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Future outlook for drug eluting stents

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Abstract: This chapter provides an overview of drug eluting stents (DES), discussing their evolution from bare metal stents. The chapter begins by addressing mechanisms of restenosis and various platforms for DES that has been developed before to overcome these complications. Also, the shortcoming of DES with respect to thrombosis has been critically reviewed, while providing an insight about the future of DES. The chapter also includes two sections discussing the biodegradable stent platform, and the prohealing approach that can improve the drug eluting stent technology.

Key words: drug eluting stents (DES), restenosis, thrombosis, biodegradable stent, prohealing.

6.1 Introduction

6.1.1 Bare metal stents (BMS)

The advent of bare metal stents (BMS) is considered a milestone in the field of coronary intervention. The arrival of this new small implant sparked a revolution in the field of biomedical engineering. No previous medical device has demanded as much scientific attention, and attracted as much marketability, as BMS. Stainless steel stents are metallic wire meshes that when stretched by an inflating angioplasty balloon holds open a clogged artery. In terms of treatment regimes, percutaneous transluminal angioplasty (PTA) such as balloon angioplasty has been established as a method for treating severe vascular obstructions. The simplicity and relatively few complications associated with angioplasty make it a suitable process. However, limited patency has plagued PTA, which led to the emergence of the metal stent. BMS, in general, overcame the complications associated with previous angioplasty techniques. However, the success of BMS was plagued by the recurrence of scar tissue (neointimal hyperplasia), which caused reconstriction of the treated artery.

6.1.2 Drug eluting stents (DES)

The concept of a drug eluting stent (DES), which is a BMS coated with a drug-containing polymer, emerged in the late 1990s as a novel combination product to

replace BMS. The success of DES is evident from the revenue of $2.6 billion generated by the first commercial DES during its first year of commercial sale in 2004.[1] No other medical product has boasted such recognition in the history of medical devices. DES showed great promise in overcoming the clinical failures of BMS including restenosis. A DES delivers a drug locally to an injured artery, thus preventing restenosis effectively. Section 6.2 in this chapter is dedicated to address mechanisms of restenosis, and various platforms for DES that have been developed before to overcome these complications. The superiority of DES in treating restenosis is evident from the positive outcome of many clinical trials. Also, the shortcoming of DES with respect to thrombosis has been critically reviewed, while providing an insight about the future of DES. Since the complexity associated with DES is multifactorial, development of the next generation DES requires delivery of multiple target agents or a combination of drug and a gene to promote healing. A lot of research has been directed towards the 'drug' part of DES, with a wide array of pharmacological agents under investigation as potential candidates for DES. Various agents that have been tried before in DES are discussed in this chapter. Brief details about their mechanistic approach at the site of action are also discussed. The two separate sections in this chapter discuss the biodegradable stent platform, and the prohealing approach. The prohealing approach is a potential approach for future DES. DES is not completely devoid of complications as reported in a few clinical trials. However, the benefit to risk ratio outweighs the drawbacks, making DES a preferred option. There is no doubt that DES has already revolutionized the field of coronary intervention. More research and long-term clinical data are essential to ascertain the safety and patency of this device, and to overcome all the limitations that are facing the current stent technology.

6.1.3 Drug delivery technologies

Drug delivery technology is a rapidly evolving field, with new technologies emerging in both delivery of pharmaceuticals and biologics (peptides, proteins, and gene based). Conventional therapeutics have been revolutionized by the emergence of nanotechnology which has high selectivity. Combination products have become the leading player in device-based technology. DES, which are used for coronary applications, hold an important place in this list of medical devices. Combination products involve pairing of a drug with a device to control the delivery of the drug. These novel drug delivery technologies have demonstrated improvement in efficacy over other routes by increasing patient compliance.[2] The rationale behind developing a device-based delivery technology is to have a controlled local delivery of drug at specific sites, where conventional approach fails to deliver a sufficient dose capable of producing the same therapeutic effect.

Combination products face extensive regulation with respect to bio-

Table 6.1 List of drug eluting stents (DES) approved by FDA

Product	Company	Description
Cypher	Cordis Inc.	Sirolimus-eluting cardiovascular stent
Taxus Express	Boston Scientific	Paclitaxel-eluting cardiovascular stent
Xience	Abbott	Everolimus-eluting cardiovascular DES
Endeavor	Medtronic	Zotarolimus-eluting cardiovascular stent

compatibility, safe degradation characteristics, ease of implantation, fabrication, and sterilization. This regulation poses a huge challenge in the development of these devices. Further, cost effectiveness and processing contribute to the economic factors that need to be considered before choosing a device-based product over the conventional therapy. In this era of increasing integration of research from material science, polymer chemistry, and biomedical engineering to the field of drug delivery, the dream of novel and sophisticated therapeutic combination product for a wide range of applications could soon be a reality.

The field of drug delivery has seen a tremendous growth in recent years through a multitude of inventions. Drug delivery through a microchip is an example. In this technology the drug is embedded in microreservoirs on a silicon microchip. The release of the active agent is controlled by the electrochemical dissolution of an anode membrane, which could be engineered to control the release kinetics of the drug.[3] Another interesting invention is the development of nanorods, which could serve as nanocapsules for delivery of biological agents.[4]

The purpose of this chapter is to provide an overview of DES, discussing its evolution from BMS, pros and cons, and its future. Table 6.1 shows the list of DESs that have been approved by the US Food and Drug Administration (FDA) or under clinical studies.

6.2 Drug eluting stunts (DES): Where are we now?

6.2.1 Various platforms for DES

For almost a decade, various approaches were developed in an attempt to deliver an adequate amount of drug to the site of interest in coronary vascular intervention. The primary concern was in achieving a high local concentration of the drug for therapeutic efficiency. Local drug concentration could be easily lost owing to the rapid wash out of drug if the coated platform failed to act as a drug carrier. This led to the advent of many coating technologies. The goal of these technologies was to deliver a successful coating on to a DES that would retain film integrity, until the drug gets released completely and thereafter. A wide range of pharmaceutical agents with specific functions have been investigated as candidates for stent therapy. This section addresses various carrier platforms along with the drugs that were tried previously to inhibit restenosis. References

Table 6.2 Mechanisms of controlled drug release

1. Diffusion
a. Reservoir system
b. Matrix system
2. Dissolution or degradation
a. Reservoir system
b. Matrix system
3. Ion exchange
4. Osmosis
5. Prodrug

to the outcome of certain clinical trials performed using these systems are also noted.

Three major components constituting DES are drug, inert polymer carrier, and stent itself. In these three components, the property of drug is critical in determining the efficiency of DES. The physicochemical properties of the drug are crucial in determining its release kinetics. Lipophilic drugs are desired to obtain a better distribution in the arterial walls with a high residence time. A hydrophilic drug might be washed away rapidly without inducing any biological effect. The therapeutic action of the biological agent is desired to last for at least a month to prevent the incidence of restenosis. This demands a prolonged zero order release kinetics to maintain the desired local action at the site of injury. Acharya *et al.* reviewed in detail various mechanisms in which a drug can be released from a polymer matrix coated on the surface of stent.[5] Table 6.2 shows the various mechanisms by which a drug can be released from a polymer matrix. Diffusion-based drug release are often found in systems carrying biostable polymers, while the release of drug from a biodegradable polymer is controlled mainly by surface or bulk erosion of the polymer. The drug release from current DES follows one of these two mechanisms.

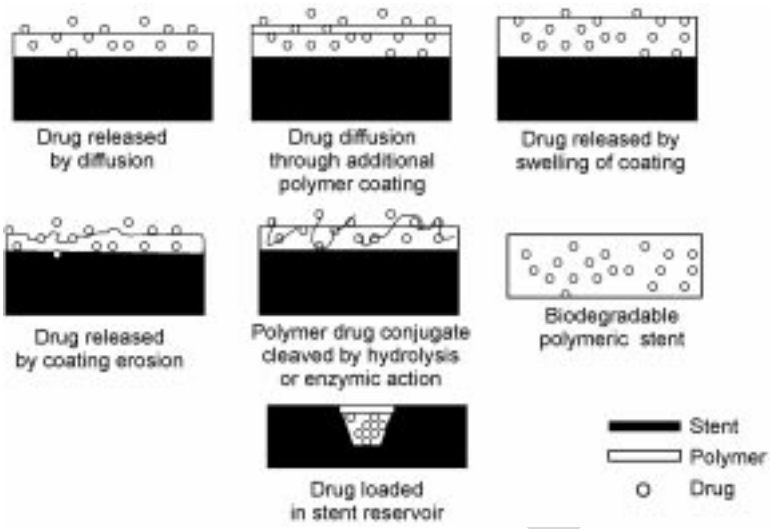
The commercially available DES, Cypher® (Johnson and Johnson) and Taxus® (Boston Scientific), contain a coated polymer platform. A number of different polymers have been investigated in an attempt to improve the biocompatibility of DES. Unger *et al.* recently published the viability of biodegradable poly(ethylene carbonate) (PEC), as a potential carrier for DES.[6] PEC tends to become hydrophilic upon incubation in media thus reducing the protein adsorption. Proteins exhibit high affinity to hydrophobic surfaces as compared with hydrophilic surfaces. This process can stimulate platelet adhesion leading to thrombosis.[7] The *in vitro* results demonstrated excellent cytocompatibility and mechanical properties of PEC-coated stent. This created a need for further investigations *in vivo*. Also, polyurethane and polytetrafluoroethane are other polymers that have been proven to reduce neointima as seen in rabbit carotid arteries.[8,9] Another promising approach in recent years has been to employ phosphorylcholine (PC)-based polymers as a carrier for DES.

PC is a constituent of the lipid bilayer of cell membranes which, when formulated as a coating, can form a biomimetic surface that can be antithrombogenic. Garcia-Touchard *et al.* investigated a PC-based copolymer which consisted of a phospholipid portion (2-methacryloyloxyethyl phosphorylcholine and lauryl methacrylate) cross-linked to hydroxypropyl methacrylate and trimethoxysilylpropyl methacrylate). This was utilized as a platform to deliver zotarolimus for reducing neointimal hyperplasia.[10] PC-based polymer was effective in reducing hyperplasia providing complete healing in a swine model. Iwasaki *et al.* review in detail, the potential biomedical applications of PC-containing polymers with specific emphasis on its ability to decrease platelet and protein adsorption.[11]

Mani *et al.* presented a broad overview of various coating platforms and metallic stent designs.[12] The underlying composition for 316L stainless steel BMS includes nickel, chromium, and molybdenum. Some of these metals may trigger allergic reactions leading to intimal hyperplasia. A coating of an inert polymer on the stainless steel substrate is a useful strategy for reducing this reaction and it has been well established. Though BMS possesses a few drawbacks, it can act as a good substrate for coating. This is clear from the fact that several DES use BMS as substrate.[12] In addition, various alloys comprising tantalum, nitinol, cobalt–chromium, titanium, platinum, pure iron, and magnesium are used in the stent manufacturing. From a material perspective, they possess excellent corrosion resistance, and mechanical properties.

Bioresorbable stents follow a 'do and disappear' strategy wherein a biostable bare metal stent is replaced by a bioresorbable platform, which serves to deliver the drug at the target site and then to degrade over a period of time. The bioresorbable stent releases the loaded drug and then disappears, leaving behind a healed natural vessel, which cannot be a locus for thrombosis.[13] Also, bioresorbable stents provide an advantage of not requiring additional therapy in the form of an antiplatelet agent, which is a necessity with the bare metal stents. Bioresorbable stents are not just limited to polymeric materials, DiMario *et al.* developed a biodegradable stent based on a magnesium alloy that provides effective antithrombotic and anti-proliferative properties.[14] Magnesium, being one of the nutrients in the human biological system, has no adverse reaction associated with this alloy with a considerable reduction in acute stent thrombosis.[14] Biodegradable polymeric stents are widely considered as the next generation of DES, albeit more improvement is desired with respect to the mechanical properties of these stents as compared to the current metallic stents. Figure 6.1 shows the different platforms that have been used for stent-based delivery.[15]

Various modalities have been investigated to deliver drugs locally from DES. Kallinteri *et al.* studied the *in vitro* release of dexamethasone-loaded liposomes lining a polyethylene terephthalate (PET)-covered metallic ureteral stent.[16] It was discovered that liposomal-based formulations could provide an effective platform for slow release of drug in the vicinity of the stent.

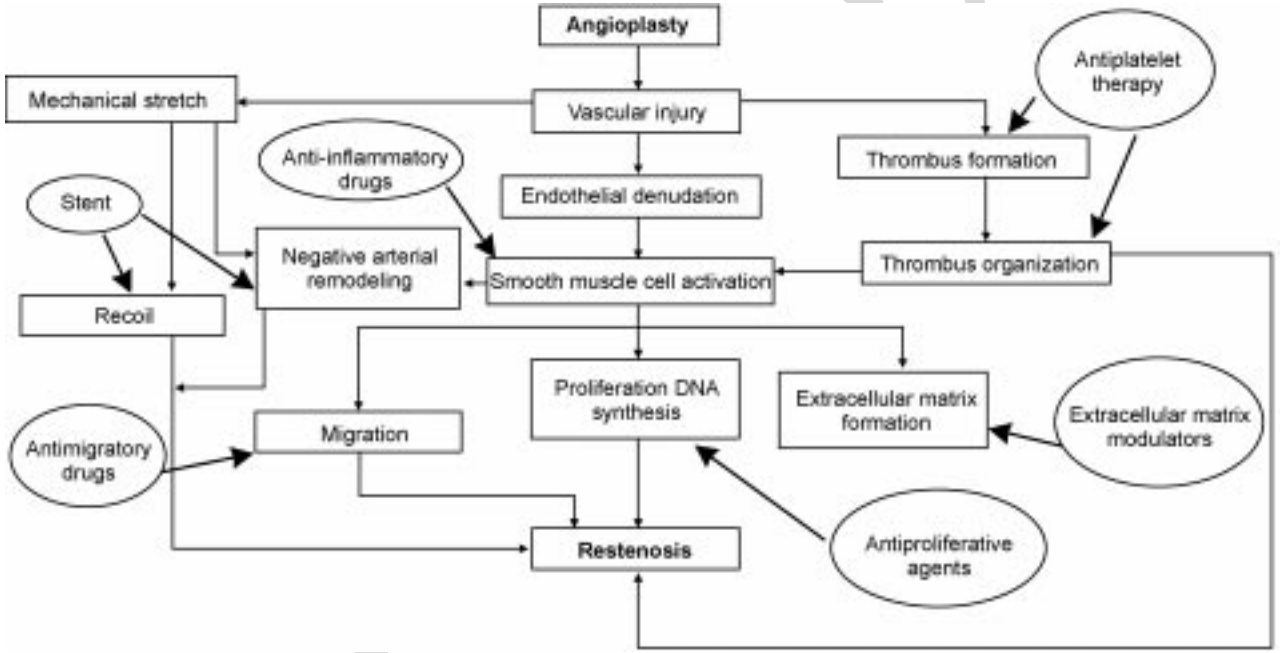


6.1 Different platforms for stent-based drug delivery (adapted from Sousa *et al.*[15]).

In another interesting study conducted by Kraitzer *et al.*, core shell fibers comprising polyglyconate and poly(DL-lactide-co-glycolide) (PDLGA) impregnated with paclitaxel, were explored as potentially basic elements for vascular stents. The core–shell fiber structures exhibited a highly controlled release kinetics.[17] Another approach demanding attention when discussing stent platforms includes the use of radionuclides for stent therapy. Stents coated with hyaluronan–diethylenetriamine pentaacetic acid conjugate which contains yttrium and indium radionuclide based drugs were tried for endovascular radiotherapy.[18] Thierry *et al.* demonstrated this approach of combining the anti-thrombogenic hyaluronic acid (HA) surface with a radionuclide that can provide an anti-proliferative effect. Naturally occurring silk fibroin is a new addendum to the list of various other carriers for DES. In a recently published article, a stent loaded with multilayers of silk mixed with heparin containing paclitaxel and clopidogrel showed a potential in terms of regulating the adhesion and growth of endothelial cells *in vitro*.[19]

6.2.2 Pharmaceutical agents

To date, numerous biological agents have also been tried as candidates for stent therapy. Although the mechanism of action for each of these drugs is different, the underlying purpose is to either prevent any adverse reaction leading to the proliferation of smooth muscle cells or to promote natural healing locally. Figure 6.2 shows the pathophysiology of restenosis and the mechanism of action of various pharmaceutical agents.[20] This section will provide an overview of



6.2 Mechanism of restenosis and action of various pharmaceutical agents (adapted from Htay and Liu[20]).

Table 6.3 Drugs and drug candidates that can be used for developing DESs

Drugs with primary mode of action			
Inhibit both SMC & EC	Inhibit mainly SMC growth	Inhibit mainly SMC, but promote EC growth	Promote EC growth
Anticancer agent (Paclitaxel)	Adenosine A_1 receptor antagonist (1,3-Dipropyl-8-cyclopentyl xanthine)	Angiotensin-converting enzyme inhibitor (Captopril)	Antihyperlipidemic (Probucol)
Immunomodulator (Sirolimus)	Beta-blocker (Isoproterenol)		Growth factor (VEGF)
Calcium channel blocker (Nifedipine)	Calcium channel blocker (Verapamil)		Hormone (17β-estradiol)
NO donor (Isosorbide dinitrate)	PDE3 inhibitor (Milrinone)		
Phosphodiesterase 3 inhibitor (Cilostazol)	PDE5 inhibitor (Sildenafil)		
Endothelin receptor antagonists (Bosentan)	Endothelin receptor antagonist (Ambrisentan)		

SMC: smooth muscle cells; EC: endothelial cells.

various agents that have been used previously, focusing on their target action *in vivo*. Table 6.3 shows the classification of drugs based on their known functions useful for stent therapy.

Paclitaxel

Paclitaxel, derived from the plant *Taxus brevifolia*, has been shown to possess strong anti-mitotic activities.[21] The first paclitaxel formulation received its approval from the FDA in 1992, and it has marked a significant advancement in cancer therapy. Paclitaxel has undergone extensive clinical development and has been approved as an effective antitumor drug. Paclitaxel enhances polymerization of microtubules and can block cell mitosis, which is responsible for the antitumor activities of the drug. Microtubules are dynamic macromolecular assemblies that are designed to be unstable. They are involved in many activities such as maintaining cell shape, motility, and cellular transport.[22,23] Paclitaxel engineers reassembly of the cellular network to form an aberrant mitotic spindle, which causes cell cycle arrest in the G2/M phase.[22,24,25] There is a great deal of literature evidence which proves that altering the microtubule dynamics can cause aberrant mitosis leading to G2/M cell cycle arrest.[26]

Sirolimus

Sirolimus, also known as rapamycin, is a macrocyclic antibiotic derived from *Streptomyces hygroscopicus*. This drug exhibits strong antitumor and immunosuppressive activities. Both *in vitro* and *in vivo* studies have reported the potent immunosuppressive activity of sirolimus.[27,28] The mechanism of action for sirolimus depends on their binding to a specific cytosolic protein family, FKBP. This binding inhibits the mammalian target of rapamycin (mTOR). The binding of the sirolimus–FKBP complex to mTOR, blocks signals from cytokines such as interleukin-2 (IL-2), which prevents cell cycle progression from G1 to S phase.[29] Gregory *et al.* have shown the efficacy of sirolimus in reducing the intimal thickening *in vivo*.[30] The commercially available Cypher® stent (Cordis Corporation-Johnson and Johnson, FL) uses sirolimus in a non-erodible polymer matrix. A number of clinical studies have been reported indicating the efficacy of sirolimus eluting stents. These studies are also discussed in detail elsewhere in this chapter.

Everolimus

Everolimus (40-*O*-(2-hydroxyethyl)-rapamycin), derived from the family of sirolimus, is an active anti-proliferative and immunosuppressant.[31] The biological mechanism of Everolimus's action is based on the inhibition of mTOR in a pathway similar to that of sirolimus. Grube *et al.* showed that oral dosing of

everolimus suppresses in-stent neointimal growth in a rabbit model. They conclude, however, by recommending longer-term studies to confirm the efficacy of this drug as a component in DES. The outcome of the first clinical trial with everolimus (FUTURE I) was promising, with reductions in restenosis and in-stent neointimal hyperplasia maintained after 6 months and 12 months in a follow-up study.[32]

Tacrolimus

Tacrolimus, yet another water-insoluble macrolide produced by *Streptomyces tsukubaensis*, is a proven immunosuppressant that inhibits cell proliferation by a calcium-dependent pathway. The mechanism of action for tacrolimus is different from that of sirolimus by the fact that tacrolimus interferes with the cytokine-mediated signal early in the G1 phase of cell cycle. Sirolimus, on the other hand, acts at a later stage of the G1 phase. Tacrolims binds to FKBPs inhibiting the formation of calcineurin, a calcium-calmodulin-dependent serine and threonine phosphatase. The inhibition of calcineurin affects the synthesis of IL-2 and other cytokines leading to the blocking of T-cell proliferation during the transition from G0 to G1 phase.[33]

Zotarolimus

Zotarolimus (formerly known as ABT-578) developed by Abbott Laboratories is a synthetic analog of rapamycin that can effectively prevent restenosis.[34] This compound is very hydrophobic, exhibiting a high octanol:water partition coefficient compared with any drugs currently used in DES. The mechanism of action of zotarolimus is also similar to rapamycin, where the cell cycle arrest occurs as a result of binding of the drug to FKBP-12 which blocks mTOR. mTOR is involved in the phosphorylation of proteins including $p27^{kip1}$ that controls various cellular events including proliferation. Zotarolimus has been tested under various clinical trials (ENDEAVOR I, II (Endeavor stent by Medtronic Inc.), PREFER (PC coated Bio*divYsio* stent by Abbott Laboratories), and the ZoMaxx trial by Abbott Laboratories).[35]

Estradiol

The endothelium is a thin layer of interface between the vessel wall and the flowing blood, and it plays a key role in regulation of the vascular system. As outlined in Section 6.3.1 on thrombosis in this chapter, a lack of natural healing process of endothelium can lead to a hostile scenario in the vasculature. Estradiol promotes endothelial layer formation by regulation of nitric oxide (NO). NO is an endothelium-derived reducing factor, which has the ability to inhibit the adhesion of leukocyte and other cytokines on to the endothelium.

(More information on NO is described on page XXX.) Estradiol can increase the production of NO, by stimulating the expression of endothelial NO synthase. This is achieved by decreasing the production of reactive oxygen species, which could otherwise react with NO to convert it to peroxynitrite.[36] The first human trial using 17-beta-estradiol (EASTER) was conducted to evaluate the feasibility of using this pharmaceutical agent in a DES to inhibit restenosis. A one year follow-up demonstrated positive outcomes suggesting that estradiol could be a useful biological agent for stent based therapy.[37] Various other studies have reported the endothelial layer regrowth in the presence of estradiol, which could lead to atheroprotection.[38,39]

Probucol

Probucol is a lipid-lowering agent that exhibits strong antioxidant properties and can act as vascular protectant.[40] Probucol is a phenolic antioxidant that promotes functional re-endothelialization and inhibits vascular smooth muscle cell (VSMC) via induction of heme oxygenase (an enzyme that catalyzes heme). The drug can block not only cell- induced changes, but also the oxidative changes and prevent endothelial cell modification. Probucol was found to promote endothelial layer formation both in rabbit and rat models *in vivo*.[41]

Probucol is more effective as a vascular therapeutic agent than other drugs because of the sulfur atoms connecting the two phenolic rings. This sulfur atom engages in the 2e-oxidation reactions with cysteine residues in the thiolate form. This helps regulate key enzymes such as tyrosine kinases, phosphatases, and transcription factors that are involved in regulating endothelial functioning.[42] This also explains why other antioxidants with phenolic moieties such as α-tocopherol are not as effective as probucol. Though probucol is an anti-cholestorolic drug, the cardioprotective effects of probucol are independent of its anti-cholestorolic properties.

Probucol has been shown to have a positive effect on controlling atherogenesis in both animals and humans.[43–45] Probucol has been proven to protect renarrowing of both small and large arteries after balloon angioplasty.[46,47] Lau *et al.* showed that probucol can promote functional endothelialization in addition to intimal proliferation of VSMC.[45]

Role of antioxidants

Nitric oxide (NO) is a major vasodilator that is formed by the action of endothelial nitric oxide synthase (eNOS). eNOS is located in the caveole of cell membranes on L-arginine.[46] The binding of calcium to calmodulin displaces the eNOS containing caveolin-1 protein, which leads to the production of NO. Production of NO prevents the adhesion and release of oxidants by neutrophils and serves as a protective agent for the endothelium.[48] A deficiency in NO can

impair normal cell function. NO acts to prevent the oxidation of low density lipoprotein (LDL), which play an important role in atherosclerosis. Oxidized LDL can trigger caveolin production, which inactivates eNOS. Also, the production of free radical superoxides, decrease NO production and destroys tetrahydrobiopterin, which is a cofactor of NO synthesis.

NO donors can be classified in to various categories depending on the way they release the NO. These include direct donors, donors requiring metabolism, and bifunctional donors. Direct NO donors include sodium nitroprusside, NO gas, *p*-nitrosophosphate, and sodium trioxydinitrate which generates a reduced form of NO^- that has a unique effect on smooth muscle cell (SMC), *S*-nitrosothiols (which spontaneously release NO^+ that make up *S*-nitroso-glutathione), *S*-nitroso-*N*-acetylpenicillamine, and *S*-nitroso-albumin. Donors requiring metabolism include organic nitrate and nitrite which comprises nitroglycerin, amyl nitrite, isosorbide dinitrate, isosorbide 5-mononitrate, and nicorandil. The mechanism of these includes guanylyl cyclase activation and inhibition of non-specific cation channels in VSMC. Bifunctional donors include nitroaspirins and *S*-nitroso-diclofenac.[49] Some of the other agents that modify endogeneous activity include angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, statins, β-blockers, and phosphodiesterase inhibitors.

6.2.3 Inhibition of restenosis

The body's response to the stent is a complex cascade of reactions. Within seconds of implantation, plasma proteins such as fibrin are deposited on the metal surfaces. Next, platelets and leukocytes adhere to the surface and subsequently, neutrophils and macrophages are seen with a thrombus that has been formed. The cellular reorganization events that follow entail smooth muscle cell proliferation, SMC migration, neointima formation (new extracellular matrix formation), and re-endothelialization.[50] These processes are mediated by the release of growth factors (such as bFGF, TGFβ, platelet derived growth factor, insulin growth factor and thrombin) by SMCs.[51,52] Furthermore, in angioplasty, the trauma is temporary and healing can take place within weeks of the surgery. However, in stenting, the stent is permanent and can elicit a delayed or prolonged healing response. The ends of the stents are particularly vulnerable to wall trauma, where perforation or tearing can occur. Additionally, endothelial dysfunction has been shown to occur with stenting. This can lead to the activation of SMC, which can migrate to intima and cause elastic recoil and remodeling of the artery. Eventually, the SMCs may thicken, causing a re-narrowing of arterial lumen termed as restenosis. Formation of neointima is the major cause of restenosis and all the anti-restenotic therapy targets smooth muscle cells preventing their proliferation. Seminal events of the restenotic process include platelet and fibrin deposition on the stent material and local denuded surfaces. Changes in the metal chemistry that may affect cell response

include different alloys, surface charge, surface area, hydrophilic and antithrombogenic coatings.[50]

The use of biological agents that are specific to SMCs is an approach that could be used to control restenosis. Currently drugs in DES are not specific to SMCs and act on endothelial cells perturbing the re-endothelialization. This can lead to thrombosis at early stage or in the late stage (discussed in the next section of this chapter). Newer therapeutic approaches include using endothelial progenitor cells or using a dual agent that can control restenosis and promote endothelialization. Table 6.4 shows the clinical trials for BMS and DES.[32,53–65]

6.3 Challenges in the use of drug eluting stents (DES)

6.3.1 Thrombosis

Endothelium is a continuous, single-layer, cell lining of the cardiovascular system between the vascular lumen and the smooth muscle tissue of the vessel wall often referred to as 'Nature's blood compatible container.'[66] Endothelium serves to maintain the balance between vasodilation and vasoconstriction, controlling the inflammation and thrombosis to maintain good vascular health. An upset in balance between vasoconstriction and vasodilation can trigger a number of vascular events such as platelet aggregation, leukocyte adhesion, and stimulation of cytokines. This could eventually play a role in atherosclerosis.[67] Endothelium has gained more attention in recent times with the emergence of many complications associated with stent therapy.

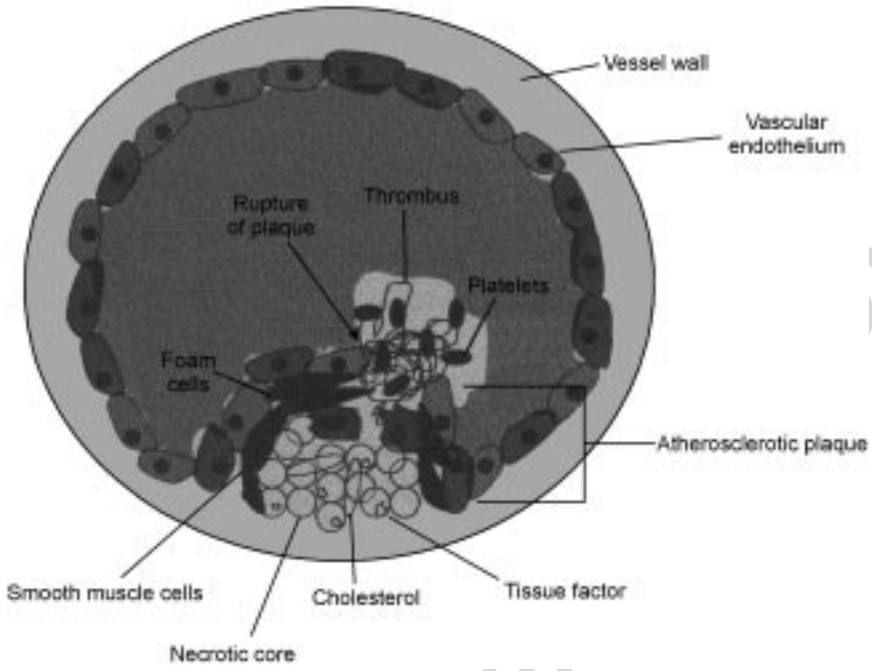
6.3.2 Mechanisms of thrombosis

Endothelium in the normal intact state is non-thrombogenic and does not react with platelets or other blood components. This state, however, can be altered by the presence of any foreign material (vascular grafts, prosthesis) that causes endothelial denudation. This leads to exposure of the subendothelial structure to blood components resulting in a hemostatic response.[68] The thrombogenic subendothelium that is essential to stop bleeding can also be involved in this adverse reaction which leads to thrombosis and atherosclerosis. The purpose of this section is to provide insights on molecular mechanisms leading to thrombosis, and therapies for arterial thrombosis as a consequence of coronary vascular stenting.

Endothelial cells have a tendency to synthesize both antithrombotic and prothrombotic agents. It is essential to maintain a balance between these two agents for proper functioning of an endothelium. An endothelial cell is a key element for maintaining vascular integrity. The failure of the endothelium to maintain a state of balance between endothelium-dependent procoagulant and

Table 6.4 Clinical trials using BMS and DES

Drug	Trials/Follow-up period	Number of patients		Restenosis rate		Reference
		BMS	DES	BMS	DES	
Sirolimus	RAVEL/6 months	118	120	26.6	0.0	61
	SIRIUS/8 months	525	533	35.4	3.2	62
	C-SIRIUS/8 months	50	50	45.5	0.0	63
	REAL/9 months	3365	872	–	–	60
	RESEARCH/3 years	450	508	–	–	55
Paclitaxel	TAXUS I/12 months	30	31	–	–	59
	TAXUS II/12 months	270	266	17.9	2.3	53
	TAXUS IV/9 months	652	662	24.4	5.5	65
	TAXUS V/9 months	579	577	31.9	13.7	64
	TAXUS VI/9 months	227	219	32.9	9.1	56
Everolimus	FUTURE I/6 months	15	27	9.1	0.0	54
	FUTURE II/6 months	21	36	19.4	0.0	58, 32
Zotarolimus	ENDEAVOR II/9 months	599	598	33.5	9.4	57



6.3 Triggers of arterial thrombosis (adapted from Mackman[69]).

anticoagulant components is termed as endothelial dysfunction. This leads to the exposure of the sub-endothelial structure comprising collagen, microfibrils around elastin, and connective tissue to flowing blood.[69,70] Figure 6.3 illustrates the triggers of thrombosis post-stenting in an artery.

Symptoms of endothelial dysfunction include decreased NO bioavailability, imbalanced synthesis of procoagulation factors, and increased secretion of prothrombic agents (e.g. von Willebrand factor (vWF), plasminogen activator inhibitor-1, and fibronectin) and procoagulant factors (e.g. factor V and tissue factor).[71,72] The endothelium acts as a barrier preventing interaction of blood with sub-endothelial tissues, which could otherwise trigger coagulation cascade. This can be attributed to the ability of endothelial cells to synthesize prostacyclin, NO, plasminogen activators, express thrombomodulin, and heparin-like molecules on their surface,[73] which inhibits platelet aggregation.[74] The injury caused as a result of stent implantation stimulates reactive oxygen species, which activates the growth factor leading to endothelial cell dysfunction. Oxidized LDL act as a stimulant for smooth muscle cell proliferation. It enhances the platelet-derived growth factor and smooth muscle cell proliferation and modification of extracellular matrix.[75,76] Also, activated platelets and neutrophils can induce chain reactions, which can result in endothelial dysfunction.

Endothelial cells in the normal state secrete NO by the action of NOS on a substrate L-arginine. NO prevents platelet and leukocyte adhesion, SMC growth, and endothelin production.[77–79] Endothelial cells synthesize fibronectin, vWF, and thrombospondin which binds to the subendothelium and engineers the adhesion of platelets. Thrombospondin, another protein released from alpha granules of platelet, binds to fibrinogen, and mediates platelet adhesion.[80]

6.3.3 Predictors of thrombosis

The major precursors of thrombosis include damage to the vessel wall, stimulation of platelet adhesion and aggregation, and activation of blood coagulation. Arterial thrombi occurs in a region of complex flow pattern created by an atherosclerotic lesion that causes narrowing of the vessel wall.[81] Vascular injury and erratic blood flow causing local turbulence are two critical factors that contribute to arterial thrombosis. Thrombus formation is a complex cascade of dynamic processes involving blood proteins and other components including thrombin, platelets, erythrocytes, and leukocytes. Gast *et al.* found that thrombin is a major mediator of thrombus growth and stabilization as observed using a rabbit model.[82] When the endothelial lining is removed by mechanical trauma, platelets interact with the subendothelial surface by adhering and aggregating. This promotes thrombin generation, which is stabilized by fibrin. Baumgartner developed a perfusion chamber that served as an *ex vivo* thrombosis model,[83] and studied the correlation of shear rate of blood flow to the adhesion of platelets on subendothelial surface. The experimental findings led to the conclusion that platelet adhesion and aggregation is predominant only at high shear rate. This finding was confirmed from a later study, where vWF-mediated platelet–subendothelium interaction at conditions of high arterial wall shear rate.[84] At a low shear rate, platelet glycoprotein (GP) Ibα controls the interaction of platelets with vWF on the endothelial surface. At high shear rate, P-selectin mediates the interaction of platelets with the endothelium through both P-selectin glycoprotein ligand, and GP Ibα.[85–87]

vWF and P-selectin are the key components for platelet and leukocyte adhesion. These are stored in a thin cell sheet lining called Weibel-Palade bodies.[88,89] vWF is a large glycoprotein that forms a non-covalent complex with coagulation factor VIII, which then causes the formation of clot.[90] vWF has gained more attention recently following reports of clinical studies of individuals with cardiovascular disease, where a correlation between high plasma levels of vWF to the occurrence of myocardial infarction was established.[91,92] This report established that the levels of vWF in plasma could be used as a suitable gauge to determine the extent of damage done to the endothelium. Wagner *et al.* provided a detailed review of the mechanism by which a thrombus is formed and stabilized at a cellular level. vWF-mediated adhesion of platelets to the loci of the injured endothelium is the first step in thrombus formation. A platelet adhered to a

dysfunctional endothelium, is stabilized by the interaction of major platelet surface integrin $\alpha_{IIb}\beta_3$ with collagen. Further, the collagen binding induces secretion of adenosine diphosphate and thromboxane A_2, which promotes the adhesion of the platelet and the growth of a thrombus.[93] Fibrinogen secreted by alpha granules binds to the GP IIb and IIIa and triggers thrombin and collagen-induced aggregation.[94] Also, fibronectin is an important protein-mediating platelet-platelet interaction. Fibronectin is known to secure thrombus stability, by the interaction of C-terminal γ chain of fibrinogen with integrin $\alpha_{IIb}\beta_3$. Zucker *et al.* reviewed the various mechanisms that cause platelet aggregation upon stimulation.[95] Endothelial cells synthesize adhesion molecules, such as monocyte chemo-attractant protein-I (MCP-1), and interleukin (IL)-8, which are involved in the recruitment of leukocytes.[96] Recent attempts are being made to develop specific antagonists that can act as therapeutic targets against MCP-1, in order to prevent the recruitment of inflammatory cells in the early stage of atheroma formation.[97] Also, IL-1 is known to inhibit the production of vWF, which can have a direct consequence on platelet adhesion to the endothelium.

A definite necessity has arisen to restore the normal biology of the vessel wall post-stenting in order to prevent thrombosis formation. Though stenting leads to a decrease in incidence of restenosis, from both paclitaxel- and sirolimus-eluting stents, life-threatening complications in the form of late thrombosis still remain a major concern.[98,99] The endothelium displays a wide array of functionality including barrier regulation of permeability, production of growth inhibitory molecules, thrombogenicity, and leukocyte adherence, all of which can impact SMC proliferation.[100,101] The time required by human vascular cells to endothelialize after injury is 3 months.[102] Though the primary cause for the delayed re-endothelialization with DES is not completely understood, a number of factors including lesion characteristics, biocompatibility of polymers, and properties of the incorporated drug, are all considered to play a role in this dysfunction.

Statistics has revealed that stent thrombosis, a life-threatening event, occurs in 0.5–1.9% of patients with bare metal stents.[103] Recently, a number of clinical trials have reported that the rate of stent thrombosis in patients treated with sirolimus-eluting stents is similar to that treated with BMS. The actual number of cases is expected to be higher the reported value. Aoki *et al.* reported a clinical trial which showed stent thrombosis associated with sirolimus-eluting stents, at a rate comparable to BMS.[104]

One universal belief for the cause of late stent thrombosis has been the discontinuation of antiplatelet therapy of aspirin. This has been confirmed from a clinical trial comprising more than 50 000 patients. Thus, discontinuation of aspirin needs to be considered only when bleeding risks outweigh the athero-thrombotic events.[105] The concern has not always been the discontinuation of antiplatelet therapy, but rather the time of discontinuation. This still remains an unsolved puzzle requiring clear answers for the patients.

6.3.4 Clinical trials of DES

The superiority of sirolimus DES over BMS for treating restenosis has been reported in several randomized clinical trials including RAVEL, SIRIUS, E-SIRIUS, and C-SIRIUS. Likewise, the efficacy of paclitaxel-eluting stents to control restenosis as compared with BMS has been also well established through trials including TAXUS I, II, IV, V, and VI.[106] TAXUS III trial, a clinical study performed with 28 patients, was conducted to evaluate the feasibility and safety of paclitaxel eluting stents for the treatment of in-stent restenosis. After a 12-month follow-up study, no cases of late stent thrombosis were reported. In this study, clopidogrel, an anti-platelet drug, was discontinued after 6 months in all cases.[107] A similar trend was seen with TAXUS-IV trial agreeing with the conclusions derived from previous clinical trials. This emphasized the efficacy of paclitaxel-eluting stents without any case of late stent thrombosis reported after the 12 month follow-up period.[65] But a long-term follow-up study was recommended to comprehend the efficacy of these stents against very late stent thrombosis beyond one year.[108] Although reports indicated that paclitaxel eluting stents and sirolimus eluting stents were superior to BMS in controlling restenosis, the same trend was not observed in terms of thrombosis. Virmani *et al.* reported the first case of late stent thrombosis at 18 months which occurred in a 58-year old male carrying two sirolimus-eluting Cypher stents.[109] The investigational reports based on pathological evidence showed that the primary cause for the late stent thrombosis was the hypersensitivity to the polymer.

A pragmatic approach for developing future DES evokes the necessity to think beyond just controlling neointimal hyperlasisa, but also consider developing a strategy that can provide intact endothelium which acts to maintain vascular integrity. The current treatment of DES does not contain agent's specific to SMC. These drawbacks can be resolved by improvement of technology based on (1) biocompatible absorbable coatings that do not cause surface reactions, (2) using multiple drugs for elution that can include both a promoter for re-endothelialization in addition to being a target agent for SMC proliferation, and (3) a prohealing approach where endothelial progenitor cells can be promoted.

6.4 Strategic approaches for future use of drug eluting stents (DES)

6.4.1 New polymers

Biodegradable polymeric stents

DES were initially developed using a metallic substrate with a thin polymer coating over it. Although DES proved superior in overcoming complications associated with BMS, a new challenge in the form of thrombosis emerged. The

Table 6.5 Desirable properties of an ideal biodegradable stent

Mechanical properties
• High radial strength
• Deformable material with minimum elasticity
Compatibility properties
• Hemocompatible
• Cell compatible surface
Stability properties
Biostable – no release of toxic agents during degradation
Bioresorbable
Sterility
• Withstand sterilization conditions

reason for this complication was denudation of the endothelium due to the metallic substrate. As a result, stents were developed using a bioresorbable polymeric substrate that can expand and act as scaffold to reopen the clogged artery. The degradation products should be eliminated either biochemically or via the renal route.[110] Table 6.5 summarizes the desirable properties for an ideal biodegradable stent. Tamai *et al.* developed the first biodegradable polymeric stent made of poly-L-lactic acid (PLA) and demonstrated the safety and efficacy for the same in humans. A successful outcome was reported from the study using a biodegradable PLA stent which demonstrated safety and efficacy in preventing restenosis after 6 months in a follow-up study.[111] In another intriguing study performed by Tsuji and coworkers, Tranilast (*N*-3,4-dimethoxycinnamoyl anthranilic acid) was delivered using the PLA Igaki–Tamai stent.[112] Tranilast is an effective cytostatic agent arresting the proliferation of vascular SMC, the mechanism of which is discussed by Miyazawa *et al.*,[113] Mishikawa *et al.*,[114] and Ishibashi *et al.*[115] Jagur-Grodzinski in his review provided an extensive list of polymers that can be applied for medical devices.[116] The degradation time for these polymers varies depending on the type of monomers (functional group), degree of polymerization, molecular weight, and surface modifications. Crystalline polymers exhibit a longer degradation time than amorphous forms owing to a higher density. It is important to note that not all polymers that serve as carriers for metallic stent can be used to form a biodegradable polymeric stent. To substantiate this statement, Van der Geissen and coworkers tested the biocompatibility of various biodegradable and non-biodegradable polymers, which had been used previously in medical devices. It was found that all the polymers tested initiated an inflammatory response after a follow-up of 4 weeks in a pig model.[117] PLA was the only bioresorbable polymer that overcame an inflammatory response without an increase in intimal hyperplasia. Though biodegradable stents have shown a potential in stent therapy, a few drawbacks need to be addressed before it can replace metallic stents in the coronary interventional procedure. PLA exhibits poor mechanical properties leading to elastic recoil irrespective of its molecular weight. PLA, being a temperature

sensitive self-expanding polymer, undergoes uncontrolled expansion even weeks after stent deployment, which could lead to perforation.[118] The current research focus on developing biodegradable stents has shifted towards improving the mechanical properties (such as radial strength) of the biodegradable stent so that they are comparable to a metallic stent. It is also important to delay the degradation until after the first few weeks as the process of degradation can trigger adverse reactions leading to restenosis.

Polymer stents for local drug delivery

The first polymeric stent developed comprised a slotted polymer fiber design, which showed great promise against thrombosis and neointimal hyperplasia. Su *et al.*, in an attempt to overcome the previous mechanical drawbacks of polymeric stents upon expansion, designed and developed a fiber-type PLA stent based on a new multiple furled lobes assembly. This assembly included both central and peripheral lobes that could convert into a single lobe upon balloon expansion. This new stent eliminated the necessity of heating to expand, and had sufficient integrity to be expanded by a balloon procedure. This approach overcame the drawback of a permanent bend of polymeric wires used in previous designs by employing highly flexible multiple loop polymeric coils. These stents were coated with di(ethylene glycol) to make them thromboresistant. This new design demonstrated the feasibility of biodegradable stents and their deployment using conventional means, although long-term safety and effects of polymer degradation still remains to be a concern.[119] Yamawaki and coworkers examined the possibility of delivering specific tyrosine kinase inhibitors from PLA stents. Tyrosine kinases regulate various extracellular signals inducing proliferation and differentiation. ST638 (α-cyano-3-ethoxy-4-hydroxy-5-phenyl-methylcinnamamide) was loaded into a PLA stent and the stent efficacy was tested in a pig model. The ST638-incorporated PLA stent was successful in suppressing geometric remodeling, but neoinitmal formation was still seen demanding further improvements of biocompatibility.[120] Grabow *et al.* developed a slotted tube biodegradable stent based on PLA and poly(4-hydroxybutyrate) (P4HB). The key finding from their study was that PLA/P4HB blend provide superior load-bearing capacity and rapid expansion over stents made of PLA alone. However, *in vivo* performance and degradation properties of these stents still need to be evaluated.[121] Ormiston *et al.* recently reported the outcome of a human clinical trial (ABSORB) conducted using bioabsorbable everolimus-eluting PLA stent. This study showed a favorable result in terms of feasibility of deploying the stent. It was also observed that in-stent late loss and hyperplasia were reduced during a one year follow-up study. Since this study was limited to patients having single short lesions, further randomized investigation and longer follow-up studies will be needed to assure the long-term benefits of this stent design.[122]

Limitations

As reported in various studies, there are discrepancies regarding the observations obtained from biodegradable stents made of polymers similar to PLA. Sharkawi and coworkers provided a review of several factors accountable for this. Table 6.6 summarizes the factors to be considered in developing a biodegradable stent. Though polymeric stents show promises in terms of decreased thrombosis and restenosis, a lack of consistency in terms of mechanical properties is one of the biggest hurdles to overcome.

Future of polymeric stents

From a materials perspective, new polymeric candidates with better biological and degradation properties are needed to develop a polymeric stent that can successfully challenge the current metallic stents. Thus far, PLA is the only polymer that has been extensively investigated as a candidate for a polymeric stent. From a mechanical point of view, newer designs should be a critical consideration while developing polymeric stents. These designs must demonstrate rapid expansion and improved radial force for appropriate stenting and drug delivery. Synergistic research among polymer chemists, engineers, biologists, and interventional cardiologists is needed to develop the successful next generation of polymeric stents.

6.4.2 Drug delivery vs. gene delivery

Gene therapy refers to introduction of a gene into the cells of tissue to treat various disorders, such as cancer, heart disease, and acquired immunodeficiency syndrome. Administration of genes prepares the cell to counter disease, without introducing any external agents such as proteins or drugs. Although toxicity is not a critical factor in developing gene-based formulations, as is typically the

Table 6.6 Considerations for polymeric stents

Polymer related
• Chemical composition
• Molecular weights
• Polydispersity
• Crystallinity
Processing related
• Manufacturing conditions
• Stent design
Intervention related
• Inflation time
• Temperature
• Pressure

case with biological drugs, efficacy remains a major challenge associated with gene therapy.

Viral and non-viral vectors for gene therapy

The delivery vehicle for gene therapy can be broadly classified in to two types, viral and non-viral vectors. Viral vectors constitute different types of viruses, including retrovirus, adenovirus, and adeno-associated virus. Non-viral vectors include delivery using liposomes, gene gun, and DNA conjugates. Delivery of genes through DES is still in an early phase of research considering safety, efficacy, and ease of development. Retroviruses function through the interaction of specific proteins with cell surface receptors, after which it integrates on the nucleus of the host cell. One of the major limitations of this type of viral vector is that they can infect cells only in mitosis. This is a major limitation as most tissues in adults consist of non-dividing cells. Adenoviruses, another type of virus used in gene delivery, are double-stranded linear DNA viruses which infect cells through interaction of viral proteins with integrin. This binding creates modification of the protein leading to further interaction of the virus with other components of the cell surface. Also, adenoviruses can infect non-dividing cells, unlike retrovirus, but are not as defective as retrovirus, leading to the formation of infectious virus when used *in vivo*.[123] The most common non-viral method of gene therapy includes delivering a gene through liposomes. These vectors are non-pathogenic and a relatively cheap mode of delivery depending on its transfection efficiency. Also, liposomes can be coupled with viral vectors as a vehicle to enhance the local delivery of a gene. A gene gun is a method in which DNA loaded onto gold particles is directly injected using an helium gun. This has been attempted *in vivo* and the uptake of DNA by tissues injected in this manner led to low turnover of encoded proteins.[124]

Gene-mediated vascular therapy

There are multiple benefits of localized gene delivery. First, it is more efficient to have a locally generated agent, as compared with the indiscriminate action of the agent in unintended sites. Second, there are decreased side effects owing to the lack of systemic dilution of the agent. Third, the agent is generated in its functionally active conformation at the very site of action. However, one of the major roadblocks in the localized delivery of cardiovascular genes has been the transfection efficiency at the target site. Further, the immunological acceptance and safety of the transfection vector itself is a concern. For example, poly(ethylenimine) (PEI), a synthetic gene delivery agent, significantly altered cellular function when tested in endothelial cells.[125] For a good review of some of the methods of gene delivery, the interested reader can refer to Nabel[126] published in 1995 and Mountain in 2000.[127] In addition, the on-demand

production of the transgene would be a significant enhancement in gene delivery.[128] This could be achieved by the use of 'smart promoters' which are activated by factors, such as altered shear stress, endothelial damage and altered synthesis of specific cytokines or growth factors in the area, and intimal hyperplasia. Several strides have been made in the area of gene-mediated vascular therapy in the last decade or so and some of these developments are discussed here as categorized below as therapies for arresting SMC proliferation, anti-thrombotic therapies, and re-endothelialization therapies.

Therapies for arresting SMC proliferation

Anti-proliferation therapies specifically tailored toward inhibiting the proliferation of SMCs will prevent restenosis of the target vessel. The challenge here is to specifically inhibit the hyperplasia of SMCs without interfering with the proliferation of endothelial cells. In the interest of space, this review will focus on some of the NO-mediated effects on the proliferation and migration of SMCs.

NO enables the dilatation of a stenosed vessel.[129] In addition, NO is known to inhibit SMC proliferation and migration and also decreases the exuberant synthesis of extracellular matrix components, which is typical of proliferative SMCs.[130] The antimitogenic effects of NO on SMCs is known to be cGMP-mediated, the latter being generated by the NO substrate soluble guanylate cyclase.[131–134] Continuous intravenous administration of NO donors, however, may result in systemic hypotension and associated side effects.[135–138] Therefore, the targeted release of NO at the site of interest appears promising. One such strategy has been engineered by Janssens *et al.* via adenovirus-mediated delivery of eNOS.[135] This method significantly inhibited neointimal formation 12 days after arterial injury.[123] This partially stemmed from the antiproliferative effect of the genetically transduced medial SMCs and adventitial cells. Along the similar lines, von der Leyen *et al.* inhibited formation of neointimal vascular lesions using a Sendai virus/liposome *in vivo* gene transfer technique. In this study, neointimal lesion formation was reduced by 70% at day 14 after balloon-mediated injury.[139] Another effect mediated via the NOS pathway is that of the kallikrein–kinin system, whereby the increase in the kinin levels exerts a protective effect after vascular injury. Sustained delivery of tissue kallikrein was achieved by Murakami *et al.* resulting in significant reduction of neointimal formation largely via suppression of the migration and proliferation of vascular SMCs by the released vasoactive kinin peptides.[140]

Anti-thrombotic therapies

Gene-mediated antithrombotic therapies are particularly attractive because the localized delivery of antithrombotic agent can eliminate the risks associated with systemic anticoagulation.[141–143] Production of vascular cyclooxygenase-1

(COX-1) is the rate-limiting step in the synthesis of prostacyclin (PGI_2), an important vasoprotective molecule. The adenoviral delivery of COX-1 gene produced a 5–8 times increase in PGI_2.[144] Numaguchi *et al.* successfully demonstrated the non-viral transfer of the PGI_2 synthase gene using the transfection agent lipofectamine.[145] Furthermore, the delivery of eNOS gene[146–148] has also been explored to combat thrombosis since eNOS upregulation has been associated with angiogenesis.[149] Another approach involves the inhibition of tissue factor (TF), the latter being a key mediator of arterial thrombosis via interaction with coagulating factors in the blood.[150] In intact endothelial cells, an anti-TF molecule, TF pathway inhibitor (TFPI), suppressed thrombin formation.[138,151,152] Therefore, in the event of endothelial denudation, the denuded vessel becomes more prone to thrombosis. Consequently, adenovirus-mediated localized expression of TFPI was found to inhibit shear stress-induced mural thrombosis without concomitant side effects of systemic thrombosis.[153] Hirudin, a highly selective inhibitor of thrombin, has also been investigated for potential antithrombotic effects. Local adenovirus-mediated hirudin delivery significantly suppressed neointimal formation after arterial injury via localized thrombin deactivation.[154] However, TFPI being an upstream inhibitor in the synthesis of thrombin has the potential to be a more effective as an antithrombotic agent. Another strategy to effect localized thrombolysis is via gene delivery of wild type tissue plasminogen activator (TPA)[155] or of glycosylphosphatidylinositol-anchored urokinase.[156] The strategy of seeding transduced endothelial cells that are overexpressing various thrombolytic agents could offer a potential route of preventing the thrombogenic responses in endothelial cell denuded vessels. In addition, cultured endothelial progenitor cells (EPCs) may offer another route of delivering anticoagulant genes at the site of vascular injury.[157] However, the grafting of scaffolds with live ECs or EPCs may face other practical challenges.

Re-endothelialization therapies

Re-endothelialization therapies have the potential to resolve the bulk of the anomalies that are caused post-arterial injury. For one, endothelial cells generate antithrombotic agents and naturally mitigate the chances of neointimal formation. Second, SMC proliferation can be indirectly suppressed if the denuded vessel can be re-endothelialized.

Localized transgene delivery of an endothelial cell-specific mitogen at the site of injury has been achieved. An attractive transgene is the vascular endothelial growth factor (VEGF) gene,[158–161] as VEGF is an effective promoter of angiogenesis.[162] However, the unregulated expression of VEGF has been associated with the emergence of endothelial cell-derived tumors at the site of implantation.[163] Therefore, the delivery kinetics of this angiogenic factor needs to be regulated possibly via the introduction of an on-demand activated promoter region.

Future directions

Though gene therapy seems to open a new gateway of treatment, the research pertaining to it is still in its early stage. More improvements are required for vector technology to be feasible. More information is needed in terms of understanding the biology of tissues and specific features of a disease to aid in designing specific vectors for effective gene transfer. In the field of DES, although the use of EPCs shows tremendous potential as a route to promote endothelialization, feasibility in development of stents with EPCs remains a concern. Also, research needs to be directed towards developing formulations that can deliver genes like VEGF with better release kinetics, for use in stent-based therapy.

6.4.3 Biological combinations for designing future DES

Prohealing strategies

The adverse consequences of the DES interfering with the natural biology of healing have been reviewed by various researchers as outlined in this chapter. The combination of DES and antiplatelet therapy is in a sense a 'marriage of convenience,' rather than a true solution to the problem. Consistent efforts are being made to incorporate biological agents that can promote endothelialization. This can provide a true solution that does not require daily administration of anti-platelet drugs.

In the first clinical study using a bioengineered stent, Aoki and coworkers used murine monoclonal anti-human CD34 antibodies capable of capturing endothelial cells, coated on to a metal stent with dextran. The CD34 coated stents showed greater than 90% of endothelial cell coverage in just 1 hour as compared with bare metal stent, which were completely devoid of cells as observed during the *in vivo* study. Also, no case of thrombosis was reported even after 6 months in a follow-up study. This prohealing strategy was shown to be both feasible and safe; however, the efficacy still needs to be determined.[164] This was followed by a second clinical trial (HEALING II), which introduced advancement in terms of sterilization, bioactivity, and stability, leading to a higher capture rate of endothelial progenitor cells. No cases of thrombosis were reported even after discontinuation of antiplatelet therapy for a month.[164] 17β-estradiol is another agent that has been administered locally from DES to promote endothelial recovery post-stenting. Chandrasekar and coworkers studied the effect of 17β-estradiol on endothelial function in a post-coronary angioplasty porcine model after 4 weeks. Vessels treated with estradiol showed enhanced endothelial function and reduction of neointima.[165]

Combination strategies

Combination of pharmaceutical agents is another approach recently adapted for use in DES. Since the complications associated with DES are multifactorial, using multiple agents to target specific complications is desired. Recently, Lin and coworkers used a combination of paclitaxel and NO, coated on a vascular stent. The *in vivo* results obtained using a rabbit model indicated that combination therapy could be effective for DES.[166] In another intriguing study performed by Patil and coworkers, combination of drug and gene was investigated in controlling angiogenesis by using a PLGA microsphere/PVA hydrogel composite.[167] Although their system did not involve a stent, the study confirmed that a concurrent delivery of dexamethasone (corticosteroid) and VEGF prevented any adverse reaction at the tissue-device interface as well as angiogenesis as confirmed using a rat model. This combination of drug and gene could be an interesting way to increase the efficacy of future DES.

6.5 Summary

After a decade of evolution, DES has provided tremendous impact on the treatment of in-stent restenosis. Several platforms of DES which were successful for treating restenosis, however, failed to prevent the manifestation of thrombosis. The further success of DES will largely depend on its ability to handle complications beyond the treatment of restenosis. Newer therapies in the form of gene therapy, combination of drug and gene, use of dual pharmaceutical agents, and the use of a polymeric stent are emerging as candidates for the next generation of DES. Although preliminary observations for these newer platforms are encouraging, more clinical data are required to determine the safety and efficacy of these technologies over a long-term basis.

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