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In-Stent Restenosis: Is Low Shear Stress to Blame?
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Abstract
Cardiovascular disease is accountable for around 31% of all deaths worldwide. Percutaneous coronary intervention, particularly the use of stents has remarkably improved the management of moderate to severe cardiovascular disease since its introduction in 1977. However, in approximately 20-30% of patients clinical in-stent restenosis (ISR) can occur. ISR has a multifactorial aetiology, in which endothelial shear stress plays a major role. We aim to review the current evidence assessing the relationship between endothelial shear stress and ISR. The introduction of a stent can lead to changes in the mechanical environment of the artery, particularly at the inlet and the outlet of the stent which are susceptible to areas of low shear stress (LSS). In vivo studies have consistently associated LSS with a higher incidence of ISR whereas higher shear stress exerts a protective effect. The mechanisms underlying this association are not fully known, but ISR is likely to occur through neointimal hyperplasia (NIH). An endothelium dependent effect of LSS, endothelium-independent effect of LSS or the effect of LSS on smooth muscle cell phenotype may contribute to NIH progression. Factors relating to the stent design, patient specific characteristics and mechanical factors may also exacerbate NIH formation. Recent advances in the methodology for in vivo shear stress profiling may allow the early identification of patients at an increased risk of developing ISR clinically. This will then help guide novel treatment strategies towards an individual’s needs.

ABBREVIATIONS
ISR: In-Stent Restenosis; NIH: Neointimal Hyperplasia; PCI: Percutaneous Coronary Intervention, BMS: Bare Metal Stent; DES: Drug-Eluting Stent; NF-κB: Nuclear Factor-κappa B; PDGF: Platelet-Derived Growth Factor; TNF-A: Tumour Necrosis Factor Alpha; PGI2: Prostacyclin; IL-1: Interleukin-1; KLF-4: Kruppel-Like Factor 4; LSS: Low Shear Stress; SMC: Smooth Muscle Cell

INTRODUCTION
Shear stress is the force per unit area that occurs when blood flow acts on the endothelium [1]. The extent of shear stress can be predicted in most vessels by Poiseuille’s law which applies to straight and circular tubes of a constant cross-sectional area. However, it may not be accurately represented in larger arteries as they are not straight, most contain branches that disturb steady flow, and the cross-sectional area varies across the artery [2-4].

The progression of atherosclerosis can be affected by both genetic predispositions and a variety of established cardiovascular risk factors [1,5]. The most striking feature of atherosclerosis is its geographic distribution along the artery tree. The atherosclerotic lesions preferentially occur in the arch and bifurcation area while rarely in the straight part [6]. According to the local geography, the blood flow patterns and forces differ significantly. In the arch and bifurcation area, it is disturbed flow with low shear stress (LSS), while in the straight it is high shear stress laminar flow [5-8] suggesting flow patterns play an important role in atherosclerosis development.

The major consequence of atherosclerosis causes vessel narrowing or occlusion, leading to blood supply insufficient or ischemia. To combat this, many techniques have been developed. Notably, percutaneous coronary intervention (PCI) with stent implantation is one of the most common procedures carried out in the UK [9]. On average one in three patients with coronary artery disease will undergo coronary angioplasty and stenting. Since its first use in 1977 by Andrea Gruntzig [10], there have been significant developments in the use of stenting. There are two main types of stent – bare metal stents (BMS) and drug-eluting stents (DES). BMS replaced balloon angioplasty which aided the long-term scaffolding of the coronary artery avoiding acute recoil and abrupt occlusion. Following this, the introduction of DES has
since shown to be an extremely effective treatment, primarily in relation to reducing but not eradicating the risk of restenosis [9].

Although these interventions have been successfully used worldwide, their clinical benefit may be compromised by the occurrence of in-stent restenosis (ISR) and stent thrombosis. The prevalence of ISR varies widely in different groups, however commonly 20-30% of patients are known to have ISR following implantation after 6-12 months [11,12]. Using procedures such as intravascular ultrasound [11], coronary angiography [12] as well as three-dimensional reconstruction of the artery during routine follow-ups [13,14], luminal changes were observed. Recently, it was shown that following successful PCI, higher differences in the corrected QT dispersion during their pre-and post-repeated PCI may be used as a predictor of restenosis [15]. Nevertheless, the underlying mechanism of ISR still unknown. While these complications may be attributed to patient-specific factors such as comorbidities or medication (anti-platelet therapies), haemodynamic factors, particularly LSS, the focus of this review, are now being shown to be important in the incidence of post stenting complications alongside the aforementioned established risk factors [1,16,17].

The mechanical implications of stented arteries

The introduction of a stent has profound effects on the stresses in the arterial wall, which may affect outcomes post intervention. As well as the high pressures that are used when introducing stents [18], balloon inflation can damage endothelial cells, compromising the integrity of the diseased artery [19]. Macroscopically, the segment undergoing intervention will return to approximately the three-dimensional shape that was present before the invasion of the plaque. This may re-establish the original low and oscillating shear stress favouring further inflammation and proliferation [17]. Studies using computational fluid dynamics [20] have shown that the introduction of stent may increase the local curvature at both the inlet and outlet of the stent [17,21,22]. Changes in shear stress are found near stent edges [22], indicating the possibility that the high curvature regions (near the stent edges) may induce separation zones, resulting in regions of LSS. In addition to this, the entrance and exit edges of the stent will be exposed to increased levels of tension due to the difference in stiffness between the artery and the stent [19]. Furthermore, the design of the stent strut including the thickness and height can lead to disturbances in blood flow resulting in small regions of disturbed shear stress [23]. This is especially true if the strut is placed at right angles (orthogonal) to the blood flow. These factors of stent design favour areas of blood stagnation, rendering these more susceptible to thrombosis and smooth muscle cell (SMC) proliferation, an important constituent of neointimal hyperplasia (NIH) [24].

LSS and ISR

The precise pathophysiological mechanism governing ISR remains unknown, however there is strong evidence suggesting that NIH is the major mechanism. This process usually occurs within or at the edges of the stent, classically due to the proliferation and migration of vascular SMCs [25]. NIH is a multifaceted process that involves the immediate action of platelets, as well as endothelial and SMCs. Although NIH can be influenced by direct insult by stent struts or endothelial dysfunction [26], the association with shear stress is an important determinant [19,27]. Low wall shear stress may promote NIH, causing structural change of vessels (increased inflammation and increased SMC proliferation) which leads to a decrease in lumen size, while a higher shear stress may oppose these effects.

In vivo studies support the hypothesis of a haemodynamic mechanism contributing to in-stent NIH. Carlier et al. introduced a flow divider (increasing shear stress) randomly in one of two stents placed in external iliac arteries of rabbits which corresponded to a reduction in NIH [28]. This association was similarly found where NIH occurred in regions where there were lowest initial values of shear stress in rabbit iliac arteries [29]. In addition, NIH occurred most in regions where blood flow was disturbed (adjacent to stent struts) and in regions where there were acute elevated gradients in shear stress at implantation. These studies show that modelling the regions susceptible to LSS could predict the distribution of ISR. However, the magnitude of shear stress at baseline may not be associated with the magnitude of ISR that requires further intervention at follow up [30].

While these in vivo studies do provide insight into mechanistic explanations, the direct clinical extrapolation to humans may be limited [29,31,32]. The first human findings after BMS implantation showed a significant inverse correlation between variations of NIH and the magnitude of shear stress [20]. However, these results are limited by the design of the study in that only patients with no clinically defined restenosis were studied (mild form of NIH), therefore whether similar results would be found in patients with restenosis is unknown. The first serial study carried out on humans showed that evidence of ISR occurred in each category of shear stress at baseline, showing no inverse correlation as found in animal studies [13]. Similarly, a study in 506 patients showed that although NIH is independently associated with LSS within the stented region, the magnitude of shear stress at baseline is not associated with ISR and further intervention [30]. These inconsistent results between shear stress and NIH in humans may be due to the differences in methodology in measuring flow (computational flow dynamics vs displacement of contrast material). More recent studies [33,14] have consistently showed an inverse relationship between shear stress and restenosis via NIH following BMS implantation although the magnitude of this relationship may be weaker than originally thought. This suggests that other factors such as inflammation, lesion complexity and patient comorbidities may also be contributing to this relationship.

Recent studies have focused on the association between shear stress and ISR in DES. Overall, DES have been shown to inhibit levels of NIH and reduce restenosis rates [9,11,34]. ISR occurred more commonly in regions of LSS following both sirolimus-eluting stent [35] and paclitaxel-eluting stent [36] deployment. In contrast to these results, in diabetic patient population treated with sirolimus-eluting stents, there was no significant association between shear stress and NIH at the stent segments [37]. This is likely to be due to other factors (hyperglycaemia and hyperinsulinemia) can accelerate restenosis other than shear stress that are governing the arterial wall response following intervention.
Mechanisms of in-stent restenosis

The mechanism of restenosis is related to the type of stent used, as well as other known risk factors relating to the patient or the disease [38]. Pathologically it is evident that there are considerable differences between ISR occurring following BMS and DES implantation [16]. The key difference is that ISR following BMS is characterised by NIH consisting of a proteoglycan rich matrix and more vascular SMCs, compared to fewer SMCs in DES [39]. Following BMS implantation, the endothelium is restored within weeks, LSS may promote ISR through interactions of endothelial cells and SMCs. On the other hand, DES attenuate vascular SMC proliferation (by pharmacological measures) hence reducing the likelihood of ISR [9,38]. In DES, shear stress may act directly on SMCs via endothelium independent mechanisms. There are three distinct mechanisms for the pathobiology of restenosis: endothelium-dependent effect of LSS, endothelium-independent effect of LSS or the effect of LSS on SMC phenotype [16].

LSS promotes inflammatory activation and apoptosis of endothelial cells, while high shear stress exerts more protective effects [19]. LSS leads to a recruitment of circulatory inflammatory cells, particularly monocytes which migrate into the intima [16,19,40]. This process is dependent on nitric oxide (NO)-regulated activation transcription factors such as nuclear factor-kappa β (NF-κB) [41]. The endothelium-independent effect of LSS involves SMCs which are responsive to changes in shear stress. Shear stress differentially regulates the activity of mitogenic molecules such as PDGF and vascular endothelial growth factor [42]. Protective high shear stress inhibits smooth muscle proliferation through the upregulation of anti-mitogenic factors, such as transforming growth factor beta (TGF-β), which inhibits DNA synthesis arresting the cell cycle at G1 phase [43]. In contrast, LSS may lead to the formation of mitogen gradients, triggering SMC migration and proliferation [16,40]. Although the exact mechanism is unknown, PDGF has been shown to activate several downstream signalling pathways, including Src (a proto-oncogene tyrosine kinase) and Ire1α/Xbp1 splicing [40,44].

Shear stress can also affect SMC phenotype. During restenosis, it is thought that vascular SMCs modulate their physiological contractile phenotype to a pathologic synthetic phenotype leading to migration into the intima and contributing to arterial thickening and narrowing of the lumen [16]. They release pro-inflammatory factors and interact with endothelial cells, producing more extracellular matrix proteins such as collagen, and proteoglycans [45]. Additionally, arterial regions that are exposed to LSS contain plaques with a reduced smooth muscle content and marked smooth muscle phenotypic modulation which promotes the formation of unstable plaques, through upregulation of PDGF and Kruppel like factor 4 (KLF 4), SMC differentiation inhibitors [46,47].

Factors that affect LSS mediated restenosis

1) Stent type and drug: Many factors have been found to affect the relationship between shear stress and ISR (Table 1). There are two main types of stent, BMS and DES. BMS were used to reduce the early elastic recoil, however, ISR was a common finding upon medium and long term follow up, due to the proliferation and migration of vascular smooth muscle cells [endothelium-dependent process] [22]. NIH is likely to be favoured in BMS by the sustained LSS that occurs following restenosis [8].

The introduction of DES decreased rates of ISR, primarily by inhibiting inflammation and vascular SMC proliferation pharmacologically [9]. This creates a more favourable haemodynamic environment within the artery. The stent drug used in DES may affect the incidence of restenosis. Most commonly sirolimus and paclitaxel have been used, however the pathways in which they inhibit SMC proliferation differs. The LSS-activated cyclin-dependent kinase contributes to SMC proliferation, sirolimus decreases the cyclin-dependent kinase activity arresting cell cycle at G1 phase, suggesting that this may be the main mechanism of sirolimus antagonising LSS-mediated restenosis [16,48]. Paclitaxel binds to microtubules and prevents their depolymerisation which inhibits SMC proliferation independently of shear stress related pathways [11]. There may be a clinical benefit of choosing sirolimus over paclitaxel elution as it leads to lower rates of revascularization in the total patient population [49]. In addition, both in vivo and in vitro studies have shown that sirolimus elution favours a contractile SMC phenotype, whereas paclitaxel promotes a synthetic SMC phenotype [50,51]. Consequently, sirolimus attenuates and paclitaxel amplifies the LSS associated synthetic phenotype which can contribute to restenosis.

2) Stent design: The stent design may also contribute to a diminished restenotic effect, as LSS areas with disturbed flow are

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<th>Table 1: The risk factors for in-stent restenosis.</th>
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<td>Patient related factors</td>
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<td>Stent type</td>
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<td>Stent design</td>
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<td>Thick strut</td>
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<td>Streamline [circular arc] strut</td>
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Abbreviations: BMS: Bare Metal Stent; DES: Drug-Eluting Stent; ISR: In-Stent Restenosis
often found distal to stent struts [24]. This leads to an increase in the concentration of the drug compound leading to an anti-proliferative effect in an area that is susceptible to LSS [16]. The thickness of the strut and the shape can affect the possibility of strut-induced flow disruption post intervention. Thicker struts increase the area within the stent that is exposed to disturbed flow and lower shear stress. On the other hand, thin struts with larger strut spacing restore shear stress to normal levels. Clinical studies have found that stents with thinner struts tend to elicit a lower incidence of ISR compared to a thicker strut [52,53].

Areas of LSS are pronounced with non-streamlined struts (rectangular) whilst they are reduced with streamlined strut (circular arc shape) [23]. A streamlined design can minimize recirculation zones and establish a protective low environment with better haemodynamic performance [54]. On the other hand, a non-streamlined strut will promote recirculation zones and high shear stress peaks which can activate platelet activity. Improvements to its design by decreasing the height can be made which lessens the effect of the height on the flow [23]. The diameter of the stent employed may affect ISR. Often clinically there is a propensity to increase the size of stents by approximately 10% more than is required to ensure accurate aligning and positioning. Gross oversizing (over 20%) of stents was found to be a strong stimulant of the restenotic process (direct damage) compared to stents that were only slightly oversized [16,55]. The oversized stents may also promote NIH by inducing changes in flow rate and increasing disturbed flow with LSS in the stented region. On the other hand, undersizing stents may lower shear stress near strut edges. A small decrease in stent diameter creates small gaps between struts and the arterial wall, increasing the resistance to flow and thus lowers the shear stress in that region. This effect becomes less obvious when there is gross undersizing (20%) due to the increased distance between the strut and the arterial wall which decreases the flow resistance, therefore LSS regions are less likely to develop [52,56]. However, the degree of undersizing affects the occurrence of restenosis with a nonlinear relationship between undersizing and shear stress [52,53,56-57].

CLINICAL IMPLICATIONS AND CONCLUSION

Although PCI has revolutionized the treatment of cardiovascular disease, the extent of complications occurring cannot be underestimated. The introduction of DES has led to a decrease in the incidence of ISR compared to BMS. However, restenosis still occurs in this population group due to biological, mechanical or technical factors. To improve clinical outcomes, it is ideal to individualize interventions having considered the stent type and design. Recent advances in the methodology in particular in vivo profiling may become suitable for use in a clinical setting [58-62]. By being able to model stented segments that are particularly susceptible to LSS, this will allow the identification of individual patients who are at an increased risk of the development of complications [16], whilst also allowing prognostic insight into how lesions may develop or change over time. Here, we reviewed the role of LSS in the pathogenesis of ISR, occurring via NIH. Whilst the exact mechanisms responsible for endothelial cell function and repair post stenting are not well understood, this may occur via an endothelium-dependent or an endothelium-independent manner. Thus, further investigation is warranted concerning both the fundamental science as well as the clinical impact in order to understand and assist the potential development of therapies that will aim to reduce ISR.

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REFERENCES

16. Koskinas KC, Chatzizisis YS, Antoniadis AP, Giannoglou GD. Role


