, again at baseline (**C**), allowing reconstruction of the 3D geometry of the vessel (**D**). Computational fluid dynamic simulation (**E**) based on the 3D geometry, showing a region of low wall shear stress that co-localised with the region of plaque progression (arrow).

Figure 3. Plaque structural stress calculations

Virtual-histology intravascular ultrasound (VH-IVUS) imaging of a coronary atherosclerotic plaque detailing tissue composition and architecture (top panels). 3D model of the same coronary plaque (middle panel), providing the geometrical basis for finite element analysis. 3D band plot for plaque structural stress (PSS)(bottom panel), illustrating two regions of high PSS. Note the highest PSS value is located in close proximity to a region of calcification.

Figure 4. Pro-inflammatory pathways regulated by mechanical sensing

The influence of wall shear stress (WSS) on inflammatory signalling pathways is depicted. The effects of high (green) and low (red) wall shear stress (WSS) are indicated.

References are indicated.

ASK, apoptosis signal-regulating kinase; eNOS, endothelial nitric oxide synthase; IKK, I-kappa B-kinase; IL, interleukin; JNK, c-Jun N-terminal kinases; KLF2, Krüppel-like factor 2; MiR10a, microRNA10a; MKKs, mitogen-activated protein kinases; MKP-1, mitogen-activated protein kinase phosphatase-1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells*; Nrf2, nuclear factor erythroid-related factor-2; TAK, transforming growth factor beta-activated kinase; TNFR, tumour necrosis factor receptor; VCAM-1, vascular cell adhesion molecule-1*

BOX 1

Anatomical features of plaques at high risk of instability and rupture:

- Large, necrotic lipid core
- Thin overlying fibrous cap (<65μm on histology[91])
- Positive vascular remodelling
- Macrophage and inflammatory cellular infiltration of the fibrous cap
- Paucity of smooth muscle cells in the fibrous cap
- Plaque neovascularization
- Spotty calcification and low attenuation plaque (on CT scanning)[161, 162]

TABLE 1

Biomechanical stress	**Scale**	**Computational method**	**Input parameters***	**Principal mechanism of action in atherosclerosis**
Blood flow-induced wall shear stress (WSS)	1-10 Pa	Computational fluid mechanics (CFD) Fluid-structure interaction (FSI) analysis	Lumen boundary from imaging, e.g., CT, IVUS, OCT Flow rate/velocity at the inlet and pressures at outlets or pressures at the inlet and outlets	Low WSS: atherogenesis High WSS: atheroprotective Supraphysiological WSS: possibly involved in plaque rupture or thrombus generation
Arterial pressure-induced plaque structural stress (PSS)	$1\text{-}10\times10^5$ Pa	Finite element analysis/method (FEA/FEM)† FSI analysis	Detailed plaque structure from high-resolution imaging, e.g. IVUS Material properties of each component Flow rate/velocity at the inlet and pressures at outlets or pressures at the inlet and outlets	High PSS: fibrous cap damage, e.g., rupture Periodic change of PSS: fibrous cap fatigue

** Inputs for WSS are for CFD and those for PSS are for the FSI analysis; † Except for structure and material properties, internal pressure is needed for FEA/FEM.*

TABLE 2

Reference	Patients, (n)	Follow-up	Imaging used for basis of CFD	Outcome description
Stone et al. (2003)[80]	8	6 months	Angiography and GS-IVUS	Low WSS associated with increased plaque thickness and outward vessel remodelling.
Stone et al. (2007)[81]	13	8±2 months	Angiography and GS-IVUS	Baseline low WSS associated with increased plaque area at follow-up.
Samady et al. (2011)[83]	20	6 months	Angiography and VH-IVUS	Low WSS regions showed increased plaque and necrotic core progression and constrictive remodelling. High WSS regions developed excessive expansive remodelling with increases in necrotic core/calcification.
Stone et al. (2012)[85]	506	6-10 months	Angiography and GS-IVUS	Baseline low WSS independently predicted decrease in luminal area, increased plaque burden and clinically relevant luminal obstruction necessitating further coronary intervention at follow-up.

CFD, computational fluid dynamics; GS-IVUS, grey-scale intravascular ultrasound; OCT, optical coherence tomography; TCFA, thin-cap fibroatheroma; WSS, wall shear stress; VH-IVUS, virtual-histology intravascular ultrasound

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