Pathophysiology of arterial restenosis

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The re-stenotic fibro proliferative disease is supported by the contribution of different phenomena, whose prevalence depends on the kind of revascularization procedure applied. The different factors contributing to vascular restenosis include constrictive remodelling, neo-intimal hyperplasia, and neoadventitia formation. Remodelling consists in a spatial reorganization of elements of the vascular wall that can be compensative, leading to vessel lumen enlargement, or constrictive, leading to lumen narrowing.

The *primum movens* of restenosis process is the interruption of the natural balance between anti- and pro-thrombotic factors; endothelial denudation, caused either by endarterectomy or PTA/stent causing endothelial damage, results in loss of antithrombotic factors (*e.g.*, nitric oxide, prostacyclin, and tissue plasminogen activator), further contributing to platelet adhesion and aggregation. Activated platelets release mitogens, including thromboxane A2, serotonin, and platelet-derived growth factor, which together promote smooth muscle cell proliferation and migration.¹ Concurrently, levels of mitogen proto-oncogenes increase in the smooth muscle cells, altering their phenotype from contractile to synthetic.² Moreover, smooth muscle cells secrete pro-migratory
proteins. Because of these changes, activated medial smooth muscle cells (SMC) migrate to the intima and synthesize extracellular matrix with the result of neointimal hyperplasia.\(^3\)

Under normal homeostatic conditions found in healthy vascular physiology, vascular smooth muscle cells (SMC) ordinarily have a low turnover rate, with low baseline levels of both proliferation and apoptosis.\(^4, 5\) Acceleration of the rate of SMC turnover, with increases in the rates of both proliferation and apoptosis, is thought to be involved in the pathogenesis of atherosclerotic lesions, restenosis after interventional therapy, and vein graft arterialization.

However, even in normal vascular tissue SMC demonstrate a heterogeneous population of cells. Spindle-shaped differentiated SMC show contractile properties, with a low frequency of proliferation, and are induced into this phenotype by heparin and transforming growth factor-\(\beta\) (TGF-\(\beta\)). Rhomboid-shape dedifferentiated SMC show a high degree of protein synthesis, proliferation and migratory activity, and are induced into this phenotype by basic fibroblast growth factor (bFGF) and platelet derived growth factor-BB (PDGF-BB).\(^6\)

The source of these different SMC phenotypes is controversial. Some of the potential sources of heterogeneous SMC populations that contribute to vascular remodelling include migration of cells from the adventitia, in situ differentiation and expansion, or accumulation from distant sources such as the bone marrow. The "myofibroblasts" (MFs) SMC phenotype is thought to be a marker of SMC that are involved in and accumulate during restenosis.\(^7\) Over the first 2 weeks after injury, the vascular smooth muscle cells multiply 3 to 5 times, accounting for 90\% of the ultimate intimal proliferation.\(^8\)

Differentiation of MFs normally occurs during the wound healing process, under concomitant mechanical and biochemical signals. The fate of recruited/activated MFs in injured tissue may ultimately determine whether normal healing occurs or progression to end-stage fibrosis ensues.

The presence and the role of MFs in vascular restenosis has been assessed in experimental animal models as well as in samples retrieved from patients during re-operation procedures. The large majority of studies highlighted the contribution to the neointima of MFs as ‘activated mesenchymal cells’ or SM-like cells.

Early studies reporting the presence and the role of MFs in animal models of vascular stenosis or in patients occasionally described these cells as ‘activated mesenchymal cells’ or SM-like cells.\(^9\) One of the first descriptions of the presence of MFs in restenosis was reported for human thrombosed grafts.\(^10\) The presence of MFs and SMCs in IH was determined based on an ultrastructural analysis of the distal anastomotic regions of thrombosed saphenous veins and prosthetic grafts removed en bloc from patients during re-operation.

The presence and the detrimental role played by MFs in restenosis progression was subsequently supported by a study, demonstrating that intravascular irradiation of porcine coronaries submitted to balloon injury had a positive effect on remodelling through the reduction of \(\alpha\)-SMA-positive cells in the adventitia and a general reduction of cell proliferation, without any increase in the apoptotic index.\(^11\) MFs contribute not only to neointima, but also induce thickening of the tunica media, adventitial fibrosis, and remodelling of the ECM, a process that can contribute to late lumen loss after vascular injury.\(^12\)

Kang et al.\(^13\) observed that expression of 44 proteins significantly changed within 3 days after artery injury, Which was relevant to phenotypic changes. Among these proteins, oxidized LDL receptor-1 (OLR1) was suggested as a regulator for VSMCs hyper-
plasia. Furthermore, the OLR1 may play dual roles in the VSMCs hyperplasia by directly mediating oxidized LDL-induced monocyte adhesion via NF-κB activation.

In addition, by improving activation of platelet-derived growth factor receptor (PDGFR). Studies also indicated that the adhesion and infiltration of monocytes to the stent-injured intima correlated with VSMCs proliferation, and activation of PDGFR could induce VSMC migration and proliferation. These results suggest that the OLR1 is a key molecule linking inflammatory responses to VSMCs proliferation and may play a vital role in neointimal hyperplasia.

The disruption of endothelial cells (ECs) in the intima, detected in a number of models of vascular injury, is of particular importance in stenosis progression, since it is the cause of a concomitant reduction of vascular-protective mediators, such as nitric oxide (NO) and prostacyclin. A number of studies indicate that an early re-endothelialisation by circulating endothelial precursors after vascular injury prevents excessive cell proliferation and restenosis. On this basis, some therapeutic strategies have been set up on the animal model. These studies aimed at increasing the number of circulating EPCs and their early homing at the injury site like promoting rapid re-endothelialisation in injured arteries through the mobilization of circulating Endothelial Progenitor Cells (EPCs) stimulated by the injection of the cytokine Granulocyte Colony-Stimulating Factor (G-CSF), or they even proposed to prevent restenosis by using stents coated with EPCs.

Fujiyama demonstrated in a model of carotid angioplasty in immunodeficient nude rats that locally transfused bone marrow-derived CD34+ CD14+ Monocyte Lineage Cells (MLCs) can also adhere to the injured endothelium when attracted by the monocyte chemotactant protein-1 (MCP-1) and induce a rapid endothelialisation like EPCs. Same authors also demonstrated that such CD14+ MLCs are more powerful than bone-marrow-derived CD34+ cells in the inhibition of neointimal hyperplasia. The trans-differentiation of bone-marrow-derived MLCs to ECs is probably related to the increased expression of VEGF in injured carotids, as supported by in vitro studies.

Interestingly, the report by Fujiyama et al. revealed striking differences of behaviour between peripheral blood-derived monocytes and bone-derived-MLCs when interacting with MCP-1, since the first ones migrate in the media and accelerate restenosis after angioplasty, the second ones only enhance re-endothelialisation and decrease neointima hyperplasia, thus demonstrating that only bone marrow-derived MLCs have a specific endothelial cell-committed property.

Some considerations about arterial restenosis can also be derived from studies on intra-prosthetic graft hyperplasia.

Tozzi et al. from Insubria University (Vascular Surgery, Circolo University Teaching Hospital, Varese) together with MIA consortium (Microscopy and Image Analysis Consortium, Bicocca University, Milan) conducted recent studies on prosthetic haemodialysis vascular access, in order to describe the various cellular populations responsible for intragraft hyperplasia. Light microscopy and SEM scans have been performed on grafts explanted between January 2015 and December 2016 (Figure 1.1).

They found significantly different findings in cohort of explanted grafts: few lymphocytes, a high number of monocytes/macrophages penetrating the graft wall with high mitotic index and, not less important, a great amount of collagen (Figure 1.2).

They analyzed different times of graft harvesting and noted two main aspects: first, a progressive cells and collagen wall penetration rate which raised from the external tissue toward the endoluminal surface of the graft.
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**Figure 1.1** Explanted vascular access prosthetic grafts.

**Figure 1.2** Immunohistochemistry and confocal images of sections of ePTFE. Some cells with different morphological features are clearly identifiable in the context of ePTFE, on the external side, only few in the endoluminal side, as a demonstration of a dynamic integration of prostheses (Microscopy and Image Analysis Consortium, Bicocca University, Milan).
Secondly, in grafts with longer permanence, this led to the development of a thin collagen layer with few cells which was homogenously spread on the endoluminal surface.

This matrix was characterized by fewer cells with low mitotic index. In appearance, we found out two distinct type of infiltration. The first, spread within the graft wall; the second inside the large wall damages due to cannulation (Figure 1.3).

Tri-layer grafts showed a significantly different appearance from normal ePTFE: cell infiltration was present only inside the external layer, probably because of the inability to penetrate the middle elastomeric membrane. This probably means that monocytes/macrophages infiltration in prosthetic graft does not come from blood circulating cells, but only from surrounding tissues (Figure 1.4).

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**Figure 1.3** Cell colonization through a cannulation hole. Sites of frequent cannulations present extensive loss of material replaced by extracellular matrix and cells infiltrate (Microscopy and Image Analysis Consortium, Bicocca University, Milan).

**Figure 1.4** Early cannulation vascular access: acidic mucins (alcian blue staining), the cells (hematoxylin eosin staining) and the collagen (picrosirius red staining). Fundamental difference from ePTFE is the lower presence of cellular infiltrate and matrix. This characteristic could be explained by the barrier that the intermediate layer of silicon opposed to cell migration. (Microscopy and Image Analysis Consortium, Bicocca University, Milan).
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CONCLUSIONS

Arterial restenosis after open surgical vascular or endovascular procedures, in either carotid or peripheral diseases, should be considered a serious complication that could limit the long-term efficacy of the revascularization.

Smooth muscle proliferation and migration after percutaneous intervention represent the result of natural healing processes triggered by vascular injury. Vascular smooth muscle cell proliferation, especially after stent implantation, plays a critical role in neointimal hyperplasia through cellular expansion and extracellular matrix deposition.

SMC are complex cells, capable of existing in heterogeneous populations and switching phenotypes upon various stimuli. The signal transduction pathways controlling SMC activation and phenotype switching are becoming established and may suggest additional points of control; the SMC signal transduction pathway control points are gradually appearing in new therapeutic modalities such as drug eluting stents and local gene transduction.

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Technologies of drug coated balloons

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Percutaneous transluminal angioplasty (PTA) is currently the main treatment of both coronary arterial disease (CAD) and for several peripheral arterial lesions. However, subsequent restenosis after percutaneous transluminal angioplasty remains too common due to intimal hyperplasia, vessel recoil or negative remodelling.

Several techniques have been proposed to treat restenosis (plain angioplasty, angioplasty with cutting balloon, rotational atherectomy, excimer laser and vascular brachytherapy) but none proved to be effective. To address the problem of vessel recoil bare metal stenting (BMS) was introduced for the treatment of restenosis in coronary arteries. The major downside of BMS is the frequent development of in stent restenosis (ISR). To prevent this complication local drug delivery of an antiproliferative drug has been designed and drug eluting stents (DES) has shown to be a successful approach to reduce the rate of restenosis after percutaneous intervention for CAD, effectively changing the standard of care for these patients.

DES have been introduced to improve long-term patency in patients affected by PAD as well, unfortunately the femoropopliteal artery is subject to multidirectional mechanical forces that could increase the risk
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of fracture and deformation. Drug coated balloon catheters (DCB) have been developed to obtain long-term patency without leaving an implant behind.

The concept of DCB is based on the combination of balloon angioplasty and local drug delivery. These devices are presumed to demonstrate a number of advantages over DES. First, short-term antiproliferative drug action may allow early intimal re-endothelialisation and vessel healing, which reduce the risk of acute or late thrombosis and the need for long-term antiplatelet therapy. Furthermore, the absence of a chronic inflammation source, such as stent strut and polymeric coat material, reduce the exaggerating vessel reparative process responsible for restenosis and acute late thrombosis.

The effectiveness of DCB depends on the type of drug used for coating and balloon coating technique. The coating technology is responsible for the efficient drug delivery to the target site; simultaneously, minimises the loss through the systemic circulation during catheter placement and balloon inflation and for the performance of a rapid, uniform local application on the vessel wall.

**DRUGS**

Several anti-proliferative therapeutic agents can be applied onto the balloon surface to inhibit intimal hyperplasia but the preferred ones are the paclitaxel (PTX) and the limus-family drugs, such as sirolimus (SRL) and zotarolimus (ZLS).

*Paclitaxel*: PTX is isolated from the Pacific Yew tree, Taxus brevifolia. It is an antimitotic drug with potent antitumor activity and its lipophilicity, and efficient uptake by and extended retention in vessel walls makes it the most preferable drug used in current DCBs. Once delivered to the vessel wall, this drug binds hydrophobic sites of the artery that disrupt normal microtubule function and cytoskeleton arrangement and thereby inhibits smooth muscle cell proliferation and prevents neointimal hyperplasia.

*Sirolimus*: SRL is a macrolide lactone, binds to FK-binding protein 12 and inhibits the mammalian target rapamycin (mTOR). mTOR inhibition prevents the degradation of p27kip1, a cyclin-dependent kinase inhibitor, restricting the migration and proliferation of SMCs.

*Zotarolimus*: ZLS is a semi-synthetic derivative of SRL. It was reported in a study to have shown good uptake and efficacy when delivered with DCBs in a familiar hypercholesterolemic swine model. A recent comparison between ZLS- and PTX-eluting DCB using *ex vivo* swine femoral arteries revealed that ZLS exhibited high arterial wall partitioning that is comparable to that of PTX, but with differences in distribution, with ZLS dominating at shorter depths and PTX at deeper depths.

The therapeutic effect of drugs used in DCBs is strictly related to its transport efficiency and tissue distribution after delivery. Lipophilic drugs easily cross the hydrophobic core of cell plasma membranes in the endothelium and therefore get transported with greater ease to the arterial wall. PTX distribution in the arterial wall is preferentially concentrated in the deep arterial tissue. This retention helps to prolong therapeutic drug levels in the tissues and is the primary reason why PTX is preferred over the limus-family drugs in DCBs. SRL main vessel target is the tunica media due to the high expression of its binding protein in the vascular smooth muscle cells, the primary mediators of hyperplasia. These findings support the rationale for using SRL to prevent restenosis.

Despite their effectiveness in inhibiting cell proliferation and their biological predisposition in spreading through cell membranes, these drugs couldn't fulfil their purpose without the proper carrier.