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SPOTLIGHT REVIEW

The effects of stenting on shear stress: relevance to endothelial injury and repair

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Abstract Stent deployment following balloon angioplasty is used routinely to treat coronary artery disease. These interventions cause damage and loss of endothelial cells (EC), and thus promote in-stent thrombosis and restenosis. Injured arteries are repaired (intrinsically) by locally derived EC and by circulating endothelial progenitor cells which migrate and proliferate to re-populate denuded regions. However, re-endothelialization is not always complete and often dysfunctional. Moreover, the molecular and biomechanical mechanisms that control EC repair and function in stented segments are poorly understood. Here, we propose that stents modify endothelial repair processes, in part, by altering fluid shear stress, a mechanical force that influences EC migration and proliferation. A more detailed understanding of the biomechanical processes that control endothelial healing would provide a platform for the development of novel therapeutic approaches to minimize damage and promote vascular repair in stented arteries.

Keywords Stent • Endothelial cell • Shear stress • Vascular repair

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1. EC damage and repair in stented arteries

Balloon angioplasty and stent implantation, interventions that are used routinely to treat coronary artery disease (CAD), lead to damage and loss of endothelial cells (EC). Given the essential role of EC in suppressing inflammation and thrombosis and in controlling vascular tone and function, the restoration of healthy vascular endothelium is an important therapeutic goal to avoid the lethal consequences of in-stent thrombosis and to prevent restenosis.[1] While both local EC and bone-marrow (BM)-derived endothelial progenitor cells (EPCs) have been suggested to participate in re-endothelialization, there is controversy surrounding the identity of the cell population(s) that are responsible. Since the discovery of EPCs by Asahara in 1997,[2] their contribution to vascular homeostasis and potential ability to contribute to the regeneration of endothelium in denuded vessels has been studied extensively. To track the fate of EPCs during vascular repair, donor BM cells labelled using LacZ or GFP markers were introduced into the circulation of mice after wire/balloon-induced vascular injury[3,4] or vein-to-artery transposition.[5] These studies suggested that BM-derived cells contribute to vascular repair and that the reparative process can be enhanced by treatment with statins[3,4] and granulocyte-colony stimulating factor (G-CSF).[6,7]

Recently, the relative contribution of local vs. BM-derived EPCs to endothelial repair following stenting was addressed directly using a murine model of stenting in the aorta.[8] The authors devised an elegant experimental approach to track EC derived from local or systemic sources by transplanting stented aortae from transgenic Tie2-LacZ mice (expressing LacZ in EC) to non-transgenic mice and vice versa, and by using chimeric mice containing Tie2-LacZ BM to track EPCs. The study revealed that repair of stented arteries involved both adjacent EC and BM-derived EC although the contribution of the latter varied between animals.[8] In contrast, recent studies of common carotid artery (CCA) allografts between Tie2-GFP transgenic and wild-type mice (of different genetic backgrounds) suggested that regeneration of the endothelium involved local cells, whereas a contribution from BM-derived cells was not demonstrated.[9,10] However, it should be noted that the conclusions from the latter studies are complicated due to strain-specific immunogenicity towards GFP. Additionally, the anastomoses required for this transplantation model may influence local repair mechanisms.[11] The seemingly conflicting results from Douglas *et al.*[8] and Hagensen *et al.*[10] may also be related to the animal models used, i.e. hypercholesterolaemic or healthy mice. Lastly, the observation that the Tie2 promoter can be activated in monocytes as well as EC[12] complicates the interpretation of studies employing Tie-2-GFP/LacZ murine models. Thus although it can be concluded that

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re-endothelialization of injured arteries can occur naturally via outgrowth of local EC or via recruitment of circulating EPCs, the exact contribution of these populations and their mechanisms of recruitment remain uncertain.

Migration of EC in injured coronary arteries may be affected by the presence of a stent since this structure provides a non-physiological surface for adhesion and generates perturbations in blood flow. Nevertheless, the effect of stent deployment on EC migration and proliferation remains poorly understood. The issue of EC repair has been brought into sharp relief in the era of drug-eluting stents (DES) which release cytostatic compounds (e.g. sirolimus/rapamycin) that inhibit the PI3K-mTOR pathway. Although DES are associated with reduced restenosis rates via inhibition of vascular smooth muscle cell (VSMC) proliferation, they have also been linked to lethal late-stage thrombotic events associated with EC injury.[13–16] Thus there is an urgent need to develop new interventions to promote EC repair in stented arteries and thereby reduce the incidence of late-stage thrombosis and avoid risks associated with prolonged administration of systemic anti-platelet treatments.

2. Novel stent designs to promote endothelialization

Several groups have attempted to promote re-endothelialization using stents that deliver growth factors[17–19] or devices that promote EPC capture.[20–27] EPC capture has been attempted by coating stents with antibodies that target EPC markers (e.g. CD34, CD133, VE-Cadherin), however, this approach has had mixed success. For example, although coating of stents with anti-VE-cadherin antibodies was shown to accelerate re-endothelialization and reduce neointimal formation in a rabbit model,[23] coating with anti-CD133 antibodies did not influence endothelialization or neointimal thickening in a porcine model.[24] In addition, coating stents with anti-CD34 antibodies enhanced early endothelialization but did not reduce neointimal thickness in animal[20] and human[25,26] studies. While some EPC capture stents have been shown to enhance endothelialization in animal models, they may have limited use in the clinic because patients with cardiovascular disease often have few and/or dysfunctional EPCs. However, this may be ameliorated by treatment with statins which has been associated with enhanced EPC number and survival in patients with cardiovascular disease.[26] Because of these considerations, new stent design strategies are urgently required including the use of novel biomaterials that enhance endothelialization. For example, a recent study by Andukuri *et al.* [27] demonstrated that a bioinspired multifunctional nanomatrix which mimicked endothelial surface characteristics by containing cell-adhesion ligands and nitric oxide donors was able to recruit EPCs and promote their differentiation towards an endothelial lineage. However, these stents have not yet been tested in pre-clinical models.

3. The mechanical environment of stented arteries

Vascular cells are exposed to a complex mechanical environment which they sense via numerous mechanoreceptors. On the luminal side, EC sense blood flow-induced frictional forces, which can be represented by the *wall shear stress*. Under the influence of blood pressure, the vessel wall deforms in a cyclic manner. The EC will follow the deformation of the sub-endothelial vessel wall and the resulting *wall strain* is the second important mechanical trigger the EC and VSMC are exposed to. In order to understand the influence of mechanics on vascular physiology, research has focussed on the following areas: *in vitro* studies of cellular response to controlled changes in the mechanical environment; *in vivo* studies of cellular responses during disease development and post-intervention using animal models; and clinical studies of patients with arterial disease and their response to intervention. Each of these approaches provides complementary information which has the potential to extend our understanding of the effects of mechanical triggers on EC and VSMC physiology following percutaneous coronary intervention (PCI).

It is well established that early plaque formation is localized in specific arterial regions.[28,29] Especially, arterial bifurcations, branch points, and curved arterial segments are prone to develop atherosclerotic lesions. From a mechanical perspective, these regions are characterized by disturbed blood flow leading to low and/or oscillating shear stress.[30,31] During early atherogenesis, outward vessel wall remodelling compensates for plaque growth, leaving the lumen essentially unchanged.[32] Since the geometry of the lumen determines blood-flow patterns, this implies that shear stress will not alter during early plaque growth. This changes once the plaque intrudes into the lumen: shear stress in the upstream and midcap plaque region increases while the downstream plaque region is exposed to low shear stress.[33,34] The development of atherosclerotic plaques is also associated with altered strain patterns, which vary as the disease progresses. Although limited information is available, clinical studies applying intravascular ultrasound techniques demonstrate that EC covering soft atherosclerotic plaques are subjected to increased wall strain.[35] If and how these strain patterns affect EC function in the context of atherosclerosis is largely unknown.

The mechanical environment in the vessel wall is greatly altered by PCI and stent placement. Balloon inflation leads to EC denudation and compromises the integrity of structures inside the diseased arterial wall. The denuded interface between the lumen and the vessel wall is exposed to altered mechanical stimuli, partly depending on the mechanical properties of the stent. On a macroscopic scale, the geometry of the stented segment returns approximately to the 3D shape it had before the plaque intruded into the lumen, resulting in the restoration of low and/or oscillating shear stress which promotes inflammation and vascular injury. Both in bare metal stents (BMS) [36] and DES,[37] this leads to an inverse correlation between shear stress and neointimal hyperplasia. Depending on the axial stiffness of the device, stent implantation might also increase the local curvature at the entrance and the exit of the stent, inducing additional disturbed shear-stress regions.[38] Furthermore, the proximal and distal edge of the stented segment (potentially the source of in-growing EC) will be exposed to elevated strain levels due to the stiffness mismatch of the artery and stent.

On a local scale, the design of the stent struts is especially relevant. The presence of stent struts leads to perturbations in the local flow patterns: thus small regions with flow reversal and disturbed shear stress will develop between the stent struts.[39] The thickness of the stent struts determines the size of the recirculation zone and strut height is associated with thrombogenicity,[40] in *in vitro* experiments. In the *in vivo* situation, the occurrence of flow reversal is determined by how far the stent struts protrude into the lumen. Stent-strut malapposition or tissue regression between the stent struts might reinforce local flow recirculation.[37,40,41] In addition to variations in shear stress, damage induced by stent placement is also determined by stent-strut design and thickness, and this has been related to subsequent restenosis.[40,41] Finally, stent design also influences the local strain distribution between the stent struts, although few data are available to quantify this effect.

4. How do vessels respond to mechanical forces?

Vascular cells are exquisitely sensitive to their mechanical environment. The application of a stent by balloon angioplasty leads to major changes in wall strain which have profound effects on VSMC proliferation and migration, thus impacting on in-stent restenosis (reviewed in Lehoux[42] and Shyu[43]). VSMCs have been estimated to be exposed under the equivalent of 1 dyn/cm^2 of shear stress due to the interstitial flow induced by the trans-vascular pressure differences. However, VSMC can be exposed to much higher levels of shear stress in a denuded blood vessel (e.g. immediately following PCI) resulting in direct interaction of VSMC with blood flow. This may have relevance to the structure and function of stented vessels since shear stress is known to modulate VSMC proliferation,[44–46] migration,[47,48] and viability.[46,49] Here we focus on the influence of stenting on endothelial responses to mechanical force.

5. The influence of shear stress on endothelial cells

EC sense shear stress via multiple mechanoreceptors, e.g. VEGFR2/VE-cadherin/Pecam-1-complex, integrins ($\alpha_v\beta_3$), the glycocalyx, and primary cilia,[50–54] that translate the mechanical signal into a biological response, resulting in the modulation of proinflammatory and cell-survival signalling pathways. Of note, the responses of EC to mechanical force differ according to the magnitude, directionality, and temporal fluctuations of the force that is applied. For example, low and/or oscillatory shear stress (at disease-prone sites) promotes inflammatory activation and apoptosis of EC, whereas high uniform shear stress exerts protective effects.[30,55–66] The fact that the endothelium is differentially affected by high or low shear stress and by unidirectional or bidirectional shear stress, governs the focal nature of atherosclerosis, but will also play a role in arterial healing after stent deployment/PCI.

In vitro and *in vivo* studies have revealed a myriad of genes that are regulated by shear stress (*Figure 1*). Of note, 70% of shear-regulated genes are dependent on the mechanosensitive transcription factors Krüppel-like factor-2 (KLF-2) and nuclear factor erythroid 2-related factor (Nrf2),[67] that are activated by high, unidirectional shear stress and co-operate to induce anti-inflammatory, anti-thrombotic, and anti-proliferative genes. Furthermore, KLF2 plays a major role in maintaining vascular tone via regulating expression of the vasoconstrictor endothelin-1 (ET-1) and endothelial nitric oxide synthase (eNOS), which produces the vasodilator NO.[68] KLF2 has suppressive effects on inflammatory activation in part by its ability to sequester critical co-activators of NF-κB,[69] thereby reducing NF-κB mediated transcription and by inhibiting the MAPK pathway. Nrf2 also protects EC exposed to high shear stress via the induction of antioxidant genes including heme oxygenase-1, glutathione S-transferase, and ferritin and via the negative regulation of p38 MAPK.[59,70,71]

In contrast, low and/or oscillatory shear stress activates the transcription factor NF-κB,[56] which controls multiple processes including immunity, inflammation, cell survival, differentiation, and proliferation. The MAPK pathway plays a role in many cellular processes, including apoptosis, proliferation, and inflammation. Low and/or oscillatory shear stress also activates c-Jun N-terminal kinase (JNK) and the p38 pathway,[55,57,59] which promote inflammation and EC apoptosis by activating transcription factors belonging to the activating protein-1 (AP-1) superfamily [including c-Jun and activating transcription factor-2 (ATF2)]. In contrast, high shear stress negatively regulates JNK/p38 MAPK by inducing MAPK phosphatase-1 (MKP-1),[57,58] reducing apoptosis signal-regulating kinase-1 (ASK-1) activation,[63] and blocking cleavage of protein kinase C zeta,[62] resulting in dampened pro-inflammatory signalling in regions of high shear stress.

In addition, shear stress plays an important role in proliferation and migration of EC. High, unidirectional shear stress increases EC migration by promoting remodelling of the actin cytoskeleton to influence cell polarity, formation, and protrusion of lamellipodia, and contractility of stress fibres that are essential for cell traction.[72–74] In contrast, low and/or oscillatory shear stress causes cell loss and migration of cells away from areas with large gradients in shear stress.[74] Shear stress regulates EC cell-cycle entry via activation of the anti-mitotic AMP-activated protein kinase (AMPK) and the proliferative Akt. High, unidirectional shear stress activates both AMPK and Akt; this counterbalance results in relatively undisturbed mTOR (mammalian target of rapamycin) and its target p70 ribosomal S6 kinase (p70S6K), attenuating EC proliferation. In contrast, low, oscillatory shear stress activates Akt in the absence of AMPK, resulting in a sustained activation of p70S6K and, consequently, EC proliferation.[75,76] DES releasing rapamycin specifically affect the Akt/mTor pathway, thereby inhibiting EC proliferation and possibly disturbing the effect of shear stress on this pathway.[77–79]

Endothelial dysfunction is associated with impaired eNOS activity or the inactivation of NO by reactive oxygen species.[80] EC dysfunction plays an important role in the arterial healing upon PCI as it leads to increased permeability, increased expression of chemotactic molecules and adhesion molecules, enhanced recruitment and accumulation of monocytes/ macrophages, decreased EC regeneration and increased SMC proliferation and migration, and increased expression of procoagulatory molecules.[29] Endothelium that has regenerated after stenting/PCI seems dysfunctional in terms of integrity and function, with areas of poor endothelialization, poorly formed EC junctions, reduced expression of anti-thrombotic molecules, and decreased nitric oxide production, and thus contributes to late stent thrombosis and development of in-stent neoatherosclerosis.[1,13–16] Maintaining a functional endothelial layer is important for the long-term health of the vessel wall. An improved understanding of the biological function of the endothelium (before and after stenting) is, therefore, crucial. In this regard, EC loss and re-endothelialization are 'black boxes'—we do not understand the mechanisms that underlie these processes but shear-stress-related changes in EC function are likely to be involved.

6. The influence of strain on endothelial function

The effect of strain on EC has received less attention than the influence of shear stress. Nevertheless, it has been demonstrated that EC sense strain via multiple mechanoreceptors including cell-adhesion sites, integrins, tyrosine kinase receptors, ion channels, and components of the lipid bilayer (reviewed in Ando and Yamamoto[81] and Anwar *et al.*[82]). Activation of these sensors results in the activation of multiple signalling pathways, including PKC, Rho, Rac, PI3K/Akt, and MAPKs. Strain affects transcription factors including AP-1 and NF-κB[81,82] and inflammatory genes including ET-1, VCAM, MMPs, and monocyte chemotactic protein-1 (MCP-1) (we refer the reader to Anwar *et al.*[82] for a more comprehensive list of strain-induced transcription factors and genes). In addition to pathway activation and subsequent gene regulation, strain was shown to induce endothelial Ang II release and AT1R

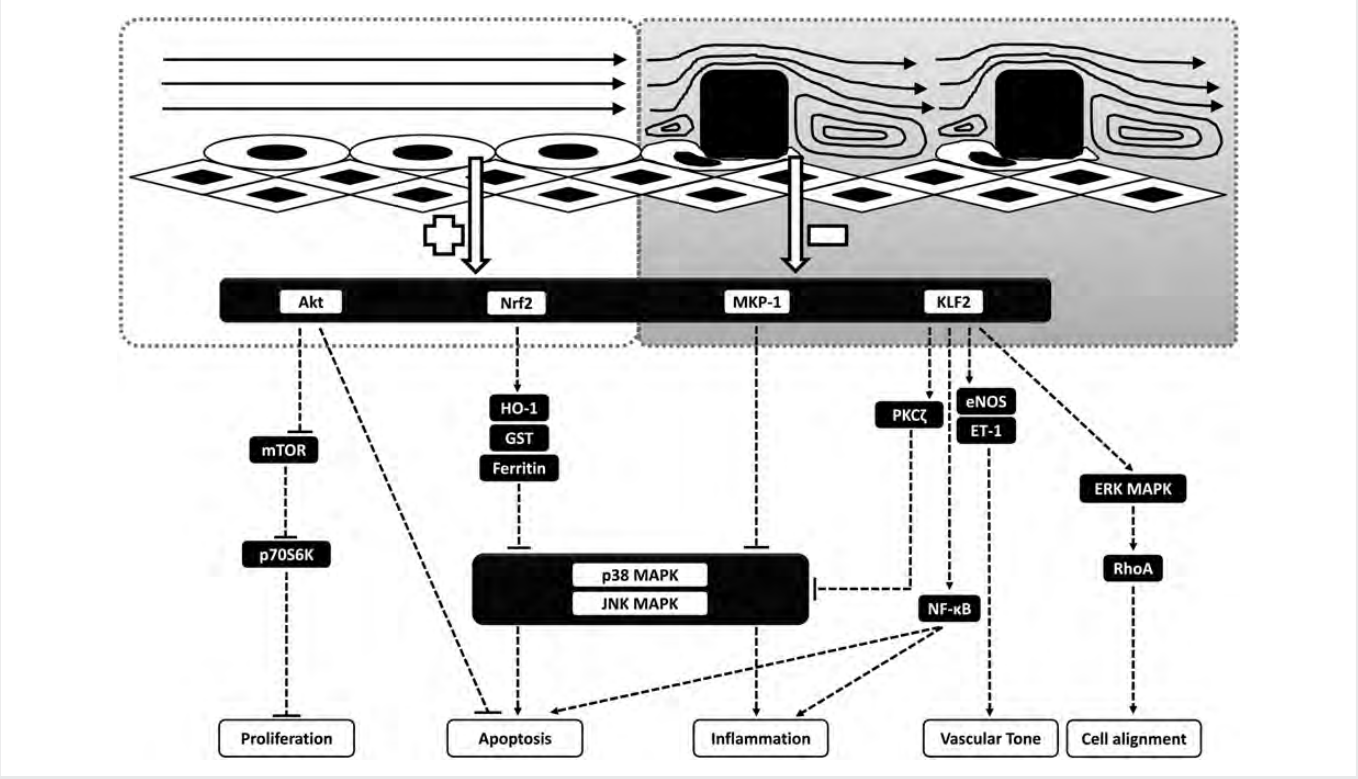

Figure 1 The contrasting effects of uniform and non-uniform flow on intracellular signalling. Uniform flow (left) activates multiple signalling pathways that protect arteries by promoting endothelial quiescence and viability, by suppressing inflammation and co-ordinating vascular tone and endothelial alignment. In contrast, non-uniform flow associated with stent struts (right) may have the opposite effects.

activation, resulting in elevated superoxide levels via activation of NADPH oxidases.[83] This increase in oxidative stress may lead to endothelial dysfunction and inflammation.

Little is known about the combined effect of shear stress and strain, however, it is likely that gene expression is regulated by an interaction between these mechanical factors. For example, ET-1 mRNA increased when EC were exposed to strain, decreased in response to shear stress, but was unchanged when the two forces were combined.[84] Similarly, shear stress and strain were shown to differentially regulate AT1R.[83] The basis for these seemingly opposing responses, and more importantly the EC responses to a combination of forces, require further study. Strikingly, several of the strain-induced genes are known players in atherogenesis and in-stent restenosis. However, studies on the role of strain in these processes are lacking.

7. Computational modelling approaches

With the advancements in computational power delivered during recent years, the use of *in silico* approaches to the study of biological systems is becoming more widespread, offering an alternative framework for integration of biological data. Numerical techniques have been employed over the past few decades to the study of both structural effects following stent placement (interactions between the stent and the vessel wall) and fluid effects (alteration in blood flow within the stented region; reviewed in Morlacchi and Migliavacca[85]). These techniques provide quantification of local variations in the structural and fluid environment, described earlier in this paper. Finite element analysis (structural) and computational fluid dynamics (fluid) are often undertaken at the length scale of the entire stented region, by dividing the geometry into a large number of discrete elements. Early application of these techniques involved simplification of the vessel and stent geometry. More recent advances have utilized image-based information (angiography, IVUS, OCT) to provide an accurate description of the vascular anatomy both pre- and post-stent implantation. As illustrated in *Figure* 2, the availability of both 3D geometry and histology data allows structural mechanics[86] and fluid dynamics[87] to be correlated with biological processes in the vessel wall (e.g. the localization of neointimal thickening).

Most models of stent implantation consider both the vessel wall and blood as a continuum, neglecting variations at the constituent level (VSMCs, EC, red blood cells, platelets etc.) or the interactions between constituents (cellular signalling etc.). However, within other biological systems, computational modelling has been applied to consider function of cellular constituents in isolation or in small groups,[88] and to study the influence of intracellular signalling on the cell-cycle.[89] A recent development in computational modelling of biological systems is the concept of multi-scale modelling, where representations of mechanical stimuli and biological function at a number of spatial and temporal scales are combined to provide a framework for examination of mechanobiology across the scales. A number of recent studies have applied the multi-scale paradigm to the study of in-stent restenosis.[90–94] For example, Evans *et al.* [95] describe the formulation of a multi-scale framework in which restenosis is simulated using an agent-based model to represent smooth muscle cell migration and proliferation. This framework has been applied to study interactions between SMC, blood flow, and pharmacological

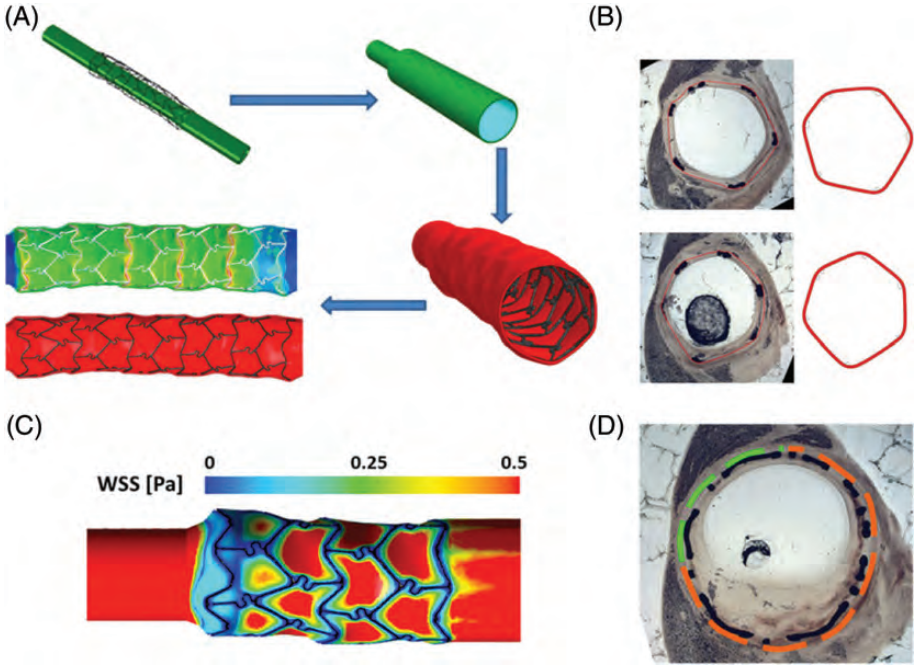

Figure 2 The influence of stenting on vascular anatomy and blood flow. (*A*) Structural model implemented to identify the geometrical configuration of the stented arteries of *in vivo* porcine coronary models: CAD model of the undeformed artery and of the stent reconstructed from micro-CT, expansion of the artery through a cylindrical surface dilatation, stent–artery coupling obtained after the recoil of the artery, and longitudinal section of the final configuration. (*B*) Comparison between two histological images and the corresponding sections obtained with the structural simulation showing the good agreement between the histological images (left) and the numerical geometrical configuration (right). (*C*) Results of the CFD simulations in terms of spatial distribution of WSS magnitude over the arterial wall. (*D*) Correlation between areas characterized by low WSS (orange lines) and the in-stent restenosis phenomenon after 14 days in a proximal section of the stented artery. Image reproduced with permission from Morlacchi and Migliavacca.[85]

agents within the vascular tissue and to examine the influence of stent design on the degree of restenosis.[96] Such approaches provide insight into potential mechanisms for interaction between mechanical stimuli generated at scales much larger than individual cells and the response of individual cells and intra-cellular signalling. However, such techniques also promote challenging research questions as the rules required to define cellular behaviour in such models are poorly understood.

Despite these challenges, computational models provide a consistent framework, allowing comparison of results from *in vitro* cellular studies, *in vivo* models of coronary pathology/response to intervention, and *in vivo* patient studies. A multi-disciplinary approach to the investigation of future research questions, combining iterative model development with study of biological responses at the molecular, cellular and tissue level, has the potential to significantly improve our understanding of arterial patho-physiology and improve our ability to develop effective treatments for arterial disease.

8. Conclusions and future perspectives

The cellular and molecular mechanisms that govern EC function and repair in stented arteries are poorly understood. In this review, we discuss the sensitivity of EC to mechanical forces and suggest that further research is required to understand the effects of stent-induced changes in shear stress and strain on EC migration and proliferation. This is important because a detailed understanding of the endogenous repair processes in stented arteries and their perturbation by flow may inform the development of novel therapies to reduce thrombosis and restenosis in stented arteries by promoting 'healthy re-endothelialization'.

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