Stents and Vascular Woundhealing

Stents en Vasculaire Wondheling

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Cover illustration: a macroscopic view into a stented artery

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A journey of a thousand miles
begins with a single step.
(Lao-Tzu)

To Shane, for your unending patience, tolerance and understanding during the compilation of this thesis, and to Keelin, who has brought us so much joy and enriched our lives beyond belief.

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Chapter 1

Introduction

Introduction.

For many years, percutaneous transluminal coronary angioplasty (PTCA) has been used to treat diseased, narrowed vessels. While it has been a great success in improving patient outcome, the technique is not free from complications: restenosis, dissections and abrupt closures are serious complications of the technique, necessitating repeat revascularization or surgery. Experimental animal studies suggested that the results of PTCA could be improved by scaffolding the internal wall of the artery with a stent, an idea first proposed in 1912 by Carrel[1]. In the late 1960's Dotter gave new impetus to this idea[2], and in 1986 the first human coronary stent implantation followed [3].

Initial stent implantations were associated with high thrombosis rates and results of stent trials were greeted cautiously or even skeptically by some. In 1994 however, the results of the European BENESTENT and American STRESS trials were published. In these trials restenosis rates after balloon angioplasty or Palmaz-Schatz stent implantation were compared, and showed significant reductions in restenosis rates after stenting (BENESTENT: 22%; STRESS: 32%) compared to angioplasty alone (BENESTENT: 32%; STRESS: 42%). These landmark trials heralded the start of an exponential growth in the use of endovascular stents, such that today, stenting has become accepted as a standard therapeutic modality in interventional cardiology.

Current clinical indications for the use of stents include: (1) the primary reduction in restenosis in de novo focal lesions in vessels greater then 3.0 mm in diameter, (2) focal lesions in saphenous vein grafts and (3) the treatment of abrupt or threatened vessel closure during angioplasty[4]. However, as the variety and sophistication of stenting devices improves, new applications are being found. Currently there are more than 55 standard or customized stent types available for use in the coronary system manufactured by more than 30 different companies.

Despite the unquestionable success of vascular stenting (reductions in restenosis rates from ±28-39% to ±12-22% and thrombosis rates from ±5-15% to ±1-4%), and the ever-increasing number of commercially available stents, the problems of restenosis and to a lesser degree thrombosis remain. While modifications of stent design, improved implantation techniques, anti-platelet therapy, together with improved operator experience have done much to improve stent performance, the above-mentioned problems still remain unresolved.

In contrast to balloon angioplasty where restenosis has been shown to be due to the combination of intimal hyperplasia, vascular remodeling, and elastic recoil, restenosis after

stenting is due solely to intimal hyperplasia. It has been proposed that intimal hyperplasia after stenting is due to an exaggerated wound healing response, thought to be brought about by an acute and chronic response to injury, together with the permanent presence of a foreign body (the stent).

Improving current stent performance therefore may further reduce restenosis rates and allow the development of a new, improved generation of stents exhibiting a reduced neointimal response. This may be achieved by modifying the blood and vessel wall response to the stent which can be done by changing the mechanical and/or non-mechanical stent properties.

Stent implantation: the sequence of events.

Implantation of stents is always accompanied by damage to the endothelial lining and stretching of the vessel wall. The extent of acute injury depends on several factors such as stent design, means of delivery (self vs balloon expandable stents), lesion morphology and the use of pre or post stent angioplasty. The induced acute injury together with the implantation of the stent triggers a sequence of events, the magnitude of which is likely to depend on the morphology of the lesion and the degree of injury.

The sequence of events following stent implantation can broadly be divided into three phases: the proteinaceous response, the cellular response and the wound healing response (Figure 1).

The proteinaceous response:

Immediately following stent implantation, the stent is covered with a layer of proteins. Which proteins adsorb to the stent surface is initially determined by their concentration in vivo, and then by their affinity for the stent surface as determined by the surface characteristics of the stent itself i.e. topography, charge and chemistry of the stent surface (Vroman effect). Adsorption is also likely to be influenced by the extent of vascular injury and the composition of the vessel wall lesion. Given that different proteins can affect the subsequent vascular response, this initial phase is thought to play an important role in determining subsequent events.

Concomitant with protein adsorption is the activation of the intrinsic coagulation system and the complement system. The intrinsic coagulation system is initiated by binding and conversion of factor XII and high molecular weight kininogen (HMWK) to the stent surface, while the complement system is activated via the alternative route.

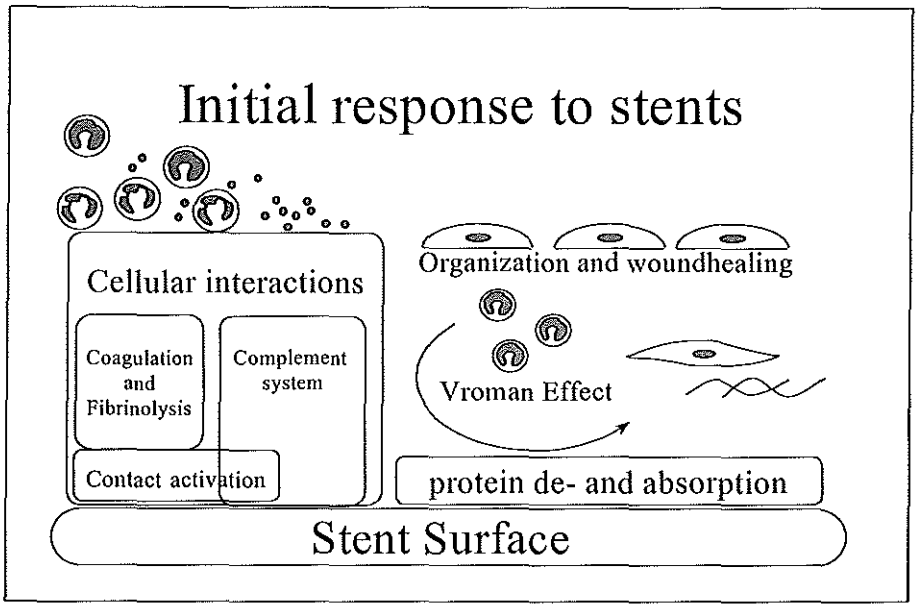

Figure 1. Schematic representation of the sequence of events which occur on the stent surface following implantation.

The cellular response.

This response constitutes the response of cellular elements from the blood stream as well as the response of the damaged cells in the vessel wall.

Platelet activation by soluble agonists such as ADP, thrombin, and tissue factor released by the injured vessel wall is the first initiated cellular response. In addition, the chemotactic C5a fragment produced by the complement system attracts neutrophils to the injury site, while C3a and C5a both act as inflammatory mediators. Thus the cellular response on the stent surface comprises the formation of a thrombus composed of platelets, trapped erythrocytes neutrophils and fibrin. It is worth noting that adhesion and aggregation of platelets, activation of the intrinsic coagulation cascade and early inflammatory responses are not separate events, but act in synergy (Figure 2).

In the vessel wall, proto-oncogenes are activated in the damaged smooth muscle cells (SMC) which play an important role in the subsequent wound healing response.

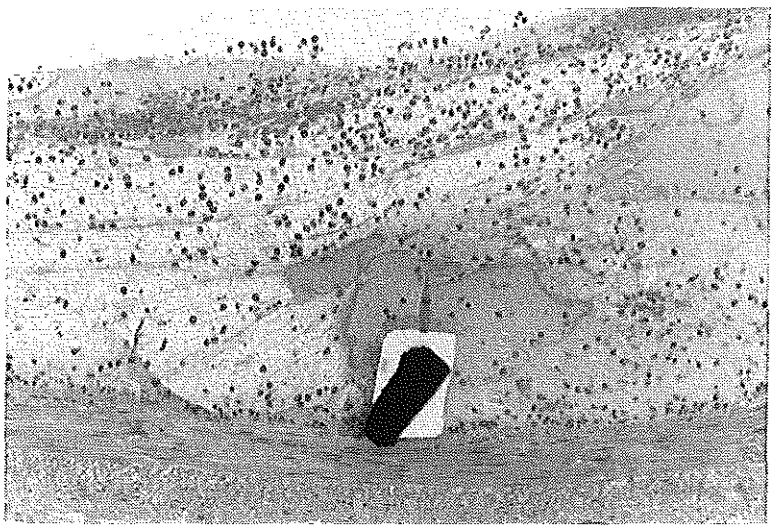

Figure 2. The vascular response to a stent 4 hours after implantation. A platelet -rich thrombus, with entrapped erythrocytes and neutrophils overlies the stent wire void and damaged vessel wall.

The wound healing response.

The healing and organization of the inflamed tissue, initiated by the action of monocytes and macrophages, is followed by proliferation of smooth muscle cells and endothelial cells. Remnant endothelial cells that remain between the stent wires, and adjacent to the stent, proliferate and spread out to cover the thrombus covered stent wires and injured vessel wall. At the same time, as macrophages move in to clear up remaining thrombus remnants around the stent wires and along the injured vessel wall surface, smooth muscle cells proliferate and migrate to the intima, where they secrete extracellular matrix. Organization of the SMCs follows to form circumferential layers of SMC, such that in time, the granulation tissue is eventually replaced by connective tissue.

Because clinically used stents are permanent implants, their presence may be a stimulus for prolonged inflammatory and foreign body responses, which could potentially interfere with the wound healing response. They may also act as a source of chronic vessel wall injury, induced by the movement of a rigid implant in a pulsating artery (degree of compliance mismatch). In humans, the vessel wall healing process is usually complete between 3 to 6 months post stenting, although a foreign body response has been observed as late as 320 days post implant[5].

The ideal stent.

Theoretically, if all aspects of stent implantation are considered, the ideal stent should have the following characteristics:

1. Flexible - have the capacity to negotiate curved and tortuous coronary segments
2. Radio-opaque - easily visible during angiography
3. Low unconstrained profile – facilitates the passing and positioning of the stent in the artery without pre-PTCA per se
4. Circumferential coverage – total coverage of the lesion
5. Low surface area – minimize surface area for potential thrombogenic interactions
6. High radial strength – capacity to hold open the artery against the vessel wall's constrictive tendencies (minimize recoil) and hold the stent at its selected implantation site
7. Show both blood and tissue compatibility
8. Not induce an excessive inflammatory or neointimal response

To date, no ideal stent exists. Depending on the lesion to be treated, there is often a compromise of one or more of the above factors e.g. stents with low surface area may not be very radio-opaque, while lesions requiring total coverage may require stents having high surface area.

Stent properties

By understanding how stent properties influence the blood and tissue response, this response can potentially be modified to reduce the thrombotic and neointimal responses. Stent properties can be divided into mechanical and non-mechanical properties (Table A).

(a) Mechanical stent properties

Studies documenting the influence of mechanical stent parameters on the vessel wall have frequently been conflicting. Radial force (an outward force that a stent itself exerts on the vessel wall) was shown by Vorwerk et al to give no differences in neointimal thickening between Wallstents with different radial forces[6]. However, in another study, stents with higher radial force were found to induce a greater neointimal response compared to stents with lower radial force[7]. Studies of stent longitudinal flexibility (the stent's ability to bend or flex in its length) and compliance (the ability of a stent to yield elastically when an external force is applied) have also shown conflicting results[8,9]. No studies of the exclusive effect of hoop strength (a measure of the external force needed to plastically deform the stent) on neointimal thickening have been published.

(b) Non-mechanical stent properties.

Numerous studies have documented the effects of non-mechanical stent parameters. Herlein et al, in a rabbit model, showed that the charge of stents did not seem to play a major role with respect to stent thrombogenicity, but that low stent charge correlated with an increased neointima formation[10]. Palmaz showed that, in vitro, grooved stent surfaces promoted an increased rate of endothelial cell migration compared to smooth, control surfaces, and therefore could theoretically enhance endothelialization of the stent[11]. Rogers et al showed that alterations in stent design and geometric configuration of the stent could affect vascular injury and neointimal hyperplasia, while surface material plays a greater role in thrombosis[12].

Table A. *Non-mechanical and mechanical characteristics of stents.*

Non-mechanical	Mechanical
Charge	Radial force
Topography	Hoop strength
Stent design	Longitudinal flexibility
Metal:artery ratio	Compliance
Type of metal used	

Manipulation of the vessel wall response to stents.

Potentially each of the three phases of the vessel wall response to stent implantation can be manipulated to improve stent performance. This can be achieved by modifying the mechanical or non-mechanical stent properties. Of these, modifications of the stent surface (topography) by polishing or the use of stent coatings have been most frequently used.

Stent coatings

Coating stents changes the outer few micrometers of the stent and therefore can be achieved with currently available stents. The ideal stent coating should meet the following criteria:

1. It should be non-thrombogenic.
2. It should not induce an inflammatory response. An inflammatory response may delay the wound healing response and contribute to an exaggerated neointimal response, or thrombotic events.
3. It should have a low profile i.e. it should form a thin, even layer on the stent surface and not significantly increase the total profile of the stent.
4. It should have mechanical integrity i.e. it should not crack of flake off during handling.

To date, no ideal stent coating exists. The majority of stent coatings used are made from synthetic polymers – both biodegradable and non-biodegradable polymers – and are frequently associated with inflammatory reactions. Table B lists several examples of each type of stent coating, while a comprehensive review can be found elsewhere[13]. Stent coatings can be divided into passive and active coatings.

Passive and active stent coatings.

Passive stent coatings are designed to influence the surface properties of the stent. Depending on the coating used e.g. fibrin or phosphorylcholine they can be used as a form of biological camouflage, or biomimicry. Although strictly speaking not a stent coating, a novel form of biomimicry is the covering of a stent with an autologous vein or artery. Animal studies have shown the technique to be feasible and in a porcine model, 6-month follow-up showed minimal intimal hyperplasia covering the graft[30,31]. Such promising results led to the use of this stent-graft in the clinic, and preliminary clinical results are encouraging[32-34]. However, given the technical complexity of the technique, it has yet to be determined whether it will find a niche in routine stent implantations.

Active stent coatings are those which present or elute drugs directly into the vessel wall. The treatment of thrombosis and restenosis using drug-eluting stents therefore offers a two-fold approach: a mechanical scaffold reduces elastic recoil and remodeling, while locally delivered therapeutic agents prevent intimal thickening and thrombosis. For local drug delivery, biodegradable polymers offer the advantage that drug release is achieved by both diffusion from the polymer and degradation of the polymer. By controlling the degradation rate of the polymer, sustained drug release can be achieved for a prolonged period of time. Since stent implantation induces both a thrombotic and tissue response, drug-loaded coated stents should ideally treat both aspects of this response. While heparin-coated stents

(although strictly speaking non-eluting), have successfully been used clinically to treat the thrombotic aspect of stent implantation, drug-eluting stents have shown limited success in reducing neointimal thickening in experimental studies.

Stents seeded with genetically modified endothelial cells are also a form of active stent coatings. By seeding stents with endothelial cells it is hoped to increase the rate of stent endothelialization such that potentially the neointimal response may be limited. Genetically modified cells may potentially improve the endothelial function of a stented vessel, through, for example, enhanced NO production or increased prostacyclin production so as to improve the barrier function.

Table B. Some examples of polymers tested as potential stent coatings.

Synthetic Polymers		***Drug-eluting polymer + drug***
Biodegradable (bioabsorbable)	*Non-biodegradable*	
PLA[14]	Biogold[15]	PLLA + Dexamethasone [16]
PGLA, PCL, PHBV, POE, PEO/PBTP[17]	PU[18,19]	PUR + Forskolin[20]
	POP[21]	PSNO/BSA+ NO[22]
	PC [23,24]	PEG-hirudin PGI2 [25-27]
	PETP, SIL[17]	PLLA+colchicine[28]
		POP+methylprednisolone [29]

PLA: poly-lactic acid, PGLA:polyglycolic/polylactic acid , PCL: polycaprolactone, PHBV: polyhydroxy-butyrate/valerate, PEO: polyorthoester, PEO/PBTP: polyethyleneoxide/ polybutylene terephthalate. PU: polyurethane, POP: polyorganophosphazene, PC: methacryloylphosphorylcholine, PETP: polyethylene terephthalate, SIL: silicone, PSNO/BSA: polynitrosated nitric oxide albumin.

Assessment of implanted stents.

A variety of techniques are available to assess the results of implanted stents. During implantation, quantitative coronary angiography (QCA) and intravascular ultrasound (IVUS) are computer-aided techniques that allow the acute effects of stents in the vasculature to be studied e.g. stent expansion, thrombus in the stent, vessel spasm. At follow-up these techniques are used to assess the performance of the stent over time e.g. stent migration, patency and tissue composition and volume. However, a limitation of QCA and IVUS is that

assessment of the cellular response to the stent cannot be determined with these techniques. Histology is therefore an important supplementary technique in helping to elucidate this vascular response by allowing assessment of cell type and density. When combined with immunohistochemistry, the proliferative response induced by a stent can also be studied. It can highlight insufficient/inadequate technical aspects of stent implantation i.e. incomplete stent expansion (the metal stent struts are not (all) in direct contact with the vessel wall), or it can show the co-implantation of contaminating factors or other foreign bodies together with the stent. Histology therefore can confirm and expand upon information gained by the use of such techniques as QCA and IVUS to give a broader and clearer picture of what exactly happens when a stent is implanted into an artery.

Aim of this thesis.

The aim of this thesis is first to elucidate the acute, but particularly the chronic vessel wall responses to endovascular stent implantation. Having determined such responses, the second aim is to see if these responses can be improved by modifying both mechanical and non-mechanical stent properties.

Outline of this thesis.

Histology plays a pivotal role in the assessment of the vessel wall response to endovascular stents. Previous histological processing techniques for assessment of coronary arteries containing metallic stents have invariably resulted in damage to the stented vessel wall, such that accurate interpretation of the vessel wall response to the stent may be hampered. Chapter 2 describes a new method of histological processing for arteries containing metallic stents, which eliminates any damage to the stented vessel induced by histological processing.

Techniques of stent implantation may, together with the stent, inadvertently introduce other foreign bodies into the vessel wall. Chapter 3 describes foreign bodies that were observed to be co-implanted with stents in the coronary arteries of pigs, and suggests ways in which such co-implantations can potentially be avoided or reduced.

The acute and chronic effects of stenting are examined in chapters 4 and 5. Specifically, the parameters of stent induced injury, endothelial (dys)function and the vessel wall proliferative response are examined at multiple time points in the coronary arteries of young swine.

The effect of the mechanical property of stent compliance on neointimal thickening is examined in Chapter 6. Two stents with different compliance are examined in a porcine femoral model and the vessel wall reactions following stent implantation compared between healthy and atherosclerotic models.

Chapters 7, 8 and 9 deal with stent modification through the use of coatings. An overview of mechanisms of drug loading and release kinetics are presented in Chapter 7, while Chapter 8 looks at the biocompatibility and short and long-term effects of a non-degrading synthetic polymer coating on the thrombotic and neointimal responses in a porcine coronary model. In contrast, the short and long-term effects of a drug-eluting, biodegradable stent coating are presented in Chapter 9. In particular, its effects on the neointimal and proliferative responses, together with endothelial function are examined.

Chapter 10 gives an overview of studies presented in this thesis and discusses the implications of these results.

References

1. Carrel A. Results of permanent intubation of the thoracic aorta. *Surg Gyn Obst.* 1912;15:245-248.
2. Dotter CT. Transluminally-placed coilsprings endarterial tube grafts: long-term patency in the canine popliteal artery. *Invest Radiol.* 1969;4:329-332.
3. Sigwart U, Puel J, Mirkovitch V, Joffre F, Kappenberger L. Intravascular stents to prevent occlusion and restenosis after transluminal angioplasty. *N Engl J Med.* 1987;316:701-6.
4. Kutryk MJB, Serruys PW. *Current State of Coronary Stenting.* Rotterdam: Barjesteh van Waalwijk van Doorn & Co's Uitgeversmaatschappij; 1997.
5. Van Beusekom HM, van der Giessen WJ, van Suylen R, Bos E, Bosman FT, Serruys PW. Histology after stenting of human saphenous vein bypass grafts: observations from surgically excised grafts 3 to 320 days after stent implantation. *J Am Coll Cardiol.* 1993;21:45-54.
6. Vorwerk D, Redha F, Neuerburg J, Clerc C, Gunther RW. Neointima formation following arterial placement of self-expanding stents of different radial force: experimental results. *Cardiovasc Intervent Radiol.* 1994;17:27-32.
7. Kobayashi Y, Bailey SR, Brown III CL, Christie LG, Matthews RV, de Franco AC, Schwartz RS, Handen CE, Fitzgerald PJ. Relationship between radial expansile force and neointimal proliferation in self-expandable stent: a serial intravascular ultrasound study. *Circulation.* 1998;17:I-163 (Abstract).
8. Barth KH, Virmani R, Froelich J, Takeda T, Lossef SV, Newsome J, Jones R, Lindisch D. Paired comparison of vascular wall reactions to Palmaz stents, Strecker tantalum stents, and Wallstents in canine iliac and femoral arteries. *Circulation.* 1996;93:2161-9.
9. Fontaine AB, Spigos DG, Eaton G, Das Passos S, Christoforidis G, Khabiri H, Jung S. Stent-induced intimal hyperplasia: are there fundamental differences between flexible and rigid stent designs? *J Vasc Interv Radiol.* 1994;5:739-44.
10. Hehrlein C, Zimmermann M, Metz J, Ensinger W, Kubler W. Influence of surface texture and charge on the biocompatibility of endovascular stents. *Coron Artery Dis.* 1995;6:581-6.
11. Palmaz JC, Benson A, Sprague EA. Influence of surface topography on endothelialization of intravascular metallic material. *J Vasc Interv Radiol.* 1999;10:439-44.
12. Rogers C, Edelman ER. Endovascular stent design dictates experimental restenosis and thrombosis. *Circulation.* 1995;91:2995-3001.
13. Bertrand OF, Sipehia R, Mongrain R, Rodes J, Tardif JC, Bilodeau L, Cote G, Bourassa MG. Biocompatibility aspects of new stent technology. *J Am Coll Cardiol.* 1998;32:562-71.
14. Schellhammer F, Berlis A, Bloss H, Pagenstecher A, Schumacher M. Poly-lactic-acid coating for endovascular stents. Preliminary results in canine experimental arteriovenous fistulae. *Invest Radiol.* 1997;32:180-6.
15. van der Giessen WJ, van Beusekom HMM, van Houten CD, van Woerkens LJ, Verdouw PD, Serruys PW. Coronary stenting with polymer-coated and uncoated endoprostheses in pigs. *Coron Art Dis.* 1992;3:631-640.

16. Lincoff AM, Furst JG, Ellis SG, Tuch RJ, Topol EJ. Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. *J Am Coll Cardiol.* 1997;29:808-16.

17. van der Giessen WJ, Lincoff AM, Schwartz RS, van Beusekom HM, Serruys PW, Holmes DR, Jr., Ellis SG, Topol EJ. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. *Circulation.* 1996;94:1690-7.

18. Rechavia E, Litvack F, Fishbien MC, Nakamura M, Eigler N. Biocompatibility of polyurethane-coated stents: tissue and vascular aspects. *Cathet Cardiovasc Diagn.* 1998;45:202-7.

19. Fontaine AB, Koelling K, Clay J, Spigos DG, Dos Passos S, Christoforidis G, Hinkle G, Hill T, Cearlock J, Pozderac R. Decreased platelet adherence of polymer-coated tantalum stents. *J Vasc Interv Radiol.* 1994;5:567-72.

20. Lambert TL, Dev V, Rechavia E, Forrester JS, Litvack F, Eigler NL. Localized arterial wall drug delivery from a polymer-coated removable metallic stent. Kinetics, distribution, and bioactivity of forskolin. *Circulation.* 1994;90:1003-11.

21. De Scheerder IK, Wilczek KL, Verbeken EV, Vandorpe J, Lan PN, Schacht E, De Geest H, Piessens J. Biocompatibility of polymer-coated oversized metallic stents implanted in normal porcine coronary arteries. *Atherosclerosis.* 1995;114:105-14.

22. Folts J, Maalej N, Kearney J, Loscalzo J. Palmaz-Schatz stents coated with a unique NO donor renders them much less thrombogenic when placed in pig carotid arteries for 28 days. *J Am Coll Cardiol.* 1996;27:86A(abstract).

23. van Beusekom HMM, Whelan DM, Krabbendam SC, van Vliet EA, Staal EE, Bac D, Serruys PW, van der Giessen WJ. Biocompatibility of phosphorylcholine coated stents in a porcine coronary model. *Circulation.* 1997;96:1609(abstract).

24. Kuiper KK, Robinson KA, Chronos NA, Cui J, Palmer SJ, Nordrehaug JE. Phosphorylcholine-coated metallic stents in rabbit iliac and porcine coronary arteries. *Scand Cardiovasc J.* 1998;32:261-8.

25. Schmidmaier G, Stemberger A, Alt E, Gawaz M, Neumann F-J, Schomig A. A new biodegradable polylactic acid coronary stent coating, releasing PEG-hirudin and a prostacyline analog, reduces both platelet activation and plasmatic coagulation. *J Am Coll Cardiol.* 1997;29:354A (abstract).

26. Prietzal K, Pasquantonio JD, Fliedner TU, Stemberger A, Janczewski M. Inhibition of neointimal proliferation with a novel, hirudin/prostacyclin analog eluting stent coating in an animal overstretch model. *Circulation.* 1996;94:1522 (abstract).

27. Alt E, Beilharz C, Preter D, Schmidmaier G, Pasquantonio J, Erhard W, Stemberger A, Schomig A. Biodegradable stent coating with polylactic acid, hirudin and prostacylin reduces restenosis. *J Am Coll Cardiol.* 1997;29:238A (abstract).

28. Eccleston D, Lincoff A, Furst J. Administration of colchicine using a novel prolonged delivery stent produces a marked local biological effect within the porcine coronary artery. *Circulation.* 1995;92:I-67(abstract).

29. De Scheerder I, Wang K, Wilczek K, van Dorpe J, Verbeken E, Desmet W, Schacht E, Piessens J. Local methylprednisolone inhibition of foreign body response to coated intracoronary stents. *Coron Artery Dis.* 1996;7:161-6.

30. Stefanadis C, Toutouzas K, Vlachopoulos C, Stratos C, Kallikazaros I, Karayannakos P, Gravanis MM, Robinson K, Toutouzas P. Stents wrapped in autologous vein: an experimental study. *J Am Coll Cardiol.* 1996;28:1039-46.

31. Stefanadis C, Toutouzas K, Tsiamis E, Vlachopoulos C, Vaina S, Tsekoura D, Haldi L, Stefanadi E, Gravanis M, Toutouzas P. Stents covered by an autologous arterial graft in porcine coronary arteries: feasibility, vascular injury and effect on neointimal hyperplasia. *Cardiovasc Res.* 1999;41:433-42.

32. Stefanadis C, Toutouzas K, Tsiamis E, Vlachopoulos C, Kallikazaros I, Stratos C, Toutouzas P. Total reconstruction of a diseased saphenous vein graft by means of conventional and autologous tissue-coated stents. *Cathet Cardiovasc Diagn.* 1998;43:318-21.

33. Stefanadis C, Tsiamis E, Vlachopoulos C, Toutouzas K, Stratos C, Kallikazaros I, Vavuranakis M, Toutouzas P. Autologous vein graft-coated stents for the treatment of thrombus- containing coronary artery lesions. *Cathet Cardiovasc Diagn.* 1997;40:217-22.

34. Stefanadis C, Tsiamis E, Vlachopoulos C, Toutouzas K, Giatrakos N, Tsioufis C, Diamantopoulos L, Toutouzas P. Arterial autologous graft-stent for treatment of coronary artery disease: a new technique. *Cathet Cardiovasc Diagn.* 1997;40:302-7.

Chapter 2

A practical and rapid method of histological processing for examination of coronary arteries containing metallic stents

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Cardiovasc Pathol 1996; 5: 69-76

A Practical and Rapid Method of Histological Processing for Examination of Coronary Arteries Containing Metallic Stents

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A practical and rapid method was developed to study vascular pathology after implantation of metal endoprostheses (stents) that are used as internal splinting devices of tube-like structures. This method obviates the need for time-consuming grinding of thick sawing sections or removal of the prosthesis prior to histological processing, allowing for detailed analysis of the tissue in general, but especially of the stent-tissue interface. The vessels, with the metal stents still in place, were dehydrated in graded series of ethanol and embedded in methyl methacrylate. Using a motor-driven rotary microtome, 3- to 5-μm sections were easily cut. After deplastination, routine and special histological stainings were performed according to standard protocols for paraffin sections. This method proved to save time, compared with sawing sections, while allowing for a more complete examination of the stent-tissue interface than is possible with routine paraffin techniques. *Cardiovasc Pathol 1996;5:69-76*

Stents as endoluminal splinting devices are increasingly used as an alternative to surgery by providing inner mechanical support for internally stenosed or externally compressed hollow structures, such as the esophagus, trachea, bile ducts, ureters, veins, aorta, and peripheral and coronary arteries. As many of these applications are still in the investigational phase, histopathological examination is an important technique in the evaluation of the short- and long-term merits of this new treatment.

Standard preparatory techniques for evaluation of stented blood vessels, or any other tissue with metal implants, have their limitations, especially when they require the time-consuming removal of the prosthesis prior to embedding. When studying metallic endovascular prostheses (stents), as with any biomaterial implant, it is important to study not only the general tissue reaction, but also the interface between the receiving tissue and the implant surface. Removal of the prosthesis prior to embedding will often result in the loss of the direct stent-tissue interface and cause excessive damage to the tissue in case of complicated designs of the prosthesis (1). In studies of acute reactions to vascular implants this is especially true, as the usually limited amount of adherent cells is often lost. Current methods of histological preparation of implanted biomaterials in hard plastics enable the prostheses to be left in place but warrant the time-consuming preparation of thick sawing sections, sometimes followed by grinding to prepare thinner sections (2,3). Although several techniques are available to stain these sections (4), the quality of the thick as well as the ground sections is not comparable to that of stained paraffin sections. Glycol methacrylate has also been described as an embedding medium for stented arteries (5), but it may not always be hard enough to allow cutting of the vessels while leaving the metal prosthesis in place, and it does not allow for a complete range of stainings to be performed.

This article describes a technique that obviates the need to remove the prosthesis prior to embedding, leaving the interface between the stent and the tissue intact. This is especially important for vascular tissue when studying the inter-

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action between blood and the vessel wall. The technique enables thin sectioning (3–5 μm) while allowing for a whole range of routine and special stains to be carried out with a quality equal to that of paraffin sections and better than that of sawing sections.

Materials and Methods

Vascular implants. Stents of various designs and materials, such as the tantalum Wiktor stent (Medtronic Inc., Minneapolis, MN), the stainless steel Wallstent (Schneider [Europe] AG, Buelach, Switzerland), Palmaz-Schatz stent (Johnson and Johnson Interventional Systems, Warren, PA), polymer stents (9), and polymer-coated metal stents (10) (Figure 1), were implanted in coronary arteries of pigs as previously described (6,7). The pigs were sacrificed at intervals ranging from several hours to months following stent implantation.

Fixation and tissue retrieval. To retrieve the stented arteries, the thorax was opened by a midsternal split, and a lethal dose of sodium pentobarbital was injected intravenously. Thereafter, the ascending aorta was cross-clamped and the aortic root punctured above the coronary ostia for in situ pressure fixation (approximately 150 cm H_2O) with 4% phosphate-buffered paraformaldehyde. Thereafter the coronary arteries were dissected free from the epicardial surface, and the tissue was kept in fixative for at least another 24 hours before methyl methacrylate embedding.

Embedding procedures. The stented coronary arteries with some surrounding tissue were dehydrated in a graded series of ethanol, impregnated in three changes of methyl methacrylate (MMA; E. Merck Nederland B. V., The Netherlands) as described in Table I, and modified from the method of Buijs and Docterom (8). After completion of the MMA impregnation, the specimens were placed in glass vials; then 5 mg/mL perkadox 16 (bis [4-*tert*-butylcyclohexyl] peroxydicarbonate; AKZO Nobel Chemicals, The Netherlands) was added to the MMA as a catalyst for polymerization and mixed well, and the specimens were oriented in the vial as required. Plasticizers were not added. After a one-hour vacuum impregnation to remove any air, the glass vials were carefully closed, and the MMA polymerized overnight in a 37° C oven. As polymerization is an exothermous reaction, the vials containing the specimen were kept in a container with water to prevent overheating. After completion of the polymerization process, the glass vials were broken and the polymerized blocks washed in water.

Sectioning and staining. After the excess MMA was trimmed off, sections were cut on a motor-driven rotary microtome (HM350, Microm GmbH, Munich, Germany) using stainless steel disposable knives (Superlap, Adamas In-

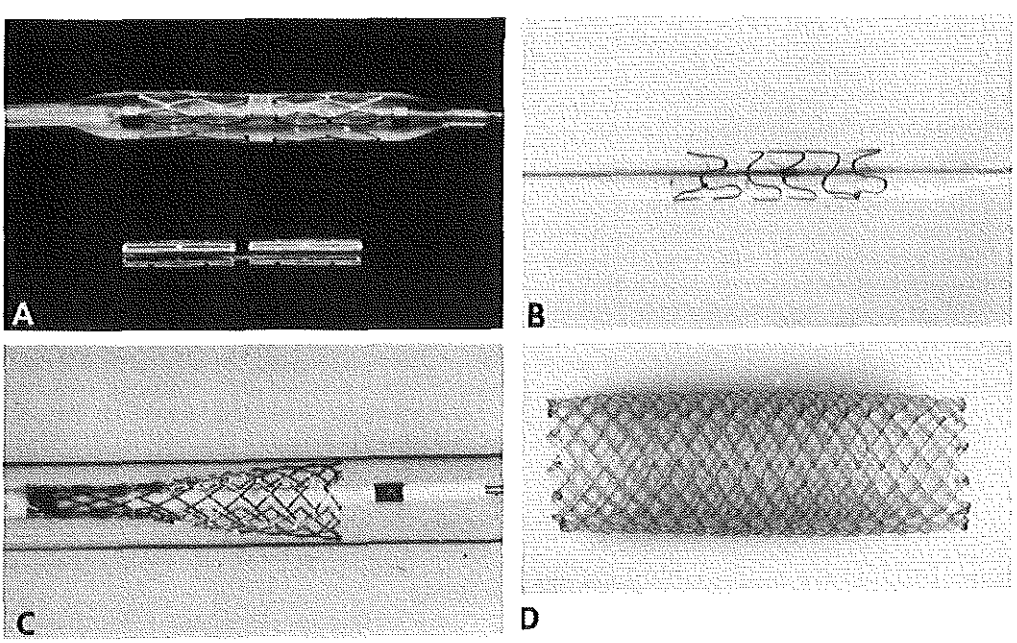


Figure 1. Various types of stents. (A) Palmaz-Schatz stent; (B) Wiktor stent; (C) Wallstent; (D) polymer stent.

Table 1. Schedule for MMA Embedding

2×	80% ethanol	1 h
2×	96% ethanol	1 h
2×	100% ethanol	1 h
1×	100% ethanol	10 h
2×	100% MMA	1 h
1×	100% MMA	5 h
Add 5 mg/mL of perkadox to the MMA, mix		
Vacuum impregnation		1 h
Oven (37°C)		≥16 h

strumenten BV, Leersum, The Netherlands). While keeping the block surface wet, sections 3 to 5 μm thick were cut. On chrome alum coated slides, sections were stretched on a hot plate at 40° C using a mixture of 60% 2-butoxyethanol and 10% ethanol in distilled water. Sections were covered by a plastic film, excess butoxyethanol-ethanol mixture was removed, and the slides were left overnight to dry in a 40° C oven.

Sections were deplasticized in a solution of equal volumes of xylene-chloroform for at least 30 to 60 minutes. Thereafter, standard staining protocols for paraffin sections were carried out, such as Goldner's trichrome stain and resorcin-fuchsin as an elastin stain.

Results

Sectioning and staining. Several different stent materials, such as stainless steel, tantalum, and metal in combination with polymeric material, could be cut without difficulty. The intact MMA-embedded vessels containing the stents were sectioned at several intervals along their lengths. For every tissue level to be studied, approximately 10 sections were cut per stain (to allow for difficulty with stretching and adhering of the sections). Additional dry sections from each level were stored in case further analysis was required. The thickness of the stent wires (70–130 μm) did not pose any problem during sectioning, although the amount of scoring in the tissue sections varied among the different types of metal stents. The average time needed for trimming and sectioning of four tissue levels (i.e., 40 sections) with subsequent stretching and adhering to glass slides is approximately two hours.

Following deplastination, hematoxylin-eosin, resorcin-fuchsin, and Goldner trichrome stainings were carried out according to standard protocols for paraffin sections. The results are illustrated in Figures 2 through 6.

Figures 2 and 3 illustrate paraffin embedding of stented vessels compared with MMA embedding. The intact neointima over the stent wires in Figure 3 is in stark contrast to that in Figure 2B. During sectioning the metal often scores the tissue, but the resulting damage is never such that it interferes with pathological assessment. As the sections are very thin, the metal may often dislodge from the section, leaving a void (Figure 4). However, small amounts of adherent cells, thrombus, and proteinaceous material remain present for evaluation of the blood and tissue response to the implant (Figures 4 and 5). The complicated and intricate design of some stents (e.g., the polymer-coated stents shown in Figures 6A and 6B) means that removal of the stent wires would cause extensive damage to the vessel. Such removal would most certainly involve the removal also of the polymer, with subsequent loss of the delicate interface between the polymer and tissue and the metal and tissue. Such interfaces are well preserved with an MMA embedding technique, while damage to the vessel caused by the operator is kept to a minimum. The cellular detail of the inflammatory response to the polymer is clearly evident in Figure 6B.

Various types of stents and stent materials were all successfully embedded in MMA and sectioned to give clear and detailed results.

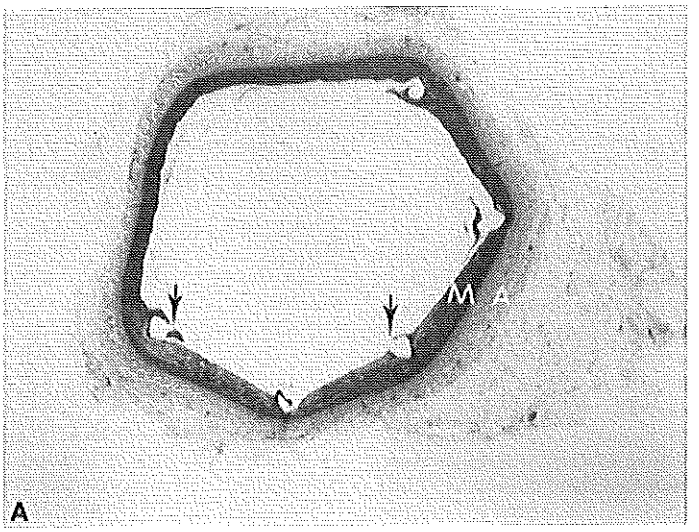
Discussion

Over the last few years there has been a dramatic increase in the use of percutaneous implants, both in patients and in the experimental setting. The desire to achieve the "perfect" implant device has led to the development of numerous stent designs, the short- and long-term effects of which must be assessed prior to patient use. Histology is one of the key techniques to assess these short- and long-term effects.

The behavior of a material as a vascular implant is dictated by the acute and chronic response to blood as well as the response of the vascular tissue to the foreign material. When studying the pathology of blood vessels containing metal or other endovascular prostheses, it is therefore important to study the interface between the prosthetic material and the surrounding tissue. This is especially true in studies of acute reactions to vascular implants, as the prosthesis is not incorporated in the vessel wall and the amount of adherent cells and proteins is often limited. Removal of the prosthesis prior to histological processing usually results in loss of the direct stent-tissue interface.

Implantation trauma can be another determinant for success or failure of an endovascular implant. As removal of the prosthesis often results in tears between the several tissue layers, this makes it difficult to assess whether the observed vascular damage is attributable to the implantation procedure or is a preparation artifact. This is a problem often encountered in the assessment of paraffin sections of stented vessels.

The procedures described in this paper obviate the need to remove the prosthesis prior to histological processing and allow for whole embedding of the arterial segments to be studied. The technique requires no special apparatus, as old automated paraffin tissue processors can easily be converted to MMA processing schedules. The microtome used in this study can also be adapted for paraffin sectioning. This therefore serves a dual function, reducing the need and the cost of having two separate instruments. The quality of the sections (3–5 μm) in terms of the staining, morphology, and architecture of the tissue is equal to that of paraffin sections but is



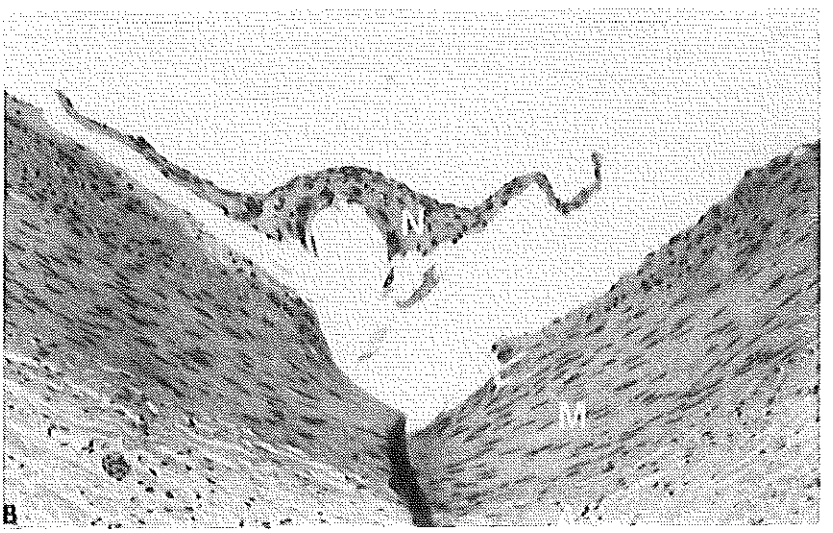


Figure 2. **(A)** View of a paraffin-embedded porcine coronary artery with the stent wires removed. Note the damage to the overlying neointima (arrows). (M = media; A = adventia. Goldner trichrome stain, original magnification ×19.) **(B)** Detail of Figure 2A illustrating tissue damage when the stent wire is removed. The overlying neointima (N) is completely detached from the media (M). (A = adventia. Hematoxylin-eosin stain, original magnification ×287.)

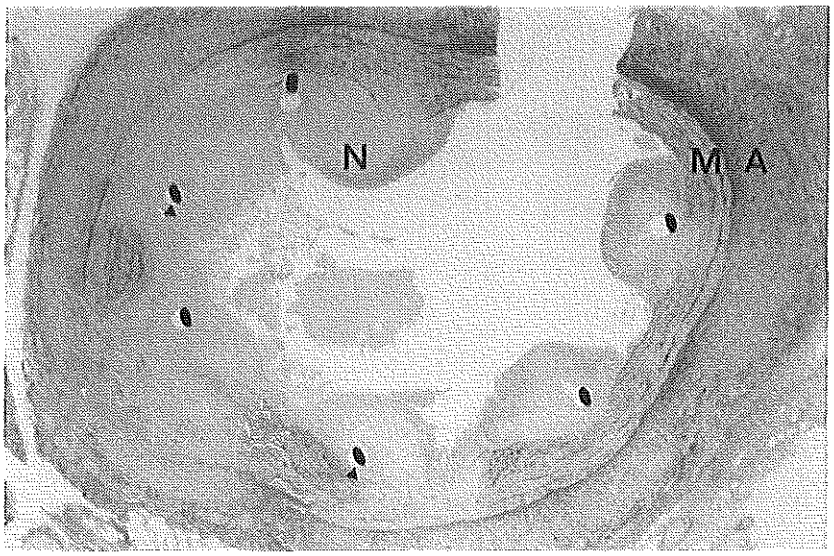


Figure 3. View of an MMA section of a plastic-embedded porcine coronary artery with the metal stent wires (arrowheads) in place and the overlying neointima (N) still intact. (M = media; A = adventitia. Resorcinol-fuchsin stain, original magnification ×29.)

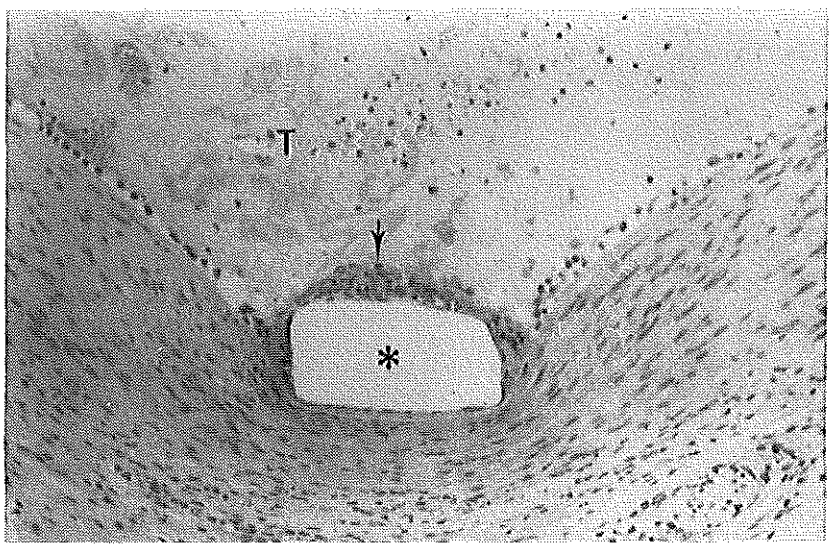


Figure 4. MMA section showing a stent wire void (*) with overlying thrombus (T) and adherent cells (*arrow*) still attached. Such detail is usually lost on removal of the wires prior to paraffin embedding. (Hematoxylin-eosin stain, original magnification ×579.)

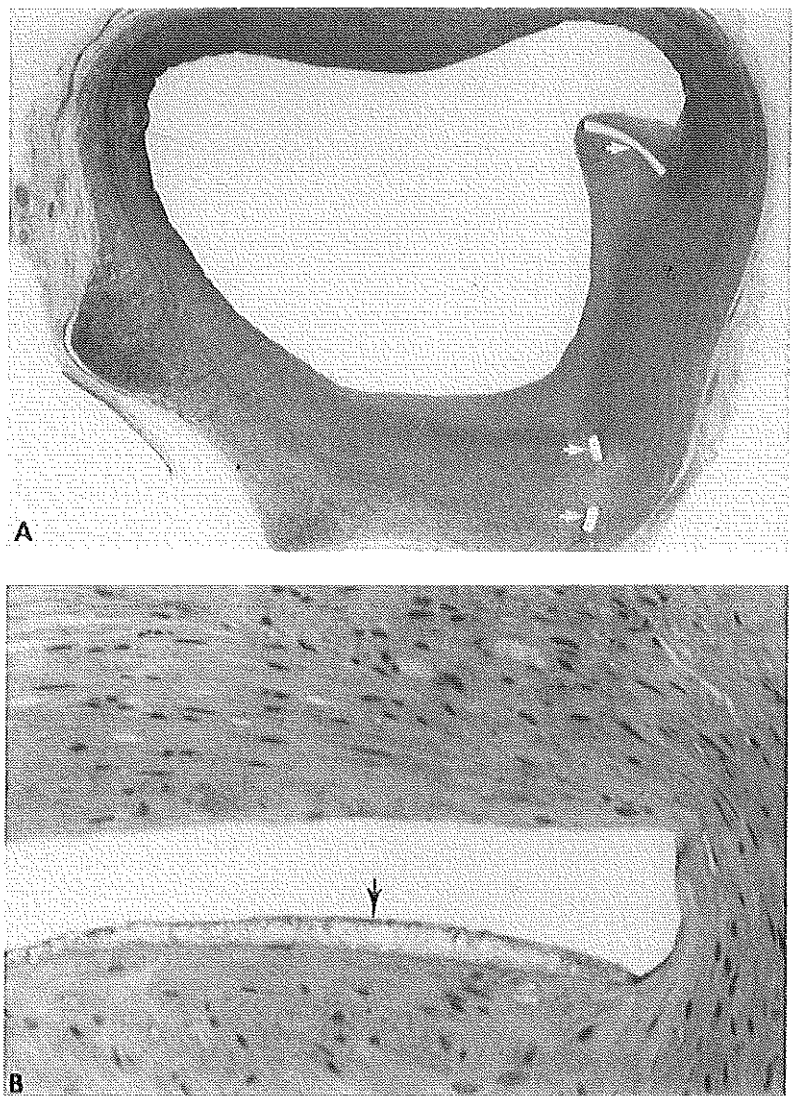


Figure 5. (A) View of an MMA section of a porcine coronary artery with a Palmaz-Schatz stent implant. Stent holes are indicated by the arrows. (Hematoxylin-eosin stain, original magnification ×29.) (B) Detail of Figure 5A showing a stent hole with proteinaceous material (*arrow*) still present. (Hematoxylin-eosin stain, original magnification ×579.)

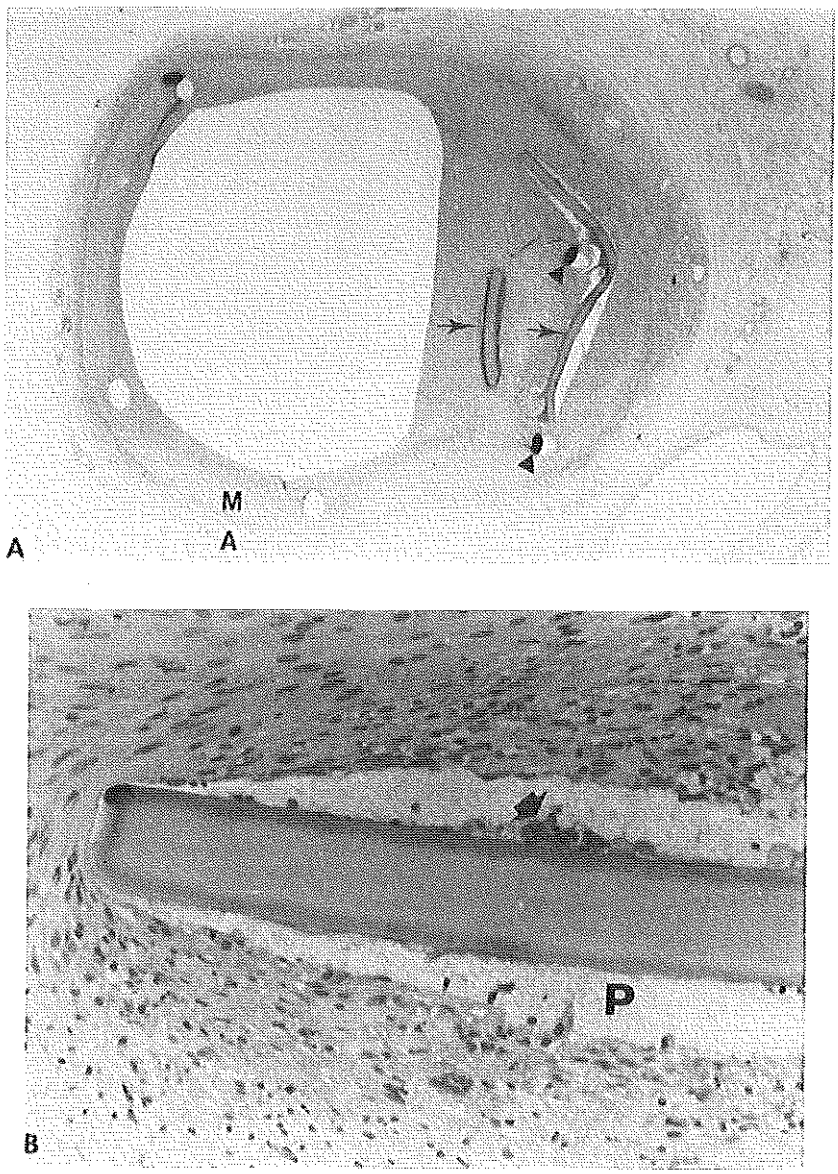


Figure 6. (A) MMA section of a porcine coronary artery with a polymer-coated Wiktor stent implant. The polymer coating (*arrows*) is clearly evident between the stent wires (*arrowheads*). (M = media; A = adventia. Hematoxylin-eosin stain, original magnification ×29.) (B) Detail of polymer with adherent cells (*arrow*) and proteinaceous material (P) still attached. (Hematoxylin-eosin stain, original magnification ×287.)

better than that of sawing sections (10 μm), for which only limited stainings are possible and magnification and resolution at higher power are not possible because of section thickness. The major advantage of this technique over paraffin techniques is that the prosthesis is not removed, allowing the delicate interface between the tissue and prosthesis to remain. It is considered to create less preparation artifact than paraffin processing and is less time consuming than sawing and grinding sections. Although the metal can score the sections to some extent, the resulting damage is never such that it interferes with pathological assessment.

Conclusion

The procedure described in this paper is a rapid and practical method for evaluating the histology of stented blood vessels, allowing a detailed analysis of the tissue reaction to vascular implants while leaving the prosthesis in place.

The authors wish to thank Pieter Derkx, Alex Nigg, and Ton de Jong of the Department of Pathology for their technical assistance and advice during the evaluation of this technique. We would also like to thank the following companies for their sponsorship: Schneider AG, Johnson and Johnson Interventional Systems, and Medtronic Inc.

References

1. Van Beusekom HMM, Van der Giessen WJ, Van Suylen RJ, Bos E, Bosman FT, Serruys PW. Histology after stenting of human saphenous vein bypass grafts: observations from surgically excised grafts 3 to 320 days after stent implantation. J Am Coll Cardiol 1993;21:45–54.
2. Murice-Lambert E, Banford AB, Polger RL. Histological preparation of implanted biomaterials for light microscopic evaluation of the implant-tissue interaction. Stain Technol 1989;64(1):19–24.
3. Van der Lubbe HBM, Klein CPAT, de Groot K. A simple method for preparing thin (10 μm) histological sections of undecalcified plastic embedded bone with implants. Stain Technol 1988;63(3):171–176.
4. Hahn M, Vogel M, Delling G. Undecalcified preparation of bone tissue: report of technical experience and development of new methods. Virchows Arch A Pathol Anat 1991;418:1–7.
5. Carter AJ, Laird JR, Farb A, Kuls W, Wortham DC, Virmani R. Morphologic characteristics of lesion formation and time course of smooth muscle cell proliferation in a porcine proliferative restenosis model. J Am Coll Cardiol 1994;24:1398–1405.
6. Van der Giessen WJ, Serruys PW, van Beusekom HMM, et al. Coronary stenting with a new, radiopaque, balloon-expandable endoprosthesis in pigs. Circulation 1991;83:1788–1798.
7. Van der Giessen WJ, van Beusekom HMM, van Houten CD, van Woerkens LJ, Verdouw PD, Serruys PW. Coronary stenting with polymer coated and uncoated self-expanding endoprostheses in pigs. Coronary Artery Dis 1992;3:631–640.
8. Buijs R, Docterom AA. An improved method for embedding hard tissue in polymethyl methacrylate. Stain Technol 1983;58(3):135–140.
9. Van Beusekom HMM, Van der Giessen WJ, Wagenvoort CA, Van Ingen Schendu DS, Huijts RA, Serruys PW. Histological features of a polymer endovascular prosthesis after transcatheter implantation in porcine arteries. Cardiovasc Pathol 1993; 2(1):41–52.
10. Lincoff AM, Schwartz RS, van der Giessen WJ, van Beusekom HMM, Serruys PW, Holmes DR, Ellis SG, Topol EJ. Biodegradable polymers can evoke a unique inflammatory response when implanted in the coronary artery. Circulation 1992; 86(4);1–801 (Abstr. 3186).

Chapter 3

Foreign body contamination during stent implantation

DM Whelan, HMM van Beusekom, WJ van der Giessen

Cathet. Cardiovasc. Diag. 1997: 40: 328-332

Basic Investigations

Foreign Body Contamination During Stent Implantation

D.M. Whelan, BSc, H.M.M. van Beusekom, PhD, and W.J. van der Giessen,* MD, PhD

The treatment of coronary artery disease using stents has become a widely accepted technique. However, the inadvertent co-implantation of contaminating factors with the stent has received little attention. We studied histological cross-sections of stented porcine coronary arteries and observed contamination of some vessels with surgical glove powder and textile fibres. The contaminating particles were associated with a foreign body reaction. Such a reaction could delay the wound-healing response of a stented vessel and thereby prolong the period in which subacute thrombosis could occur. It is also proposed that air contamination could affect the thrombogenicity of the stent. Appropriate measures should be followed to reduce the chance of contamination occurring. *Cathet. Cardiovasc. Diagn. 40:328–332, 1997.*

Key words: coronary artery; glove powder; textile fibres; coronary angioplasty; stents

INTRODUCTION

The last 15 years have seen the rapid growth of interventional techniques in cardiology, particularly during the last two to three years in stenting. From the first stent implantation in 1986 there has been an exponential increase in the number of patients receiving stents in the clinical setting. However, concerns still exist regarding the problem of subacute thrombosis and the longer-term problem of restenosis. Whereas new stent designs and coatings, along with improved biomaterials and local drug delivery, all aim to improve stent performance, there still exist several basic factors that could further compromise the use of stents. In this paper, which is based on observations made during preclinical stent research, we raise the possibility of the inadvertent introduction of other foreign bodies in conjunction with the stent, i.e., starch granules, textile fibres, and air.

METHODS

Coronary Interventions

The Palmaz-Schatz stent (Johnson & Johnson Interventional Systems, Warren, NJ), the Wallstent (Schneider (Europe) AG, Bülach, Switzerland), and the Wiktor stent (Medtronic Inc., Minneapolis, MN) were implanted into the coronary arteries of cross-bred Landrace X Yorkshire pigs. Such implantations formed the basis of several projects carried out in our laboratory over the last number of years. A detailed description of the implantation and follow-up procedures have previously been described (1,2,3). In brief, under sterile conditions an arteriotomy of the left carotid artery was performed and a 9F introduction sheath was placed. Sodium heparin (10,000 IU) and isosorbide dinitrate (1 mg) were administered, followed by left coronary angiography using the non-ionic contrast Iopamidol. The angiograms were analysed on-line using a quantitative coronary angiography analysis system to allow for precise sizing of the stent. Stent placement was performed as previously described (1). After repeat angiography of the treated coronary arteries, the arteriotomy was repaired and the skin closed in two layers. To assess luminal narrowing within the treated segments, angiography of the treated arteries at follow-up was performed using the same settings of the X-ray equipment as that used during implantation. The implanted stents were studied at varying time intervals from several hours up to 12 weeks.

Histological Analysis

The stented coronary arteries were fixed in 4% phosphate-buffered paraformaldehyde for a minimum of 24

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TABLE I. The Different Animal Studies From Which the Random Sample Was Taken

Stent	No. of animals	No. of stents implanted	No. of animals randomly sampled
Palmaz-Schatz	64	64	15
Wiktor	15	20	14
Wallstent	32	63	17
Total:	111	147	46

TABLE II. Percentage of Starch and Textile Fibre Contamination In the Animal Groups Studied

Animal group	% Starch contamination	% Textile fibre contamination
Total (n = 46)	19.6	6.5
Short-term follow up (n = 12)	41.6	0
Long-term follow up (n = 34)	11.8	8.8

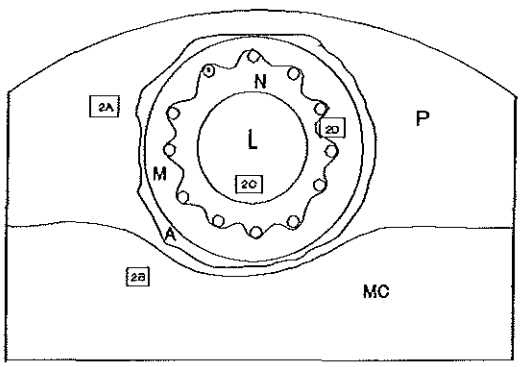

Fig. 1. A schematic representation of a stented coronary artery, indicating the areas from which photographs 2A, 2B, 2C, and 2D are derived. L: lumen; N: neointima; M: media; A: adventitia; MC: myocardium; P: periadventitia; *: stent wire.

hours. For paraffin embedding, the stented vessel was cut into several pieces and the stent wires carefully removed using a pair of fine forceps. For plastic embedding, the stented vessels were embedded intact. The embedding techniques have been described in detail elsewhere (4,5). A total of 125 randomly sampled stained sections of stented coronary arteries from 46 animals were examined for contamination with starch granules and textile fibres (Table 1).

RESULTS

The 46 animals were randomly sampled from a total of 111 animals used in various studies, in which a total of 147 stents had been implanted (Table 1). These 46 animals were divided into two groups: group A consisted of animals with a short-term follow-up (due to stent thrombosis); group B consisted of animals with a longer-term follow-up. Groups A and B showed, respectively, 41.6% and 11.8% starch contamination and 0% and 8.8% textile fibre contamination (Table 2). A schematic representation of a stented artery indicating where contamination was found is shown in Figure 1.

Starch granule deposition in histological sections was identified using polarising microscopy, the granules appearing as birefringent bodies, and illustrating the typical Maltese cross associated with starch. Their presence was also confirmed by positive periodic acid schiff (PAS) staining. Starch granule deposition was observed within the capillary beds of stented vessels. The granules vary in size, but some are so large that they could easily clog the capillary in which they are lodged (Fig. 2A), while others are surrounded by inflammatory cells, most probably macrophages trying to engulf them (Fig. 2B). Granules were also found in the lumen of a stented vessel with adherent proteins and platelets attached (Fig. 2C).

Textile fibre deposition was also observed in stained histological sections by polarizing light microscopy, and was identified as such by their characteristic morphology. We have seen fibres in the vessel lumen with adherent cells, proteins, and platelets attached and pieces of fibres incorporated into the newly formed neointimal layer in sections from chronic experiments (Fig. 2D).

DISCUSSION

While the co-implantation of contaminating factors during stent implantation may seem harmless enough, it is possible that their presence may influence the wound-healing response, i.e., by the development of a foreign body reaction; this may increase the period during which subacute thrombosis can occur.

Starch Granules and Textile Fibres

The literature of the last 40 years or more have reported many cases of starch-granule deposition in tissue samples and the subsequent development of starch granulomatosis. The deposition of starch granules in stented coronary arteries is most likely due to contamination from the operator's gloves during crimping of the stent onto the balloon, or to general handling of the stent/balloon/catheter assembly. A comprehensive review of the hazards of surgical-glove dusting powders was published in 1990 (6). Although conflicting data appear as to whether starch can induce a granulatomous response, or whether the observed granuloma is due to the presence of talc (used as a mould-release agent) on the patient contact surface of the surgical gloves, it is clear that starch will induce a foreign body reaction. The long-term follow-up group showed a smaller incidence of starch contamina-

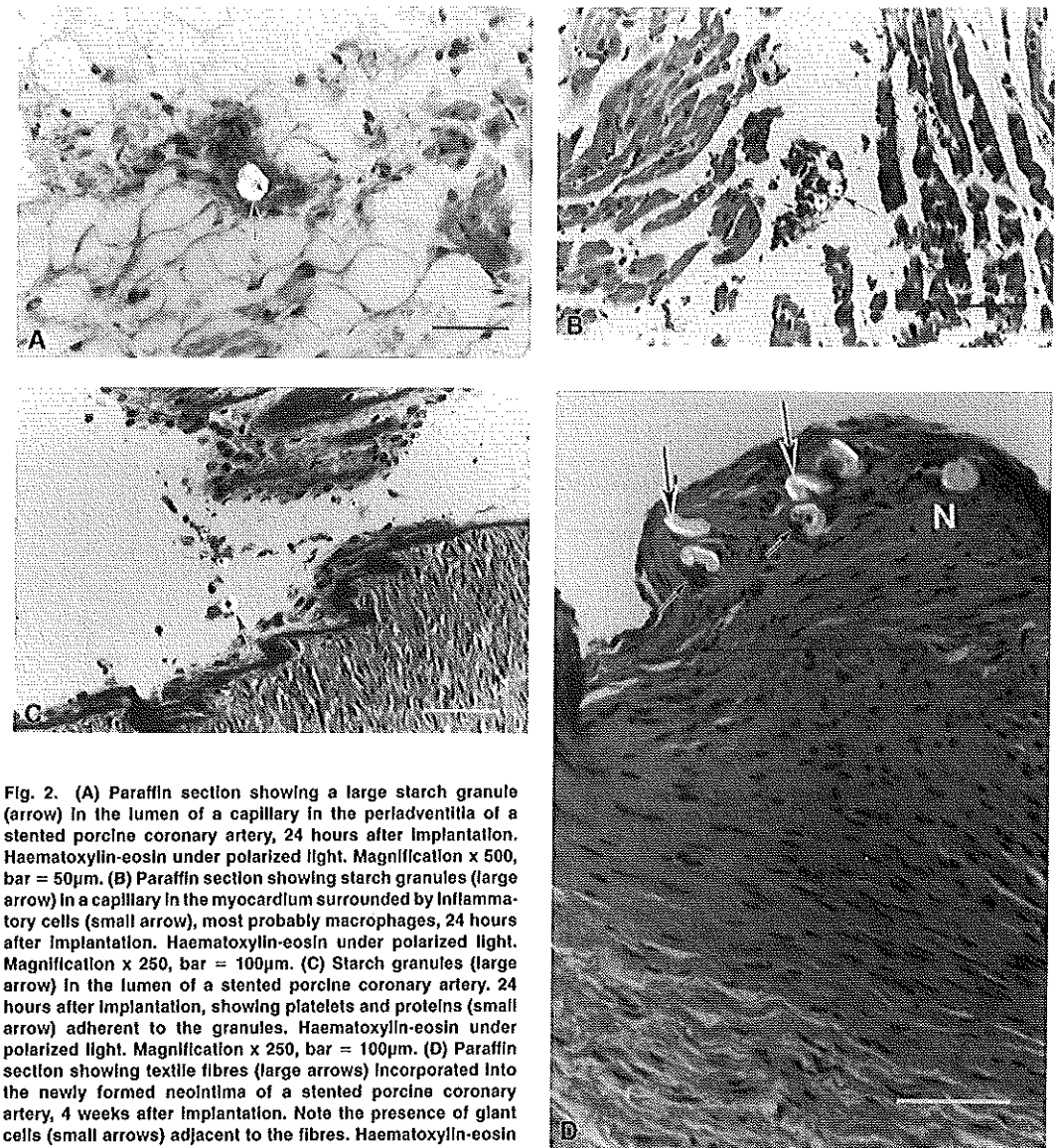

Fig. 2. (A) Paraffin section showing a large starch granule (arrow) in the lumen of a capillary in the periadventitia of a stented porcine coronary artery, 24 hours after implantation. Haematoxylin-eosin under polarized light. Magnification x 500, bar = 50μm. (B) Paraffin section showing starch granules (large arrow) in a capillary in the myocardium surrounded by inflammatory cells (small arrow), most probably macrophages, 24 hours after implantation. Haematoxylin-eosin under polarized light. Magnification x 250, bar = 100μm. (C) Starch granules (large arrow) in the lumen of a stented porcine coronary artery. 24 hours after implantation, showing platelets and proteins (small arrow) adherent to the granules. Haematoxylin-eosin under polarized light. Magnification x 250, bar = 100μm. (D) Paraffin section showing textile fibres (large arrows) incorporated into the newly formed neointima of a stented porcine coronary artery, 4 weeks after implantation. Note the presence of giant cells (small arrows) adjacent to the fibres. Haematoxylin-eosin under polarized light. Magnification x 500, bar = 50μm.

tion compared to the short-term follow-up group. This may be explained by the fact that the starch is eliminated by phagocytosis over a period of time and its presence therefore becomes undetectable.

Contamination of apparatus or instruments with textile fibres, most probably gauze, may occur during the implantation procedure: e.g., during the cleaning off of blood from operators' hands or from guidewires, or when wiping off the balloon before crimping, or during crimping of the stent onto the balloon. Microscopic fibres may adhere to the stent, balloon, or guidewire, and then be carried into the vessel, where they can lodge in the vessel wall.

The deposition of gauze in tissues can also result in granuloma formation. Again the literature contains many examples of granuloma formation; for example, in pa-

tients undergoing gauze reinforcement of berry aneurysm (7). Gauze fibres are known to persist for several weeks after implantation and thereby lead to a long and persistent foreign body reaction.

Talc and textile-fibre contamination were demonstrated in sections at short-term and long-term follow-up. However, it may be necessary to study sections at more frequent time intervals to see the full course of the inflammatory reactions against the contaminants.

It is unclear whether inflammations induced by the presence of contaminants do in fact interfere with the wound-healing response in the clinical setting. However, we believe this to be a possibility for two main reasons: a litany of previously published reports on the complications induced by contaminants after surgical procedures, and the fact that while most subacute thrombosis occurs three to five days post-implant, it has been reported up to 28 days post-implant (8).

Possible contamination with starch granules and textile fibres as a secondary factor of histological processing can be excluded due to the presence of a cellular reaction to the contaminants.

Air

While such contaminating factors as starch granules and textile fibres can be histologically localized, the demonstration of microscopic air bubbles (by angioscopy) suggests that air can also be introduced as a contaminating factor during stent implantation. Microscopic air bubbles can potentially adhere to the guidewire during its introduction into the vessel, or bubbles may be caught on the surface of the stent itself before, or during, flushing with saline. Such inadvertent introduction of air bubbles into the circulation may potentially affect the thrombogenicity of the stent. It is well known that microscopic air bubbles on the surface of materials introduced into the body are potentially thrombogenic through their activation of the complement pathway (9). Should these microscopic bubbles be caught on the stent surface or between the stent struts, they are then highly likely to cause further activation of the complement pathway and thereby contribute to the stent thrombogenicity. If, for example, a heparin-coated stent is being used, microscopic bubbles caught on its surface could then interfere with its function as a non-thrombogenic surface and render its coating useless.

In 1993 Markus et al. (10) described how larger air bubbles can potentially be introduced when the contrast medium is drawn up and then injected. In a recent report it has been suggested that the estimated incidence of total air emboli events during diagnostic and interventional catheterization procedures is approximately 0.3% (11). Such air bubbles can temporarily stop coronary flow with subsequent complications such as myocardial infarction or cardiac arrest. Air emboli are also known to be associated with neurological complications and central nervous system damage post-procedure.

TABLE III. Comparison of Percentages of Starch and Textile Fibre Contamination Using Previous and Revised Implantation Techniques

Animal group	% Starch contamination	% Textile fibre contamination
Previous implantation technique (n = 46)	19.6	6.5
Revised implantation technique (n = 28)	5.6	3.5

The questions still exist—is contamination a real problem in patients, and can air introduction affect the thrombogenicity of the stent? And is contamination a stent-related problem or a procedure-related problem? To satisfactorily address these problems, a controlled study would have to be set up to compare the current implantation technique with a revised technique. Such a revised technique would involve undertaking measures to reduce contamination; these measures could include allowing the contrast medium to stand prior to injection (up to 90% of air is gone after standing for at least 10 minutes); reducing the speed of injection and saline washes; thorough and frequent washing of gloved hands (different methods of hand washing have been suggested [12]); and minimal handling and cleaning of catheters and guidewires, particularly with cotton gauze. Such measures have now been undertaken in this laboratory and indicate that contamination can be reduced (Table 3). It would also be interesting to see whether contamination is affected by different stent designs, such as sheathed versus non-sheathed, or by manual crimping versus automated crimping.

CONCLUSION

From our observations, we conclude that contamination *can possibly* be a problem during stent implantation. While it may be some time yet before our speculations are proved or disproved, in the meantime it would seem prudent to implement measures to reduce the chances of contamination occurring. Prevention is always better than cure!

REFERENCES

1. Härdhammer PA, van Beusekom HMM, Emanuelsson HU, Hofma SH, Albertsson PA, Verdouw PD, Boersma E, Serruys PW, van der Giessen WJ: Reduction in thrombotic events with heparin-coated Palmaz-Schatz stents in normal porcine coronary arteries. Circulation 93(3):423–430, 1996.
2. van der Giessen WJ, van Beusekom HMM, van Houten CD, van Woerkens LJ, Verdouw PD, Serruys PW: Coronary stenting with polymer-coated and uncoated Wallstent self-expanding endoprostheses in pigs. Cor Art Dis 3:631–640, 1992.

3. van der Giessen WJ, Serruys PW, van Beusekom HMM, van Woerkens LJ, van Loon H, Soei LK, Strauss BH, Beatt KJ, Verdouw PD: Coronary stenting with a new radiopaque, balloon-expandable endoprosthesis in pigs. Circulation 83:1788–1798, 1991.
4. Beusekom MHH, van der Giessen WJ, Post JC, Serruys PW, Verdouw PD: Stenting or balloon angioplasty of stenosed autologous saphenous vein grafts in pigs. Am Heart J 127:273–281, 1994.
5. van Beusekom HMM, Whelan DM, van der Plas M, van der Giessen WJ: A practical and rapid method of histological processing for examination of coronary arteries containing metallic stents. Cardiovasc Pathol 5(2):69–76, 1996.
6. Ellis H: The hazards of surgical glove dusting powders. Surg Gynecol Obstet 171:521–527, 1990.
7. Chambi I, Tasker RR, Gentili F, Lougheed WM, Smyth HS, Marshall J, Young I, Deck J, Shrubb J: Gauze-induced granuloma ('gauzoma'): An uncommon complication of gauze reinforcement of berry aneurysms. J Neurosurg 72:163–170, 1990.
8. van Beusekom HMM, Serruys PW, van der Giessen WJ: Coronary stent coatings. Cor Art Dis 5:590–596, 1994.
9. Ward CA, Koheil A, Johnson WR, Madras PN: Reduction in complement activation from biomaterials by removal of air nuclei from the surface roughness. J Biomed Mat Res 18:255–269, 1984.
10. Markus H, Loh A, Israel D, Buckenham T, Clifton A, Brown MM: Microscopic air embolism during cerebral angiography and strategies for its avoidance. Lancet 341:784–787, 1993.
11. Khan M, Schmidt DH, Bajwa T, Shalev Y: Coronary air embolism: Incidence, severity, and suggested approaches to treatment. Cathet Cardiovasc Diagn 36:313–318, 1995.
12. Fraser I: Simple and effective method of removing starch powder from surgical gloves. BMJ 284:1835, 1982.

Chapter 4

Increasing arterial wall injury after long-term implantation of two types of stent in a porcine coronary model

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Increasing arterial wall injury after long-term implantation of two types of stent in a porcine coronary model

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Aims There is increased late loss in luminal diameter following long-term coronary stenting, compared with balloon angioplasty. We evaluated short- and long-term vessel wall injury after experimental implantation of two stent designs as well as balloon angioplasty and their relationship to neointimal hyperplasia.

Methods and Results Wiktor stents and Palmaz-Schatz stents were implanted in normal coronary arteries of pigs (balloon/artery ratio: 0·9–1·1). In control coronary arteries, balloon angioplasty was performed. At 1, 4 and 12 weeks, the vessel injury score, neointimal thickness and inflammatory response were assessed by histology.The vessel injury score increased over time in both Wiktor and Palmaz-Schatz stents: $0·9 \pm 0·1$, $1·5 \pm 0·5$ and $1·7 \pm 0·6$ (mean ± SD) for Wiktor stents and $0·7 \pm 0·2$, $1·0 \pm 0·1$ and $1·2 \pm 0·3$ for Palmaz-Schatz stents at 1, 4 and 12 weeks follow-up, respectively. No increase in injury was seen in balloon angioplasty controls. Inflammation was seen in both stented groups but was absent 12 weeks after balloon angioplasty. No strong correlation between injury and neointimal thickness was apparent.

Conclusion Stents induce chronic injury in contrast to balloon angioplasty. Stent design (coil vs slotted tube) as well as inflammation may influence vessel response.
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Key Words: Stent, coronary arteries, vascular injury, pigs, angioplasty, histology.

Introduction

A greater acute gain in luminal diameter is the mechanism for favourable late restenosis rates in stenting compared to balloon angioplasty[1,2], despite increased late luminal loss[3]. With minimal stent recoil, and remodelling, this late loss is exclusively due to neointimal thickening[4–6], while elastic recoil and remodelling are both major components of restenosis after balloon angioplasty[7–13]. A correlation between neointimal thickening and arterial damage after balloon angioplasty and stenting has been reported[14–18].

Schwartz *et al.*[14] have developed a vessel injury score for stents enabling analysis of arterial damage and neointimal thickening. This score has been validated in porcine coronary arteries 4 weeks after implantation of tantalum coil stents, which were over-sized to create arterial injury. A high correlation was reported between vessel injury score and neointimal thickening. However, creating deep arterial injury by over-sizing will cause a non-specific tissue response which might blur more subtle changes in tissue reaction, making the comparison of different stents difficult. Whether vessel injury score at 4 weeks represents acute damage at implant or includes additional chronic damage by the presence of the stent in the vessel wall cannot be evaluated.

The importance of stent design in arterial injury and neointimal thickening was recently demonstrated by Rogers *et al.* in rabbit iliac arteries[19]. The goal of the present study was therefore to investigate the relationship between coronary arterial wall damage and neointimal hyperplasia in two different stent designs without over-sizing. Furthermore, several time points were studied, from 1 up to 12 weeks. We also evaluated the inflammatory response in each stented segment. To find out whether the observed arterial injury, neointimal thickening and inflammatory reaction are specific features after stenting, or a general healing response

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to the acute implantation trauma, additional animals underwent balloon angioplasty only.

Methods

Animal preparation

Domestic pigs (n=53, weight: 26–46 kg, HVC, Hedel, The Netherlands) underwent the experimental procedures according to the *Guide for the Care and Use of Laboratory Animals*[20], and after approval by the Committee on Experimental Animals of Erasmus University Rotterdam. Experiments were performed as previously described[21]. Briefly, after an overnight fast animals were sedated with ketamine hydrochloride (20 mg . kg^{-1}). Following endotracheal intubation, pigs were mechanically ventilated with 30% oxygen in nitrous oxide. Anaesthesia was maintained with 1–4 vol% enflurane. An intramuscular injection of procaine penicillin G (200 000 I.E./ml) and dihydrostreptomycin sulphate (200 mg per 10 kg body weight) was administered as antibiotic prophylaxis. Arteriotomy of the left carotid artery was performed under sterile conditions and a 9 F introduction sheath was inserted. Heart rate and arterial blood pressure were monitored and arterial blood was sampled to control blood gases and acid-base balance. After administration of 200 IU . kg^{-1} of heparin sodium and 250 mg acetyl salicylic acid, a 9 F guiding catheter was advanced into the ascending aorta. Left coronary angiography was performed using iopamidol (Iopamiro 370, Dagra, Diemen, the Netherlands) as contrast agent after injection of 1 mg of isosorbide dinitrate.

Stents

The Wiktor stent (Medtronic Inc., Minneapolis, Minn., U.S.A.) and the Palmaz-Schatz Coronary Stent (PS 153, Johnson & Johnson Interventional Systems Co., Warren, NJ, U.S.A.), were studied. The Wiktor stent consists of a single tantalum wire (0·127 mm diameter) formed into a sinusoidal wave and wrapped into a helical coil structure[21]. The Palmaz-Schatz Coronary Stent is composed of two segments (7 mm each) of slotted tubes (strut thickness: 0·064 mm), connected by a short (1 mm) coupler[22].

Stent implantation

Coronary angiograms were measured on-line, with a quantitative analysis system using the edge-detection method (CMS, Medis Inc., Nuenen, The Netherlands)[23]. A segment with a mean diameter of approximately 2·5 mm (for 3·0 mm balloon) or 3·0 mm (for 3·5 mm balloon) was selected from the left anterior descending or left circumflex coronary artery. The stent-mounted catheter was advanced to this pre-selected segment over a steerable guide-wire. A single 30 s inflation was performed at 6–8 atmospheres and the maximally inflated contrast-filled balloon was measured to determine the balloon/artery ratio. Angiography was repeated immediately after implantation for assessment of patency and acute result. Finally, the introduction sheath was removed, the carotid artery ligated, the skin closed and the animals were allowed to recover from anaesthesia.

Balloon angioplasty

In two groups of five animals, only balloon angioplasty was performed with a balloon inflation of 30 s at 6–8 atmospheres. The sites of balloon injury were chosen at anatomical landmarks (side branches) which could easily be identified at follow-up.

Follow-up procedure

At 1, 2, 4 or 12 weeks post-implant, animals were anaesthetized as described above. The thorax was opened by a mid-sternal split, and the ascending aorta was cross-clamped after injection of a lethal dose of sodium pentobarbital and fibrillation of the heart with a 9 V battery. Saline (300 ml) was infused, followed by 400 ml of buffered (pH 7·3) formaldehyde under a pressure of 120 mmHg just above the coronary ostia. Finally, the heart was excised, the coronary arteries dissected free from the epicardial surface and the stented or ballooned segments placed in 4% formaldehyde for at least 24 h in preparation for microscopy. After removal of the stent struts, the tissue was processed for paraffin embedding. Haematoxylin-eosin was used as a routine stain while resorcin-fuchsin was used as an elastin stain.

Morphometry and injury score

From each stented or ballooned coronary artery segment, three transverse sections from the proximal, middle and distal part were used for histological analysis. Neointimal thickness was measured on top of the stent struts using a calibrated microscope reticle, as used for standard microscopic measurements. Individual thickness at each stent strut from all three sections was averaged to obtain the mean neointimal thickening per stent. In the ballooned vessels, neointimal thickening was measured at areas of fragmentation of the internal elastic lamina or medial proliferation. The mean for all three sections was taken as mean neointimal thickening.

To evaluate the vessel wall damage caused by the stent, the same elastin stained transverse sections used for morphometry were used for analysis of injury. At each stent strut, damage was quantified by the vessel injury score, according to Schwartz *et al.*[14]. This score

Table 1 Values for vessel injury score according to Schwartz et al.*[14] (with permission)*

Score	Description of vascular injury
0	Internal elastic lamina intact; endothelium denuded; media compressed, not lacerated
1	Internal elastic lamina lacerated; media compressed, not lacerated
2	Internal elastic lamina lacerated, media visibly lacerated, external elastic lamina intact but compressed
3	External elastic lamina lacerated; large lacerations of media extending through external elastic lamina; coil wires sometimes residing in adventitia

grades wall damage from 0 when the internal elastic lamina is intact to 3 when even the external elastic lamina is disrupted (Table 1). Individual scores of the stent struts of the three sections of one stent were averaged to obtain the mean vessel injury score per stented segment. For the balloon angioplasty groups, the vessel injury score cannot be applied because the grading of injury is directly coupled to the presence of a stent strut. Therefore, we graded the injury in the balloon angioplasty groups in analogy to the fracture length method[24], but for a non-over-sized model. Fragmentation of the internal elastic lamina, often accompanied with some degree of medial hypertrophy occurred in one or more areas; it was rare to see one area with a totally ruptured and disintegrated internal elastic lamina. The circumferential lesion length (L_{lesion}) divided by the total circumferential internal elastic lamina length (L_{tot}) was used as a measure of magnitude of damage (L_{lesion}/L_{tot}).

Inflammatory response

Inflammation was assessed in the HE-stained sections corresponding to those used for analysis of vessel injury score and neointimal thickening measurements, according to the following semi-quantitative score: 0: non-existent inflammatory response; 1: inflammatory infiltrates in the adventitia; 2: diffuse, clearly recognizable inflammatory infiltrates in the adventitia; 3: severe, often granulomatous, inflammatory response in the adventitia, sometimes extending to the intima.

Statistical analysis

All data were expressed as mean ± SD. Differences in the balloon/artery ratio, vessel injury score and neointimal thickening between the different stent and balloon groups at the same point in time were evaluated with the non-parametric Wilcoxon Rank Sum Test. A P value $<0{\cdot}05$ (two-tailed) was considered statistically significant. To evaluate differences in vessel injury score within the same groups at different points in time, Kruskal–Wallis one way Analysis of Variance was used. Because of multiple testing, the Bonferroni correction was applied to correct for increasing type I error and significance was stated at the 0·025 level. After curve fitting, regression analysis was used to investigate progression over time of injury response and differences between stent types[25]. Regression analysis was also performed to describe the correlation between vessel injury score and neointimal thickening. (Statistical package: SPSS, release 6·0, SPSS Inc. Chicago, Illinois, U.S.A.).

Results

Systemic haemodynamics and blood gases during intervention

During interventions, heart rate (94 ± 14 beats . min^{-1}, 99 ± 14 beats . min^{-1} and 97 ± 14 beats . min^{-1}) and mean arterial blood pressures (82 ± 17 mmHg, 74 ± 14 mmHg and 73 ± 8 mmHg) were similar for the Wiktor stent, Palmaz-Schatz stent and balloon groups, respectively, while arterial blood gases remained within the normal range (pH: 7·35–7·45; Po_2: 120–160 mmHg; Pco_2: 35–45 mmHg).

Stent implantation

Twenty-one Wiktor stents were placed (one stent per artery) in 16 pigs. Three stented arteries were excluded from final analysis: one stent migrated during implant, a second was erroneously oversized in a small marginal branch, while a third stented animal died suddenly after 23 days without macroscopic evidence of stent occlusion. In total, 18 Wiktor stents were analysed.

Twenty-eight Palmaz-Schatz stents were implanted in 27 animals. Nine stents were excluded from analysis following the death of three animals from arrhythmia during the implantation procedure and six (six stents) from stent thrombosis within 48 h post-implantation. Thrombosis, confirmed by light microscopy, was not accompanied by vascular damage, and was therefore excluded from analysis. In total 19 Palmaz-Schatz stents were analysed.

Angiography during the implantation procedure showed that stents were properly sized, as demonstrated by balloon-artery ratios of 0·9–1·1 (Table 2(a)).

Balloon angioplasty

Ten coronary artery segments in 10 pigs underwent balloon angioplasty with a balloon/artery ratio of $1{\cdot}0 \pm 0{\cdot}1$ (Table 2(b)). Further augmentation of injury up to 12 weeks could not be demonstrated.

At 1 week, the vessel injury score was lower in the Palmaz-Schatz stent group despite a slightly higher mean balloon/artery ratio compared to the Wiktor

Table 2(a) Morphological parameters and balloon/artery ratios of the Wiktor and Palmaz-Schatz stent groups at 1, 4 and 12 weeks follow-up

Follow-up (weeks)	Wiktor Stent					Palmaz-Schatz stent				
	#	VIS	NT μm	Inflammation score	B/A	#	VIS	NT μm	Inflammation score	B/A
1	6	0·9 ± 0·1*	61 ± 10	0·6 ± 0·7	0·9 ± 0·1*	8	0·7 ± 0·2	61 ± 35	1·0 ± 0·9	1·0 ± 0·1
4	7	1·5 ± 0·5	151 ± 50	0·6 ± 0·6	1·1 ± 0·1	5	1·0 ± 0·1	103 ± 13	0·5 ± 0·5	1·0 ± 0·1
12	5	1·7 ± 0·6†	305 ± 155‡	1·6 ± 0·9	1·1 ± 0·1*	6	1·2 ± 0·3‡	198 ± 54†	0·2 ± 0·4	1·0 ± 0·1

Data are mean ± SD; #=number of animals; VIS=vessel injury score; NT=mean neointimal thickness; B/A=balloon/artery ratio. *=$P<0·05$ W vs PS for same time-point; †=$P<0·025$ vs other time-points of same stent design. ‡=$P<0·025$ vs 1 week of same stent design.

Table 2(b) Morphological parameters and balloon/artery ratios of the balloon angioplasty groups at 2 and 12 weeks follow-up

Follow-up (weeks)	(#)	Balloon angioplasty			
		L_{lesion}/L_{tot}	NT μm	Inflammation score	B/A
2	5	0·29 ± 0·29	23 ± 24	0·4 ± 0·5	1·0 ± 0·1
12	5	0·26 ± 0·23	12 ± 15	0 ± 0	1·0 ± 0·1

Data are mean ± SD; #=number of animals; L_{lesion}/L_{tot}=length of fragmented IEL (L_{lesion}) divided by total circumferential IEL length; NT=mean neointimal thickness at L_{lesion}; B/A=balloon/artery ratio.

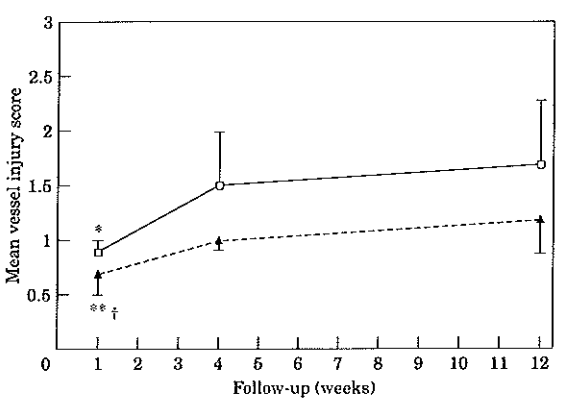

Figure 1 **Progression of vessel wall injury between 1 and 12 weeks for both stent designs. *$P<0·025$ vs 4 and 12 weeks Wiktor; †$P<0·05$ vs 1 week Wiktor; **$P<0·01$ vs 12 weeks Palmaz-Schatz. Linear regression showed a significant progression of vessel injury score between 1 and 12 weeks ($P=0·0004$) as well as a difference in vessel injury score between both stent designs ($P=0·0013$). □=Wiktor; ▲=Palmaz-Schatz.**

stents. Although the increase in the vessel injury score between 1 and 4 weeks in the Palmaz-Schatz stents showed only a trend, the progress of the vessel injury score between 1 and 12 weeks was significant in this analysis ($P=0·0004$) (Fig. 1). In both stent designs linear regression analysis revealed a continued difference in vessel wall injury at follow-up ($P=0·0013$) (Fig. 1). The slope of increase in the vessel injury score over time was not significantly different ($P=0·62$) between the stent types.

After balloon angioplasty, vessel wall damage was very mild, expressing itself at follow-up as

fragmentation of the internal elastic lamina accompanied by an increase in medial thickness. There was no increase in injury from 2 to 12 weeks (Table 2(b)).

Inflammatory response

Table 2(a) shows the inflammation score for both stent groups. Extensive inflammation was not observed in either group, with most scores being <1. The balloon angioplasty vessels showed a very mild inflammatory reaction at 2 weeks, but at 12 weeks the inflammatory response was absent in all vessels studied.

Morphometry

Mean neointimal thickening at the stent wires in the Wiktor stent groups increased from 61 ± 10 μm at 1 week to 151 ± 50 μm at 4 weeks and 305 ± 155 μm at 12 weeks ($P<0{\cdot}025$). In Palmaz-Schatz stented coronary arteries, the neointimal thickening also increased significantly from 61 ± 35 μm at 1 week to 103 ± 3 μm at 4 and 198 ± 54 μm at 12 weeks respectively (Table 2(a)).

Table 2(b) shows that neointimal thickening at 2 weeks after balloon angioplasty was limited (23 ± 24 μm) and significantly less than after stent implantation. There was no progression of neointimal thickening between 2 and 12 weeks.

Correlation between injury and neointimal response

The correlation between vessel injury score and the neointimal thickening was poor in each of the stent groups (Fig. 2). The correlations for all Wiktor stents together ($y=155x-29$, $r=0{\cdot}69$, $P=0{\cdot}001$) and all Palmaz-Schatz stents together ($y=86x+47$, $r=0{\cdot}36$, $P=0{\cdot}14$) were not significantly different from each other. To increase the power of the analysis, all data were pooled. Even then correlation between vessel injury score and neointimal thickening remained weak (Fig. 3; $y=107x+17$, $r=0{\cdot}49$, $P=0{\cdot}002$).

Discussion

Background and purpose of study

The use of stents is increasing exponentially worldwide. However, concerns remain as regards thrombogenicity and vessel wall tissue response to the stent. Tissue response and thrombosis have been strongly related to acute vessel wall damage during the procedure[14,17,18,26]. Stent injury in the porcine model has therefore been used to study restenosis[27–31]. Schwartz *et al.*[14], by oversizing the stent (Wiktor at 4 weeks) and thereby creating deep arterial injury, showed a strong correlation between vessel injury score and neointimal thickening.

Rogers *et al.*[19] were able to reduce vessel wall injury and neointimal thickening after stenting by modifying the geometric configuration of the stent. However, their data derive from peripheral rabbit arteries and are also limited to one time point at 14 days. Colombo *et al.* have emphasized the importance of correct sizing of the stent using high-pressure inflation guided by intravascular ultrasound[32]. Applying these rules of stent deployment, Serruys *et al.* observed no subacute thrombosis and a restenosis rate of only 6% at 6 months after implantation of a heparin coated Palmaz-Schatz stent in 50 patients of the Benestent II pilot trial[33].

We investigated the vessel injury score concept in two different stent designs, at various time points and without deliberately creating deep arterial damage (mean balloon/artery ratio: 0·9–1·1). The data were compared with a control group that underwent balloon angioplasty alone.

Main findings

The major finding of the present study is that in properly sized stents, but not after balloon angioplasty alone, vessel wall injury increases over time. Although neointimal thickening increases concurrently, no strong correlation could be found between vessel injury score and neointimal thickening. In all stent groups the inflammatory response was very mild. However, in contrast to the balloon angioplasty group, inflammation was still visible after 12 weeks and may have influenced the progression of vessel injury score over time.

Acute vs chronic injury

Acute vessel wall damage is caused during the interventional procedure. If this damage is predominantly caused by stretching of the vessel wall, then this damage is comparable in balloon angioplasty and stenting (with a balloon-expandable stent), except for the profile of the stent on the outer surface of the balloon. For the Palmaz-Schatz stent this implies an extra profile of 2 times 64 μm (128 μm), and for the Wiktor stent 2 times 127 μm (254 μm). In stenting a 3·0 mm coronary artery with a 3·0 mm stent-mounted balloon, this would mean at 4 or 8% increase in diameter, respectively. However, in our data this increase in profile was not accompanied by a proportional increase in damage within the given ranges of balloon/artery ratios of 0·9 to 1·1 (± 8%). Stent strut geometry (round vs rectangular) is an additional factor which may modify vessel wall injury caused during stretching[19]. We found a significant lower mean vessel injury score in the Palmaz-Schatz stent compared to the Wiktor stent at 1 week follow-up, which might represent lower acute damage at

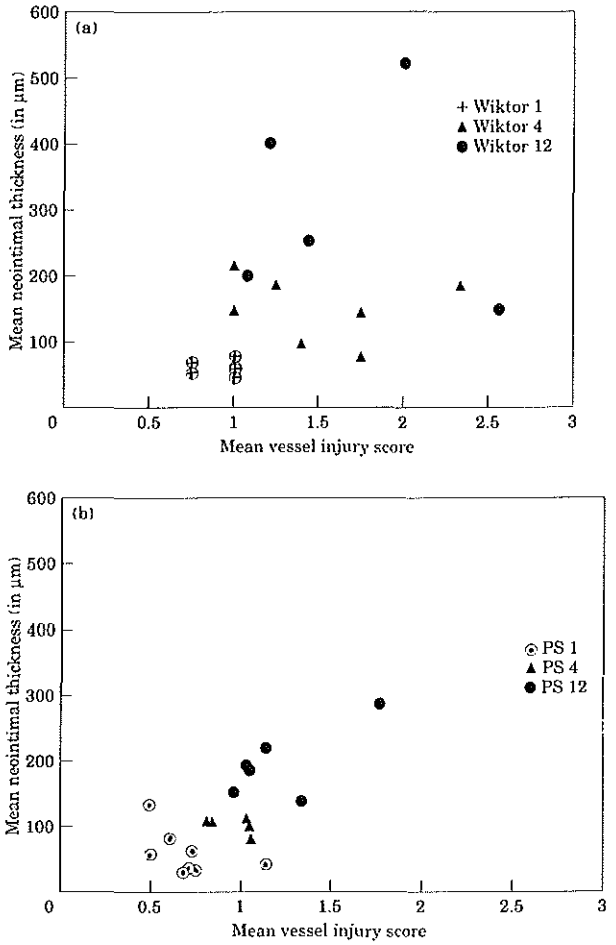

Figure 2 **(a) Correlation between mean vessel injury score and mean neointimal thickness per individual Wiktor stent at 1, 4 and 12 weeks follow-up. No significant correlation can be found in either group. (b) Correlation between mean vessel injury and mean neointimal thickness per individual Palmaz-Schatz (PS) stent at 1, 4 and 12 weeks. No significant correlation can be found in either group.**

implant. Further studies assessing damage directly after implantation might elucidate this further.

Chronic damage was defined as vessel wall damage occurring during follow-up. The present data show a significant increase in damage, as assessed by the vessel injury score, between 1 and 12 weeks post-stenting (P=0·0097). This was probably caused by the continued presence of the stents, as chronic damage was not observed after balloon angioplasty alone. Unfortunately, it was not possible to use the same injury scoring system in stented and ballooned arteries. However, we feel that the large difference in outcome in this study allows for the above conclusion.

Possible implications of chronic injury

In this study, the progressive damage caused by the stent in the first weeks after stenting may act as a direct stimulus for smooth muscle cell proliferation through the release of several growth factors and chemotactic agents from the damaged cells[34 37]. However, the

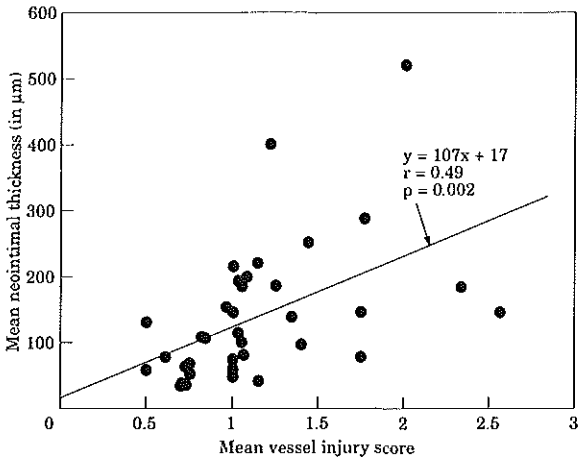

Figure 3 **Correlation between mean vessel injury score and mean neointimal thickness plotted for both stents in all pigs. (y=107x+17, r=0·49, *P*=0·002).**

resultant neointimal thickening is probably not influenced by damage alone as no strong correlation was evident between vessel injury score and neointimal thickening in the present study. Our data indicate that the largest increase in vessel injury score occurs between 1 and 4 weeks, while the largest increase in neointimal thickening occurs between 4 and 12 weeks.

The weak correlation between vessel injury score and neointimal thickening may also be due to the very low mean neointimal thickenings (151 µm for Wiktor and 103 µm for Palmaz-Schatz, both at 4 weeks). These values, however, are comparable to other studies by our group[21,38]. It is unlikely that an increase in the number of experiments in this study will result in a better correlation, as pooling all the data did not show a better correlation (Fig. 3). Therefore, in the present study, damage is probably only one of the contributing factors to the resultant neointimal thickening. The study by Schwartz *et al.* indicated that damage played a more important role than in ours, but this does not contradict our results. Their model was characterized by immense acute damage, resulting in neointimal thicknesses of up to 1400 µm.

Rogers *et al.*[19] have shown that the difference in geometry or surface characteristics between stents may be important in relation to the injury inflicted to the vessel wall. Our results show that this effect is most pronounced during the first weeks after stenting.

The role of inflammation

In all groups, mild inflammation was seen in the first weeks. Although a correlation between inflammation score and vessel injury score or neointimal thickening could not be observed, the persisting mild inflammatory response in the stent groups at 12 weeks may have contributed to the progression of vessel wall injury, as this was not observed in the group who received balloon angioplasty alone. Theoretically, persistent inflammation may have facilitated the increased morphological injury by allowing deeper stent strut penetration into the vessel wall, resulting in a higher vessel injury score. Furthermore, by releasing growth factors and cytokines, inflammatory cells may also influence neointimal thickening and chronic endothelial dysfunction[39].

Study limitations

Our first time point of follow-up was chosen at one week. We are aware that this does not represent true acute damage at implant. However, histological assessment of acute injury requires removal of stent struts from 'freshly' injured vessel wall tissue, which may induce more handling damage than removal after several days to weeks. After one week, a measurable neointima is present, which data could be included in this analysis.

In the Wiktor stent group, no early stent thrombosis was seen while six Palmaz-Schatz stents thrombozed in the first 48 h post implantation, causing the death of the animals. These six Palmaz-Schatz stents were not included in the analysis, because increased vessel wall damage could not be found in either of these cases and therefore bias was not likely to be introduced.

In this study, non-atherosclerotic coronary arteries of juvenile pigs were stented. Vessel wall injury and tissue response may be different when stenting atherosclerotic lesions. However, in the pig model, hypercholesterolaemic diets or endothelial abrasion before stenting do not significantly change the tissue

response[31]. Moreover, our model has extensively been used in pre-clinical stent testing, and it seems valid to assess vessel wall injury, tissue response, thrombotic response and restenosis in the same model.

Conclusions

This study shows progression of vessel wall injury up to 12 weeks after stenting, but not after balloon angioplasty alone. Different stent designs cause different degrees of acute injury and this difference persists at longer follow-up.

As (mild) inflammatory response was persistent in the stent groups in contrast to balloon angioplasty, this may be important in influencing progression of vessel wall injury subsequent to mechanical injury caused by stenting. In this study, no strong correlation between injury score and neointimal hyperplasia could be demonstrated.

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References

[1] Serruys PW, de Jaegere P, Kiemeneij F *et al.* for the BENESTENT Study Group. A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. N Engl J Med 1994; 331: 489–95.

[2] Fischman DL, Leon MB, Baim DS *et al.* for the Stent Restenosis Study Investigators. A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease.. N Engl J Med 1993; 331: 496–501.

[3] Jaegere de PPT, Hermans WR, Rensing BJ, Strauss BH, Feyter de PJ, Serruys PW. Matching based on quantitative coronary angiography as a surrogate for randomized studies: Comparison between stent implantation and balloon angioplasty of native coronary artery lesions. Am Heart J 1993; 125: 310–9.

[4] Painter JA, Mintz GA, Chin Wong S *et al.* Serial intravascular ultrasound studies fail to show evidence of chronic Palmaz-Schatz stent recoil. Am J Cardiol 1995; 75: 398–400.

[5] Mintz GS, Pichard AD, Kent KM *et al.* Endovascular stents reduce restenosis by eliminating geometric arterial remodeling: A serial intravascular ultrasound study (Abstr). J Am Coll Cardiol 1995; Febr: 36A.

[6] Gordon PC, Gibson CM, Cohen DJ, Carrozza JP, Kuntz RE, Baim DS. Mechanisms of restenosis and redilation within coronary stents-Quantitative angiographic assessment. J Am Coll Cardiol 1993; 21: 1166–74.

[7] Rensing BJ, Hermans WRM, Beatt KJ *et al.* Quantitative angiographic assessment of elastic recoil after percutaneous transluminal coronary angioplasty. Am J Cardiol 1990; 66: 1039–44.

[8] Hjemdahl-Monsen CE, Ambrose JA, Borrico S *et al.* Angiographic patterns of balloon inflation during percutaneous transluminal coronary angioplasty: role of pressure-diameter curves in studying distensibility and elasticity of the stenotic lesion and the mechanism of dilation. J Am Coll Cardiol 1990; 16: 569–75.

[9] Rensing BJ, Hermans WR, Strauss BH, Serruys PW. Regional differences in elastic recoil after percutaneous transluminal coronary angioplasty: a quantitative angiographic study. J Am Coll Cardiol 1992; 17: 34B–38B.

[10] Hanet C, Wijns W, Michel X, Schroeder E. Influence of balloon size and stenosis morphology on immediate and delayed elastic recoil after percutaneous transluminal coronary angioplasty. J Am Coll Cardiol 1991; 18: 506–11.

[11] Hermans WRM, Rensing BJ, Strauss BH, Serruys PW. Methodological problems related to the quantitative assessment of stretch, elastic recoil, and balloon-artery ratio. Cathet and Cardiovasc Diagn 1992; 25: 174–85.

[12] Post MJ, Borst C, Kuntz RE. The relative importance of arterial remodeling compared with intimal hyperplasia in lumen renarrowing after balloon angioplasty. Circulation 1994; 89: 2816–21.

[13] Lafont A, Guzman LA, Whitlow PL, Goormastic M, Cornhill JF, Chisolm GM. Restenosis after experimental angioplasty: Intimal, medial and adventitial changes associated with constrictive remodeling.. Circ Res 1995; 76: 996–1002.

[14] Schwartz RS, Huber KC, Murphy JG Edwards WD, Camrud AR, Vlietstra RE, Holmes DR. Restenosis and the proportional neointimal response to coronary artery injury: Results in a porcine model. J Am Coll Cardiol 1992; 19: 267–74.

[15] Carter AJ, Laird JR, Farb A, Kufs W, Wortham DC, Virmani R. Morphologic characteristics of lesion formation and time course of smooth muscle cell proliferation in a porcine proliferation restenosis model. J Am Coll Cardiol 1994; 24: 1398–405.

[16] Waller BF, Orr CM, Pinkerton CA, VanTassel JW, Pinto RP. Morphologic observations late after coronary balloon angioplasty: Mechanisms of acute injury and relationship to restenosis. Radiology 1990; 174: 961–7.

[17] Ellis SG, Muller DWM. Arterial injury and the enigma of coronary restenosis. J Am Coll Cardiol 1992; 19: 275–7.

[18] Beatt KJ, Serruys PW, Luijten HE *et al.* Restenosis after coronary angioplasty: the paradox of increased lumen diameter and restenosis. J Am Coll Cardiol 1992; 19: 258–66.

[19] Rogers C, Edelman ER. Endovascular stent design dictates experimental restenosis and thrombosis. Circulation 1995; 91: 2995–3001.

[20] Guide for the Care and Use of Laboratory Animals. Office of Science and Health Reports (DRR/NIH). 1985. Department of Health, Education and Welfare publication no. NIH 85-23.

[21] van der Giessen WJ, Serruys PW, van Beusekom HMM *et al.* Coronary stenting with a new, radiopaque, balloon-expandable endoprosthesis in pigs. Circulation 1991; 83: 1788–98.

[22] Schatz RA, Palmaz JC, Tio FC, Garcia d, Reuter SR. Balloon-expandable intracoronary stents in the adult dog. Circulation 1987; 76: 450–7.

[23] Reiber JHC, Serruys PW, Kooijman CJ *et al.* Assessment of short-, medium- and long-term variations in arterial dimensions from computer-assisted quantification of coronary cineangiograms. Circulation 1985; 71: 280–8.

[24] Waksman R, Robinson KA, Crocker IR, Gravanis MB, Cipolla GD, King III SB. Endovascular low-dose irradiation inhibits neointima formation after coronary artery balloon injury in swine. A possible role for radiation therapy in restenosis prevention. Circulation 1995; 91: 1533–9.

[25] Glantz S, Slinker B. Primer of Applied Regression and Analysis of Variance. New York: McGraw-Hill, 1990.

[26] Lam JYT, Chesebro JH, Steele PM *et al.* Deep arterial injury during experimental angioplasty: Relation to a positive indium-111-labeled platelet scintigram, quantitative platelet deposition and mural thrombosis. J Am Coll Cardiol 1986; 8: 1380–6.

[27] Karas SP, Gravanis MB, Santoian EC, Robinson KA, Anderberg KA King III SB. Coronary intimal proliferation after balloon injury and stenting in swine: an animal model of restenosis. J Am Coll Cardiol 1992; 20: 467–74.

[28] Steele PM, Chesebro JH, Stanson AW *et al.* Balloon angioplasty: Natural history of the pathophysiological response to injury in a pig model. Circ Res 1985; 57: 105–12.

[29] Bonan R, Paiement P, Scortichini D, Cloutier MJ, Leung TK. Coronary restenosis: Evaluation of a restenosis injury index in a swine model. Am Heart J 1993; 126: 1334-40.

[30] Schwartz RS, Murphy JG, Edwards WS, Camrud AR, Vlietstra RE, Holmes DR. Restenosis after balloon angioplasty: A practical proliferative model in porcine coronary arteries. Circulation 1990; 82: 2190-200.

[31] Grinstead WC, Rodgers GP, Mazur W *et al.* Comparison of three porcine restenosis models: the relative importance of hypercholesterolemia, endothelial abrasion, and stenting. Cor Art Dis 1994; 5: 425-34.

[32] Colombo A, Hall P, Nakamura S *et al.* Intracoronary stenting without anticoagulation accomplished with intravascular ultrasound guidance. Circulation 1995; 91: 1678-88.

[33] Serruys PW, Emanuelsson H, Van der Giessen W *et al.* Heparin-coated PalmaZ-Schatz stents in human coronary arteries. Early outcome of the Benestent-II pilot study. Circulation 1996; 93: 412-22.

[34] Liu MW, Roubin GS, King III SB. Restenosis after coronary angioplasty: potential biologic determinants and role of intimal hyperplasia. Circulation 1989; 79: 1374-87.

[35] Baird A, Ling N. Fibroblast growth factors are present in the extracellular matrix produced by endothelial cells in vitro: implications for a role of heparinase-like enzymes in the neovascular response. Biochem Biophys Res Commun 1987; 142: 428–35.

[36] McNeil PL, Muthukrishnan L, Warder E, D'Amore PA. Growth factors are released by mechanically wounded endothelial cells. J Cell Biol 1989; 109: 811-22.

[37] Lindner V, Lappi DA, Baird A, Majack RA, Reidy MA. Role of fibroblast growth factor in vascular lesion formation. Circ Res 1991; 68: 106-13.

[38] Hårdhammar PA, van Beusekom HMM, Emanuelsson HU *et al.* Reduction of thrombotic events using heparin-coated Palmaz-Schatz stents in normal porcine coronary arteries. Circulation 1996; 93: 423-30.

[39] Van Beusekom HMM, Hofma SH, Whelan DMC, Verdouw PD, van der Giessen WJ. Stents but not balloon angioplasty induce chronic neointimal permeability (Abstr). J Moll Cell Cardiol 1995; 27: A98.

Chapter 5

Long-term endothelial dysfunction is more pronounced after stenting than after balloon angioplasty in a porcine coronary model

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Long-Term Endothelial Dysfunction Is More Pronounced After Stenting Than After Balloon Angioplasty in Porcine Coronary Arteries

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Objectives. To compare percutaneous transluminal coronary angioplasty (PTCA) and stent implantation with respect to the long-term changes they induce in the newly formed endothelium in porcine coronary arteries by studying both morphological and functional parameters of the endothelium at 2 weeks and 3 months after intervention.

Background. Problems affecting PTCA or stent implantation have been overcome to a large extent by means of better techniques and the availability of new drugs. Late problems, however, still exist in that restenosis affects a large number of patients. With an increasing number of patients being treated with stents, the problem of in-stent restenosis is of even greater concern, as this seems difficult to treat. A functional endothelial lining is thought to be important in controlling the growth of the underlying vascular tissue. We hypothesized that the enhanced neointimal hyperplasia observed after stenting is associated with a more pronounced and prolonged endothelial dysfunction.

Methods. Arteries were analyzed using a dye-exclusion test and planimetry of permeable areas. Thereafter, the arteries were processed for light and scanning electron microscopy for assessment of morphology and proliferative response.

Results. Leakage of the endothelium for molecules such as Evans blue-albumin as well as prolonged endothelial proliferation is observed as late as 3 months after the intervention, and is more pronounced after stenting. Permeability is associated with distinct morphologic characteristics: endothelial retraction, the expression of surface folds, and the adhesion of leukocytes.

Conclusions. Stenting especially decreases long-term vascular integrity with respect to permeability and endothelial proliferation, and is associated with distinct morphologic characteristics.

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Problems affecting percutaneous transluminal coronary angioplasty (PTCA) or stent implantation such as acute closure, stent thrombosis, or bleeding complications due to the stringent anticoagulation protocols from the early days of stent use have been overcome to a large extent by means of better techniques and the availability of new drugs (1–4). Late problems, however, still exist in that restenosis affects approximately 15–20% of patients after primary stenting and 30–50% after PTCA alone. With an increasing number of patients being treated with stents (up to 50%), the problem of in-stent restenosis is of even greater concern as this seems difficult to treat.

Every intervention aimed at increasing lumen size inevitably leads to damage of the vessel wall. The subsequent healing response, necessary to pacify the inflicted wound, triggers the growth of a neointimal thickening (NI). Excessive growth of this NI is only one of the contributors to restenosis after PTCA, but is likely the sole responsible factor when dealing with in-stent restenosis. PTCA mechanically damages the endothelial cells and induces endothelial dysfunction that persists for several weeks both in the laboratory animal and in patients (5–7). A functional endothelial lining is important in controlling the growth of the underlying vascular tissue (8), and it may well be that the enhanced neointimal hyperplasia observed after stenting is associated with a more pronounced and prolonged period of endothelial dysfunction.

The objective of the present study was, therefore, to compare PTCA and stent implantation with respect to the long-term changes they induce in the newly formed endothelium in porcine coronary arteries. We therefore studied both morphological (as assessed by general pathology, morphometry, histochemistry, electron microscopy [EM]) and functional parameters of the endothelium (barrier function and proliferative status).

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Abbreviations and Acronyms
BrdU = bromodeoxy uridine
EB = Evans blue
EM = electron microscopy
LM = light microscopy
NI = neointimal thickening
PTCA = percutaneous transluminal coronary angioplasty

Methods

Animal care. Experiments were performed under the regulations of the animal care committee of the Erasmus University Rotterdam and in accordance with the "Guide for the Care and Use of Laboratory Animals" (9).

Animal preparation. Experiments were performed in Yorkshire pigs (25–30 kg; HVC). After an overnight fast, the animals were sedated with 20 mg/kg ketamine hydrochloride. After induction of anesthesia with thiopental (12 mg/kg) and after endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2 [vol/vol]). Anesthesia was maintained with 0.5–2.5 vol% isoflurane. Antibiotic prophylaxis was administered by an intramuscular injection of 1,000 mg of a mixture of procaine penicillin-G and benzathine penicillin-G.

Under sterile conditions, an arteriotomy of the left carotid artery was performed and a 9-F introduction sheath was placed. Then 10,000 IU heparin sodium were administered followed by left coronary angiography using the nonionic contrast agent iopamidol (Iopamiro 370) after intracoronary administration of 1 mg isosorbide dinitrate.

Coronary interventions. From the angiograms (analyzed on-line using a quantitative coronary angiography analysis system), arterial segments of 2.5–3.5 mm in diameter were selected in the left anterior descending and/or left circumflex coronary arteries. Typically, balloon sizes were chosen 0.2–0.5 mm larger than the recipient artery. The stents (PS 153, Palmaz-Schatz Coronary Stent; JJIS, and Wiktor stent; Medtronic) were placed as described before (10,11). PTCA was performed in a similar way, using identical inflation parameters. After repeat angiography of the treated coronary arteries, the guiding catheter and the introducer sheath were removed, the arteriotomy was repaired, and the skin was closed in two layers. The animals were then allowed to recover from anesthesia.

Experimental groups and follow-up. Interventions were performed in four groups of animals, as shown in Table 1. In groups 1 and 2 (a subset of animals from a previously published study [10]) the animals received a Palmaz-Schatz stent only and were followed for 4 and 12 weeks, respectively, to assess: 1) the "molecular window," i.e., to which extent the barrier function of the endothelial lining was impaired, and 2) whether this "window" of permeability changed in time. In groups 3 and 4, both PTCA and stent implantation were performed in each animal. These animals were followed for 2 and 12 weeks to assess the morphologic determinants correlating with the impaired barrier both in morphologically immature and mature endothelium, and to study differences between the two types of intervention.

Assessment of cell proliferation. To assess the proliferative response to stent implantation and PTCA in comparison with control coronary arteries, five animals each in groups 3A, 3B, 4A, and 4B were given three intramuscular injections of BrdU (Sigma Chemical Co.) at 100, 50, and 50 mg/kg at 8-h intervals, starting 24 h before sacrifice. Using light microscopy (LM), the total number and number of BrdU-positive cells were counted for each section in several high-power fields both proximal and distal in the treated arteries. The right coronary artery served as a control.

Assessment of intimal permeability at follow-up. In this test (Fig. 1, dye-exclusion test) Evans blue (EB) (Sigma Chemical Co.) was used in two configurations (12,13).

EB-albumin. To subject the arteries to the large molecular marker (70 kD), 300 mL of EB in saline (0.3% [wt/vol]) was administered intravenously, to allow for EB binding to albumin. The infusion was given for 30 min, and then 1 h was allowed for recirculation of the EB-albumin complex.

Binding control. During and after the EB infusion, arterial blood samples were taken, proteins precipitated with trichloric acid (final concentration 20%), and then spun down to check the supernatant for unbound dye.

EB-saline. To subject the arteries to the small molecular marker (1 kD), 300 mL of EB in saline (0.3% [wt/vol]) was administered directly into the coronary circulation after a saline flush. After completion of the EB infusions, the coronary arteries were flushed with approximately 300 mL saline

Table 1. Study Groups

Group	Intervention	Follow-up	n	Molecular Weight	Study Objective
1	Palmaz-Schatz stent	4 weeks	10	1 and 70 kD	Window of permeability
2	Palmaz-Schatz stent	12 weeks	10	1 and 70 kD	Window of permeability
3A	Wiktor stent	2 weeks	9	70 kD	Morphologic determinants
3B	PTCA	2 weeks	9	70 kD	Morphologic determinants
4A	Wiktor stent	12 weeks	5	70 kD	Morphologic determinants
4B	PTCA	12 weeks	5	70 kD	Morphologic determinants

n = number of arteries.

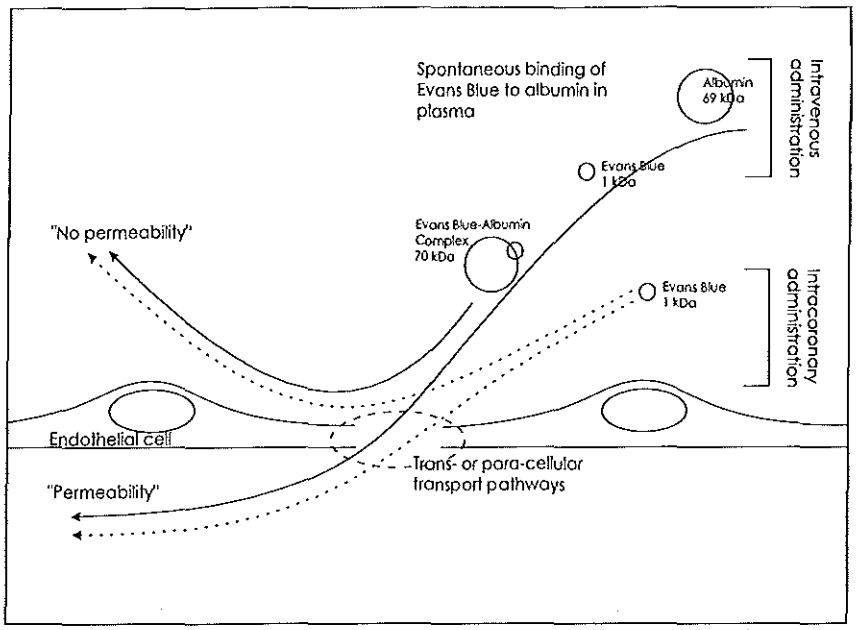

Figure 1. EB can be administered both intravenously and intracoronarily. Intravenous administration results in the spontaneous binding of EB to albumin, and subjection of the arterial wall to the 70-kD large complex. Intracoronary administration after a saline flush to remove serum proteins results in subjection of the arterial wall to the smaller 1-kD molecule. Blue staining of the arterial wall indicates a breach in the luminal barrier.

before pressure fixation in situ (approximately 100 mm Hg) with 500 mL 4% buffered formaldehyde. Then the heart was excised, and the treated and control coronary arteries (not treated with balloon or stent) were dissected from the epicardial surface.

Macroscopic assessment. The excised treated and control coronary arteries were opened longitudinally and checked under a dissection microscope for penetration of the blue dye. The arteries were documented on film and used for planimetric analysis of the permeable areas. Thereafter, both proximal and distal areas of the specimen were divided for EM and LM.

Routine histology. To check for abnormal vascular reactions to the interventions and for a general assessment of the histological appearance, all specimens were processed for routine histology as described before (10). Sections were stained with hematoxylin-eosin as a routine stain and resorcin-fuchsin as a collagen and elastin stain.

Histochemistry. *Lectin- and immunocytochemistry.* This was performed to confirm the identity of the endothelium and smooth muscle cells as described before (14).

Detection of BrdU incorporation. After acid DNA denaturation and elimination of endogenous tissue peroxidase activity, rehydrated paraffin sections were exposed to mouse anti-BrdU antibody (Becton Dickinson & Co.), dilution 1:80, to detect BrdU-positive cells. As a second antibody, HRP-labeled rabbit anti-mouse antibody (Dakopatts) was used, with 670 μg/ml Di Amino Benzidine (Sigma Chemical Co.) in phosphate-buffered saline as a detecting reagent.

Morphometry. Intimal and medial thickness was determined along the length of the treated arterial segments. A distinction was made between intimal and medial thickness within and outside the PTCA lesion area and the media underneath or between the stent struts. Data were analyzed using elastin-stained sections and assessed on a microscopy image analysis system (Impak C, Clemex vision Image analysis system; Clemex Technologies Inc.) as described before (11). In addition, lesion length was determined in the arteries treated with PTCA, which was defined as the percentage of the internal elastic lamina containing discontinuities or associated with an intimal and/or medial thickening (15).

Scanning and transmission EM. To study endothelial morphology (scanning EM) and to assess endothelial cell-cell contact (transmission EM), selected tissues were fixed with 2.5% glutaraldehyde in 0.15 M cacodylate buffer, postfixed with 0.1 M cacodylate buffer containing 1% OsO_4 and 50 mM ferricyanide ($K_3[Fe\{CN\}_6]$), and further processed as described before (18). Specimens were examined in a JSM25 scanning electron microscope (Jeol Ltd.) and a CM100 transmission microscope (Philips).

Statistical analysis. Analysis was performed using Sigmastat (versions 1.0 and 2.0, Jandel Scientific). Data are given as mean ± standard deviation. Morphometry was analyzed with a

Table 2. QCA Assessment of Arterial and Balloon Diameter at the Site of Intervention

Group	Pre	Balloon	Ratio	Post	Fu
1 (n = 10)	3.05 ± 0.28	2.98 ± 0.38	0.95 ± 0.07	3.06 ± 0.33	2.37 ± 0.39
2 (n = 10)	3.11 ± 0.21	3.03 ± 0.30	0.97 ± 0.06	3.02 ± 0.17	3.12 ± 0.27
3A (n = 9)	2.87 ± 0.27	3.02 ± 0.41	1.02 ± 0.09	2.74 ± 0.29	2.65 ± 0.28
3B (n = 9)	2.95 ± 0.31	2.85 ± 0.32	0.95 ± 0.05	2.66 ± 0.34	2.79 ± 0.48
4A (n = 5)	2.61 ± 0.46	2.92 ± 0.39	1.08 ± 0.06	2.67 ± 0.30	2.80 ± 0.25
4B (n = 5)	2.58 ± 0.25	2.65 ± 0.42	1.04 ± 0.06	2.28 ± 0.28	3.08 ± 0.42

Data are in mm and given as mean ± SD. Pre, Post, Fu: mean coronary lumen diameter at the site of intervention before, directly after, and at follow-up, respectively. Balloon: mean diameter of the contrast filled balloon during maximal inflation. Ratio: balloon to artery ratio.

one-way ANOVA, the planimetry and angiography with a one-way repeated measures ANOVA, and followed by an all-pairwise comparison in case of statistical significance using a Student Neuman Keuls or Tukey test. A p value of <0.05 was considered statistically significant.

Results

Procedural outcome. A total of 39 animals were enrolled in the study. In group 3, one animal died suddenly within 1 h after the procedure due to stent thrombosis. In group 4, four animals died: one animal died during the procedure due to ventricular fibrillation, one died within a few hours after the procedure due to stent migration and subsequent stent thrombosis, one animal due to respiratory problems during recovery from anaesthesia, and one animal died at 3 weeks after the procedure ex causa ignota (not stent related). The remaining 34 animals were used for analysis, as summarized in Table 1. Quantitative angiographic measurements are shown in Table 2. Quantitative coronary angiography (QCA) confirmed that all stent and balloon sizes closely matched coronary artery vessel size with a balloon-artery ratio between 0.95 and 1.1.

Intimal permeability. Binding control confirmed that complete binding of EB to the albumin was achieved.

The "window" of permeability (groups 1 and 2). Macroscopy (Fig. 2A and B) revealed that both the 1- and 70-kD markers were able to stain the vessel wall at 4 as well as at 12 weeks post stenting. This indicates that there is a wide window of permeability that does not change during the first 3 months.

The "extent" of permeability (all groups). Except for occasional small areas distal to side branches, where the endothelium is often subject to hemodynamic stress, the control arteries did not reveal staining (Fig. 2A). Both stent- and balloon-treated arteries, however, did reveal a distinct staining of the vessel wall (Fig. 2B–D). In the stented arteries staining of the intima was generally observed over the stent wires, while between the stent struts a much lower level of staining was seen (Fig. 2B and C). Both the Wiktor stent and the Palmaz-Schatz stent, however, revealed a specific staining pattern. In the Palmaz Schatz stent staining was seen over the stent struts in the area of the stent ends and in the area of the coupler (Fig. 2B), while in the Wiktor stents staining was observed over the wire along the whole length of the stent (Fig. 2C). These patterns did not change during the observation period. In group 3B (2 weeks after PTCA alone) there were also areas that revealed staining albeit less prominently (Fig. 2D). In group 4B (12 weeks after PTCA) the pattern was the same but the staining intensity was less.

Planimetry. Planimetry of the area permeable to EB at 2 weeks showed that the percentage was 35.4 ± 19.7% for the stented arteries ($p < 0.05$ vs balloon and control), 10.1 ± 6.8% for the PTCA treated arteries, and 2.5 ± 2.2% for the control arteries.

Electron microscopy and the endothelial barrier function. Both scanning and transmission EM were performed on groups 3 and 4. It confirmed that, in contrast to normal endothelium (Fig. 3A), at 2 weeks after either stent implantation or PTCA the endothelial covering was still incomplete. The areas covering the stent struts especially showed missing cells, often in association with adhesion of leukocytes and platelets. These areas were highly permeable to the EB dye. The more diffusely permeable areas were characterized by an endothelial layer where the cells appeared "retracted" (Fig. 3B). Transmission EM showed small or nonexistent intercellular junctional complexes (Fig. 3C) in the areas with retracted cells. Twelve weeks after the interventions, scanning EM showed that the endothelial covering was complete in all groups, and transmission EM now showed more extensive junctional complexes even with tight junctions (Fig. 3D). Permeability was now associated with a different phenomenon, namely an endothelial layer with adhesion of leukocytes with a rounded morphology that were also often seen penetrating the endothelium as well as surface folds (Fig. 3E), which indicates an increased endocytotic activity, although endothelial retraction was still observed occasionally.

Morphometry and assessment of cell proliferation. Morphometric analysis is summarized in Table 3. At 2 weeks after stenting (group 3A) there is a trend ($p > 0.05$) toward a larger NI over the stent struts than after PTCA at the site of the lesion (group 3B). At 12 weeks both the Wiktor stent (group 4A) and the Palmaz-Schatz stent (group 2) do induce a statistically significant larger NI than after PTCA. Also, the Wiktor stent induces a significantly more pronounced NI as compared with the Palmaz-Schatz stent. Morphometric analysis of the medial layers show that the stents significantly impress the media, whereas at the site of the PTCA lesion the media has thickened. At 4 and 12 weeks (groups 1, 2, and 4A) the media has thickened between the stent struts and is now

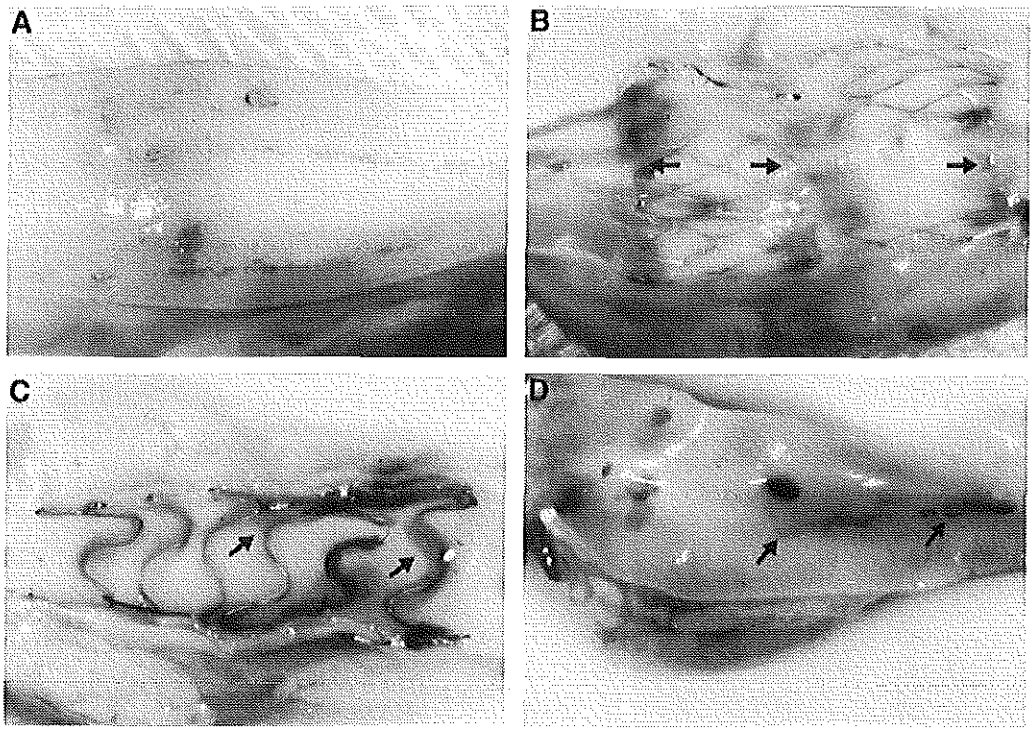

Figure 2. Macroscopy of the dye-exclusion test. A, Control right coronary artery, shows a clean surface with occasional small blue areas. B, Group 2, Palmaz-Schatz stent, 1 kD. For both molecular weights, blue staining is found mainly at the stent ends and in the area of the coupler (arrows). C, Group 3A Wiktor stent. Blue staining is seen especially in the region of the intimal tissue covering the stent struts (arrows). D, Group 3B, PTCA. Randomly stained areas (arrows) are found in the arterial segment treated with PTCA and are more apparent and intense in group 3B than 4B.

similar to the lesion area in the balloon-treated arteries at 2 and 12 weeks.

For assessment of cell proliferation, the BrdU-positive and total number of cells were counted in several sections. For each artery this amounted to 200–400 endothelial cells; 200–400 (balloon group) and 1500–5500 (stent group) intimal cells; 1000–4000 medial and adventitial cells. The percentage of BrdU incorporation is summarized in Table 4, and shows that at 2 weeks after intervention the stent (group 3A) induced a higher percentage of BrdU incorporation as compared with PTCA in all tissue layers ($p < 0.05$). In the PTCA vessels (both lesion area and nonlesion area) there was a trend ($p > 0.05$) toward a higher percent proliferating cells as compared with control (not injured) values. Twelve weeks after intervention the levels of BrdU incorporation had decreased in all groups. Only the endothelium overlying the stent struts was still significantly higher as compared with control values ($4.8 \pm 2\%$; $p < 0.05$).

Microscopy. There were no adverse or unexpected vascular reactions to the interventions as performed in groups 1–4, and in general the tissue response was as described before (10,11). In short, the stented arteries in groups 1, 2, and 4 were covered by a variable intimal thickness consisting of smooth muscle cells in a collagenous matrix and covered by endothelium. Inflammatory reactions were limited for groups 1 and 2. In group 4A, there were areas where the stent strut lacerated the media, a phenomenon associated with a diffuse inflammatory response. At 2 weeks (group 3A), the stent was embedded in a mass of organizing thrombus, containing leukocytes and macrophage giant cells, and was covered by an incomplete layer of endothelial cells. There was medial hyperplasia (medial thickening and longitudinal orientation of smooth muscle cells) in association with BrdU incorporation between the stent struts.

PTCA. At 2 weeks after PTCA (group 3B), there were focal areas with a limited amount of neointima, which consisted of smooth muscle cells in a collagenous matrix. In these areas we observed fragmentation of the Lamina Elastica Interna (LEI) and significant medial hyperplasia in association with incorporation of BrdU (Fig. 4A). These lesions encompassed approximately 30% of the measured circumference. The endothelial covering (as confirmed by lectin histochemis-

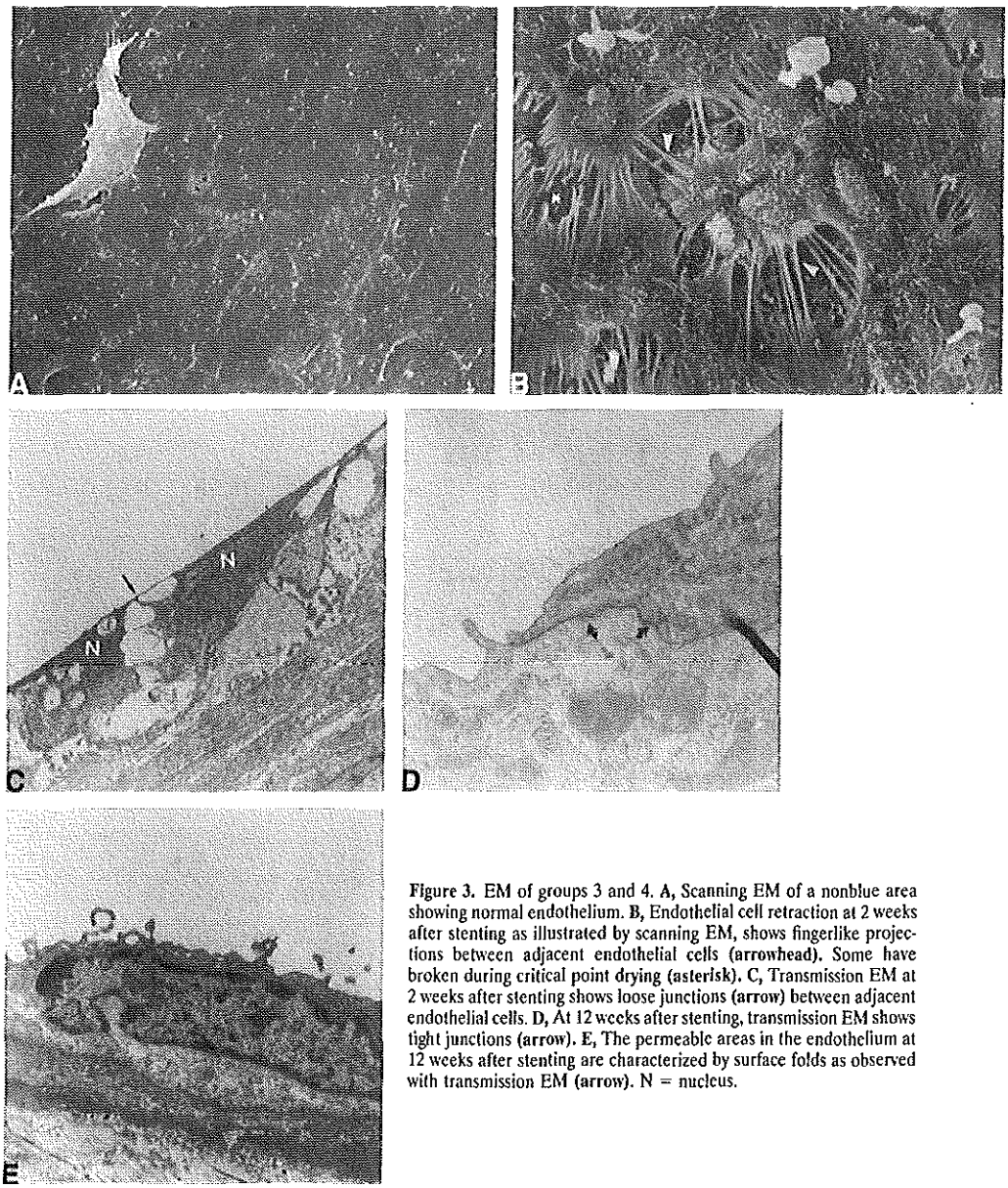

Figure 3. EM of groups 3 and 4. A, Scanning EM of a nonblue area showing normal endothelium. B, Endothelial cell retraction at 2 weeks after stenting as illustrated by scanning EM, shows fingerlike projections between adjacent endothelial cells (arrowhead). Some have broken during critical point drying (asterisk). C, Transmission EM at 2 weeks after stenting shows loose junctions (arrow) between adjacent endothelial cells. D, At 12 weeks after stenting, transmission EM shows tight junctions (arrow). E, The permeable areas in the endothelium at 12 weeks after stenting are characterized by surface folds as observed with transmission EM (arrow). N = nucleus.

try) was incomplete and had a variable morphology. At 12 weeks after PTCA (group 4B), there was still a limited amount of intimal hyperplasia. Although the media was still thickened in these areas and the lesions still encompassed approximately 30% of the measured circumference, there was no incorporation of BrdU, nor a clear fragmentation of the LEI. On the contrary, we often observed an additional internal elastic membrane under the newly formed endothelium (Fig. 4B). The adventitia was unremarkable.

Discussion

In contrast to restenosis after PTCA, which is dictated both by constrictive remodeling and tissue growth (16,17), resteno-

Table 3. Morphometry

Group	NI	Media	
	Stent or PTCA Lesion	Underneath PTCA Lesion or Stent Struts	Between Stent Struts or PTCA Lesion/Nonlesion
1 (stent)	259 ± 104*3a,3b,4b	99 ± 22	187 ± 28*3a,3b,4a
2 (stent)	192.1 ± 63.3*3b,4b	109.8 ± 21.6	184.3 ± 16.3*3a,3b,4b
3A (stent)	114 ± 60	71 ± 40	122 ± 26
3B (PTCA)	33 ± 11	221 ± 77*1,2,3a,4a	119 ± 15
4A (stent)	305 ± 155*3a,3b,4b	73 ± 29	182 ± 29*3a,3b,4b
4B (PTCA)	19 ± 12	181 ± 53*1,3a,4a	128 ± 33

*$p < 0.05$ vs group . . ., ANOVA-Tukey test. Data are in μm and given as mean ± SD. The NI was measured on top of the stent struts and compared with the NI in the PTCA lesion. Media thickness was measured underneath and between the stent struts and compared with the media thickness in the PTCA lesion and nonlesion area.

sis after stenting is the result of tissue growth alone. With increasing numbers of patients being treated with stents (up to 50%), the problem of in-stent restenosis is becoming more and more of a problem, as this seems more resistant to effective treatment.

Vascular dysfunction and in particular endothelial dysfunction has been described after PTCA both in humans and animals (5–8). As one of the first changes in the etiology of atherosclerosis, endothelial dysfunction might also be involved in the ongoing tissue growth after angioplasty procedures (8,18). The aim of our study, therefore, was to investigate endothelial function after stenting and compare this with PTCA alone by assessing both morphologic and functional parameters.

The main finding in our study is that both PTCA and stent implantation result in an impairment of the vascular barrier function at least up to 3 months after the procedure as evidenced by the uptake of the EB dye. This loss of barrier function is more pronounced after stenting than after PTCA, and showed a stent-specific pattern. This breach was characterized by specific endothelial morphologic correlates: in the early phase by incomplete endothelialization and endothelial retraction or loose intercellular connections. The late phase was characterized by the expression of surface folds and the adhesion of leukocytes. Both phenomena were also observed in stented human vein grafts (19).

Transport routes across the endothelial lining. The extravasation of (macro)molecules, such as EB bound to albumin, proceeds mainly by two routes. One is through diffusion via the cellular junctions, i.e., paracellular exchange (20,21), and the other is by vesicle-mediated transport, i.e., transcellular transport (22).

Paracellular exchange is morphologically associated with small interendothelial gaps caused by contractile forces in the cell and by disintegration of cell–cell junctions (23,24). This process is regulated by actin fibers, which are connected to other proteins anchoring the cells to their neighbors and to the extracellular matrix (25,26). Vasoactive agents and thrombin can affect the integrity of the endothelium through phosphorylation of specific target proteins. As a consequence, actin reorganization may occur through RhoA– and protein kinase C–activated pathways. The interaction between actin and nonmuscle myosin, activated by phosphorylation of the myosin light chain, subsequently causes contraction and gap formation (24,27). Interaction of leukocytes with the endothelium, which is enhanced by inflammatory mediators, can enhance this response (28). In addition to the effects of vasoactive agents, paracellular permeability can also be enhanced by the lingering proliferative response of the endothelium itself. Cell retraction associated with cell mitosis causes paracellular gaps in arterial endothelial cells in vivo (29). Because cell division is found until at least 3 months after intervention, particularly in areas overlying stent struts, it may contribute to the observed leakage of EB-albumin complex. Both endothelial barrier function and proliferation are under control of the extracellular matrix. Remodeling of the extracellular matrix by proteases can re-

Table 4. Assessment of Cell Proliferation (% BrdU-Positive Cells)

Group (n)	EC	NI	M	ADV
Control (n = 6)	0.4 ± 0.1	Nonexistent	0.9 ± 0.1	1.07 ± 0.1
3A (n = 6)	22.2 ± 7.5*	18.8 ± 2.7*	6.5 ± 1.2*	7.9 ± 2.1*
3B: lesion area (n = 5)	5 ± 3.8	6.14 ± 8.2	2.66 ± 2.62	3.98 ± 4.41
3B: non-lesion area (n = 6)	2.64 ± 2.65	Nonexistent	0.86 ± 0.43	0.98 ± 0.78
4A (n = 5)	3.5 ± 1.1	2.7 ± 1.1	1.6 ± 1	2.3 ± 1.1
4B: lesion area (n = 4)	1.3 ± 1.5	1.2 ± 0.91	1.5 ± 0.67	0.75 ± 0.76
4B: non-lesion area (n = 5)	0.3 ± 0.6	Nonexistent	0.99 ± 0.91	0.64 ± 0.54

EC = endothelial cells; M = media; ADV = adventitia. *$p < 0.05$ vs. all groups, ANOVA-student neuman keuls test. Data are mean ± SEM.

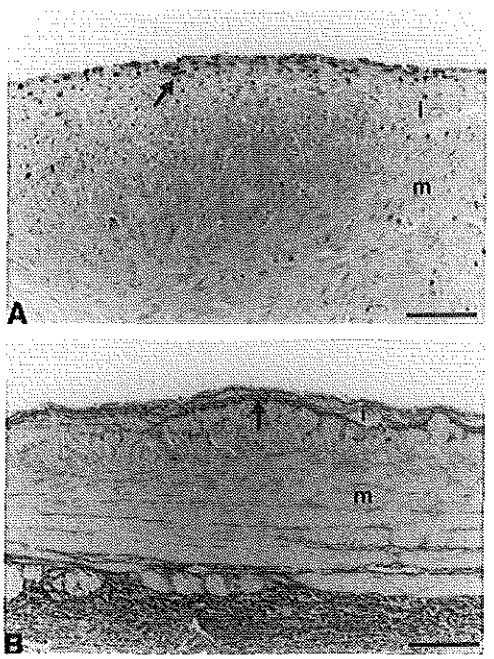

Figure 4. LM. A, Group 3B. At 2 weeks after PTCA, focal lesions can be found with BrdU incorporation (arrow), especially underneath the endothelial lining but also elsewhere in the intima (i) and media (m). HRP-DAB with hematoxylin counterstain. Bar = 50 μm. B, Group 4B. At 12 weeks after PTCA, there is still a limited NI, sometimes with an additional elastic membrane underneath the endothelium (arrow). Resorcin-Fuchsin, i = intima, m = media. Bar = 50 μm.

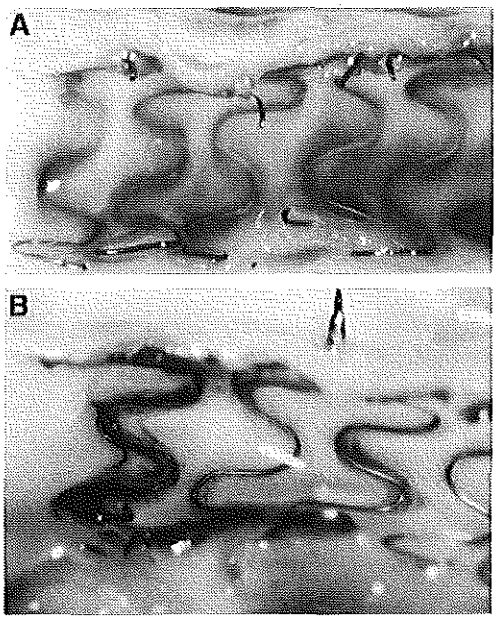

Figure 5. Macroscopy c-AMP-treated arteries. Macroscopy of the coronary arteries at 2 weeks after implantation of a Wiktor stent. While a "control" artery (A) was not exposed to db-c-AMP, but only to EB, the c-AMP-treated artery was first exposed to 1 mM db-c-AMP and then to EB with a molecular weight of 1 kD (B), showing an improvement especially in the areas between the stent struts.

duce the firm interaction between cell and matrix. This reduction may enhance the efficacy of endothelial permeability-increasing agents (25,30).

Transcellular (vesicle-mediated) transport is the second major route for macromolecular exchange across the endothelial lining. Both paracellular and transcellular exchange of albumin are increased by VEGF (31), a growth factor that is induced in injured arteries (32,33). Further studies have to elucidate whether and for how long VEGF is induced in stented coronary arteries.

In vivo (34,23) and in vitro studies (35,36) have shown that an increase in endothelial permeability in the microcirculation can be reversed by exposing the endothelium to cAMP-elevating agents. In preliminary experiments we found that intracoronary administration of 1 mM dibutyryl-cAMP within 10 min indeed partly normalizes the barrier function in areas of increased permeability covering and adjacent to the implanted stents (Fig. 5). The areas that were permeable because of missing endothelial cells were not affected by this treatment. A reduced cellular cAMP concentration/content has been reported in arterial cells in intimal tissue affected by atherosclerosis (37). However, it remains to be established whether this also occurs in the new layer of endothelial cells in stented arteries. The reduction in endothelial permeability by elevation of cellular cAMP levels indicates that a major part of the leakage of EB-albumin occurs via impaired junctional complexes between endothelial cells. However, the current state of knowledge does not permit exclusion of a minor contribution by vesicular transport.

Permeability after PTCA takes place preferentially in the area designated as the lesion area containing intimal hyperplasia. Clearly these are areas where the endothelium is attached to a changed basement membrane or extracellular matrix. In the stented segments, the intima covering the stent struts is also constantly changing during the process of scar maturation, which might explain permeability over the stent struts in general but not the differences between stent designs. Whereas the Wiktor stent shows preferential dye-uptake over all of the stent wires throughout the whole stent, the Palmaz-Schatz stent shows dye-uptake preferentially over the stent struts at both stent extremities and over the area of the coupler. It would seem that these specific patterns of dye-uptake are a reflection of the design of the stent. The Wiktor stent has a more open design than the Palmaz-Schatz stent, and as permeability seems to occur in areas where theoretically movement between the tissue and the stent struts may occur,

this may be a factor influencing the chronic vascular irritation by the stents. From the literature it is known that mechanical instability of healing bone fractures, for instance, affects the composition of the extracellular matrix with respect to the level of sulfate incorporation in the glycosaminoglycans (38). If these processes also take place in the vessel wall it would certainly influence endothelial function and permeability.

Conclusions. This study indicates that especially stenting decreases long-term vascular integrity with respect to permeability. Leakage is observed with molecules such as EB and EB-albumin complex, and is associated with prolonged endothelial proliferation and distinct morphologic characteristics such as endothelial retraction, the expression of surface folds, and the adhesion of leukocytes.

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References

1. Colombo A, Ferraro M, Itoh A, et al. Results of coronary stenting for restenosis. J Am Coll Cardiol 1996 Oct;28:830–6.
2. Karrillon GJ, Morice MC, Benveniste E, et al. Intracoronary stent implantation without ultrasound guidance and with replacement of conventional anticoagulation by antiplatelet therapy. 30-day clinical outcome of the French Multicenter Registry. Circulation 1996;94:1519–27.
3. Serruys PW, Emanuelsson H, van der Giessen W, et al. Heparin-coated Palmaz-Schatz stents in human coronary arteries. Early outcome of the Benestent-II Pilot Study. Circulation 1996;93:412–22.
4. Lincoff AM, Califf RM, Anderson KM, et al. Evidence for prevention of death and myocardial infarction with platelet membrane glycoprotein IIb/IIIa receptor blockade by abciximab (c7E3 Fab) among patients with unstable angina undergoing percutaneous coronary revascularization. EPIC Investigators. Evaluation of 7E3 in preventing ischemic complications. J Am Coll Cardiol 1997;30:149–56.
5. Shimokawa H, Flavahan NA, Shepherd JT, Vanhoutte PM. Endothelium dependent inhibition of ergonovine-induced contraction is impaired in porcine coronary arteries with regenerated endothelium. Circulation 1989; 80:643–50.
6. Weidinger FF, McLenachan JM, Cybulsky MI, et al. Persistent dysfunction of regenerated endothelium after PTCA of rabbit iliac artery. Circulation 1990;81:1667–79.
7. McLenachan JM, Vita J, Fish DR, et al. Early evidence of endothelial vasodilator dysfunction at coronary branchpoints. Circulation 1990;82:1169–73.
8. Badimon L, Badimon J, Penny W, et al. Endothelium and atherosclerosis. J Hyperten 1992;10(Suppl 2):43–50.
9. NIH publication 85-23, revised 1985.
10. Hårdhammar P, van Beusekom HMM, Emanuelsson HU, et al. Reduction of thrombotic events with heparin-coated Palmaz-Schatz stents in pigs. Circulation 1996;93:423–30.
11. Van der Giessen WJ, Serruys PW, Van Beusekom HMM, et al. Coronary stenting with a new, radiopaque, balloon expandable endoprosthesis in pigs. Circulation 1991;83:1788–98.
12. Björkerud S, Bondjers G. Endothelial integrity and viability in the aorta of the normal rabbit and rat as evaluated with dye exclusion tests and interference contrast microscopy. Atherosclerosis 1972;15:285–300.
13. Caplan BA, Schwartz CJ. Increased endothelial cell turnover in areas of in vivo evans blue uptake in pig aorta. Atherosclerosis 1973;17:401–17.
14. Van Beusekom HMM, van der Giessen WJ, Wagenvoort CA, et al. Histological features of a polymer endovascular prosthesis after transcatheter implantation in porcine arteries. Cardiovascular Pathology 1993;2:41–52.
15. Hofma SH, Whelan MC, van Beusekom HMM, Verdouw PD, van der Giessen WJ. Increasing arterial wall injury after long term implantation of two stent types in a porcine coronary model. Eur Heart J 1998;19:601–9.
16. Post MJ, Borst C, Kuntz RE. The relative importance of arterial remodeling compared with intimal hyperplasia in lumen renarrowing after PTCA: a study in the normal rabbit and the hypercholesterolemic Yucatan micropig. Circulation 1994;89:2816–2.
17. Mintz GS, Pichard AD, Kent KM, et al. Intravascular ultrasound comparison of restenotic and de novo coronary artery narrowings. Am J Cardiol 1994;74:1278–80.
18. Ross R. The pathogenesis of atherosclerosis. An update. N Engl J Med 1986;314:488–500.
19. Van Beusekom HMM, Van der Giessen WJ, Van Suylen RJ, et al. Histology after stenting of human saphenous vein bypass grafts: Observations from surgically excised grafts 3 to 320 days after stent implantation. J Am Coll Cardiol 1993;21:45–54.
20. Rippe B, Haraldsson B. Transport of macromolecules across microvascular walls: the two pore theory. Physiol Rev 1994;74:163–219.
21. Michel CC. Transport of macromolecules through microvascular walls. Cardiovasc Res 1996;32:644–53.
22. Palade GE. The microvascular endothelium revisited. In: Simionescu N, Simionescu M, eds. Endothelial Cell Biology in Health and Disease. New York: Plenum Publishing Corp., 1988:3–21.
23. Baluk P, McDonald DM. The B2-adrenergic receptor agonist formoterol reduces microvascular leakage by inhibiting endothelial gap formation. Am J Physiol 1994;L461–8.
24. Van Hinsbergh VWM. Endothelial permeability for macromolecules. Mechanistic aspects of pathophysiological modulation. Arterioscl Thromb Vasc Biol. 1997;17:1018–23.
25. Lum H, Malik AB. Regulation of vascular endothelial barrier function. Lung Cell Mol Physiol 1994;11:L223–41.
26. Drenckhahn D, Ness W. The endothelial contractile cytoskeleton. In: Born GVR, Schwartz CJ. Vascular Endothelium. Physiology, Pathology and Therapeutic Opportunities. Stuttgart: Schattauer, 1997:1–25.
27. Garcia JGN, Davis HW, Patterson CE. Regulation of endothelial cell gap formation and barrier dysfunction: role of myosin light chain phosphorylation. J Cell Physiol 1995;163:510–22.
28. Granger DN, Korthuis RJ. Physiological mechanisms of postischemic tissue injury. Ann Rev Physiol 1995;57:311–32.
29. Lin SJ, Jan KM, Weinbaum S, Chien S. Transendothelial transport of low density lipoprotein in association with cell mitosis in rat aorta. Arteriosclerosis 1989;9:230–36.
30. Meredith JE, Faxeli B, Schwartz MA. The extracellular matrix as a cell survival factor. Mol Biol Cell 1993;4:953–61.
31. Roberts WG, Palade GE. Increase microvascular permeability and endothelial fenestrations induced by vascular endothelial growth factor. J Cell Sci 1995;108:2369–79.
32. Lindner V, Reidy MA. Expression of VEGF receptors in arteries after endothelial injury and lack of increased endothelial regrowth in response to VEGF. Arterioscler Throm Vasc Biol 1996;16:1399–405.
33. Tsurumi Y, Murohara T, Krasinski K, et al. Reciprocal relation between VEGF and NO in the regulation of endothelial integrity. Nature Medicine 1997;3:879–86.
34. Rippe B, Grega GJ. Effects of isoprenaline and cooling on histamine induced changes of capillary permeability in the rat hindquarter vascular bed. Acta Physiol Scand 1978;103:252–62.
35. Stelzner TJ, Weil JV, O'Brien RF. Role of cyclic adenosine monophosphate in the induction of endothelial barrier properties. J Cell Physiol 1989;139: 157–66.
36. Langeler EG, van Hinsbergh VWM. Norepinephrine and iloprost improve barrier function of human endothelial cell monolayers: role of cAMP. Am J Physiol 1991;260:C1052–9.
37. Tertov VV, Orekhov AN, Grogorian G, et al. Disorders in the system of cyclic nucleotides in atherosclerosis: cyclic AMP and cyclic GMP content and activity of related enzymes in human aorta. Tissue Cell 1987;19:21–8.
38. Page M, Ashhurst DE. The effects of mechanical stability on the macromolecules of the connective tissue matrices produced during fracture healing. II. The glycosaminoglycans. Histochem J 1987;19:39–61.

Chapter 6

Stent compliance does not affect the degree of neointimal thickening in non-atherosclerotic and atherosclerotic porcine femoral arteries

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PD Verdouw, BJGL de Smet, MJ Post, WJ van der Giessen

Stent compliance does not affect the degree of intimal thickening in non-atherosclerotic and atherosclerotic porcine femoral arteries

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ABSTRACT.
Background: From studies of implants in general, it is known that compliance mismatch between the implant and the surrounding tissue is an important factor in the resultant degree of tissue response. We therefore sought to determine if stent compliance played a role in the degree of intimal thickening post stenting.
Methods: Following measurement of stent compliance in vitro, paired implantations of Cordis Perflex stents of different compliance i.e. compliant single crosslinked (SC, n=21) or less compliant multiple crosslinked (MC, n=21) stents, were performed under guidance of quantitative coronary angiography (QCA). Stents were implanted into the femoral arteries of both normolipemic and atherosclerotic Yucatan micropigs. After a follow-up period of 6 weeks, repeat QCA was performed, after which the animals were sacrificed, and the vessels processed with stent in situ for histology and morphometry.
Results: In the normolipemic model all animals survived the follow-up period without stent thrombosis or other adverse events, while one animal died of non-cardiovascular causes in the atherosclerotic model. QCA showed no significant differences in late loss between the SC and MC stents within each model. Morphometry showed no significant differences in intimal thickening between the compliant SC and less compliant MC stents neither in the normolipemic nor the atherosclerotic animal models. However when the two models were compared there were significant differences in neointimal area, and neointimal and medial thickness. In addition, the vessel wall response showed greater inflammation and frequency of dissections in the atherosclerotic model compared to the normolipemic model.
Conclusion: Stent compliance does not affect the degree of neointimal thickening at 6 weeks in normolipemic and atherosclerotic porcine femoral arteries.
Key words: stent, compliance, atherosclerotic, intima

Introduction

Vascular stenting has significantly improved patient prognosis compared to balloon angioplasty alone. However, stent performance must be further improved if current restenosis rates are to be reduced and the use of stents is to be widened to also treat more unfavorable types of lesions.

Stent performance can be enhanced by improving both non-mechanical and mechanical stent properties. Previous studies have proposed that non-mechanical stent properties such as charge and topography[1], type of metal used[2] and stent design[3,4] can influence the neointimal response. However there is a paucity of information relating to how the mechanical properties of stents can be improved, since it is unclear which mechanical properties contribute to the resultant degree of neointimal thickening post stenting.

Mechanical properties of stents include compliance, hoop strength, longitudinal flexibility and radial force. While the terms are all inter-related, they each essentially refer to separate mechanical properties of the stent. Compliance refers to the ability of a stent to yield elastically

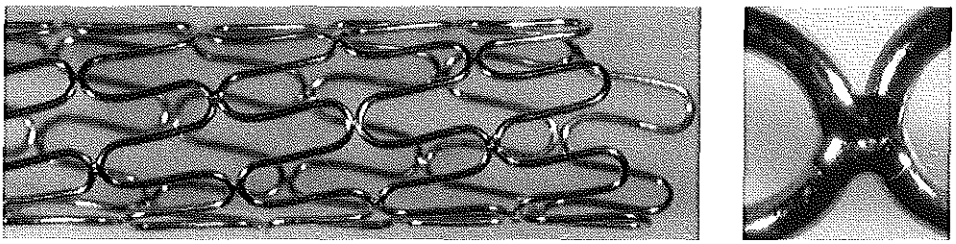


Figure 1. Macroscopic view of the Cordis Perflex stent (left) with detail (right) of a crosslink.

when an external force is applied. A compliant stent is one that is pliable, while a non-compliant stent is more rigid. Hoop strength on the other hand is a measure of the external force needed to plastically deform the stent. Longitudinal flexibility refers to the stent's ability to bend or flex in its length, while radial force is an outward force that a stent itself exerts on the vessel wall.

From studies of implants in general, it is known that compliance mismatch between the implant and the surrounding tissue is an important factor in the resultant degree of tissue response i.e. mismatch of the implant and recipient tissue causes an increased degree of fibrotic encapsulation of the implant. Indeed, studies of compliance in arterial grafts have shown that, in certain situations, compliance mismatch promotes intimal hyperplasia[5]. If such a hypothesis is also true for arterial stenting, then improved matching of the compliance of the stent with that of the vessel (i.e. reducing the degree of compliance mismatch) should lead to attenuation of the neointimal response.

The compliance of a stent can be changed by altering the material properties or design of the stent. For stents with a sinusoidal loop design, this can be achieved by crosslinking the loops within the stent i.e. a stent with a single cross-link is more compliant (pliable) than a stent containing multiple crosslinks (rigid). By using stents that differed only in their number of crosslinks, but were otherwise identical, we studied the effect of stent compliance on neointimal thickening in a non-oversized, normolipemic porcine model. To see whether this response differed between healthy arteries and the more clinically relevant diseased atherosclerotic arteries, experiments were also performed in an atherosclerotic model induced by balloon injury in combination with an atherogenic diet. To our knowledge this is the first study to investigate, in two different animal models, the contribution of stent compliance alone to neointimal thickening, without the confounding effects of, amongst others, stent design, type of metal used and stent profile.

Materials and Methods

The Stent.

The stent used in the current study was the stainless steel Cordis Perflex stent (Figure 1). The Perflex stent was produced in two forms that differed only in their number of crosslinks, but were otherwise identical. The multiple cross-linked stent (MC) had 4 times as many cross-links as the single cross-linked stent (SC).

In-vitro measurement of compliance.

Measurement of both arterial and stent compliance was performed in phantoms and explanted femoral arteries, with all measurements being performed in triplicate. The apparatus used for in-vitro measurements of stent compliance is illustrated in Figure 2A and consisted of an isolated vessel suspended in a pressure

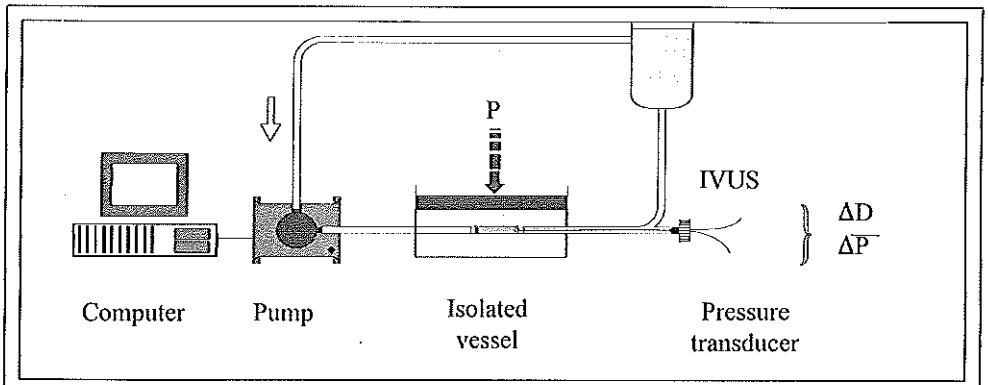
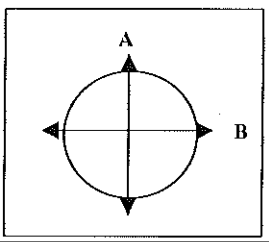


Figure 2A. Apparatus for *in-vitro* measurement of stent and vessel compliance.

Figure 2B. Directions in which vessel wall diameter changes were measured for estimation of compliance.



chamber, where both the inner and outer pressure of the vessel could be varied. For measurement of arterial compliance, the internal diameter of the arteries was first measured by varying the internal pressure in the artery from 80-140mmHg at incremental changes of 10mmHg/measurement. For measurement of stent compliance, stent and artery diameter were matched from vessel diameter measurements when the internal pressure was 80mmHg (representative of in-vivo mean arterial pressure). The appropriately sized stent was chosen and implanted under guidance of IVUS. The internal pressure of the artery was maintained at 80mmHg while the external pressure was varied from 0-200mmHg. For measurements of both arterial compliance and stent compliance the cross-sectional area of each vessel or stent was recorded at incremental changes of pressure (10mmHg/measurement) and documented by IVUS, with measurements being performed in two directions (Figure 2B). Compliance (C) was expressed as the change in diameter (given as a diameter ratio D_i / D_o) with incremental changes in external pressure. D_i is the stent diameter after incremental changes in external pressure, while D_o is the initial stent diameter i.e. the stent diameter at zero pressure difference between internal and external pressures.

Animal models.

Two animal models were used for the experiments, (a) a non-injured, normolipemic Yucatan model and (b) a high cholesterol, balloon injured atherosclerotic Yucatan model. In the latter, 11 seven-month old Yucatan micropigs (25kg) were started on an atherogenic diet consisting of essential nutrients, vitamins, salts, 1.5% cholesterol, 17.5% casein, 14% lard and 6% peanut oil[6]. Induction of lesions by balloon injury has previously been described[7]. In brief, two weeks after initiation of the diet, Fogarty balloon denudation of the femoral arteries was performed. Under fluroscopic guidance 3-4cm segments in the femoral

Animal Model	Right femoral	Left femoral
	N	N
Normal		
SC	6	4
MC	5	5
Atherosclerotic		
SC	5	6
MC	6	5

Table A. Distribution of SC and MC stents in femoral arteries in normolipemic and atherosclerotic animal models.N= number of stents. SC= single cross-linked stent. MC= multiple cross-linked stent.

arteries were denuded by triple withdrawal of a 4F Fogarty catheter. Following denudation the animals were returned to the care facilities where the atherogenic diet was continued for an additional 4 months.

Animal care.

Experiments were performed under the regulations of the animal care committee of the Erasmus University Rotterdam and in accordance with the "Guide for the care and use of laboratory animals" (NIH publication 85-23).

Animal preparation.

Starting one day prior to the procedure and throughout the follow-up period, all animals received 300mg Ascal (Carbasalatum calcium, Asta Medica B.V. The Netherlands) p.o. daily. After an overnight fast the animals were sedated with 20mg/kg ketamine hydrochloride. Anesthesia was induced by 11mg/kg thiopental and following endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2, v/v). Anesthesia was maintained with 1-2.5vol % isoflurane. Antibiotic prophylaxis was administered by an intramuscular injection of 1ml/25kg Streptoprocpen (A.U.V., Cuijk, The Netherlands, containing 200mg procaine benzylpenicillin and 250mg dihydrostreptomycin sulfate per ml) as a standard laboratory procedure. Under sterile conditions an arteriotomy of the left carotid artery was performed and an 8F-introduction sheath was placed. After measurement of arterial blood pressure and heart rate, and withdrawal of an arterial blood sample for the measurement of activated partial thromboplastin time (APTT), blood gases and acid-base balance (settings of the ventilator corrected, if necessary), 10,000 IU heparin sodium and 250mg acetylsalicylic acid were administered through the sheath. An 8F guiding catheter was advanced to the descending aorta. An APTT of at least three times baseline was maintained throughout the procedure. Angiography was performed using Omnipaque (Nycomed Ireland Ltd., Cork, Ireland) as contrast agent, and quantitative angiographic analysis was performed using the edge-detection method (Cardiovascular Measurement System, Medis Inc., Nuenen, The Netherlands).

Stent implantation.

From the angiograms, a femoral segment with a diameter of 3.5 mm was selected in the right or left femoral artery. One SC (3.5mm) and one MC (3.5mm) stent were randomly implanted in the right or left femoral arteries per pig (Table A). After repeat angiography, the guiding

catheter and the introducer sheath were removed, the arteriotomy repaired and the skin closed in two layers. At the end of the procedure, the animals were allowed to recover and returned to the animal care facilities where they remained for 6 weeks.

Follow-up angiography.

The anesthesia and catheterization procedures at follow-up were similar as described above, while angiography was performed in the same projection, and using identical settings of the X-ray equipment as during implantation.

Microscopical examination.

After angiography at follow-up, a lethal dose of sodium pentobarbital was injected intravenously. The femoral artery tree was dissected free from surrounding tissue and immersion fixed for at least 24 hours in 4% buffered formaldehyde after which vessels containing the stent were processed intact for light microscopy (LM)[8]. Haematoxylin-eosin was used as a routine stain, while resorcin-fuchsin was used as an elastin stain.

Morphometry.

Morphometric analysis of the tissue layers was performed in the proximal, middle and distal stent segment on elastin stained sections by tracing the external and internal elastic laminae and the endothelial lining using a microscopy image analysis system (Impak C, Clemex vision Image analysis system, Clemex Technologies Inc., Quebec, Canada). In the normolipemic model, the media was defined as the layer between the internal and external elastic laminae. The area between the endothelial lining and the internal elastic lamina was taken as the intima. In the atherosclerotic model the media + plaque was defined as the layer between the stent struts and external elastic laminae. The intima was taken as the area between the endothelial lining and the stent struts.

Statistical analysis.

Data were analyzed using Jandel Sigmastat statistical software, version 2.0 (Jandel Scientific Corporation, California, USA). All data are expressed as mean $\pm$ SD or as median and range. The angiographic data was evaluated using a two way repeated measures ANOVA, followed, when necessary, by a Tukey test. Morphometric data was evaluated by a two way ANOVA followed by a Tukey test. A p value of <0.05 was considered significant.

Results

In-vitro measurement of compliance.

Assessment of stent compliance was limited to that in healthy porcine femoral arteries. From multiple in-vitro measurements of compliance (n=3), an average value for each stent was estimated. Stent compliance is represented graphically in Figure 3, and shows a clear difference in compliance between the two stents i.e. the SC stent is more compliant than the MC stent.

In-vivo stent implantation and procedural success.

Number of animals: A total number of 21 SC stents (normal n=10, atherosclerotic n=11) and 21 MC stents (normal n=10-, atherosclerotic n=11) were implanted successfully (Table A). In the normal model all animals survived the follow-up period without stent thrombosis or other adverse events. In the atherosclerotic model, one animal died of non-cardiovascular causes.

Quantitative angiographic measurements.

Quantitative angiographic measurements are summarized in Table B. There were no significant differences in mean late loss between the MC and SC stents within each animal model 0.0mm(range: -0.4 - 1.2mm) versus -0.1mm (range: -0.3 - 0.7mm), $p>0.05$ (MC versus SC, normal model), and 1.09±0.45mm versus 0.97±0.51mm, $p>0.05$ (MC versus

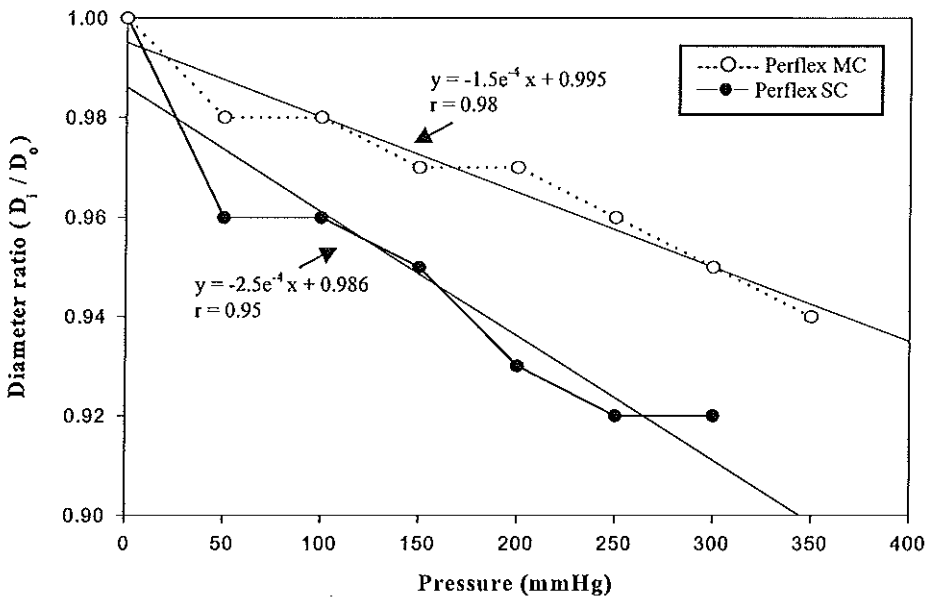


Figure 3. Changes in stent diameter of the SC and MC stent with changes in external pressure

	Pre (mm)	Post (mm)	FU (mm)	LL (mm)	N	B/A ratio
Normal						
SC	3.6±1.2	3.9±1.4	3.9±1.1	-0.1 (-0.3 - 0.7)*	10	1.2±0.1
MC	3.8±1.2	4.0±1.4	4.0±1.1	0.0 (-0.4 - 1.2)*	10	1.2±0.1
Atherosclerotic						
SC	3.6±0.7	4.1±0.5	3.1±0.6	1.0±0.5	9	1.2±0.1
MC	3.4±0.4	4.1±0.5	3.0±0.4	1.1±0.5	10	1.2±0.1

Table B. QCA results of mean or median luminal diameter of SC and MC stents in normolipemic and atherosclerotic animal models.

	No. of Stents	Inflammation			Dissection
		Neointima	Media	Adventitia	
Normal					
SC	10	3	1	3	0
MC	10	3	1	2	0
Atherosclerotic					
SC	10	7	5	6	3
MC	10	8	2	6	6

Table C. Summary of histological features for SC and MC stents in both animal models.

SC, atherosclerotic model). Late loss was significantly greater in atherosclerotic versus normal animals ($p<0.05$).

Histology

Table C summarizes the assessment of histological features for both SC and MC stents in the two animal models with respect to inflammation and the frequency of dissection.

Normolipemic model

In both the SC and MC groups, morphologic assessment by light microscopy showed a completely endothelialized luminal surface at six weeks. The internal elastic lamina (IEL) was fragmented in all vessels. The thin, and frequently minimal neointima, consisted of vascular smooth muscle cells in an extracellular matrix (Figure 4). In 6 vessels a small localized area of inflammation in the neointima was observed, but this was generally associated with the presence of thrombus remnants around or between the wires. There was compression of the media under the stent wires, but no medial rupture. The external elastic lamina was intact, and except for mild, localized adventitial inflammation in 6 vessels, the adventitia of both groups was otherwise unremarkable.

Atherosclerotic model

In both the MC and SC groups, histology showed arteries that were clearly damaged by the initial balloon injury to induce lesions as illustrated by medial ruptures, medial and intimal fibrosis and areas of calcification. The tissue contained foam cells and occasionally cholesterol crystals, both in the resulting plaque as well as in the media.

Stent implantation apparently enlarged the artery lumen, due to both stretching of the media and plaque, and also through tearing of both plaque and normal media (Figure 5). The luminal surface was completely endothelialized. Fragmentation of the internal elastic lamina was observed in all arteries. An asymmetrical neointima consisted of fibrotic tissue containing thrombus remnants. Inflammatory cells were observed in the NI of 15 arteries, in the media of 7 arteries and adventitia of 12 arteries. Foam cells were interspersed throughout the neointima and in the border-zone between stent and neointimal tissue in 19 arteries, and calcium deposits in 13 arteries. Dissections were noted in 9 arteries, while areas of neovascularization were found in 12 arteries.

Normolipemic versus atherosclerotic model

Within each model there was little difference in the overall degree of inflammation between SC and MC stents. However, comparison of the two models showed more inflammation in the neointima, media and adventitia of the atherosclerotic model compared to the normal model. Within the atherosclerotic model, dissections were seen in 6MC and 3SC stents (P=ns), while no dissections were observed in the normolipemic model.

Morphometry

Normolipemic model

In the normolipemic model, neointimal area (SC: $1.68\pm1.47mm^2$, MC $1.32\pm0.98mm^2$), together with lumen and medial areas showed no significant differences between the two stents ($p>0.05$) (Table D). Neointimal thickness at (SC: $0.24\pm0.12mm$, MC: $0.19\pm0.04mm$) and between the stent struts (SC: $0.11\pm0.11mm$, MC: $0.08\pm0.05mm$), together with medial thickness under and between the stent were also not significantly different when both stents were compared ($p>0.05$).

Atherosclerotic model

In the atherosclerotic model, neointimal area (SC: $4.44\pm1.48mm^2$, MC: $4.36\pm1.16mm^2$), lumen, plaque and medial areas showed no significant differences between the two stents ($p>0.05$). Neointimal thickness at (SC: $0.65\pm0.19mm$, MC: $0.65\pm0.17mm$) and between the stent struts (SC: $0.49\pm0.19mm$, MC: $0.48\pm0.17mm$), together with plaque thickness at the stent

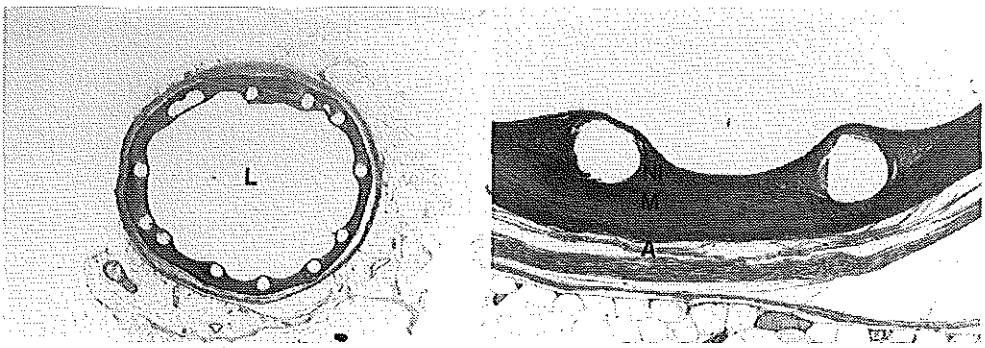


Figure 4: Overview (left) of a stented porcine femoral artery from the normolipemic group at 6 weeks, with detail (right) showing a minimal neointimal response to the stent. (Resorcin Fuchsin.)

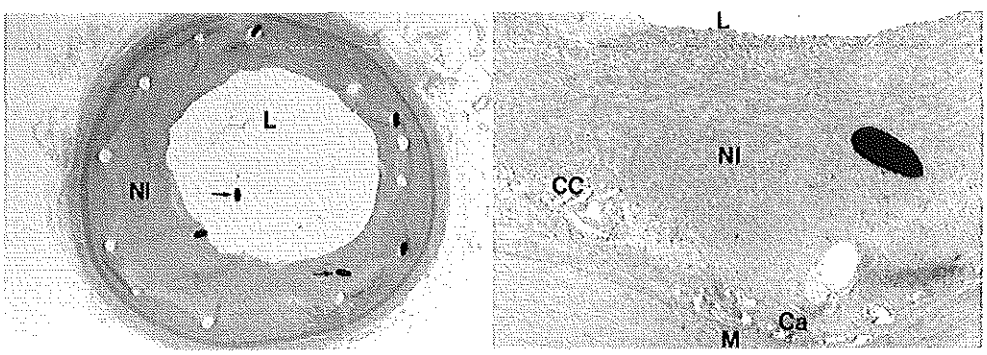


Figure 5: Overview (left) of a stented porcine femoral artery from the atherosclerotic group at 6 weeks, showing an asymmetric neointimal thickening. Detail (right) showing the stent embedded in a thick neointima containing foam cells, calcified spots, neo-vessels ans inflammatory cells. (Haematoxylin-Eosin.)

	Lumen area (mm^2)	NI-area (mm^2)	Plaque area (mm^2)	Media area (mm^2)	NI thickness at stent struts (mm)	Plaque thickness at stent struts (mm)	Media thickness under stent struts (mm)	NI between stent struts (mm)	Plaque between stent struts (mm)	Media between stent struts (mm)
Normal										
SC	8.74±7.94	1.68±1.47	NA	1.72±1.34	0.24±0.12 *	NA	0.08±0.04	0.11±0.11	NA	0.15±0.05
MC	8.74±5.77	1.32±0.98	NA	1.36±0.82	0.19±0.04 *	NA	0.06±0.02	0.08±0.05	NA	0.13±0.02
Atherosclerotic										
SC	4.09±1.58	4.44±1.48	1.80±1.01	2.14±0.62	0.65±0.19 *	0.10±0.07	0.15±0.03	0.49±0.19	0.18±0.09	0.20±0.05
MC	4.01±0.94	4.36±1.61	1.82±0.50	1.91±0.36	0.65±0.17 *	0.09±0.04	0.13±0.02	0.48±0.17	0.20±0.05	0.17±0.03

Table D. Morphometric results of SC and MC stents in normal and atherosclerotic animal models. *Including area occupied by the stent struts. NA = not applicable

struts and medial thickness under and between the stent were also not significantly different when both stents were compared ($p>0.05$).

Normolipemic versus atherosclerotic model.

When morphometric parameters for each stent type were compared between the animal models, both stent types showed a 2-3 times greater neointimal area in the atherosclerotic model compared to the normolipemic model ($p<0.05$). Neointimal thickness over and between the stent, together with medial thickness under and between the stents also showed significant differences between the two models ($p<0.05$) i.e. neointimal thickness at the stent was 2.5-3.5 (SC, MC) times greater in the atherosclerotic model then in the normolipmeic model. Similarly, neointimal thickness between the stents was 4-6 times greater (SC, MC), medial thickness at the stent 2 times greater (SC, MC), while medial thickness between the stent was 1.4 times thicker (SC, MC) in the atherosclerotic model than in the normolipemic model.

Discussion

Objective and main findings

From studies of vascular grafts, compliance mismatch has been reported as a cause of graft failure[9,10]. Indeed, better matching of the elastic properties of grafts and vessels has been shown to improve graft performance[11]. Given such evidence, we sought to determine if such a theory also holds true for vascular stenting. We examined the effect of stent compliance on vessel wall reaction both in normolipemic and atherosclerotic porcine femoral arteries and found that within both animal groups there were no differences in morphometric parameters between the compliant SC and less compliant MC stents. However when the two models were compared there were significant differences in neointimal area, and neointimal and medial thickness, at, under and between the stents ($p<0.05$). In addition, the vessel wall response showed greater inflammation and frequency of dissections in the atherosclerotic model compared to the normolipemic model.

QCA results

QCA results showed no significant difference in late loss between the SC and MC stent in the normolipemic model (Table B). Similarly, no differences in late loss were observed for the atherosclerotic animal group when MC and SC were compared. Late loss was however greater in the atherosclerotic model compared to the normolipemic model and this was due to an increased neointimal response in the atherosclerotic model.

Comparison of the two animal models

Our study was performed in both normolipemic and atherosclerotic porcine femoral arteries. While stent compliance did not affect the resultant degree of neointimal thickening in either animal model, the vessel wall response to the stents was different (Table C). In an atherosclerotic model, implanted stents come to rest on a matrix that is essentially more reactive (richer in growth factors etc.) than a healthy vessel wall. It is not surprising therefore that there was a greater neointimal and inflammatory response induced in the atherosclerotic model compared to the normolipemic model. In this model, the MC stent also induced more dissections than the SC stent (MC 6; SC 3), but no dissections were observed for both stents in the normolipemic model. It may be that the less compliant MC stent is easier able to rupture a foam cell, lipid-rich porcine atherosclerotic lesion, than the same stent implanted in a healthy porcine vessel.

Differences in the vessel wall reaction between the two animal models illustrate the merits of both models. Testing of stents in healthy arteries can be

Non-mechanical	Mechanical
Charge	Radial force
Topography	Hoop strength
Stent design	Longitudinal flexibility
Metal:artery ratio	Compliance
Type of metal used	

Table E. Non-mechanical and mechanical characteristics of stents.

used to demonstrate the biocompatibility of an implant without such reactions being complicated by an exaggerated response to injury induced by an oversized, balloon injury model. In our study the inherent biocompatibility of the stent is apparent from the minimal neointimal and inflammatory responses. In contrast the atherosclerotic model allows implants to be studied in a more clinically relevant situation.

Mechanical stent properties and comparison with other studies

Stent properties or stent characteristics can be broadly divided into non-mechanical and mechanical properties (Table E). While experimental studies have elucidated the effects of non-mechanical stent properties on neointimal thickening[1-4] , studies of mechanical stent properties are incomplete or inconclusive.

A study by Vorwerk et al[12] showed no differences in neointimal thickening between Wallstents with different radial forces, but a more recent study showed that for the Radius stent, higher radial expansile force induced a greater neointimal response compared to stents with lower radial expansile force[13]. No study to date has looked solely at the property of hoop strength and its relationship to neointimal response

A few studies have looked at the effect of stent compliance on neointimal thickening (although the term compliance was substituted with the terms stent rigidity and flexibility). A study by Barth[14] found that flexible stents induced the greatest neointimal response. However, the results of this study, with respect to the neointimal thickness due to stent compliance alone, are difficult to interpret, given the confounding effects of stent design, type of metal used, surface to area ratio etc. on the resultant neointimal response. In a study by Fontaine et al[15], the authors performed their study in an oversized artery model. The main problem with this study was that directly post stenting there was a significant difference in stent lumen, which taken together with the heterogeneity of a neointima induced through oversize injury, makes it difficult to assess the contribution of stent compliance alone to the resultant neointimal response. To look exclusively therefore at the effect of compliance, we chose two stents which, except for differences in their number of crosslinks (and thus their compliance), were identical and carried out the experiments

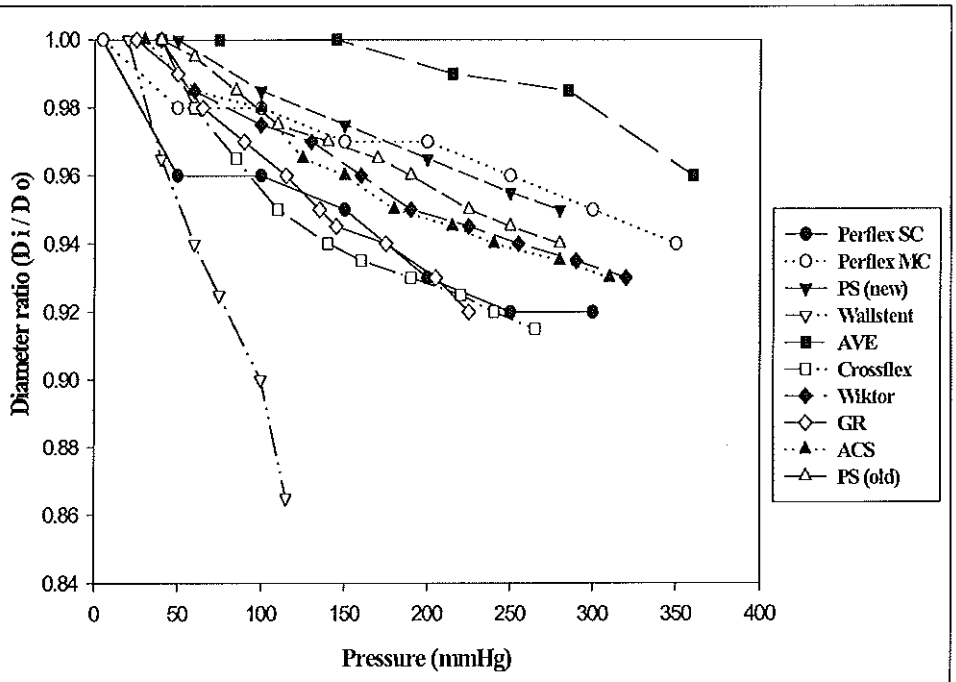


Figure 6. Changes in stent diameter with increasing external pressure. Perflex SC and MC stents compared to other stent types. (Values for other stent types calculated from Figure 5, Keane et al[16]).

in non-oversized normolipemic and atherosclerotic animal models.

Comparison with other stents

It is known both from animal studies and patient stent trials, that the subsequent degree of intimal thickening which develops post stenting varies between the different stents used. While there is little data available documenting the compliance of stents, a study in 1995 by Keane et al[16] compared the changes in stent diameter of 8 commercially available stents as the external pressure was varied (Figure 6). They showed that the AVE stent was the least compliant, followed by the Palmaz-Schatz (PS, new), Palmaz-Schatz (PS, old), Wiktor, ACS, Gianturco-Rubin (GR), Crossflex, and the Wallstent being the most compliant. For comparative purposes, data for the SC and MC stents have been included in Figure 6. The stents presented in the graph can be broadly divided into 3 groups: the compliant stents (Wallstent, Crossflex, GR), intermediate-compliant stents (ACS, Wiktor, old PS), and least compliant stents (newPS, AVE). The SC stent falls into the group of compliant stents, while the MC stent falls into the least compliant stent group. Indeed, the compliant SC stent may be suitable for lesions in flexing arteries or arteries subject to extensive motion, whereas given the reduced vessel compliance when hard, calcified plaque is present, the lesser-compliant MC stent may be more suitable.

Figure 6 shows clear differences in compliance between the Wallstent, Palmaz-Schatz and Wiktor stent. However, in 1994 a comparison of these three stents

in the clinical situation showed that at 6-month follow-up, angiographic results for all 3 stents were comparable[17]. Indeed results of the NIRVANA (NIR Vascular Advanced North American Trial) trial, which compared the NIR stent and Palmaz-Schatz stent, showed no differences in restenosis rates at 6 month follow-up between the stents[19].

In contrast however, results of a recent study which compared five different slotted-tube stents did show significant differences in restenosis rates (6 month follow-up) and in the incidence of major adverse cardiac events (MACE – 1 year follow-up) between the 5 stents studied[18]. However, given that no data is available on the compliance of these stents, and taking the results of the NIRVANA trial into consideration, it is more likely that differences in neointimal responses induced by the different stents are more probably attributable to stent design than stent mechanics[3].

Where stent mechanics might be important however, is in the incidence of medial rupture and dissection. In our study the less compliant MC stent had a higher frequency of dissection than the compliant SC stent in the atherosclerotic model. In patients, differences in the compliance of stents implanted into fragile and friable diseased vessel walls may be such that they could, theoretically, also lead to differences in the incidence of dissection and associated complications. In theory therefore, stent compliance may have contributed to differences in the incidence of MACE seen between the 5 stents in the above mentioned study, although until data is available for the compliance of these stents, such a proposal remains purely speculative.

Conclusion

Stent compliance does not affect the degree of neointimal thickening at 6 weeks in normolipemic and atherosclerotic porcine femoral arteries.

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References

1. Hehrlein C, Zimmermann M, Metz J, Ensinger W, Kubler W. Influence of surface texture and charge on the biocompatibility of endovascular stents. *Coron Artery Dis.* 1995;6:581-6.
2. Schurmann K, Vorwerk D, Kulisch A, Stroehmer-Kulisch E, Biesterfeld S, Stopinski T, Gunther RW. Experimental arterial stent placement. Comparison of a new Nitinol stent and Wallstent. *Invest Radiol.* 1995;30:412-20.
3. Rogers C, Edelman ER. Endovascular stent design dictates experimental restenosis and thrombosis. *Circulation.* 1995;91:2995-3001.
4. Tominaga R, Kambic HE, Emoto H, Harasaki H, Sutton C, Hollman J. Effects of design geometry of intravascular endoprostheses on stenosis rate in normal rabbits. *Am Heart J.* 1992;123:21-8.
5. Ballyk PD, Walsh C, Butany J, Ojha M. Compliance mismatch may promote graft-artery intimal hyperplasia by altering suture-line stresses. *J Biomech.* 1998;31:229-37.
6. de Smet BJ, van der Zande J, van der Helm YJ, Kuntz RE, Borst C, Post MJ. The atherosclerotic Yucatan animal model to study the arterial response after balloon angioplasty: the natural history of remodeling. *Cardiovasc Res.* 1998;39:224-32.
7. de Smet BJ, Pasterkamp G, van der Helm YJ, Borst C, Post MJ. The relation between de novo atherosclerosis remodeling and angioplasty- induced remodeling in an atherosclerotic Yucatan micropig model. *Arterioscler Thromb Vasc Biol.* 1998;18:702-7.
8. van Beusekom HMM, Whelan DM, van der Plas M, van der Giessen WJ. A practical and rapid method of histological processing for examination of coronary arteries containing metallic stents. *Cardiovasc Pathol.* 1996;5:69-76.

9. Abbott WM, Megerman J, Hasson JE, L'Italien G, Warnock DF. Effect of compliance mismatch on vascular graft patency. *J Vasc Surg.* 1987;5:376-82.
10. Hasson JE, Megerman J, Abbott WM. Increased compliance near vascular anastomoses. *J Vasc Surg.* 1985;2:419-23.
11. Walden R, L'Italien GJ, Megerman J, Abbott WM. Matched elastic properties and successful arterial grafting. *Arch Surg.* 1980;115:1166-9.
12. Vorwerk D, Redha F, Neuerburg J, Clerc C, Gunther RW. Neointima formation following arterial placement of self-expanding stents of different radial force: experimental results. *Cardiovasc Intervent Radiol.* 1994;17:27-32.
13. Kobayashi Y, Bailey SR, Brown III CL, Christie LG, Matthews RV, de Franco AC, Schwartz RS, Handen CE, Fitzgerald PJ. Relationship between radial expansile force and neointimal proliferation in self-expandable stent: a serial intravascular ultrasound study. *Circulation.* 1998;17:I-163 (Abstract).
14. Barth KH, Virmani R, Froelich J, Takeda T, Lossef SV, Newsome J, Jones R, Lindisch D. Paired comparison of vascular wall reactions to Palmaz stents, Strecker tantalum stents, and Wallstents in canine iliac and femoral arteries. *Circulation.* 1996;93:2161-9.
15. Fontaine AB, Spigos DG, Eaton G, Das Passos S, Christoforidis G, Khabiri H, Jung S. Stent-induced intimal hyperplasia: are there fundamental differences between flexible and rigid stent designs? *J Vasc Interv Radiol.* 1994;5:739-44.
16. Keane D, Schuurbiers J, Slager CJ, Ozaki Y, den Boer A, Bruining N, Serruys PW. Comparative quantitative mechanical, radiographic, and angiographic analysis of eight coronary stent designs. *In: "Coronary stenting: a quantitative angiographic and clinical evaluation". PhD Thesis, Erasmus University Rotterdam, The Netherlands.* 1995.
17. Eeckhout E, Goy JJ, Stauffer JC, Vogt P, Kappenberger L. Comparison of the Wallstent, Palmaz-Schatz stent, and Wiktor stent late after intracoronary stenting. *Am J Cardiol.* 1994;74:609-12.
18. Dirschinger J, Schuhlen H, Boekstegers P, Hausleiter J, Kastrati A, Giehrl W, Pache J, Hadamitzky M, Neumann F-J, Steinbeck G, Schomig A. Equivalence or Difference? One-year follow-up of a randomized trial of five different slotted-tube stents. *Circulation.* 1998;17:I-661 (Abstract).
19. Baim DS, Cutlip DE, Lansky AJ, Ricci GB, Fitzpatrick M. Results of the Nirvana equivalency trial comparing the NIR Primo stent to the Palmaz-Schatz stent. *Circulation.* 1998;98:I-661 (Abstract).

Chapter 7

Mechanisms of drug loading and release kinetics

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Mechanisms of drug loading and release kinetics

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In an effort to overcome the limitations of local drug delivery associated with the use of catheters, drug-loaded stents have been developed. Loading of such stents is achieved through either drug absorption (incorporation into a matrix) or drug adsorption (surface layering). The type of drug binding determines the elution profile/release kinetics of the drug, while the therapeutic target determines both the choice of drug used and the manner in which it is bound, i.e. eluting or non-eluting. While non-eluting stents have clinically reduced thrombotic complications following stent implantation, current experimental work concentrates on the use of eluting stents to combat restenosis.

Key words: stent, drug, release kinetics, coatings

Introduction

Systemic administration of anti-thrombotic, anti-restenotic or anti-inflammatory drugs following stent implantation is associated with two main problems. Firstly, if therapeutic levels of the drug are to be achieved at the site of the stent, then systemic doses may be associated with toxicity. Secondly, there is the problem of targeting the drug to the vascular injury site and maintaining sufficient drug concentrations for a prolonged period of time such that it can achieve its effect. The use of catheters to locally deliver drugs to the implant site has not eliminated the above problems and therefore, in an effort to improve local delivery, drug-loaded stents have been developed.

Considerations of drug-eluting stents

In considering the use of stents as vehicles for local drug delivery, several issues must be addressed. The therapeutic target dictates not only which drug is used, but also the type of drug binding and/or elution profile. For example, if the desired effect of local delivery is to interfere in the early thrombotic response or the adhesion of neutrophils etc., it may be advantageous to have rapid release of the drug, i.e. dose dumping [Fig. 1(a)]. If, however, the aim is to intervene at a later stage in the wound healing process, then drug release over a longer period of time [Fig. 1(b)], or slow release after an initial lag phase [Fig. 1(c)], may be desirable. Alternatively, a combination of slow- and rapid-releasing drugs which bridge both the early and later phases of wound healing following stent implantation may be achieved by combining two drugs in a single coating [Fig. 1(d)]. A second consideration is whether a non-eluting or eluting stent is required. While coverage of a non-eluting stent with plasma proteins, thrombus or tissue may limit the availability of the drug, rapid release from an eluting stent may be associated with drug washout. Finally, the question of the amount of drug that can be incorporated into the stent has to be addressed. The amount of drug incorporated into a pure polymer stent for example, must be such that it does not affect the physical and mechanical characteristics of the stent. In the case of a stent coating, the amount of incorporated drug is limited by the thickness of the applied coating. This may be important, since this alters the profile of the stent. Increases in stent profile may enhance neointimal formation [1].

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Methods of drug loading

Several methods exist by which a stent can be loaded with a therapeutic agent. First, the drug can be *ab*sorbed into a polymer matrix [Fig. 2(a)]. *Ab*sorption refers to

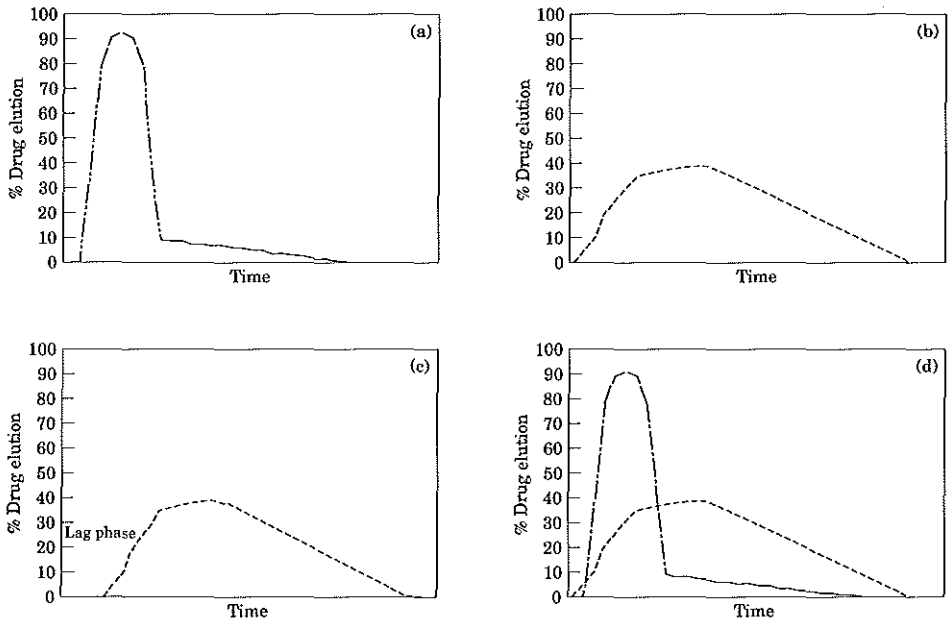

Figure 1. Theoretical elution curves from a drug-loaded stent. (a) Dose dumping—an initial rapid elution of drug in a short period of time. (b) Slower, more controlled release with a lower initial drug elution compared to (a), but elution continues for a longer period of time. (c) An initial delay period or lag phase, before the drug elutes. Elution is then as for (b). (d) Theoretical elution profile of two drugs combined in a single coating. One drug elutes rapidly, while the second has a slower more controlled release.

the incorporation of the drug into the matrix, which is then applied as a coating to the stent surface. Alternatively, the matrix can be used to manufacture the stent itself, i.e. a pure polymer stent. Secondly, the agent can be *ad*sorbed to the stent surface. *Ad*sorption refers to the surface layering of the stent with the drug. In both *ab*sorption and *ad*sorption the drug is 'held' through either covalent or non-covalent bonds [Figs 2(b), 2(c), Table 1].

Drug absorption

Currently, the development of techniques to provide sustained local drug delivery has focused on polymers as matrices for drug absorption and elution. The matrix is formed by compressing or blending the drug and polymer material together. The rate of drug release may be controlled by blending two or more polymers together. By increasing the proportion of the more hydrophilic polymer, the rate of drug release can be increased. Alternatively, the degree of cross-linking and the type of plasticizer used provide additional means for modifying the release rate of the drug [2].

To date, both biodegradable (poly-L-lactic acid [3], polycaprolactone [4], cellulose [5]) and non-biodegradable polymers (polyurethane [6, 7], poly-organophosphazene [8]) have been used to absorb drugs from which a stent coating or the stent itself has been manufactured. A comprehensive review was recently published by Bertrand *et al.* [9]

Drug adsorption

While there is a spectrum of drugs that can be bound or immobilized to foreign surfaces, e.g urokinase [10], prostacyclin [11], heparin is most frequently the drug of choice, with the heparin-coated stent being widely used in the clinical setting [12]. Depending on the way in which heparin is attached, surfaces can be divided into two categories, i.e. heparin can be immobilized either chemically or physically. Chemically-bound heparin can be divided into ionically- or covalently-bound heparin. Physically-bound heparin includes blend systems in which heparin is mixed with the surface material and surfaces onto which heparin is physically adsorbed.

Ionic binding

Ionic binding of heparin to medical devices began as early as the 1960s [13]. Surfaces were precoated with a graphite paste, treated with a cationic surfactant such as benzalkonium chloride (BZC), and this charged surface was then complexed with heparin. The disadvantage of ionically bound heparin is the rapid desorption of the

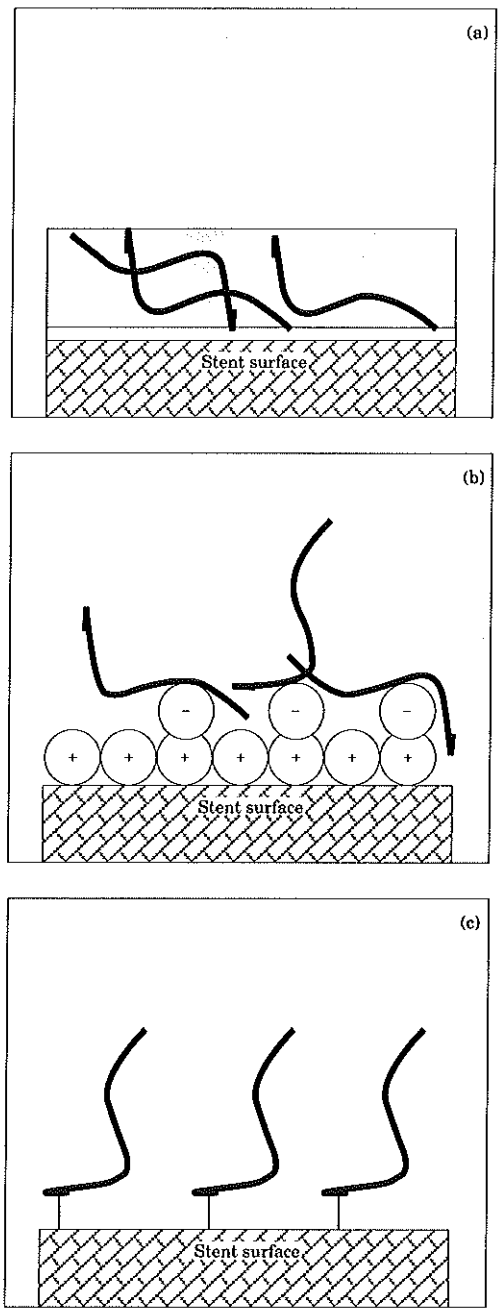

Figure 2. (a) Drug (∿∿) absorbed into a polymer matrix (▭) applied as a coating to the stent surface. The drug is released by diffusion from the polymer, or by degradation of the polymer. (b) Ionic binding of a negatively charged drug (∿∿) to a positively charged stent surface. (c) Drug (∿∿) end-point attached to the stent surface by a covalent bond.

Table 1. Examples of the different types of non-covalent and covalent bonds

- Non-covalent binding e.g.
  - Electrostatic bonds—ionic bonds, salt linkage, salt bridge, ion pair
  - Hydrogen bonds
  - Van der Waals forces
- Covalent binding e.g.
  - Sulphur bridges
  - C–C bonds

drug upon exposure to blood. Its application, therefore, in binding heparin to a metal stent is limited, since weakly-bound heparin is easily released or displaced from the BZC, thereby requiring high concentrations of stent bound heparin to achieve a therapeutic level at the site of implant.

Covalent binding

Immobilization of heparin to surfaces through covalent bonds has previously been described [14, 15]. By attaching heparin in this manner, a stable bond is achieved compared to ionically-bound heparin. A clinical application of covalently bound heparin is the heparin-coated Palmaz–Schatz stent [16]. The coating applied to the stent consists of fragmented heparin molecules that have been end-point covalently coupled to a polymer matrix (Fig. 3). The drug remains attached to the surface of the stent, with the efficiency of the coating based on the repeated interaction between the active site of the immobilized heparin and circulating antithrombin III. Animal studies have shown that up to 4 weeks post implant, 20–50% of the heparin activity remains [16]. Other heparin-coated stents include the Medtronic® Wiktor Hepamed® stent and the Jomed heparin-coated stent.

Drug elution

Coated stents can be divided into non-eluting or eluting stents. For eluting stents, the manner in which the drug is bound or incorporated into the stent or its coating determines the way in which the drug is released.

Elution of matrix bound drugs

The elution of matrix bound drugs can be either random or controlled. Random release is associated with diffusion of the drug from the polymer matrix, the rate of diffusion being dependent on the thickness of the coating and the amount of drug incorporated. This type of release was demonstrated as early as 1994 by Lambert *et al.* [6] They demonstrated the feasibility of

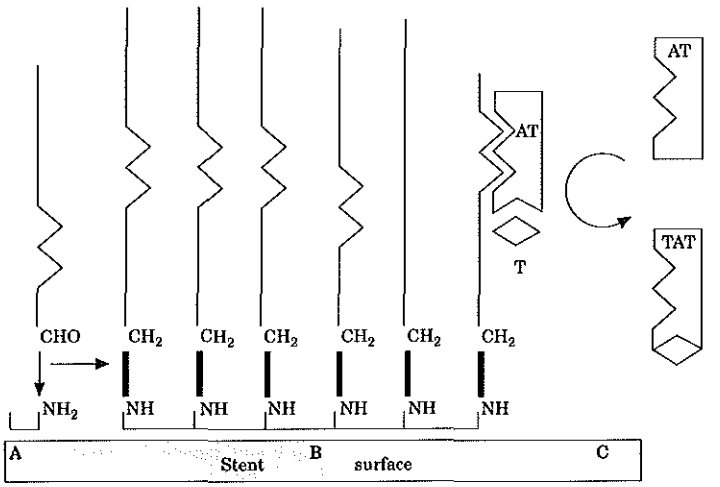

Figure 3. Principle of heparin coating and mechanism of anti-thrombotic action of heparin-coated stent surface. (Modified from Hårdhammer *et al.* [16]). (A) The metal surface has been conditioned with functional amino groups that can bind covalently with the aldehyde group of fragmented heparin molecules. (B) End-point attached heparin molecules form a heterogeneous population, some without and some with the epsilon-shaped antithrombin III (AT)-binding region. (C) Circulating antithrombin can bind to the active site, which catalyses the inhibition of thrombin (T). The resultant inactive thrombin/antithrombin complex (TAT) is released into the bloodstream, thereby enabling the active site on the heparin to repeatedly interact with T and AT.

locally administering forskolin to the vessel wall and the kinetics thereof. Forskolin is a drug with poor water solubility, such that systemic administration is limited. However, it can readily be dissolved by organic solvents and incorporated into non-polar polymers. Metal stents were spray-coated with polyurethane to a thickness of 50 µm and forskolin was then incorporated by incubating the coated stent in the drug solution. Release of the drug over a 24-h period was proportional to the mass of drug remaining in the polymer matrix, and thus can be modeled on a first-order or diffusion-limited system.

In contrast, however, controlled release is achieved when the matrix used is a biodegradable polymer. The rate of matrix degradation determines the rate of drug release. To date, most stent-related studies of local drug delivery have focused on the use of biodegradable polymer coatings. A recent example of this was the study by Lincoff *et al.* who demonstrated the administration of dexamethasone from a metallic stent coated with low- and high-molecular weight poly lactic acid [3].

It has previously been reported that not only was it feasible to administer a single drug to the vessel wall, but potentially two drugs could be incorporated into a single coating. In 1997, Schmidmaier *et al.* [17] reported the development of a polymer stent coating consisting of poly-L-lactic acid containing 5% PEG-Hirudin and 1% Illoprost. *In vitro* studies had demonstrated that, up to at least 30 days, both drugs were released. PEG-Hirudin showed an initial rapid release (random release), while the release of Iloprost appeared to be dependent on the rate of degradation of the carrier (controlled release).

While the amount of drug released from coatings is limited by the thickness of the coating, and therefore the amount of drug that can be incorporated, the use of a completely synthetic, biodegradable stent may allow for the incorporation of a larger amount of drug. Recently Ye *et al.* [18] described the use of a purely bioresorbable microporous stent to deliver adenoviral gene transfer vectors to the vessel wall. (While gene transfer vectors are strictly speaking not drugs, in considering the release of compounds from stents and their coatings, they can be included in this group.) This biodegradable stent is manufactured from poly-L-lactic acid/polycaprolactone, with polyethylene oxide being incorporated to improve its hydrophilic character, allowing the uptake of recombinant adenovirus.

Recently, a biodegradable poly lactic acid stent, coated with a poly-ε-caprolactone coating loaded with a tyrosine kinase inhibitor has been described which appears to reduce neointimal hyperplasia in a porcine model [4].

Conclusion

Drug loaded stents in the form of the non-eluting heparin coated stent have made significant improvements in reducing thrombotic complications following stent implantation. While such stents have been less successful in tackling the problem of restenosis, it is hoped that developments in stent design and/or polymer chemistry will lead to the clinical use of eluting stents in the very near future.

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References

1 Schurmann K, Vorwerk D, Kulisch A, Stroehmer-Kulisch E, Biesterfeld S, Stopinski T, Gunther RW. Neointimal hyperplasia in low-profile Nitinol stents, Palmaz stents, and Wallstents: a comparative experimental study. *Cardiovasc Intervent Radiol* 1996; **19:** 248–254.

2 Shargel L, Yu ABC. *Applied Biopharmaceutics and Pharmacokinetics*. Third ed. Prentice-Hall International Inc., 1993.

3 Lincoff AM, Furst JG, Ellis SG, Tuch RJ, Topol EJ. Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. *J Am Coll Cardiol* 1997; **29:** 808–816.

4 Yamawaki T, Shimokawa H, Kozai T, Miyata K, Higo T, Tanaka E, Egashira K, Shiraishi T, Tamai H, Igaki K, Takeshita A. Intramural delivery of a specific tyrosine kinase inhibitor with biodegradable stent suppresses the restenotic changes of the coronary artery in pigs *in vivo*. *J Am Coll Cardiol* 1998; **32:** 780–786.

5 Aggarwal RK, Ireland DC, Azrin MA, Ezekowitz MD, de Bono DP, Gershlick AH. Antithrombotic potential of polymer-coated stents eluting platelet glycoprotein IIb/IIIa receptor antibody. *Circulation* 1996; **94:** 3311–3317.

6 Lambert TL, Dev V, Rechavia E, Forrester JS, Litvack F, Eigler NL. Localized arterial wall drug delivery from a polymer-coated removable metallic stent. Kinetics, distribution, and bioactivity of forskolin. *Circulation* 1994; **90:** 1003–1011.

7 Dev V, Eigler N, Sheth S, Lambert T, Forrester J, Litvack F. Kinetics of drug delivery to the arterial wall via polyurethane-coated removable nitinol stent: comparative study of two drugs. *Cathet Cardiovasc Diagn* 1995; **34:** 272–278.

8 de Scheerder I, Wang K, Wilczek K, van Dorpe J, Verbeken E, Desmet W, Schacht E, Piessens J. Local methylprednisolone inhibition of foreign body response to coated intracoronary stents. *Coron Artery Dis* 1996; **7:** 161–166.

9 Bertrand OF, Sipehia R, Mongrain R, Rodes J, Tardif JC, Bilodeau L, Cote G, Bourassa MG. Biocompatibility aspects of new stent technology. *J Am Coll Cardiol* 1998; **32:** 562–571.

10 Ohshiro T, Kosaki G. Urokinase immobilized on medical polymeric materials: fundamental and clinical studies. *Artif Organs* 1980; **4:** 58–64.

11 Ebert CD, Lee ES, Kim SW. The antiplatelet activity of immobilized prostacyclin. *J Biomed Mater Res* 1982; **16:** 629–638.

12 Serruys PW, Emanuelsson H, van der Giessen W, Lunn AC, Kiemeney F, Macaya C, Rutsch W, Heyndrickx G, Suryapranata H, Legrand V, Goy JJ, Materne P, Bonnier H, Morice MC, Fajadet J, Belardi J, Colombo A, Garcia E, Ruygrok P, de Jaegere P, Morel MA. Heparin-coated Palmaz–Schatz stents in human coronary arteries. Early outcome of the Benestent-II Pilot Study. *Circulation* 1996; **93:** 412–422.

13 Gott VL, Whiffen JD, Valiathan SM. Graphite-benzalkonium-heparin coatings on plastics and metals. *Ann N Y Acad Sci* 1968; **146:** 21–29.

14 Hennink WE, Feijen J, Ebert CD, Kim SW. Covalently bound conjugates of albumin and heparin: synthesis, fractionation and characterization. *Thromb Res* 1983; **29:** 1–13.

15 Goosen MF, Sefton MV. Properties of a heparin-poly(vinyl alcohol) hydrogel coating. *J Biomed Mater Res* 1983; **17:** 359–373.

16 Hårdhammar PA, van Beusekom HM, Emanuelsson HU, Hofma SH, Albertsson PA, Verdouw PD, Boersma E, Serruys PW, van der Giessen WJ. Reduction in thrombotic events with heparin-coated Palmaz–Schatz stents in normal porcine coronary arteries. *Circulation* 1996; **93:** 423–430.

17 Schmidmaier G, Stemberger A, Alt E, Gawaz M, Schömig A. Non-linear time release characteristics of a biodegradable polylactic acid stent coating releasing PEG-hirudin and a PGI_2 analog. *Eur Heart J* 1997; **18:** 3316 (Abstract).

18 Ye YW, Landau C, Willard JE, Rajasubramanian G, Moskowitz A, Aziz S, Meidell RS, Eberhart RC. Bioresorbable microporous stents deliver recombinant adenovirus gene transfer vectors to the arterial wall. *Ann Biomed Eng* 1998; **26:** 398–408.

Chapter 8

Biocompatibility of phosphorylcholine-coated stents in normal porcine coronary arteries

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In Press

Biocompatibility of Phosphorylcholine-Coated Stents In Normal Porcine Coronary Arteries.

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ABSTRACT

Objective. To improve the biocompatibility of stents using a phosphorylcholine-containing synthetic polymer stent coating (PC) as a form of biomimicry. ***Interventions.*** Implantation of PC (n=20) and non-coated (NC, n=21) *divYsio* stents was performed in the coronary arteries of 25 swine. After 5 days (n=6), 4 weeks (n=7) and 12 weeks (n=8), the animals were sacrificed and the vessels harvested for histology, scanning electron microscopy and morphometry. ***Main outcome measures.*** Stent performance was assessed by studying early endothelialisation, neointima formation and the vessel wall reaction to the synthetic coating. ***Results.*** Stent thrombosis did not occur in either group. Morphometry showed no significant differences between the two study groups at any time point. At 5 days both PC and NC were equally well endothelialised (91 *v* 92%). At 4 and 12 weeks there was no difference in intimal thickness between the PC and NC stents. Up to 12 weeks post implant the PC coating was still discernible in the stent strut voids, and did not appear to elicit an adverse inflammatory response. ***Conclusion.*** In this animal model the PC coating showed excellent blood and tissue compatibility unlike a number of other polymers tested in a similar setting. Given that the coating was present up to 12 weeks post implant with no adverse tissue reaction, it may be a potential candidate polymer for local drug delivery.

Key Words: Phosphorylcholine, stents, coatings, biocompatible materials

INTRODUCTION

Significant advances have been made in the field of interventional cardiology through the use of coronary stents. A restenosis rate of 30-50% after balloon angioplasty has been reduced to a current rate of 10-30% after stenting. Such a reduction has led to the use of stents in wider patient populations with improved clinical outcome. In addition, improvements in implantation techniques together with enhanced anti-platelet regimes have significantly reduced acute ischemic events[1] (<2% when stents are implanted electively[2] and approximately 5% in bailout situations[3]). However, both anti-restenosis and anti-thrombotic aspects must be addressed if the stent's therapeutic potential is to be expanded even further. Such improvements may be achieved by enhancing the biocompatibility of the implant.

Modification of a stent with respect to blood and tissue compatibility can be achieved by changing the stent material itself, or by modifying the outer layer of the stent surface. Changing the stent material may influence the mechanical behaviour of the stent, but since only the outer few micrometers of the metal interact with the blood and tissue wall, it is more practical to modify the outer stent surface through coating it with a biocompatible molecule. The ideal coating would be haemocompatible, thereby not inducing stent thrombosis, and could potentially reduce neointimal thickening through improved tissue compatibility.

Previous studies have highlighted the success of phosphorylcholine (PC) in improving the biocompatibility of surfaces

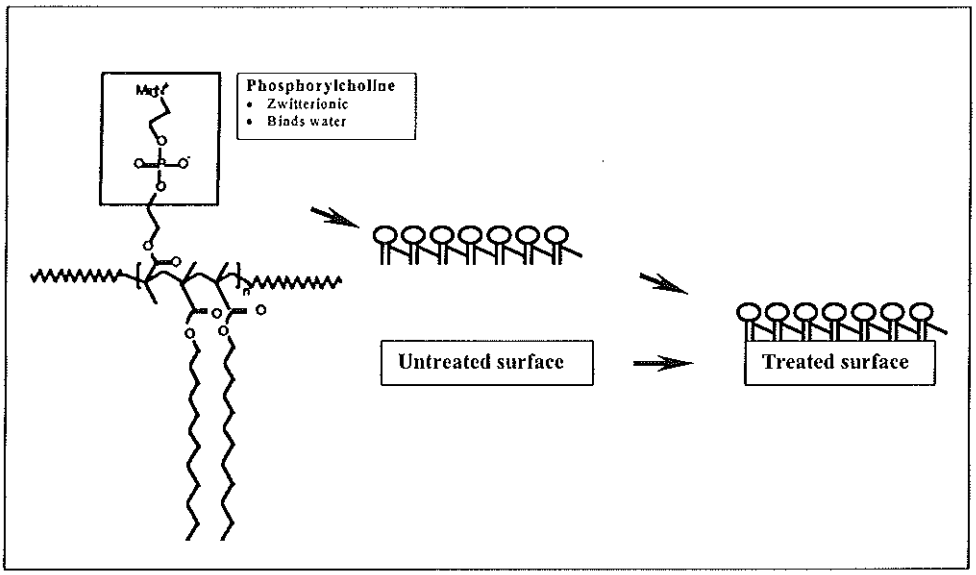

Fig 1. A schematic representation of the PC coating applied to the *divYsio* stent (modified from Campbell et al.[8])

coated with PC.[4][5][6][7] In this study the PC coating applied to a metallic coronary stent consists of a copolymer of laurylmethacrylate and methacryloylphosphorylcholine, the latter as the PC component (Fig 1).[8] The zwitterionic head group of PC provides a hydrophilic surface whilst retaining an overall neutral charge. The PC head group that is held responsible for improving biocompatibility, accounts for a small part of the coating, while the majority of the coating consists of components to give the required adhesion to the metal surface. Although the PC component is based on a naturally occurring phosphatidylcholine (a constituent of the lipid bilayer of the cell membrane), the current coating must be regarded as entirely synthetic. As many synthetic polymers have been associated with aggressive inflammatory reactions and excessive neointimal growth[9] it is important to study both the early and late response to the coating.

The aim of this study was therefore to assess the blood and tissue biocompatibility of the coating, particularly its influence on the wound healing response, its effect on subsequent neointima formation and the vessel wall reaction to the synthetic coating in a porcine coronary stent model.

METHODS

Balloon-expandable stent.

The stent used in the current study is the balloon-expandable *divYsio* stent (Biocompatibles Cardiovascular, Farnham, UK) (Fig 2). This stent, with a length of 15mm, has an interlocking arrowhead design and is constructed of medical grade stainless steel 316L. Both phosphorylcholine coated (PC) and non-coated (NC; bare) stents were used.

Phosphorylcholine coating.

The coating applied to the *divYsio* stent consists of a copolymer of methacryloylphosphorylcholine and laurylmethacrylate (Fig 1). The stents were dip coated from a solution of the polymer in ethanol to give a coating that is approximately 50nm thick. The stents were received pre-coated and sterilised from Biocompatibles Cardiovascular, UK.

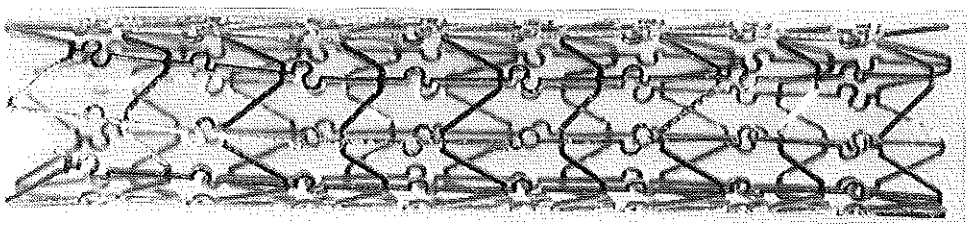

Fig 2. A macroscopic view of the *divYsio* stent.

Animal care.

Experiments were performed under the regulations of the animal care committee of the Erasmus University Rotterdam and in accordance with the "Guide for the care and use of laboratory animals" (NIH publication 85-23).

Animal preparation.

Experiments were performed in crossbred Landrace - Yorkshire pigs (30kg) as previously described.[10] Starting one day prior to the procedure and throughout the follow-up period, all animals received 300mg Ascal (Carbasalatum calcium, Asta Medica B.V. The Netherlands) p.o. daily. After an overnight fast the animals were sedated with 20mg/kg ketamine hydrochloride. Anaesthesia was induced by 11mg/kg thiopental and following endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2, v/v). Anaesthesia was maintained with 1-2.5vol % isoflurane. Antibiotic prophylaxis was administered by an intramuscular injection of 1ml/25kg Streptoprocpen (A.U.V., Cuijk, The Netherlands, containing 200mg procaine benzylpenicillin and 250mg dihydrostreptomycin sulphate per ml) as a standard laboratory procedure. Under sterile conditions an arteriotomy of the left carotid artery was performed and an 8F introduction sheath was placed. After measurement of arterial blood pressure and heart rate, and withdrawal of an arterial blood sample for the measurement of activated partial thromboplastin time (APTT), blood gases and acid-base balance (settings of the ventilator corrected, if necessary), 10,000 IU heparin sodium and 250mg aspirin were administered through the sheath and an 8F guiding catheter was advanced to the ascending aorta. An APTT of at least three times baseline was maintained throughout the procedure. Coronary angiography was performed using Omnipaque (Nycomed Ireland Ltd., Cork, Ireland) as contrast agent, and quantitative angiographic analysis was performed using the edge-detection method (Cardiovascular Measurement System, Medis Inc., Nuenen, The Netherlands).[11]

Stent implantation.

From the angiograms, a coronary segment with a diameter of 2.5 mm to 3.5 mm was selected in the proximal left anterior descending coronary artery (LAD), left circumflex coronary artery (LCX) or right coronary artery (RCA) using on-line quantitative coronary angiography. Stents were randomised and implanted in a paired fashion in separate coronary arteries. While a preference was given for the LAD/LCX (14 animals) stents were also implanted in

the LAD/RCA (1 animal) and LCX/RCA (1 animal). Due to vessel size or tapering in the remaining 9 animals, stents were implanted in single vessels (Tables 1A, 1B). Side branches and curved coronary artery segments were not avoided. A PC or NC stent was crimped onto a deflated balloon and advanced over a 0.014-inch steerable guidewire to the preselected site for implantation. The balloon was then inflated for 30 seconds (mean inflation pressure 9 atm), deflated and negative pressure maintained for 60 seconds. Matched implantation of the stent and artery was performed, such that the intended balloon/artery ratio was as near to 1 as possible. The catheter was then slowly withdrawn while leaving the stent in place. After repeat angiography, the guiding catheter and the introducer sheath were removed, the arteriotomy repaired and the skin closed in two layers. At the end of the procedure, the animals were allowed to recover and returned to the animal care facilities.

In total, 41 stents (20 PC, 21 NC) were implanted into 25 animals and these animals were assigned to 3 groups (Table 1A, 1B) to study the progress of wound healing and neointima formation at 5 days, 4 weeks and 12 weeks.

Follow-up angiography.

The anaesthesia and catheterisation procedures at follow-up were similar as described above, while coronary angiography was performed in the same projection, and using identical settings of the X-ray equipment as during implantation.

Microscopical examination.

After angiography at follow-up, the thorax was opened by a midsternal split and a lethal dose of sodium pentobarbital was injected intravenously, immediately followed by cross-clamping of the ascending aorta. After puncturing the aortic root above the coronary ostia, 300ml of saline was infused under a pressure of 150 cm H_2O, followed by 500ml of 2.5% glutaraldehyde in 0.15 mol/L cacodylatebuffer for scanning electron microscopy (SEM) or 500ml of 4% buffered formaldehyde for light microscopy (LM). The heart was then excised and the coronary arteries dissected free from the epicardial surface. The stented and adjacent unstented (5mm) segments were kept in fixative for at least 24 hours and thereafter were further processed for LM examination[12] or for SEM as previously described.[10] For LM microscopy, stented vessels were embedded intact in methylmethacrylate, sectioned and stained with haematoxylin-eosin as a routine stain, and resorcin-fuchsin as an elastin stain. Lectin histochemistry was performed to confirm the identity of the regenerated endothelium.[13]

Table 1A. Distribution of stents in coronary vessel pairs at different time points.

Vessel pairs	5 day	4 week	12 week
LAD/LCX	5	4	5
LAD/RCA	1	0	0
LCX/RCA	0	1	0
LAD only	0	3	4
LCX only	0	0	2

Table 1B. Distribution of PC and NC stents in coronary arteries at different time points.

	LAD	LCX	RCA
5 DAY			
PC	4	2	0
NC	3	2	1
4 WEEK			
PC	5	1	0
NC	2	4	1
12 WEEK			
PC	5	3	0
NC	4	4	0

Morphometry.

Morphometric analysis of the neointimal formation in vessels processed for light microscopy was performed in the proximal, middle and distal stent segment on elastin stained sections by tracing the

	QCA pre (mm)	QCA post (mm)	QCA FU (mm)	B/A Ratio	N
5 Days					
Coated	2.9±0.5	3.1±0.3	3.0±0.3	1.1±0.1	6
Non-coated	3.2±0.3	3.3±0.3	3.2±0.3	1.0±0.0	6
4 Weeks					
Coated	3.2±0.2	3.3±0.2	2.8±0.5	1.0±0.1	7
Non-coated	2.9±0.4	3.1±0.2	3.0±0.3	1.2±0.2	6
12 Weeks					
Coated	3.0±0.3	3.1±0.5	3.2±0.5	1.0±0.1	8
Non-coated	3.0±0.3	3.0±0.3	2.9±0.4	1.0±0.1	8

Table 2. Quantitative Coronary Angiography at 5 days, 4 and 12 weeks, after implantation of the phosphorylcholine coated and non-coated *divYsio* stents. Data as Mean ± SD. B/A ratio: balloon/artery ratio. N= number of stented coronary arteries.

external and internal elastic laminae and the endothelial lining using a microscopy image analysis system (Impak C, Clemex vision Image analysis system, Clemex Technologies Inc., Quebec, Canada). The media was defined as the layer between the internal and external elastic laminae. The area between the endothelial lining and the internal elastic lamina was taken as the intima.

Scanning electron microscopy and planimetry.

SEM was performed to study endothelial regrowth at 5 days. After fixation with 2.5% glutaraldehyde in 0.15 mol/L cacodylatebuffer, the arteries were further processed as previously described.[10] Assessment of endothelial regrowth was performed by studying 8 areas in the proximal, middle and distal stent segments of vessels by SEM. From photographs of these areas, the stent struts were traced, the endothelium marked and the percentage endothelial coverage of the stent struts quantified using computer assisted planimetry.

Statistical analysis.

Data were analysed using Jandel Sigmastat statistical software, version 2.0 (Jandel Corporation). All data are expressed as mean (SD) or as median (range). The angiographic and morphometric data were evaluated by a two way repeated measures analysis of variance (ANOVA) and a two way ANOVA respectively, followed by a Student-Neuman Keuls test. A p value of 0.05 was considered significant.

RESULTS

Procedural success.

A total number of 20 PC and 21 NC stents (25 animals) were implanted successfully. All animals survived the follow-up period without stent thrombosis or other adverse events. However, in the 4-week group there was one incidence of stent collapse, where the excised stented vessel appeared to be elliptical rather than round.

Quantitative angiographic measurements.

Quantitative angiographic measurements are summarised in Table 2. With a mean balloon diameter of 3.1±0.3mm at maximal pressure, stent recoil was 2 ± 7%. The results at 5 days, 4 and 12 weeks showed no significant difference in late loss (post versus follow-up) between the PC and NC groups. However, there appeared to be a

trend towards an increased late loss in the coated group at 4 weeks which is explained by one incidence of stent collapse in this group (angiographic late loss then seems abnormally large). Such a trend was not observed in the morphometric results and therefore deemed unimportant. Indeed, the neointimal thickness in this group was identical to that of the control group at 4 weeks (Table 3).

General histopathology and performance of the stent coating.

Histopathology 5 days.

In this group, 2 vessels (1 animal; 1PC, 1NC) were examined by LM, the remainder by SEM for evaluation of endothelialisation. In the two arteries examined by LM the intimal thickening consisted of organising thrombus or granulation tissue containing inflammatory cells (macrophages, macrophage-giant cells, granulocytes)(Fig 3A). In both vessels, macrophage giant cells were frequently seen surrounding the stent struts voids. In the PC group, the purple stained coating was still discernible in the stent strut voids and did not appear to elicit an adverse inflammatory response. Both arteries showed adhesion and infiltration of leukocytes to an incomplete endothelial layer, the identity of the endothelium being confirmed by lectin histochemistry. The medial and adventitial layers were normal. In both the PC and NC groups, SEM showed an advanced, but incomplete endothelial covering, with macrophages frequently occupying the areas that were devoid of endothelium (Fig 3B).

Histopathology at 4 weeks.

At four weeks in both the PC and NC groups, the asymmetric neointima (Fig 4) consisted of smooth muscle cells in an extracellular matrix, covered by an endothelial layer with some adherent leukocytes. Towards the intimal/medial borderzone the cellular density was distinctly less and the cells were in a haphazard orientation with some containing thrombus remnants, inflammatory cells and foam cells. Macrophage giant cells were observed surrounding the stent strut voids in both groups. In areas of maximal intimal thickening there was often abundant extracellular matrix. The medial and adventitial layers were only affected by inflammation in the case of medial ruptures or complete dissection (4 vessels: 2PC, 2NC), but these inflammatory changes were discrete. In the PC group, the purple stained coating in the stent strut voids was discernible in 5 of the 7 vessels.

Histopathology 12 weeks.

In both groups the neointima consisted of smooth muscle cells in an extracellular matrix with an organisation similar to that observed in the 4 - week group, except that the intimal/medial borderzone sometimes contains acellular areas. In this borderzone, inflammation and neovascularisation were observed concomitant with medial damage. Some diffuse inflammatory infiltrates were seen in the adventitia (3 vessels). In the PC-stented arteries purple stained coating was still observed in the stent strut voids in 6 out of 8 vessels (Fig 5A).

Morphometry.

Endothelialisation.

At 5 days the percentage of endothelium covering the stent struts was determined using SEM (Fig 3B) and amounted to 91% (range 76-93) for the PC stent and 92% (range 70-98) for the NC stent which was not significantly different.

Neointimal formation.

The data for the 4 and 12- week study groups are summarised in Table 3. There is no significant difference in intimal thickness and area between the PC and NC stents at four weeks. While it was attempted to implant the stents with minimal vascular damage (balloon-artery ratio of approximately 1), on 3 occasions stent implantation resulted in excessive vascular damage, with a concomitant inflammatory response in the 12-week group. Analysis of this group was therefore performed with (PC n=8, NC n=8) and without (PC n=6, NC n=7) inclusion of these vessels in an attempt to ascertain whether the inflammatory reaction somehow masked any small

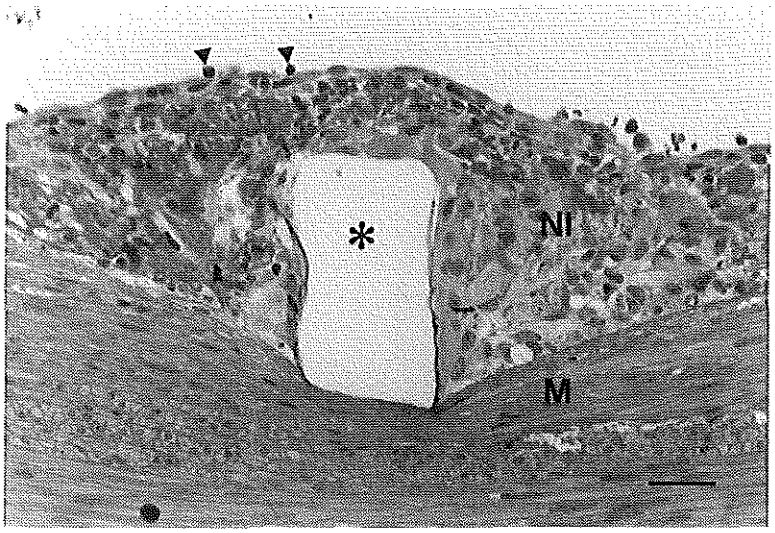

Fig 3A. Light microscopy of the intimal thickening at 5 days post implant, showing granulation tissue over the stent wire void (*), with occasional leucocytes (arrowheads) attached to the endothelium. NI = neointima; M = media. Haematoxylin-eosin. Bar = 30μm

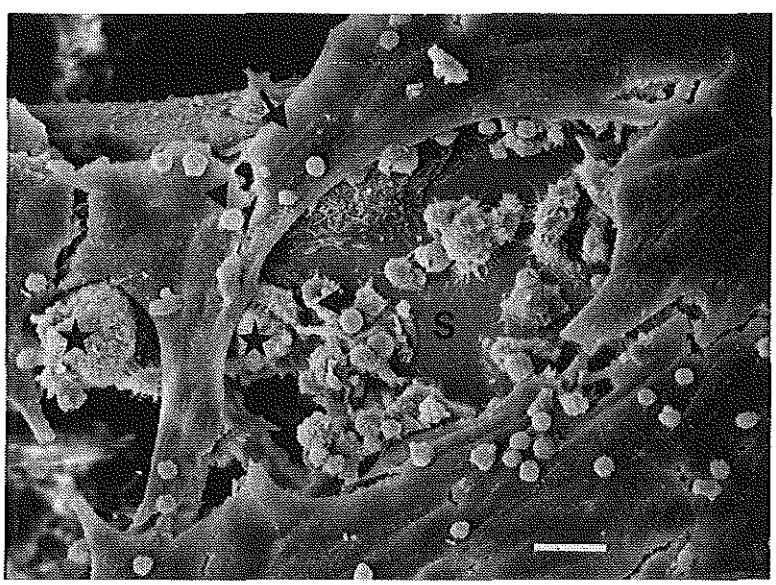

Fig 3B. A scanning electron micrograph of a stent strut (S) at 5 days, showing an incomplete endothelial lining (arrow), with leucocytes (arrowheads) and macrophages (★) occupying areas devoid of endothelium. Bar = 20μm

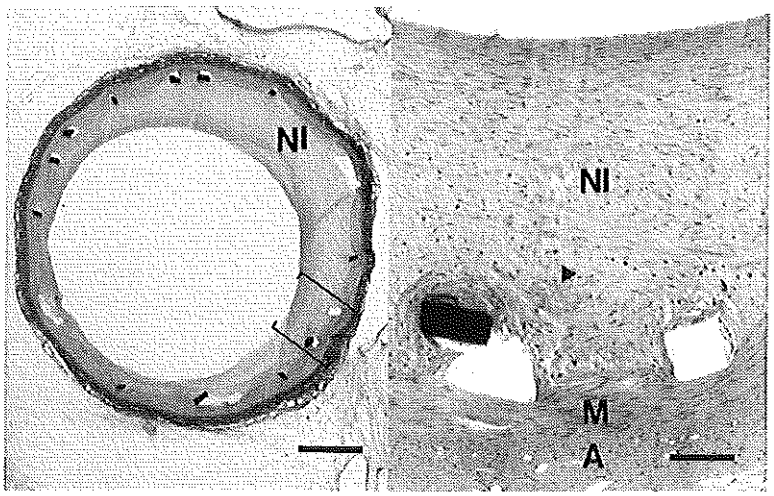

Fig 4. Overview (left) of a stented artery at 4 weeks showing an asymmetric neointima (Bar = 500μm), with detail (right) showing cells in a haphazard orientation with thrombus remnants (arrowhead) in the intimal/medial border zone. NI=Neointima; M=media, A=adventitia. Haematoxylin-eosin. Bar = 100μm

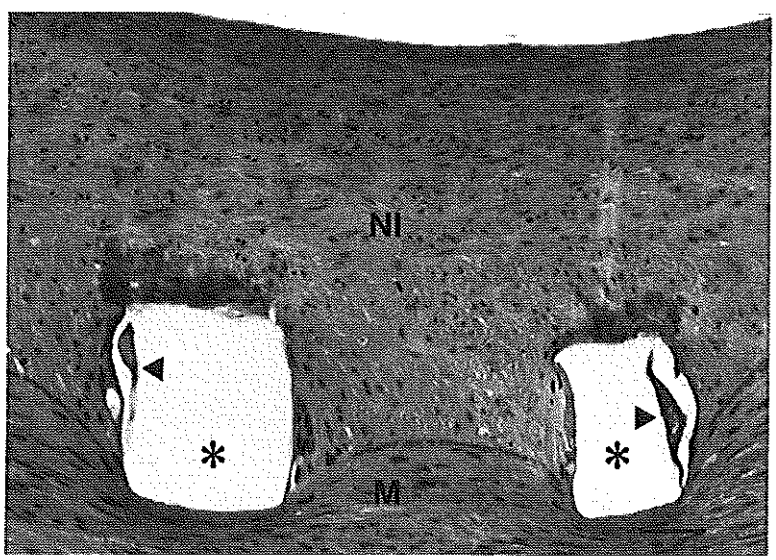

Fig 5A. Detail of purple stained coating (arrowheads) observed in the stent strut void (*) 12 weeks after implantation of a PC stent. NI= Neointima; M=Media. Haematoxylin-eosin. Bar = 45μm.

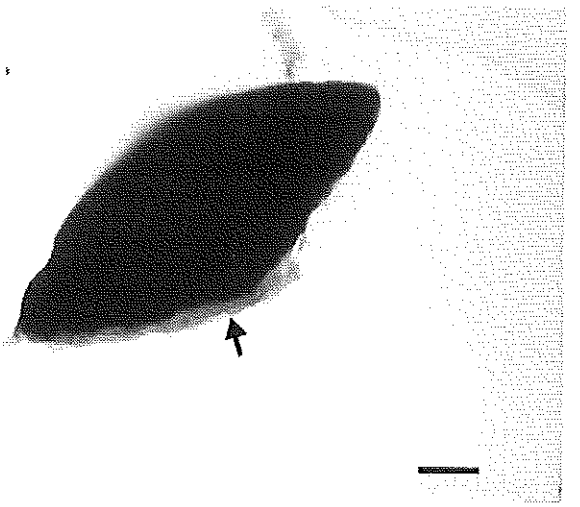

Fig 5B. Detail of the cut surface of a stent strut from a non-implanted PC stent. The PC coating (arrow) seen at the strut edge is similar in appearance to that seen in Fig 5A. Haematoxylin-eosin. Bar = 20μm.

differences in intimal thickness or area in this group. The results in Table 3 show that when the oversized vessels are excluded from analysis, there is no significant difference in the intimal thickness and area between the PC and NC groups at 12 weeks.

DISCUSSION

Although coronary stenting is regarded as a major success in interventional cardiology, the problem of restenosis, and to a lesser extent that of stent thrombosis, remain limiting factors that restrict its use in a wider patient population.

Clinically approved stents are all made of metal. In an effort to reduce the above-mentioned problems, coating the metal with a molecule that mimics a biological, naturally occurring molecule (a form of biological camouflage or biomimicry) has raised hopes that these problems can be reduced or eliminated.[14][15] In this respect, our study addressed the effects of coating a stainless steel metal stent with a synthetic polymer containing a phosphorylcholine head group. Previous work has demonstrated its success in improving biocompatibility of surgical equipment and guide wires[4][5][6][7] and in reducing neointimal hyperplasia in synthetic vascular grafts in a canine model.[16]

Objective and main findings:

The principle objective of this study was to determine the biocompatibility of the PC coating over a time period of up to 12 weeks, comparing it to a non-coated equivalent. The main finding was that the coating has an excellent biocompatibility. The PC coating did not induce stent thrombosis, had no adverse effect on the rate of reendothelialisation and was present for the duration of the study. The wound healing response for PC and NC stents was similar at both 4 and 12 weeks.

Animal model and injury:

In the porcine model of oversized stent implantation, the degree of vascular injury that accompanies stent implantation induces a variable amount of inflammation and neointimal growth. In this respect,

Table 3. Morphometric analysis at 4 and 12 weeks after implantation of the phosphorylcholine-coated and non-coated *divYsio* stents.

	Lumen area	NI area*	Media area	NI thickness at stent struts*	Media thickness at stent struts	NI thickness between stent struts	Media thickness between stent struts
4 weeks							
Coated (n=7)	5.76±1.63	1.71±0.67	1.46±0.55	0.24±0.09	0.09±0.04	0.18±0.08	0.13±0.03
Non-coated (n=6)	6.82±2.27	1.81±0.99	1.24±0.11	0.25±0.13	0.08±0.02	0.18±0.13	0.13±0.02
12 weeks							
Coated (n=8) N=6	5.89±1.37	2.22±1.54 1.38±0.54†	1.18±0.29	0.32±0.22 0.20±0.08†	0.06±0.02	0.23±0.18 0.14±0.07†	0.12±0.04
Non-coated (n=8) N=7	6.16±1.79	1.41±0.77 1.19±0.48†	1.13±0.15	0.22±0.12 0.19±0.07†	0.06±0.01	0.15±0.11 0.12±0.06†	0.12±0.02

Data as Mean ± SD. Area in mm^2. Thickness in mm. *Including area occupied by the stent strut. † Data when aberrant results due to rupture of EEL and excessive inflammatory reaction have been excluded.

variation in vessel wall response due to the effect of coatings and the induced vessel injury are less easily distinguished from one another. In our study therefore, we chose a matched-size model for stent implantation, which allowed us to detect small differences in the vessel wall response to the PC coating without being overshadowed by a considerable response to injury. In the 12 week group however, 3 stents were clearly oversized (2PC, 1NC). Inclusion of the morphometric data for these animals would appear to suggest that the NI increased in the PC group between 4 and 12 weeks. However if the data is excluded for those animals in which oversized stents were implanted, then there is normal regression of the NI in both groups at 12 weeks.

Blood biocompatibility and early wound healing:

In a previous study using the stainless steel Palmaz-Schatz stent (same material, different geometry and surface characteristics), stents thrombosed irrespective of whether acetylsalicylic acid was post-operatively administered or not.[17][18] Absence of thrombosis in our study in both the PC and NC groups may be explained by the fact that the geometry and/or surface characteristics of the stent are such that it is inherently less thrombogenic. In our animal model both the stent and the PC coating showed excellent blood compatibility.

Following stent implantation, it is desirable that the vascular wall heals as quickly as possible, thereby incorporating the stent into the vessel wall. Histological parameters of completed vessel wall healing are re-endothelialisation and the progression of granulation tissue to a mature neointima. In a previous study, data suggested that a heparin coating delayed re-endothelialisation at 4 weeks, such that the neointimal thickening was also affected.[17] We therefore used the rate of endothelialisation as a parameter of the early wound healing process. The effect of PC on wound healing and neointimal formation after stenting is unknown. Theoretically the PC group might be expected to retard the rate of re-endothelialisation since it inhibits the deposition of plasma proteins onto the stent surface.[19] The absence of a protein layer may make the stent surface less "attractive" for the endothelial cells to grow on.[20] In this model however, the percentage endothelial covering after 5 days as assessed by planimetry was similar in both the PC and NC groups (91% *v* 92%). Therefore the PC coating did not delay the early wound healing response.

Tissue biocompatibility:

At 4 weeks there was no difference in neointimal thickness between the PC and NC groups (PC: 0.14±0.09mm; NC: 0.15±0.13mm). These results correlate with those from Kuiper et al[21] in so far that he also found no difference in neointimal thickness between the PC and NC stents. However his study restricted it's follow-up to 28 days only. In studying the biocompatibility of synthetic polymers it is important to study the reaction at multiple time points, given the potential for synthetic polymers to show delayed inflammatory reactions. We therefore studied the vessel wall reaction to the coating at multiple timepoints up to 12 weeks.

At 12 weeks in both the PC and NC groups, a morphologically mature neointima was formed. In the area of the stent strut voids macrophage giant cells were occasionally associated with the implant. Such cells were observed in both PC and NC groups, and are part of the normal reaction to foreign implants. In the PC group, evidence of the coating was observed in the stent struts voids up to 3 months post implant. Given that such purple stained material is similar to that observed when stained cross-sections of a non-implanted PC stent were examined for the presence of the coating (Fig 5B), we conclude that this coating remains present up to 3 months post implantation. Indeed, our findings have recently been corroborated by Tolhurst[22] who reported results showing that the coating does not degrade and is stable up to six months post implantation. (Although the

Table 4. Coated stents

Author (ref)	Stent	Animal Model	Coating	Control	NI thickness (mm)		Duration (Weeks)
					Coated	Control	
-	*divYsio*	Porcine coronary, matched	PC	Bare stent	0.14±0.09[§]	0.15±0.13[§]	4
				Bare stent	0.10±0.08[§]	0.09±0.07[§]	12
Kuiper [21]	Palmaz-Schatz	Rabbit iliac, Porcine coronary, Matched	PC	Bare Stent	0.20±0.05 0.44±0.27	0.23±0.11 0.47±0.38	4 4
van der Giessen [9]*	Wiktor	Porcine coronary, matched	PGLA	Bare stent	0.46±0.18	0.08±0.03	4
			PEO	Bare stent	2.36±0.60	0.38±0.17	4
Hardhammer [17]	Palmaz-Schatz	Porcine coronary, matched	Heparin	Bare stent	0.26±0.10	0.11±0.04	4
				Bare stent	0.15±0.06	0.20±0.05	12
van der Giessen [26]	Wallstent	Porcine coronary,matched	Biogold	Bare stent	0.11 (0.04-0.17)[†]	0.10 (0.07-0.19)[†]	12
de Scheerder [27]	Not described	Porcine coronary, oversized injury model	POP/MPD	POP stent	0.99±0.28	1.74±0.84	6
Cox [28]	Cook	Porcine coronary, oversized injury model	CEL/HEP/MET	Bare stent	-	-	4
Lincoff [23]	Wiktor	Porcine coronary, oversized injury model	HMWPLLA/DEX	PLLA stent	0.79±0.44[‡]	0.81±0.23[‡]	4
				Bare stent		0.88±0.31[‡]	
Aggarwal [34]	Cook	Rabbit iliac, balloon injury model	CEL/GPIIb/IIIa	CEL ± anti CMV	0.12±0.04	0.11±0.07	4

PGLA = polyglycolic/lactic acid; PEO = polyethyleneoxide. *Representative values for the best and worst performing polymers are given; POP/MPD = polyorganophosphazene/methylpredisnolone; CEL/HEP/MET = cellulose/heparin/methotrexate; HMWPLLA/DEX = high molecular weight poly-L lactic acid/dexamethasone. †(Range). ‡ Values calculated from Figure 6, Lincoff *et al.* [23] §For comparative purposes, the thickness of the wire struts have been deducted from the data.

PC coating on the stent is thin, some swelling of the polymer occurs during histological processing, such that it is visible with light microscopy). The fact that there was a minimal inflammatory response to the coating is a reassuring finding, since the PC coating is composed of synthetic polymers and to date most synthetic materials used to coat stents have often been associated with intense inflammatory reactions and variable neointimal growth.[9][23][24][25]

PC compared to other coatings

Table 4 compares the neointimal thickness (NI) from our study with those of other studies using coated stents in both matched sized models and oversized injury models.

In matched sized models of stent implantation[9][17][26], when results of NI for the control stents are grouped, the range of NI is 0.10mm-0.40mm. The NI of the PC group is well within this range. Indeed the NI of the PC group is comparable to that of the heparin-coated Palmaz-Schatz stent, which is expected to be approved by the FDA in 1999. Given that the PC coating can be regarded as a synthetic polymer, the results appear to be considerably better than those using PGLA and PEO strips (amongst others) applied to the Wiktor stent.[9]

In the 12-week PC group, 2 stents were oversized at implantation. If the mean NI for these vessels (0.62±0.11mm) is compared to the grouped range in the oversized injury group (0.11mm-1.74mm) [23][27][28], then the PC result (oversized group) again falls within this range. In the study of de Scheerder[27], the NI of the eluting stent is reduced compared to the control POP stent (0.99±0.28mm *v* 1.74±0.84mm), but this NI is still almost twice as large as that of the NI for the oversized PC stents in the current study (0.62±0.11mm *v* 0.99±0.28mm). Therefore, in comparison to other studies of coated stents and irrespective of the model used, the synthetic PC coating performs well.

Potential of the coating

The complex processes that lead to restenosis can probably never be resolved by a single therapy alone. The possibility of using a coated stent as a carrier of drugs has therefore great therapeutic potential. To date studies involving synthetic polymer-coated stents can be divided into those used (a) to explore the potential of various materials as polymer stent coatings,[9][29][30][31] (b) *in-vitro* drug-release kinetic studies,[32][33] and (c) for *in-vivo* efficiency studies of local drug delivery to the vessel wall.[23][27][34] The results of such experiments have generally shown that while drug release is feasible, the drug may be effective in reducing only the unwanted side effects of the polymer used, but not in reducing neointimal hyperplasia per se. In the present study the coating remained present up to 12 weeks post implant, without excessive neointimal growth (compared to the control). While the amount of polymer remaining cannot be quantified in this study, nor can it be ascertained if there is a gradient of polymer depletion over time, the long-term therapeutic potential of this particular coating in it's present form is significantly improved over currently available synthetic polymer stent coatings. Such findings suggest that this polymer can potentially be used as a vehicle for local drug delivery, and initial drug release studies are underway using this PC coated stent. By controlling the physiochemical properties of the coating (crosslinked density, water content, isoelectric point, molecular weight) the stent coating can be tailored to the specific drugs under investigation.

CONCLUSION

Both PC-coated and NC *divYsio* stents were implanted with a 100% success rate without incidence of acute stent thrombosis, despite an anti-platelet regime of only aspirin. Phosphorylcholine applied to the *divYsio* stent as a coating does not interfere with endothelialisation as measured at 5 days after implantation. During the subsequent process of wound healing, the PC and NC stents elicit a tissue-response that is similar in nature. The only observed

difference between the two stents is the presence of the PC coating in the stent strut voids up to 12 weeks after implantation. Given the long-term presence of a coating that shows no adverse inflammatory reaction, the PC coating shows potential as a suitable candidate for local drug delivery.

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REFERENCES

1. Moussa I, Di Mario C, Di Francesco L, *et al.* Subacute stent thrombosis and the anticoagulation controversy: changes in drug therapy, operator technique, and the impact of intravascular ultrasound. *Am J Cardiol* 1996;**78(3A)**:13-7.

2. Keane D, Haase J, Slager CJ, *et al.* Comparative validation of quantitative coronary angiography systems. Results and implications from a multicenter study using a standardised approach. *Circulation* 1995;**91(8)**:2174-83.

3. Goods CM, Al-Shaibi KF, Yadav SS, *et al.* Utilisation of the coronary balloon-expandable coil stent without anticoagulation or intravascular ultrasound. *Circulation* 1996;**93(10)**:1803-8.

4. von Segesser LK, Olah A, Leskosek B, *et al.* Coagulation patterns in bovine left heart bypass with phospholipid versus heparin surface coating. *Asaio J* 1993;**39(1)**:43-6.

5. Hunter S, Angelini GD. Phosphatidylcholine-coated chest tubes improve drainage after open heart operation. *Ann Thorac Surg* 1993;**56(6)**:1339-42.

6. Veil KR, Chronos NAF, Palmer SJ, *et al.* Phosphorylcholine: a biocompatible coating for coronary angioplasty devices. *Circulation* 1995;**92(8)**:2337 (abstract).

7. Plante S, Juneau C. Thrombogenicity of PTCA guide wires: a scanning electron microscopy study. *Circulation* 1997;**96(8)**:4243 (abstract).

8. Campbell EJ, O'Byrne V, Stratford PW, *et al.* Biocompatible surfaces using methacryloylphosphorylcholine laurylmethacrylate copolymer. *Asaio J* 1994;**40(3)**:M853-7.

9. van der Giessen WJ, Lincoff AM, Schwartz RS, *et al.* Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. *Circulation* 1996;**94(7)**:1690-7.

10. van der Giessen WJ, Serruys PW, van Beusekom HM, *et al.* Coronary stenting with a new, radiopaque, balloon-expandable endoprosthesis in pigs. *Circulation* 1991;**83(5)**:1788-98.

11. Reiber JH, Serruys PW, Kooijman CJ, *et al.* Assessment of short-, medium-, and long-term variations in arterial dimensions from computer-assisted quantitation of coronary cineangiograms. *Circulation* 1985;**71(2)**:280-8.

12. van Beusekom HMM, Whelan DM, Van der Plas M, *et al.* A practical and rapid method of histological processing for examination of coronary arteries containing metallic stents. *Cardiovasc. Pathol* 1996;**5**:69-76.

13. van Beusekom HMM, van der Giessen WJ, Wagenvoort CA, *et al.* Histological features of a polymer endovascular prosthesis after transcatheter implantation in porcine arteries. *Cardiovasc Pathol* 1993;**2(1)**:41-51.

14. Baron JH, de Bono DP, Azrin MA, *et al.* Adsorption and elution of c7E3Fab from polymer-coated stents in vitro: local delivery of an anti-thrombotic agent that also may inhibit restenosis. *Circulation* 1997;**96(8)**:2254 (abstract).

15. McKenna CJ, Camrud AR, Wolff R, *et al.* Fibrin-film stenting in a porcine coronary injury model: efficacy and safety compared to uncoated stents. *Circulation* 1997;**96(8)**:81 (abstract).

16. Chen C, Lumsden AB, Ofenloch JC, *et al.* Phosphorylcholine coating of ePTFE grafts reduces neointimal hyperplasia in canine model. *Ann Vasc Surg* 1997;**11(1)**:74-9.

17. Hardhammar PA, van Beusekom HMM, Emanuelsson HU, *et al.* Reduction in thrombotic events with heparin-coated Palmaz-Schatz stents in normal porcine coronary arteries. *Circulation* 1996;**93(3)**:423-30.

18. Rogers C, Edelman ER. Endovascular stent design dictates experimental restenosis and thrombosis. *Circulation* 1995;**91(12)**:2995-3001.

19. Ikada Y. Surface modification of polymers for medical applications. *Biomaterials* 1994;**15(10)**:725-36.

20. van Wachem PB, Vreriks CM, Beugeling T, *et al.* The influence of protein adsorption on interactions of cultured human endothelial cells with polymers. *J Biomed Mater Res* 1987;**21(6)**:701-18.

21. Kuiper KKJ, Robinson KA, Chronos NAF, *et al.* Phosphorylcholine-coated metallic stents in rabbit iliac and porcine coronary arteries. *Scand Cardiovasc J* 1998;**32**:261-8.

22. Tolhurst LA. The analysis of post implant biodivYsio stents. *Proceedings of the Medical Device Technology Conference, March 1999, London, UK.* 1999:427-41.

23. Lincoff AM, Furst JG, Ellis SG, *et al.* Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. *J Am Coll Cardiol* 1997;**29(4)**:808-16.

24. Wilczek K, Scheerder ID, Wang K, *et al.* Comparison of self-expanding polyethylene

terephthalate and metallic stents implanted in porcine iliac arteries. *Cardiovasc Intervent Radiol* 1996;**19(3)**:176-80.
25. Murphy JG, Schwartz RS, Edwards WD, *et al.* Percutaneous polymeric stents in porcine coronary arteries. Initial experience with polyethylene terephthalate stents. *Circulation* 1992;**86(5)**:1596-604.
26. van der Giessen WJ, van Beusekom HMM, van Houten CD, *et al.* Coronary stenting with polymer-coated and uncoated self-expanding endoprostheses in pigs. *Cor Art Dis* 1992;**3**:631-40.
27. de Scheerder I, Wang K, Wilczek K, *et al.* Local methylprednisolone inhibition of foreign body response to coated intracoronary stents. *Coron Artery Dis* 1996;**7(2)**:161-6.
28. Cox DA, Anderson PG, Roubin GS, *et al.* Effect of local delivery of heparin and methotrexate on neointimal proliferation in stented porcine coronary arteries. *Cor Art Dis* 1992;**3**:238-48.
29. De Scheerder IK, Wilczek KL, Verbeken EV, *et al.* Biocompatibility of polymer-coated oversized metallic stents implanted in normal porcine coronary arteries. *Atherosclerosis* 1995;**114(1)**:105-14.
30. De Scheerder IK, Wilczek KL, Verbeken EV, *et al.* Biocompatibility of biodegradable and nonbiodegradable polymer-coated stents implanted in porcine peripheral arteries. *Cardiovasc Intervent Radiol* 1995;**18(4)**:227-32.
31. Fontaine AB, Koelling K, Passos SD, *et al.* Polymeric surface modifications of tantalum stents. *J Endovasc Surg* 1996;**3(3)**:276-83.
32. Dev V, Eigler N, Sheth S, *et al.* Kinetics of drug delivery to the arterial wall via polyurethane-coated removable nitinol stent: comparative study of two drugs. *Cathet Cardiovasc Diagn* 1995;**34(3)**:272-8.
33. Lambert TL, Dev V, Rechavia E, *et al.* Localised arterial wall drug delivery from a polymer-coated removable metallic stent. Kinetics, distribution, and bioactivity of forskolin. *Circulation* 1994;**90(2)**:1003-11.
34. Aggarwal RK, Ireland DC, Azrin MA, *et al.* Antithrombotic potential of polymer-coated stents eluting platelet glycoprotein IIb/IIIa receptor antibody. *Circulation* 1996;**94(12)**:3311-7.

Chapter 9

Early and late reactions to PLA coated and drug loaded PLA coated stents in porcine coronary arteries

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Early and late reactions to PLA coated and drug-loaded PLA coated stents in porcine coronary arteries.

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ABSTRACT

Introduction. The treatment of restenosis using coated stents offers a two-fold approach: the stent acts as a mechanical scaffold reducing elastic recoil and remodeling, while locally delivered therapeutic agents eluting from the coating may prevent intimal thickening and thrombosis. We compared the effects of non-coated (NC), poly lactic acid (PLA) coated, and PLA-hirudin/iloprost (PLA-HI) coated metal stents on vessel wall healing and intimal thickening in a porcine coronary model. All stents were initially coated with a layer of iridium oxide to reduce thrombogenicity. The PLA-HI stent contained 5% hirudin and 1% iloprost.

Methods. Matched implantation of PLA-HI (n=20) with either C (n=10), or PLA (n=10) stents were performed in a paired fashion in the coronary arteries of 20 swine. After a follow up period of 2 or 12 weeks, repeat angiography was performed, the animals sacrificed, and the vessels processed for histology. Prior to sacrifice all animals received BrdU to assess the proliferative response, and at follow-up a tracer dye was injected to evaluate the permeability of the vessels. Angiographic late loss, neointimal thickness, % thrombus deposition, vessel injury score and inflammatory score were measured for all groups at both time points. Planimetry was used to assess the % thrombus deposition and % permeable areas.

Results. The results show that there were no significant differences in morphometric parameters, percentage thrombus deposition, vascular proliferative response (BrdU), inflammation score and injury score between the coated and bare stent groups at 2 or 12 weeks. Vascular permeability at 2 weeks was significantly different in the PLA-HI group only. The inflammatory and injury scores together with the vascular permeability increased in all groups over time. Due however to its larger group size, such changes were significant only for the PLA-HI stent.

Conclusion. While the eluting drugs had no effects on thrombus deposition or neointimal thickening, all 3 stents performed well in this animal model. Although the PLA coating was well tolerated up to 12 weeks, only longer-term studies will elucidate the true potential of this coating for vascular applications.

INTRODUCTION

The treatment of significant coronary artery stenosis using polymer coated stents offers a two-fold approach: the stent acts as a mechanical scaffold reducing elastic recoil and remodeling, while locally delivered therapeutic agents eluting from the coating may prevent intimal thickening and thrombosis. In the search for candidate materials to use as stent coatings, both biodegradable (bioabsorbable) and non-biodegradable synthetic polymers have been used[1,2]. For local drug delivery, biodegradable polymers offer the advantage that drug release is achieved by both diffusion from and degradation of the polymer. By controlling the degradation rate of the polymer, sustained drug release can be achieved for a prolonged period of time.

Biodegradable polymers must meet certain criteria: they must show blood and tissue compatibility, and their degradation products must neither accumulate in the body and be toxic, nor induce inflammatory reactions. A potential candidate polymer therefore is poly-lactic acid (PLA), which degrades to lactic acid, and is then metabolized to H_2O and CO_2. PLA has found medical application as screws and pins in orthopedics and has been used in vitro and in animal models for local delivery of antibiotics[3], chemotherapeutic agents[4] and hormones[5,6]. However, although it has been used for medical applications for many years now, it is not uncommon for very late inflammatory reactions (years post implant) to occur[7,8]. Given such potential for late inflammatory responses, it is essential that for vascular applications, both short and long-term studies of the polymer are performed.

Since stent implantation induces both a thrombotic and tissue response, drug loaded coated stents should ideally treat both aspects. In this respect, two possible candidate drugs are hirudin and iloprost. Hirudin is a potent anti-thrombotic, and from animal experiments it has been shown to significantly reduce platelet and fibrin deposition on coronary stents in comparison to heparin, dextran and aspirin[9]. It has also shown a reduction in neointimal proliferation after balloon angioplasty[10], although such an effect was not seen from clinical trials[11]. However in the latter study hirudin was administered intravenously and not locally. Iloprost, a stable prostacyclin analogue with a half-life of 30 minutes, suppresses platelet activation and aggregation and has a strong vasodilatory and anti-inflammatory effect. It has also been shown to inhibit PDGF-induced proliferation of smooth muscle cells[12].

Previous studies using PLA for vascular applications have shown variable results. While one study showed that PLA, applied as a stent coating on a metallic stent, had excellent blood and tissue compatibility up to 40 weeks post implant[13], its use as a drug-loaded stent coating showed less favorable short-term results[14]. In-vitro studies using PLA-coated stents loaded with hirudin and iloprost have shown that drug release is feasible up to 90 days post implant[15], and in vivo studies suggest that these drugs can attenuate the neointimal response post stenting[16,17]. However, the latter studies examined only the short-term effects of the coating on the vessel wall response, and given that biodegradable polymers may be associated with delayed inflammatory responses due to degradation of the polymer, it is imperative to also study the long-term biocompatibility of such a coating.

The aim of this study was to examine the long and short-term biocompatibility of PLA-coated and PLA-hirudin/iloprost coated stents (PLA-HI). The effects of both stent coatings, as well as the eluting drugs, on the vessel wall response were compared to that of a non-coated stent at both 2 and 12 weeks. Specifically, we examined the effects on both the neointimal and proliferative responses and assessed the endothelial function at two time points. In this respect we aim to expand upon the results of previous short-term studies[16,17].

METHODS

The stent.

The stent used in the current study is the balloon-expandable InFlow Dynamics stent (InFlow Dynamics AG, Munich, Germany). This stent is 7mm long and is constructed of medical grade stainless steel 316L. All stents were initially coated with a layer of iridium oxide to reduce thrombogenicity. Coated stents were dip-coated in either a solution of PLA only (D,L polylactide, 30kDa), or in a solution of PLA containing 5% polyethyleneglycol-hirudin (PEG-hirudin)and 1% Iloprost. Hirudin was coupled to PEG in order to prolong the stability of hirudin, without affecting the pharmacological properties[18]. The dip-coated stents had 200±102μg PLA per stent,

containing therefore 10μg PEG-hirudin and 2μg Iloprost. The stents were received pre-coated and sterilized from the manufacturer.

Animal care.

Experiments were performed under the regulations of the animal care committee of the Erasmus University Rotterdam and in accordance with the "Guide for the care and use of laboratory animals" (NIH publication 85-23).

Animal preparation.

Experiments were performed in crossbred Landrace - Yorkshire pigs (30kg) as previously described[19]. Starting one day prior to the procedure and throughout the follow-up period, all animals received 300mg Ascal (Carbasalatum calcium, Asta Medica B.V. The Netherlands) p.o. daily. After an overnight fast the animals were sedated with 20mg/kg ketamine hydrochloride. Anesthesia was induced by 11mg/kg thiopental and following endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2, v/v). Anesthesia was maintained with 1-2.5vol % isoflurane. Antibiotic prophylaxis was administered by an intramuscular injection of 1ml/25kg Streptoprocpen (A.U.V., Cuijk, The Netherlands, containing 200mg procaine benzylpenicillin and 250mg dihydrostreptomycin sulfate per ml) as a standard laboratory procedure. Under sterile conditions an arteriotomy of the left carotid artery was performed and an 8F introduction sheath was placed. After measurement of arterial blood pressure and heart rate, and withdrawal of an arterial blood sample for the measurement of activated partial thromboplastin time (APTT), blood gases and acid-base balance (settings of the ventilator corrected, if necessary), 10,000 IU heparin sodium and 250mg acetylsalicylic acid were administered through the sheath and an 8F guiding catheter was advanced to the ascending aorta. An APTT of at least three times baseline was maintained throughout the procedure. Coronary angiography was performed using Omnipaque (Nycomed Ireland Ltd., Cork, Ireland) as contrast agent, and quantitative angiographic analysis was performed using the edge-detection method (Cardiovascular Measurement System, Medis Inc., Nuenen, The Netherlands).

Stent implantation.

Using on-line quantitative coronary angiography, a coronary segment with a diameter of 2.5 mm to 3.5 mm was selected in the proximal left anterior descending coronary artery (LAD), left circumflex coronary artery (LCX) or right coronary artery (RCA). Matched, paired implantation of PLA-HI with either PLA or NC was performed. A preference was given for the LAD/LCX (19 animals), but in 1 animal stents were implanted in the LAD/RCA. Side branches and curved coronary artery segments were not avoided. Stents were crimped onto a deflated balloon and advanced over a 0.014 inch steerable guidewire to the preselected site for implantation. The balloon was then inflated for 30 seconds (mean inflation pressure 9 atm), deflated and negative pressure maintained for 60 seconds. The catheter was then slowly withdrawn while leaving the stent in place. After repeat angiography, the guiding catheter and the introducer sheath were removed, the arteriotomy repaired and the skin closed in two layers. At the end of the procedure, the animals were allowed to recover and returned to the animal care facilities.

In total, 40 stents (20 PLA-HI, 10 PLA, 10 NC) were implanted into 20 animals and these animals were assigned to 2 groups (Table A) to study the progress of wound healing and neointima formation at 2 and 12 weeks.

Follow-up angiography.

The anesthesia and catheterization procedures at follow-up were similar as described above, while coronary angiography

Vessel	*LAD*	*LCX*	*RCA*
	N	N	N
2 Weeks			
PLA	-	*5*	-
PLA-HI	*6*	*4*	
NC	*4*	*1*	-
12 Weeks			
PLA	*3*	*2*	-
PLA-HI	*4*	*5*	*1*
NC	*3*	*2*	-

Table A. Distribution of PLA, PLA-HI and NC stents in coronary arteries at 2 and 12 weeks.

was performed in the same projection, and using identical settings of the X-ray equipment as during implantation.

Assessment of vascular permeability at follow-up.

At both 2 and 12 weeks follow-up, intimal permeability was assessed using the dye Evan's Blue (Sigma Chemical Company, St Louis, USA) in a dye exclusion test. A full description of the procedure has been previously published[20]. In short, 300 ml of 0.3% (w/v) Evan's Blue in saline was administered intravenously over 30 minutes, and then allowed to recirculate for 30 minutes, before proceeding with the sacrifice. In this way all EB will bind to albumin and this EB-albumin complex (Mw 70kDa) was used as a test molecule for endothelial permeability. The permeable areas (blue stained areas) are expressed as a percentage of the total (explanted) opened vessel lumen area.

Assessment of cell proliferation.

To assess the proliferative response to stent implantation, all animals were given three intramuscular injections of 100, 50 and 50 mg/kg 5-Bromo-2-Deoxyuridine (BrdU, Sigma Chemical Company, St Louis, USA) at 8-hour intervals, starting 24h before sacrifice as previously described[20].

Macroscopic and microscopic examination.

After angiography at follow-up and infusion of the dye, the thorax was opened by a midsternal split and a lethal dose of sodium pentobarbital was injected intravenously, immediately followed by cross-clamping of the ascending aorta. The aortic root was punctured above the coronary ostia, and 300ml of saline infused under a pressure of 150 cm H_2O. The heart was then excised and the LAD, LCX and RCA dissected free from the epicardial surface. The non-stented vessel was used as a control. The excised arteries were opened longitudinally and checked under a dissection microscope for penetration of the blue dye and the presence of thrombus. Digital images of the arteries were made using an image analysis system (Impak C, Clemex Vision Image analysis system, Clemex Technologies Inc., Quebec, Canada). The images were stored and then used for planimetric assessment of the percentage permeable areas and the percentage thrombus deposition. Vessels were fixed in 4% buffered formaldehyde for 24 hours and then further processed for light microscopic (LM) examination[19]. Haematoxylin-eosin was used as a routine stain, while resorcin-fuchsin was used as an elastin stain. Lectin histochemistry was performed to confirm the identity of the regenerated endothelium as described earlier[21].

Estimation of % permeability and % thrombus deposition.

Planimetry was used to assess both the % thrombus deposition and % permeable areas from the digitized images of the opened vessels. From these images, the areas of thrombus deposition or blue areas (permeable areas) were marked and expressed as a percentage of the total area.

Detection of BrdU incorporation.

After acid denaturation and elimination of endogenous tissue peroxidase

activity, rehydrated paraffin sections were exposed to mouse anti-BrdU antibody (Becton Dickinson, San Jose, California, USA), diluted 1:80, to detect positive BrdU cells. As a secondary antibody, HRP-labeled rabbit anti-mouse antibody (Dako A/S, Glostrup, Denmark) was used, with 670 µg/ml diaminobenzadine (Sigma Chemical Company, St Louis, USA) in phosphate-buffered saline as a detecting reagent. Using light microscopy (LM), the total number of BrdU-positive cells were counted for each section using an automated computer program (Impak C, Clemex Vision Image analysis system, Clemex Technologies Inc., Quebec, Canada). The BrdU positive cells were expressed as a percentage of the total cell population per vessel. For each group an average cell density, together with an average percentage BrdU positivity was calculated in the intima, media and adventitia at each time point.

Morphometry.

Morphometric analysis of the neointimal formation in vessels processed for LM was performed at two levels in the stent. On elastin stained sections the external and internal elastic laminae and the endothelial lining were traced using a microscopy image analysis system (Impak C, Clemex vision Image analysis system, Clemex Technologies Inc., Quebec, Canada). The media was defined as the layer between the internal and external elastic laminae. The area between the endothelial lining and the internal elastic lamina was taken as the intima.

Inflammatory response

Inflammation was assessed in HE-stained sections. A distinction was made between the presence of foreign body giant cells (GC) and inflammatory infiltrates in the tissue layers. The foreign body reaction to the wires was graded as mild: 0-5 GC/section (average 12 stent voids counted per section); intermediate: 5-10 GC/section, and aggressive: >10 GC/section. Both the neointimal and adventitial inflammatory responses were graded as: 0 = no inflammatory infiltrates; 1 = occasional single inflammatory cell scattered in the neointima or adventitia; 2 = localized areas of concentrated inflammatory cells in the intima surrounding < 1/3 of stent wire, or in the adventitia; 3 = localized areas of concentrated inflammatory cells in the intima surrounding the majority of stent wires, or in the adventitia. An average score was calculated for each stent group at both time points.

Response to injury.

To evaluate the vessel wall damage caused by the stent, RF elastin-stained sections were analyzed and the vessel wall damage quantified by the vessel injury score, according to Schwartz[22]. Individual scores of the stent struts of 2 sections from one stent were averaged to obtain the mean vessel injury score per stented segment. An average score was calculated for each stent group at both time points.

Statistical analysis.

Data were analyzed using Jandel Sigmastat statistical software, version 2.0 (Jandel Corporation). All data are expressed as mean $\pm$ SD or as median (range). The angiographic data was analyzed by a one way repeated measures analysis of variance (ANOVA) and morphometric data was evaluated by a two way ANOVA respectively, followed by a Student-Neuman Keuls test. The inflammatory and injury scores, together with data for the percentage vascular permeability, thrombus deposition and proliferative response (BrdU) were analyzed by a one way ANOVA or Kruskal-Wallis ANOVA on Ranks (when normality test failed), followed if necessary by a Student-Neuman Keuls test. Differences in the above mentioned parameters between 2 and 12 weeks were analyzed using a t-test, or by a Mann-Whitney rank sum test (when normality test failed). A P value of <0.05 was considered significant.

	QCA Pre (mm)	QCA Post (mm)	QCA FU (mm)	B/A ratio	LL (mm)	N
2 Weeks						
PLA	3.0±0.3	3.4±0.3	3.0±0.3	1.1±0.1	0.3±0.2	5
PLA-HI	3.1±0.3	3.4±0.4	2.9±0.3	1.1±0.1	0.5±0.3	10
NC	3.2±0.3	3.3±0.3	2.9±0.3	1.1±0.1	0.4±0.1	5
12 Weeks						
PLA	2.9±0.3	3.0±0.2	2.9±0.2	1.1±0.1	0.1±0.1	5
PLA-HI	2.9±0.2	3.0±0.3	2.9±0.2	1.1±0.1	0.1±0.3	10
NC	3.1±0.2	3.3±0.2	3.1±0.3	1.1±0.1	0.2±0.3	5

Table B. Quantitative Coronary Angiography at 2 and 12 weeks after implantation of PLA, PLA-HI and NC stents. Data as mean lumen diameter ± SD. B/A ratio: balloon/artery ratio. N= number of stented coronary arteries.

RESULTS

Procedural success

A total of 10 NC (2 weeks n=5; 12 weeks n=5), 10 PLA (2 weeks n=5; 12 weeks n=5) and 20 PLA-HI coated stents (2 weeks n=10; 12 weeks n=10) were successfully implanted (Table A). There was no incidence of thrombotic occlusion, and all animals survived the follow-up period without adverse events.

Quantitative angiographic measurements.

Quantitative angiographic measurements are summarized in table B. The balloon:artery ratio in all groups was similar i.e. 1.0:1.1. Late loss (2 weeks: ~0.4mm; 12 weeks ~0.13mm) was comparable between the 3 stent groups and did not show any statistically significant difference at 2 or 12 weeks (p>0.05).

Macroscopic and microscopic examination.

Vascular permeability

The percentage permeable areas are represented graphically in Figure 2. Planimetric analysis of the opened stented vessels showed no significant difference in percentage blue areas (permeable areas) between the PLA, NC and control vessels at 2 weeks (p>0.05). However, there was a significant difference in permeability when the PLA and PLA-HI groups were compared at 2 weeks (PLA: 0.00(0.00-0.18); PLA-HI: 2.17(0.00-15.03), p=0.02).

At 12 weeks there was no significant difference in permeability between the groups (p>0.05). In comparison to the 2 week group however, the permeability had significantly increased at 12 weeks and was 20-30 times greater in the NC, PLA and PLA-HI groups (p=0.008).

Thrombus deposition.

Planimetric analysis was used to assess (red) thrombus deposition on the stent and was expressed as a percentage thrombus area of the (explanted) opened vessel surface area. At 2 weeks, the percentage red thrombus deposition on the 3 stent groups as observed through the translucent intimal tissue showed greatest thrombus deposition on the PLA stent, followed by the PLA-HI and NC stents respectively (Figure 3). The differences between the groups were however not statistically significant (p>0.05). There was no thrombus deposition on the control vessels. No thrombus deposition was

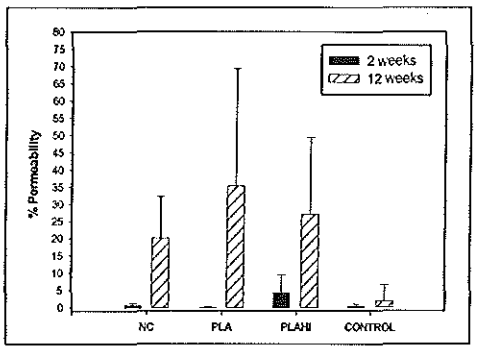

Figure 2. Area showing permeability as % of total area.

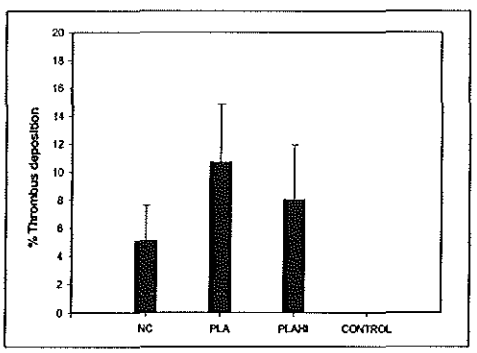

Figure 3. % thrombus deposition for all groups at 2 weeks.

observed on the stents through the intimal tissue of the stented vessels in the 12 week groups.

General histology.

Histological features of the stented vessels are summarized in Table C.

2 weeks.

In general, histology showed all stents embedded in an asymmetric neointima, partially covered with an incomplete layer of endothelial cells and frequently showing neutrophils attached to the endothelial surface. All vessels showed a fragmented internal elastic lamina (IEL). The neointima consisted of smooth muscle cells in a collagenous-like matrix, organizing fibrin/thrombus around the wire voids, inflammatory cells, and extravascular erythrocytes between the wire voids. Neovascularization in the intima was observed in the PLA and PLA-HI groups only. The cellular reaction to the stents was varied - giant cells (GC), which form part of the normal foreign body reaction to implants, were observed in all groups, but the reaction was most aggressive in the PLA-HI group. In this group, adventitial inflammatory responses, together with medial rupture (the stent wire lies deep in the medial layer, but the external elastic lamina (EEL) is intact) and dissection (the stent wire has penetrated the EEL) were observed. In contrast, there were no inflammatory responses in the PLA and NC groups, despite associated medial ruptures. Localized areas of adventitial fibrosis were observed in all vessels where medial rupture was present (all groups).

12 weeks

The vessel wall response at 12 weeks showed all vessels completely endothelialized. In the neointima, fibrin-like thrombus remnants were still observed in or around the stent wire voids, while neovascularization was observed in all groups adjacent to or between the wires. While the foreign body response and neointimal inflammation were comparable between the groups, the adventitial inflammatory response was greatest in the PLA-HI group. The incidence of medial rupture was similar for all groups, but dissections were observed in the PLA-HI and PLA groups. Adventitial fibrosis was observed in all vessels where medial rupture was present.

2 and 12 week histology compared.

Comparison of the histological features of all groups at 2 and 12 weeks show that the general foreign body reaction remained the same, while neointimal and adventitial inflammation appeared to increase in all groups over time. There was a higher frequency of medial rupture in all groups at 12 weeks compared to 2 weeks, and the frequency of dissections was increased in the PLA and PLA-HI groups, although not significant.

Vascular proliferation: BrdU incorporation and cell density

The percentage BrdU incorporation in the intima, media and adventitia of all groups at 2 and 12 weeks are shown in Figure 4. Table D summarizes the cell density in the intima, media, and adventitia of all groups.

At 2 weeks, while there appeared to be a trend towards a higher BrdU incorporation in all 3 tissue layers (both over and between the stent struts) of the PLA-HI group compared to the PLA and NC groups, such differences are not statistically significant ($p>0.05$). At 12 weeks differences in BrdU incorporation were also not significantly different ($p>0.05$).

From 2 to 12 weeks the percentage BrdU incorporation decreased. This was significant in the intima only of the PLA ($p<0.02$), in all tissue layers of the PLA-HI ($p\leq 0.001$) but not in the NC stent group ($p>0.05$).

At both 2 and 12 weeks there were no significant differences in cell density in all 3 tissue layers between the groups ($p>0.05$).

Morphometric results.

Morphometric results are summarized in Table E. At both 2 and 12 weeks there were no significant differences in neointimal, medial and adventitial thickness (both at and between the stent struts) between the 3 different stents used ($p>0.05$, all groups). There were no significant increases (or decreases) in the thickness of any tissue layers between 2 and 12 weeks.

Inflammatory and injury scores.

Table F summarizes the inflammation and injury scores for the three groups at both time points.

Inflammation score

Analysis of inflammation in the neointima (around the wires) and in the adventitia showed no significant differences at 2

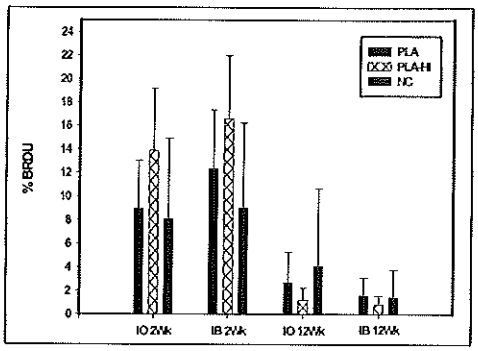

Figure 4a. % BrdU positive cells in the intima of all groups at 2 and 12 weeks. IO = intima over, IB = intima between stent struts

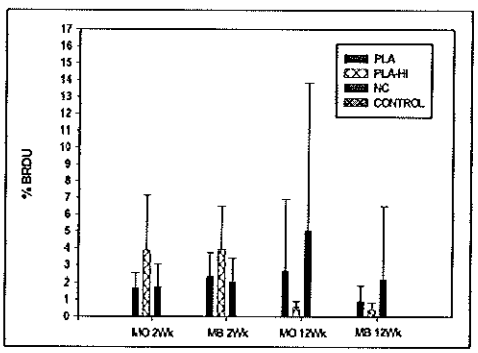

Figure 4b. % BrdU positive cells in the media of all groups at 2 and 12 weeks. MO = media over, MB = media between stent struts. Values for control vessels are negligible.

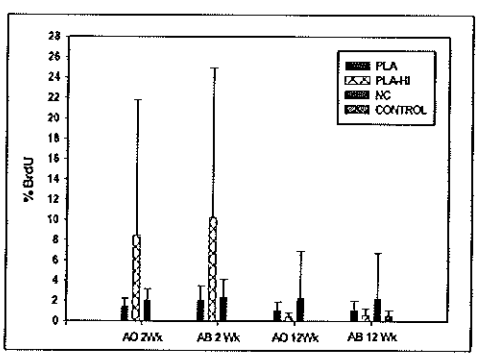

Figure 4c. % BrdU positive cells in the adventitia for all groups at 2 and 12 weeks. AO=adventitia over. AB=adventitia between stent struts.

Table C. Histological features associated with the 3 stent groups at 2 and 12 weeks.

Stent	Foreign Body Reaction (around wires)			Inflammation		Medial Rupture	Dissection
	Mild	Intermediate	Aggressive	NI	A		
2 WEEKS							
PLA (n=5)	1	4	0	0	0	2	0
PLAHI (n=10)	0	7	3	0	2	4	3
NC (n=5)	5	0	0	0	0	1	0
12 WEEKS							
PLA (n=5)	1	3	1	3	1	4	1
PLAHI (n=10)	1	8	1	6	8	7	4
NC (n=5)	2	2	1	3	2	2	0

Table D Cell density in tissue layers of all groups at 2 and 12 weeks

	2 weeks			12 weeks		
	Intima	*Media*	*Adventitia*	*Intima*	*Media*	*Adventitia*
RCA	NA	3.1±0.7	3.1±0.5	NA	2.9±0.3	1.7±0.3
Over wires						
PLA	4.1±3.6	3.0±0.4	3.0±0.9	3.6±0.8	3.1±0.5	2.2±0.4
PLA-HI	3.6±0.6	2.7±0.3	3.2±1.1	3.5±0.7	3.3±0.7	2.2±0.5
NC	3.9±0.7	2.6±0.5	3.1±0.4	3.8±0.9	3.5±0.8	2.3±0.9
Between wires						
PLA	3.9±0.7	3.2±0.3	2.8±0.5	3.9±0.7	3.4±0.5	2.0±0.3
PLA-HI	3.4±0.8	2.8±0.5	3.1±1.0	3.5±0.4	3.7±0.5	2.1±0.5
NC	3.8±0.9	2.8±0.5	3.1±0.1	3.5±0.8	3.7±0.6	2.2±0.9

Cell density measurements are the number of cells/mm^2*1000. P=ns, all groups.

or 12 weeks within the groups ($p>0.05$). When inflammation around the wires or in the adventitia were compared within the groups between 2 and 12 weeks, only the larger PLA-HI group showed a significant increase ($p=0.025$ and $p=0.031$). However if the data (mean values) from this group is compared to that of the PLA and NC groups, then it would appear that all groups actually increased by approximately the same amount i.e. neointimal and adventitial inflammation 2 v 12 weeks: PLA-HI: 1.0, 0.5; PLA: 0.8, 0.2; NC: 1.0, 0.6 respectively. Due therefore to the small sample size in the PLA and NC groups ($n=5$), we must cautiously interpret any significance in the PLA-HI group.

Injury score.

Injury scores for the PLA, PLA-HI and NC stents at 2 weeks were comparable and differences were not statistically significant ($p>0.05$). Similarly, at 12 weeks injury scores were comparable within the groups and were not significantly different ($p>0.05$). From 2 to 12 weeks the injury score increased for all three stent groups, but was only significant for the PLA-HI group ($p=0.018$). However as for the inflammation score, if the data (mean values) from this group is compared to that of the PLA and NC groups, then it would appear that all groups actually increased by approximately the same amount i.e. 2 v 12 weeks PLA-HI: 0.6, PLA: 0.7, NC: 0.7 (approximate values). Thus therefore due to the small sample size in the PLA and NC groups ($n=5$), we must again cautiously interpret any significance in the PLA-HI group.

Correlation of parameters

While both the % permeability and injury scores increased for all groups from 2 to 12 weeks, there was no correlation between the two parameters. Similarly, no correlation was found between neointimal thickness, injury scores and inflammation in any group at both time points.

DISCUSSION

Treatment of coronary lesions by the combined use of a mechanical scaffold (stent) and local pharmacological therapy administered via a stent coating is a promising therapeutic modality.

Numerous materials, including both synthetic and natural, biodegradable and non-biodegradable, have been tested as suitable stent coatings and drug delivery polymers, a comprehensive review of which was published by Bertrand in 1998[2]. Few however, have shown promising results due to the generally associated inflammatory responses, particularly those associated with synthetic polymers[23]. Biodegradable synthetic polymers however, are particularly attractive as potential stent coatings as drugs incorporated into the matrix itself can be efficiently released through diffusion and polymer degradation. Given that the degradation products of biodegradable polymers must neither be toxic nor induce an inflammatory response, PLA is a potentially suitable synthetic polymer worthy of further investigation.

In this study the vessel wall reactions to a PLA coated stent and a PLA-coated stent eluting hirudin and iloprost were compared to a non-coated stent at 2 and 12 weeks. The results show that there were no significant differences in morphometric parameters, percentage thrombus deposition, vascular proliferative response (BrdU), inflammation score and injury score between the coated and bare stent groups at 2 or 12 weeks. The % permeability at 2 weeks was significantly different in the PLA-HI group only. Vascular permeability, inflammatory score and injury score increased in all groups over time. Due to its larger group size, such changes were significant only for the PLA-HI stent. In general however, all 3 stents performed well in this animal model.

Vascular permeability

At 2 weeks, results show a significantly higher permeability in the PLA-HI group compared to the PLA group. Given

Table E Morphometric data for NC, PLA and PLA-HI groups at 2 and 12 weeks

Stent	N	NI thickness at stent struts	NI thickness between stent struts	Media thickness at stent struts	Medial thickness between stent struts	Adventitial thickness at stent struts	Adventitial thickness between stent struts
2 Weeks							
PLA	5	0.24±0.07	0.12±0.05	0.14±0.04	0.19±0.03	0.15±0.07	0.16±0.09
PLA-HI	10	0.25±0.08	0.15±0.08	0.14±0.04	0.20±0.03	0.17±0.10	0.18±0.11
NC	5	0.23±0.09	0.13±0.07	0.15±0.04	0.22±0.02	0.16±0.03	0.16±0.03
12 Weeks							
PLA	5	0.29±0.11	0.20±0.09	0.11±0.04	0.20±0.03	0.08±0.03	0.09±0.04
PLA-HI	10	0.38±0.14	0.25±0.10	0.14±0.05	0.24±0.04	0.07±0.02	0.08±0.03
NC	5	0.40±0.20	0.26±0.17	0.11±0.05	0.23±0.08	0.07±0.02	0.07±0.02

Table F Injury and inflammation scores at 2 and 12 weeks after implantation

Stent	Injury score		Inflammation score			
			Neointima		Adventitia	
	2 weeks	12 weeks	2 weeks	12 weeks	2 weeks	12 weeks
PLA	0.19±0.22	0.91±0.70	0.00	0.80±0.84	0.00	0.20±0.45
(n=5)	0.11(0.00-0.52)	1.06(0.1-1.73)		1.00(0.00-2.00)		0.00(0.00-1.00)
PLA-HI	0.32±0.41	0.92±0.61*	0.00	1.00±1.05†	0.5±1.8	1.00±0.94‡
(n=10)	0.17(0.00-1.30)	0.95(0.14-2.00)		1.00(0.00-3.00)	0.00(0.00-3.00)	1.00(0.00-3.00)
NC	0.11±0.09	0.86±0.82	0.00	1.00±1.00	0.00	0.60±0.89
(n=5)	0.13(0.00-0.24)	1.17(0.00-1.78)		1.00(0.00-2.00)		0.00(0.00-2.00)

Values are given as mean ± SD and as median (range). PLA-HI: * p=0.018 2 *v* 12 week injury score. † p=0.025 2 *v* 12 week neointimal inflammation. ‡ p=0.031 2 *v* 12 week adventitial inflammation.

that there was no correlation between the percentage permeability and inflammatory score to explain the increased permeability in the PLA-HI group (inflammation causes increased endothelial permeability), it would seem that the eluting drugs somehow increased the vascular permeability. From the literature, previous studies have shown iloprost-induced up-regulation of vascular permeability factor mRNA (or VEGF - vascular endothelial growth factor) in rat aortic smooth muscle cells[24] and iloprost-induced VEGF gene expression in human monocytic cell lines[25]. It is possible therefore that iloprost eluting from the stent at two weeks causes a VEGF-induced increased permeability in this group. By 12 weeks however, the amount of eluting iloprost may not be great enough to show a significant difference in permeability between the groups.

Figure 5 shows the cumulative elution of Iloprost in saline[26]. If the iloprost release at 16 days is calculated from Δ iloprost / Δ time, then a release rate of 0.1μg/16 days is achieved, or 0.09μg/hr. Assuming that intimal tissue (~200μm thick) in a 3.5mm coronary vessel weighs 50mg per 7mm, then there is a release rate of 1.8ng/g/hr iloprost into the vessel wall. For simplicity, we have assumed that because the stent is embedded in tissue at this stage, any drug released is taken up by the intima. We have neither taken into account the half-life of the drug, nor the basal tissue levels reached from release prior to 16 days. A previous study reported that a concentration of 10μM Iloprost was enough to improve the barrier function of endothelial cells in vitro[27], equivalent to ~3.7μg/g. This concentration of iloprost is almost 2000 times greater than that achieved with the PLA coating. Thus failure of iloprost to show an improvement in endothelial barrier function at 2 weeks may be due to insufficient tissue levels of the drug. Given that some drugs have multiple effects which are concentration dependant, it may be that at these low tissue levels, the drug causes a VEGF-induced increase in permeability.

The NC, PLA and PLA-HI groups showed an increased permeability over time i.e. from 2 to 12 weeks. This may however be due to the chronic presence of the implant since previous work by our group has shown that stenting causes a prolonged vascular permeability up to 3 months post implantation[20].

Chronic inflammation and vessel injury

Although there appeared to be a trend towards an increasing inflammatory and injury score in all 3 stent groups from 2 to 12 weeks, such scores appeared to be significant only for the PLA-HI stent. Drugs eluting from a polymer matrix can change the surface topography of the stent i.e. spaces or compartments where the drug has been now become empty and give a crater-like appearance to the stent surface. It has previously been reported that the surface topography of PLA itself can influence the inflammatory response i.e. porosity of the polymer enhances an inflammatory response[28]. It is also known that macrophages favor rough surfaces as opposed to smooth surfaces[29]. Taking both factors into account, they may explain why the PLA-HI at 12 weeks has a significantly different inflammatory score to that at 2 weeks, and maybe why the PLA and NC stents show no significant differences between these time points.

While Iloprost is generally reported to have an anti-inflammatory effect, it has been known to also have pro-inflammatory effects[30]. In this respect, it may be that Iloprost in combination with the degradation of the PLA polymer induces a pro-inflammatory effect. Theoretically, inflammation may facilitate increasing arterial injury through the release of inflammatory mediators and enzymes, causing weakening of the vessel wall and subsequent further penetration of the stent struts into the vessel wall and chronic vessel wall damage.

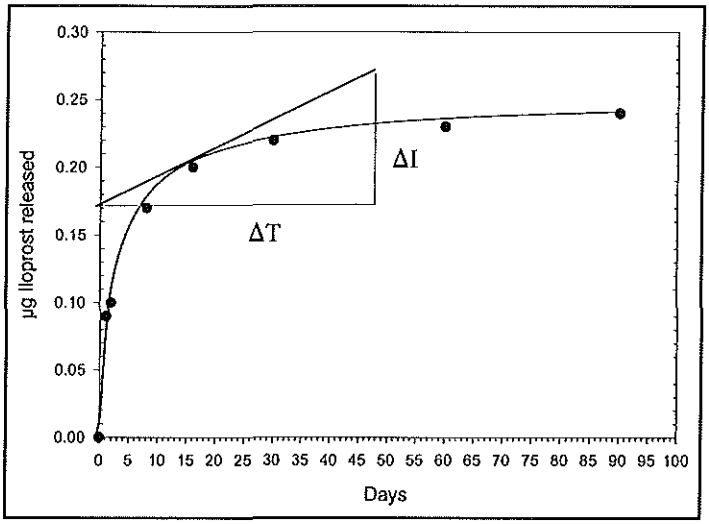

Figure 5. Elution of Iloprost from a PLA-HI coated stent in saline. Modified from Alt[26]

The neointimal response.

5% hirudin and 1% iloprost were both combined in a PLA coating. Given previous positive reports of the effects of the drugs on neointimal proliferation after balloon angioplasty[10] and on SMC proliferation[12] respectively, it was hoped that combined, they would reduce the neointimal response after stenting. Indeed, a PLA-HI coated stent had previously shown a 24% reduction in neointimal response after stenting in an overstretch porcine coronary model[17]. However, in our study morphometric results did not show any differences in morphometric parameters measured between the three stent groups at both time points. This discrepancy in results may be due to the fact that in an overstretch model, (balloon:artery ratio 1.5) over-expansion of the stent may result in cracking or fragmentation of the coating such that larger quantities of drugs are released into the vessel wall at an earlier time point. Alternatively, in may be that in an oversized model the inducement of injury and subsequent neointimal response are so great that only then do the eluting drugs have a measurable effect on reducing thrombus deposition and neointimal thickening.

Comparison with other studies.

The use of PLA in interventional cardiology was proposed in the early 1990's when it was investigated as a possible material for the manufacture of a pure, biodegradable stent[31]. While a group from Duke University published favorable results using pure PLA stents in a canine model, no subsequent published results followed[32]. In the mid-1990's, given the problems associated with pure polymer stents, interest in them waned and shifted in favor of the use of polymer coated stents. Such stents offered the combined mechanical strength of a metal stent with the drug-carrying capacity of a polymer coating.

In 1997 two studies reported apparently favorable results using stents coated with PLA. Lincoff et al[14], in a short-term study, showed that high molecular weight PLLA (321kDa), but not low molecular weight PLLA- coated stents (80kDa) were well tolerated in a porcine coronary model up to 4 weeks post implant. In a longer-term study, Scheldhammer et al[13] reported no adverse inflammatory reactions

to PLA-coated nitinol stents implanted in arteriovenous fistulae in a canine model up to 40 weeks post implant

In Lincoff's study the low molecular weight poly-L-lactic acid polymer (80kDa) induced an aggressive inflammatory response at 1 month, while in our study no adverse response was seen to the 30kDa poly-DL-lactic acid polymer we used. Such a discrepancy may be due to three main differences between the studies. (a) It has previously been reported that in vivo degradation rates of polylactides are inversely proportional to the molecular weight of the polymer i.e. the rate of degradation increases with decreasing molecular weight[33]. This would seem to imply therefore that low molecular weight polymers of PLA may be associated with an increased inflammatory response. However, the inflammatory response is also dependant on the form of PLA used i.e. the inflammatory response at 1 month is less for materials containing the D-unit in the polymer chain[33]. Thus although both studies used low molecular weight polymers, the form of PLA used may have been the determining factor in the induction of an inflammatory response. (b) The proportion of drug:polymer used by Lincoff was 2:1. Elution of the drug therefore resulted in a sponge-like matrix, containing many compartments and holes that the drug had occupied. Given that macrophages favor rough surfaces, such a matrix may have induced an aggressive inflammatory response. (c) In Lincoff's study, the stents were spray-coated with PLLA to a thickness of 11-27μm, while the stents in our study were dip-coated with PLA to a thickness of ~10μm. The thickness of the coating is a measure of the bulk of polymer present, and it is known that the polymer bulk can also determine/affect the inflammatory response[1]. Taken together therefore, this may explain why we found no excessive inflammatory response with a low molecular weight PLA coating at 2 and 12 weeks.

Scheldhammer et al[13] reported no adverse inflammatory reactions to PLA-coated nitinol stents implanted in arteriovenous fistulae in a canine model up to 40 weeks post implant. Our results at 12 weeks follow-up also showed no excessive inflammatory response to the polymer. However, we must be careful in extrapolating the results of Scheldhammer to the current study since species variation in reactions are known to exist. Also, at 12 weeks there is only 10% polymer degradation[15] such that the potential exists for a delayed inflammatory reaction. Longer-term follow-up studies are therefore needed to assess the true potential of this polymer for vascular applications.

Conclusion

While the eluting drugs had no effect on thrombosis and intimal thickening, the PLA coating in our animal model was well tolerated up to 12 weeks after implantation. In this respect, we are optimistic of its use as a biodegradable stent coating for vascular applications, although only longer-term studies can elucidate its true potential.

References

1. van Beusekom HMM, Schwartz RS, van der Giessen WJ. Synthetic polymers. *Semin Intervent Cardiol.* 1998;3:145-148.
2. Bertrand OF, Sipehia R, Mongrain R, Rodes J, Tardif JC, Bilodeau L, Cote G, Bourassa MG. Biocompatibility aspects of new stent technology. *J Am Coll Cardiol.* 1998;32:562-71.
3. Tsai DC, Howard SA, Hogan TF, Malanga CJ, Kandzari SJ, Ma JK. Preparation and in vitro evaluation of polylactic acid-mitomycin C microcapsules. *J Microencapsul.* 1986;3:181-93.
4. Kumanohoso T, Natsugoe S, Shimada M, Aikou T. Enhancement of therapeutic efficacy of bleomycin by incorporation into biodegradable poly-d,l-lactic acid. *Cancer Chemother Pharmacol.* 1997;40:112-6.
5. Okada H, Doken Y, Ogawa Y, Toguchi H. Preparation of three-month depot injectable microspheres of leuprorelin acetate using biodegradable polymers. *Pharm Res.* 1994;11:1143-7.

6. Rothen-Weinhold A, Besseghir K, Vuaridel E, Sublet E, Oudry N, Gurny R. Stability studies of a somatostatin analogue in biodegradable implants. *Int J Pharm.* 1999;178:213-21.
7. Bergsma EJ, Rozema FR, Bos RR, de Bruijn WC. Foreign body reactions to resorbable poly(L-lactide) bone plates and screws used for the fixation of unstable zygomatic fractures. *J Oral Maxillofac Surg.* 1993;51:666-70.
8. Bostman OM. Osteoarthritis of the ankle after foreign-body reaction to absorbable pins and screws: a three- to nine-year follow-up study. *J Bone Joint Surg Br.* 1998;80:333-8.
9. Buchwald AB, Sandrock D, Unterberg C, Ebbecke M, Nebendahl K, Luders S, Munz DL, Wiegand V. Platelet and fibrin deposition on coronary stents in minipigs: effect of hirudin versus heparin. *J Am Coll Cardiol.* 1993;21:249-54.
10. Buchwald AB, Hammerschmidt S, Stevens J, Goring J, Nebendahl K, Unterberg C. Inhibition of neointimal proliferation after coronary angioplasty by low-molecular-weight heparin (clivarine) and polyethyleneglycol-hirudin. *J Cardiovasc Pharmacol.* 1996;28:481-7.
11. Serruys PW, Herrman JP, Simon R, Rutsch W, Bode C, Laarman GJ, van Dijk R, van den Bos AA, Umans VA, Fox KA, et al. A comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty. Helvetica Investigators. *N Engl J Med.* 1995;333:757-63.
12. Zucker TP, Bonisch D, Hasse A, Grosser T, Weber AA, Schror K. Tolerance development to antimitogenic actions of prostacyclin but not of prostaglandin E1 in coronary artery smooth muscle cells. *Eur J Pharmacol.* 1998;345:213-20.
13. Schellhammer F, Berlis A, Bloss H, Pagenstecher A, Schumacher M. Poly-lactic-acid coating for endovascular stents. Preliminary results in canine experimental arteriovenous fistulae. *Invest Radiol.* 1997;32:180-6.
14. Lincoff AM, Furst JG, Ellis SG, Tuch RJ, Topol EJ. Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. *J Am Coll Cardiol.* 1997;29:808-16.
15. Herrmann R, Schmidmaier G, Markl B, Resch A, Hahnel I, Stemberger A, Alt E. Antithrombogenic coating of stents using a biodegradable drug delivery technology. *Thromb Heamost.* 1999;82:51-57.
16. Alt E, Beilharz C, Preter D, Schmidmaier G, Pasquantonio J, Erhard W, Stemberger A, Schomig A. Biodegradable stent coating with polylactic acid, hirudin and prostacylin reduces restenosis. *J Am Coll Cardiol.* 1997;29:238A (Abstract).
17. Prietzal K, Pasquantonio JD, Fliedner TU, Stemberger A, Janczewski M. Inhibition of neointimal proliferation with a novel, hirudin/prostacyclin analog eluting stent coating in an animal overstretch model. *Circulation.* 1996;94:1522 (Abstract).
18. Schmidmaier G, Stemberger A, Alt E, Gawaz M, Neumann F-J, Schomig A. A new biodegradable polylactic acid coronary stent coating, releasing PEG-hirudin and a prostacyline analog, reduces both platelet activation and plasmatic coagulation. *J Am Coll Cardiol.* 1997;29:354A (Abstract).
19. van der Giessen WJ, Serruys PW, van Beusekom HM, van Woerkens LJ, van Loon H, Soei LK, Strauss BH, Beatt KJ, Verdouw PD. Coronary stenting with a new, radiopaque, balloon-expandable endoprosthesis in pigs. *Circulation.* 1991;83:1788-98.
20. van Beusekom HM, Whelan DM, Hofma SH, Krabbendam SC, van Hinsbergh VW, Verdouw PD, van der Giessen WJ. Long-term endothelial dysfunction is more pronounced after stenting than after balloon angioplasty in porcine coronary arteries. *J Am Coll Cardiol.* 1998;32:1109-17.
21. Van der Giessen WJ, Danser AHJ, van Beusekom HMM, Derkx FHM, Verdouw PD, Lamers JMJ, Serruys PW. Enhanced angiotensin II degradation in porcine coronary neointimal hyperplasia induced by stent implantation. *Cor Art Dis.* 1992;3:730-737.
22. Schwartz RS, Huber KC, Murphy JG, Edwards WD, Camrud AR, Vlietstra RE, Holmes DR. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. *J Am Coll Cardiol.* 1992;19:267-74.
23. van der Giessen WJ, Lincoff AM, Schwartz RS, van Beusekom HM, Serruys PW, Holmes DR, Jr., Ellis SG, Topol EJ. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. *Circulation.* 1996;94:1690-7.
24. Pueyo ME, Chen Y, D'Angelo G, Michel JB. Regulation of vascular endothelial growth factor expression by cAMP in rat aortic smooth muscle cells. *Exp Cell Res.* 1998;238:354-8.
25. Hoper MM, Voelkel NF, Bates TO, Allard JD, Horan M, Shepherd D, Tuder RM.

Prostaglandins induce vascular endothelial growth factor in a human monocytic cell line and rat lungs via cAMP. *Am J Respir Cell Mol Biol.* 1997;17:748-56.

26. Alt E, Seliger C. Antithrombotic stent coatings: hirudin/iloprost combination. *Semin Intervent Cardiol.* 1998;3:177-183.
27. Langeler EG, van Hinsbergh VW. Norepinephrine and iloprost improve barrier function of human endothelial cell monolayers: role of cAMP. *Am J Physiol.* 1991;260:C1052-9.
28. Lam KH, Schakenraad JM, Groen H, Esselbrugge H, Dijkstra PJ, Feijen J, Nieuwenhuis P. The influence of surface morphology and wettability on the inflammatory response against poly(L-lactic acid): a semi-quantitative study with monoclonal antibodies. *J Biomed Mater Res.* 1995;29:929-942.
29. Tang L, Eaton JW. Inflammatory responses to biomaterials. *Am J Clin Pathol.* 1995;103:466-71.
30. Ekerdt R, Muller B. Role of prostanoids in the inflammatory reaction and their therapeutic potential in the skin. *Arch Dermatol Res.* 1992;284:S18-21.
31. Agrawal CM, Haas KF, Leopold DA, Clark HG. Evaluation of poly(L-lactic acid) as a material for intravascular polymeric stents. *Biomaterials.* 1992;13:176-82.
32. Chapman GD, Gammon RS, Bauman RP, Howell S, Clark H, Mikat EM, Palmos L, Buller CE, Stack RS. A bioabsorbable stent: initial experimental results. *Circulation.* 1990;82:283(abstract).
33. Gogolewski S, Jovanovic M, Perren SM, Dillon JG, Hughes MK. Tissue response and in vivo degradation of selected polyhydroxyacids: polylactides (PLA), poly(3-hydroxybutyrate) (PHB), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/VA). *J Biomed Mater Res.* 1993;27:1135-1148.

Chapter 10

Discussion

Discussion

Aim of this thesis.

The acute vessel wall damage that occurs during stent implantation, together with the presence of a permanent foreign body in the bloodstream initiates a complex set of interactions involving the damaged and diseased vessel wall, the stent and the flowing blood. Understanding how such interactions contribute to subsequent thrombotic complications and excessive tissue growth (in the form of neointimal thickening) is important for developments in stent technology or future devices.

The aim of this thesis was therefore twofold. First to understand the vascular woundhealing response following stent implantation, particularly the chronic effects of the stent on the vessel wall. With this knowledge, the second aim was to see if modulating stent characteristics could modify or improve the vascular response. Modulation of stent properties was achieved by changing both the mechanical properties of the stent, as well as the non-mechanical stent properties through altering the stent surface by the use of coatings, with and without the incorporation of drugs.

Contamination and stent implantation.

Techniques of stent implantation may, together with the stent, inadvertently introduce other foreign bodies into the vessel wall. In Chapter 3 we found starch powder from surgical gloves, together with gauze fibers implanted along with the stent. Such particles induce a foreign body reaction, and could thereby theoretically interfere with the wound healing response. A delayed wound healing response can prolong the period in which sub-acute thrombosis occurs. The potential for stent contamination can be reduced in several ways – the use of non-powdered gloves, thorough washing of gloved hands before handling the stent, minimum handling of the stent and contact of the guidewire, balloon and other instruments with gauze. Crimping the stent onto the balloon is a major source of potential stent contamination with glove powder and we suggested in Chapter 3 that the use of pre-crimped stents may reduce this contamination. This was confirmed in a recent study that showed the potential for contamination of stents with glove powder was greater for manually crimped stents than for pre-mounted stents[1].

Other possible contaminants of stents include endotoxins and bacteria. Endotoxin contamination of stents can result in excessive intimal tissue growth in vessels where for example, no apparent oversizing of the stent has occurred. In a series of experiments carried

out by our group (unpublished data), unexplained excessive neointimal growth resulted after non-oversized implantation of stents in porcine coronary arteries. It was eventually discovered by the manufacturer that a water-bath in which the stents had undergone in-vitro testing had been contaminated with bacterial endotoxin. Despite sterilization of the stents, the endotoxin remained and resulted in excessive tissue growth. It highlighted an up to now unheard of source of contamination.

Bacterial contamination of stents is a frequently described problem associated with stents implanted in the urinary or esophageal tracts. Although not expected as a complication of vascular implanted stents, Thibodeaux et al[2] in a series of animal experiments performed in 1996, showed that up to 4 weeks after implantation, vascular stents had the potential to become colonized by bacteria. Infection of the stented vessel was associated with acute inflammation of the arterial wall and vessel thrombosis. In patients, while stent infection is generally rare, infections of femoral and iliac arteries have been reported. Complications arising from such infections have been serious, and include amputation and even death[3-5]. The most common cause of stent infection is contamination of the implant with *Staphylococcus aureus*. Although not a frequent complication of peripheral stent implantation, it has been proposed that antibiotic prophylaxes be administered as part of routine post implantation care[6].

Acute and chronic effects of stenting.

Many studies have previously described the acute effects of stenting – stretching of the vessel wall, damage to the endothelium, the initiation of a thrombotic response followed by reendothelialization together with healing and incorporation of the stent into the injured vessel wall. In comparison however, fewer studies have looked at the longer-term effects of stents, such that much still has to be learned[7-9].

In Chapters 4 and 5 we examined the acute and chronic effects of stent-induced vessel wall injury, together with their effects on the proliferative response and on endothelial permeability, and compared these results to PTCA alone.

Chapter 4 showed that in a non-oversized porcine coronary model, the Wiktor and Palmaz-Schatz stents both induced a progressive and prolonged vessel wall injury, together with an inflammatory response up to 12 weeks post implant. After PTCA no increase in injury, or prolongation of the inflammatory response was observed. Chapter 5 showed that PTCA and stent implantation both resulted in impairment of the vascular barrier function up to 3 months after the interventions, although it was more pronounced in the stent groups.

Endothelial permeability was observed at both 2 and 12 weeks, and was characterized by specific endothelial morphological features. Up to 3 months after stent implantation, the endothelium showed a significantly higher proliferation compared to the PTCA group. Taken together these results show that in contrast to PTCA, the presence of a stent in the vessel wall induces chronic injury and inflammation, together with an impaired barrier function and an increased proliferative response.

The continued presence of an inflammatory response up to 12 weeks post stenting may contribute to the progressive vessel wall injury. Stents induce a foreign body response with macrophage giant cells frequently observed surrounding the wire struts. Given that such cells can never engulf the metal struts, it leads to a type of "frustrated" phagocytocis with the continued release of enzymes and inflammatory mediators. It has been proposed that such inflammatory mediators may cause further influx of inflammatory cells which then release growth factors, enzymes and cytokines that may weaken the vessel wall and facilitate further penetration of the wire struts through the vessel wall.

An endothelial layer showing increased cell proliferation may contribute to the increased vascular permeability, since cell retraction associated with mitosis causes paracellular gaps in arterial endothelial cells in vivo[10]. A leaky endothelial layer permits the continued influx of growth factors and cells into the healing vessel wall, which may contribute to the development of the neointima.

Some of the long-term effects of stents seen in our porcine model as mentioned above, are comparable to those found from pathology of human stented vessels[11-13]. Although the barrier function of human stented vessels has never been tested in vivo, we know that up to 6 months post stenting, the functional capacity of the endothelium adjacent to the stent is impaired[14,15].

Modulation of the vessel wall response to stenting.

Changing mechanical stent properties.

From studies of implants in general, it is known that the degree of compliance mismatch between the implant and surrounding tissue is a determining factor of the subsequent tissue response. The study in Chapter 6 showed that in both healthy and atherosclerotic porcine femoral arteries, stent compliance had no effect on neointimal thickening. These results confirm results of recent clinical trials of "stent versus stent" comparisons which also show that despite mechanical difference between clinically used stents, there is no difference in restenosis rates between them[16,17]. Variations therefore in

lesion characteristics, operator experience and non-mechanical stent properties are most probably more responsible for the degree of intimal thickening observed with clinically used stents.

Although in our study changing stent compliance had no effect on the lumen area, modulation of other mechanical stent parameters may have an effect. In a study by Carter et al[18], implantation of a thermoelastic self-expanding stent in a swine model was shown to give less neointima, but more importantly a greater lumen area, compared to a balloon-expandable stent. After deployment, this stent gave a slow and continuous expansion over 28 days, which significantly increased the vessel lumen area and appeared to limit the neointimal response. The consequence therefore of modulating the mechanical property of radial force was inducement of a greater lumen area that then compensates for any reduction in lumen area caused by neointimal growth. Thus while stent mechanical parameters cannot be modulated to reduce the neointimal response per se, they can be modulated to achieve the overall desired effect i.e. a restored patent vessel lumen.

Coated and drug-loaded coated stents.

Another way to modulate the vessel wall response to stents is by the use of stent coatings, both passive coatings (Chapter 8) and active coatings (Chapter 9).

In Chapter 8 we used a phosphorylcholine-coated stent to attempt to tackle both thrombosis and restenosis. Although the coating appeared to have no effect on either thrombosis or restenosis rates, the coating itself showed excellent biocompatibility at least up to 12 weeks post implant. This was a surprising finding, given that the coating is purely synthetic, and most synthetic polymers have been associated with variable degrees of inflammatory responses and/or a delayed inflammatory response[19,20].

In Chapter 5 we saw that stenting induced a problem at the level of the endothelial barrier function i.e. prolonged endothelial permeability. From pilot experiments performed we demonstrated that within 10 minutes, this barrier function could be partially improved by intracoronary administration of 1mM dibutyryl c-AMP. In Chapter 9 therefore we incorporated iloprost into a PLA stent coating in an effort to address the problem of permeability. However, contrary to the expected improvement in barrier function, the eluting drugs appeared to increase the endothelial permeability at 2 weeks relative to that of the other stents.

Recently data has become available on the elution profile of iloprost from the PLA coating (Figure 1)[21]. If from the graph we calculate the amount of iloprost eluting from the

coating as Δ iloprost / Δ days, then at 2 weeks, an elution rate of approximately 2ng/g tissue/hr is released. For simplicity, we have assumed that because the stent is embedded in tissue at this stage, any drug released is taken up by the intima. We have neither taken into account the half-life of the drug, nor the basal tissue levels reached from release prior to 16 days. A previous study reported that a concentration of 10μM iloprost was enough to improve the barrier function of endothelial cells in vitro[22]. A 10μM solution is equivalent to approximately 4μg/g and this is thus a concentration almost 2000 times greater than that achieved with the PLA coating. Thus failure of iloprost to show an improvement in endothelial barrier function at 2 weeks may be due to insufficient tissue levels of the drug. Given that some drugs have multiple effects which are concentration dependant, it may be that at these low tissue levels, the drug causes a VEGF-induced increase in permeability.

Implications of these results for future studies.

Potential for foreign body contamination of stents

As seen in Chapter 3, the technique of stent implantation lends itself to potential contamination. While we cannot say if contamination is a problem in the clinical setting, the potential for such contamination exists. It would seem prudent therefore that steps be undertaken to minimize such contamination occurring. Such steps include thorough washing of gloved hands or the use of non-powdered gloves, minimal handling of the stent and if possible the use of pre-crimped stents, and minimal wiping of guide-wire with gauze. Given also the potential for bacterial contamination of stents, and the current interest in an infective agent/factor as cause or additional effect in the development of atherosclerosis and restenosis, a potential treatment may be the incorporation of antibiotics into stent coatings.

Persistent vessel wall injury and inflammation.

In Chapter 4 it was proposed that the vessel wall injury may be due in part to the persistent inflammatory response in the vessel wall. Macrophages are a necessary constituent of the initial vessel wall response to stents, in that they are required for the cleaning-up process i.e. clearing up thrombus remnants and dead cells. However their persistent presence around the wires and their potential for prolonged "frustrated phagocytosis" may contribute to the vessel wall weakening and the associated injury. A potential approach therefore to try to minimize their effect may be the incorporation of an anti-inflammatory drug or free radical scavenger into a stent coating. Such a coating would need to have time-delay release characteristics such that in the initial period after implantation, no drug release occurs,

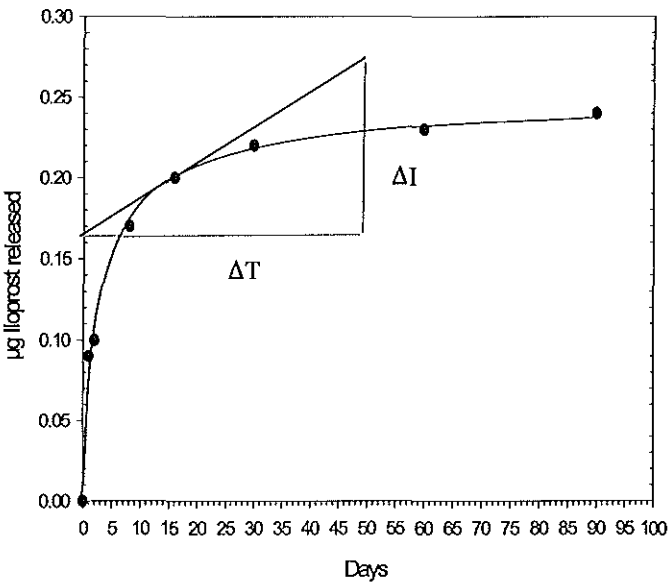

Figure 1. Elution of Iloprost from a PLA-coated stent. Modified from Alt et al[21]. The graph shows the cumulative release of Iloprost in saline over 90 days.

allowing macrophages and neutrophils to perform their tasks. After a period of time, slow drug release would occur to inhibit the later effects of the inflammatory cells.

Endothelial dysfunction

In Chapter 5 we showed a stent-induced problem at the level of the endothelial barrier i.e. increased permeability. In Chapter 9 we incorporated both Iloprost and hirudin into a PLA stent coating. It was hoped that iloprost would improve the endothelial barrier function of the stented vessels, but this was not the case, due most likely to insufficient tissue levels being achieved. The incorporated hirudin also had no effect on thrombus deposition. If only iloprost was incorporated into the coating, then it may be possible to achieve high enough tissue levels, so as to improve the endothelial barrier function.

Currently, the use of brachytherapy is in vogue. While clinical trials have reported successful short-term outcome with this technique, the longer-term effects of such treatment are unknown. We know from Chapters 4 and 5 that the longer-term effects of stents are chronic injury and endothelial dysfunction. Recent animal studies have reported incomplete endothelialization 3 months after placement of radioactive stents[23] and that the vascular

wound healing response is delayed up to 6 months after combined angioplasty and brachytherapy[24]. If this also holds true after stenting in patients, then an incomplete and dysfunctional endothelium, together with a delayed healing response may lead to an increased incidence of thrombotic complications within this group of patients. Indeed, a recent report by Costa et al[25] suggests that intracoronary brachytherapy is associated with late coronary occlusion. In his study he reports a 6.6% incidence of late thrombosis i.e. thrombosis 2-15 months after treatment in 91 patients that underwent either PTCA and/or stenting. Has a "theoretical" problem become reality? If this is the case, then maybe the use of brachytherapy should necessitate as routine, the incorporation of anti-thrombotics into a stent coating. Such a coating would need to have time-delay characteristics, such that drug release occurs over an extended period of time.

Mechanical stent properties

In Chapter 6 we saw that changing the compliance of the stent had no effect on intimal thickening. Therefore, in the future assessment or comparison of stent performance, we can exclude the compliance of the stent as a contributory factor to the neointimal growth and focus our efforts on alternative ways to reduce this tissue growth.

Stent coatings.

Chapters 8 and 9 showed that synthetic polymers could be well tolerated in the vasculature up to 3 months post implant. The inflammatory and neointimal response to both polymers was not excessive, such that both the PC and PLA coatings show potential as biocompatible drug carriers. While the currently incorporated drugs showed no positive effects, incorporation of other drugs in the same coatings may show improved results.

The eluting drugs in Chapter 9 had no effect on neointimal thickness, most probably due to insufficient tissue levels being achieved. This highlights the importance of theoretically estimating effective tissue levels prior to incorporation of drugs into a stent coating, whereby greater amounts of the drug can be incorporated should the desirable tissue levels not be reached.

Conclusion.

From the animal studies performed in this thesis, the following conclusions can be drawn:

1. The technique of stent implantation lends itself to contaminating the stent, such that contaminating particles can potentially interfere with the wound healing response.
2. Stent implantation induces a chronic irritation of the vessel wall as illustrated by a persistent vascular injury and long-term endothelial dysfunction.
3. Modulating the compliance of a stent has no effect on the neointimal response.
4. Modulating stent properties through the use of passive and active stent coatings does not reduce the neointimal response.
5. Not all synthetic polymer stent coatings are associated with aggressive inflammatory responses.

References

1. Jung J, Bach R, Franke RP. Effect of crimping on contamination of coronary stents. *Eur Heart J.* 1999;20:628.
2. Thibodeaux LC, James KV, Lohr JM, Welling RE, Roberts WH. Infection of endovascular stents in a swine model. *Am J Surg.* 1996;172:151-4.
3. Latham JA, Irvine A. Infection of endovascular stents: an uncommon but important complication. *Cardiovasc Surg.* 1999;7:179-82.
4. Muller G, Stockmann H, Markert U, Heise S. The infected arterial stent. *Chirurg.* 1998;69:872-6.
5. Schachtrupp A, Chalabi K, Fischer U, Herse B. Septic endarteritis and fatal iliac wall rupture after endovascular stenting of the common iliac artery. *Cardiovasc Surg.* 1999;7:183-6.
6. Deiparine MK, Ballard JL, Taylor FC, Chase DR. Endovascular stent infection. *J Vasc Surg.* 1996;23:529-33.
7. Froelich JJ, Alfke H, Wilke A, Ramaswamy A, Barth KH, Hoppe M, Wagner HJ, Klose KJ. Effects of nitinol Strecker stent placement on vascular response in normal and stenotic porcine iliac arteries. *J Vasc Interv Radiol.* 1999;10:329-38.
8. Virmani R, Kolodgie FD, Dake MD, Silver JH, Jones RM, Jenkins M, Gillespie DL. Histopathologic evaluation of an expanded polytetrafluoroethylene- nitinol stent endoprosthesis in canine iliofemoral arteries. *J Vasc Interv Radiol.* 1999;10:445-56.
9. Barth KH, Virmani R, Froelich J, Takeda T, Lossef SV, Newsome J, Jones R, Lindisch D. Paired comparison of vascular wall reactions to Palmaz stents, Strecker tantalum stents, and Wallstents in canine iliac and femoral arteries. *Circulation.* 1996;93:2161-9.
10. Lin SJ, Jan KM, Weinbaum S, Chien S. Transendothelial transport of low density lipoprotein in association with cell mitosis in rat aorta. *Arteriosclerosis.* 1989;9:230-236.
11. Van Beusekom HM, van der Giessen WJ, van Suylen R, Bos E, Bosman FT, Serruys PW. Histology after stenting of human saphenous vein bypass grafts: observations from surgically excised grafts 3 to 320 days after stent implantation. *J Am Coll Cardiol.* 1993;21:45-54.
12. Farb A, Sangiorgi G, Carter AJ, Walley VM, Edwards WD, Schwartz RS, Virmani R. Pathology of acute and chronic coronary stenting in humans. *Circulation.* 1999;99:44-52.
13. Anderson PG, Bajaj RK, Baxley WA, Roubin GS. Vascular pathology of balloon-expandable flexible coil stents in humans. *J Am Coll Cardiol.* 1992;19:372-81.
14. Monnink SHJ, van Boven AJ, Peels H-OJ, Tio RA, den Heijer P, van der Geissen WJ, van Gilst WH, Grijns HJGM. Do we know the long-term effects of stent implantation on coronary endothelial function? *Circulation.* 1998;98:I-977(Abstract).
15. Caramori PRA, Lima VC, Seidelin PH, Newton GE, Adelman AG. Endothelial dysfunction distal to stents implanted for more than six months. *Circulation.* 1997;96:I-756 (Abstract).
16. Dirschinger J, Schuhlen H, Boekstegers P, Hausleiter J, Kastrati A, Giehrl W, Pache J, Hadamitzky M, Neumann F-J, Steinbeck G, Schomig A. Equivalence or Difference? One-year follow-up of a randomized trial of five different slotted-tube stents. *Circulation.* 1998;17:I-661 (Abstract).

17. Eeckhout E, Goy JJ, Stauffer JC, Vogt P, Kappenberger L. Comparison of the Wallstent, Palmaz-Schatz stent, and Wiktor stent late after intracoronary stenting. *Am J Cardiol.* 1994;74:609-12.

18. Carter AJ, Scott D, Laird JR, Bailey L, Kovach JA, Hoopes TG, Pierce K, Heath K, Hess K, Farb A, Virmani R. Progressive vascular remodeling and reduced neointimal formation after placement of a thermoelastic self-expanding nitinol stent in an experimental model. *Cathet Cardiovasc Diagn.* 1998;44:193-201.

19. van der Giessen WJ, Slager CJ, van Beusekom HM, van Ingen Schenau DS, Huijts RA, Schuurbiers JC, de Klein WJ, Serruys PW, Verdouw PD. Development of a polymer endovascular prosthesis and its implantation in porcine arteries. *J Interv Cardiol.* 1992;5:175-85.

20. van der Giessen WJ, Lincoff AM, Schwartz RS, van Beusekom HM, Serruys PW, Holmes DR, Jr., Ellis SG, Topol EJ. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. *Circulation.* 1996;94:1690-7.

21. Alt E, Seigler C. Antithrombotic stent coatings: hirudin/iloprost combination. *Semin Intervent Cardiol.* 1998;3:177-183.

22. Langeler EG, van Hinsbergh VW. Norepinephrine and iloprost improve barrier function of human endothelial cell monolayers: role of cAMP. *Am J Physiol.* 1991;260:C1052-9.

23. Farb A, Tang AL, Virmani R. Neointima is reduced but endothelialization is incomplete 3 months after 32P b-emitting stent placement. *Circulation.* 1998;98:I- 779 (Abstract).

24. Marijianowski MM, Crocker IR, Styles T, Forestner DM, Waksman R, Cipolla GD, King SB, 3rd, Robinson KA. Fibrocellular tissue responses to endovascular and external beam irradiation in the porcine model of restenosis. *Int J Radiat Oncol Biol Phys.* 1999;44:633-41.

25. Costa MA, Sabat M, van der Giessen WJ, Kay IP, Cervinka P, Ligthart JM, Serrano P, Coen VL, Levendag PC, Serruys PW. Late coronary occlusion after intracoronary brachytherapy. *Circulation.* 1999;100:789-92.

Summary

The use of endovascular stents has revolutionized the field of interventional cardiology - restenosis rates have been significantly reduced compared to results of PTCA alone. Although major advancements have been made in this field since the first human stent implantation 13 years ago, restenosis post stenting remains a serious problem. In this respect, much has still to be learned, particularly about the longer-term effects of a permanent metallic implant in the vasculature. Only by understanding the effects of the stent on the vessel wall, can future generations of stents be modified or manipulated so as to reduce or minimize this response.

The basic characteristics of stents are dealt with in Chapters 1 and 2. Specifically the development and current status of stents and the importance of histological techniques in the evaluation of the vascular healing response are described.

The technique of stent implantation together with the presence of a metallic prosthesis and the possible unwanted co-implantation of contaminating particles together with the stent in the vasculature, invariably induces an acute degree of vascular irritation and injury with a subsequent inflammatory response. However, as well as having acute effects on the vessel wall, a stent can also induce chronic vessel wall damage, as seen in Chapters 3 to 5. Such effects include the inducement of an increasing vessel wall injury over time, and decreased vascular integrity in the form of a permeable endothelial layer as well as a prolonged proliferative activity of the cells in the vessel wall.

Having highlighted the long-term effects of stents, Chapters 6 to 9 deal with the modification of both mechanical and non-mechanical stent properties in an effort to influence the vessel wall response. The mechanical stent property of compliance is looked at in Chapter 6, while the use of stent coatings as a means of modifying the non-mechanical stent properties are presented in Chapters 7, 8 and 9. Specifically Chapter 7 gives an overview of ways in which drugs can be incorporated into a stent coating, while both non-biodegradable and biodegradable stents coatings, the latter in combination with drugs, are presented in Chapters 8 and 9 respectively.

The results of Chapters 2 to 9 and the implications of these results for future studies are summarized and discussed in Chapter 10.

Samenvatting

Het gebruik van endovasculaire stents heeft een enorme verbetering betekend voor de interventie cardiologie – het optreden van restenose is significant verminderd vergeleken met de resultaten van PTCA alleen. Ondanks grote vorderingen die zijn geboekt sinds de eerste humane stent implantatie 13 jaar geleden, blijft het optreden van restenose na stentplaatsing een serieus probleem. In dit opzicht is er een gebrek aan kennis, met name betreffende de lange termijn effecten van deze permanente metalen implantaten op de vaatwand. Alleen door het begrijpen van deze effecten kunnen toekomstige generaties stents worden verbeterd om zo deze respons te reduceren of te minimaliseren.

De basis karakteristieken van stents worden besproken in hoofdstuk 1 en 2. Speciek wordt de ontwikkeling, het huidige gedrag van stents, en het belang van histologische technieken in de evaluatie van vasculaire wondheling beschreven.

De techniek van stent implantatie alsmede de aanwezigheid van een metalen prothese met mogelijke ongewilde contaminerende partikels, induceren acute vasculaire irritatie en verwonding met een daaropvolgende inflammatoire respons. Naast acute effecten op de vaatwand kan een stent ook chronische vaatwandschade induceren, zoals beschreven in hoofdstuk 3 tot en met 5. Zulke effecten omvatten een toegenomen schade aan de archituur van de vaatwand in de tijd, een afgenomen vasculaire integriteit in de vorm van een permeabele endotheellaag, als ook een verlengde proliferatieve activiteit van cellen in de vaatwand.

Modificatie van mechanische en niet-mechanische stent eigenschappen, om te trachten de vaatwandreactie te beïnvloeden, wordt behandeld in hoofstukken 6 tot en met 9. Compliantie, een mechanische stent eigenschap, wordt beschreven in hoofdstuk 6. Het gebruik van stent coatings ter modificatie van de niet-mechanische stent eigenschappen wordt gepresenteerd in de hoofdstukken 7, 8 en 9. Hoofdstuk 7 geeft een overzicht van de manieren waarop een medicament geïncorporeerd kan worden in een stent coating. Experimenten met biostabiele en bioafbreekbare coatings, waarvan de laatstgenoemde in combinatie met medicamenten, worden beschreven in hoofdstukken 8 en 9.

De resultaten van de hoofdstukken 2 tot en met 9 en de implicaties van deze resultaten voor toekomstige studies, zijn samengevat in hoofdstuk 10.

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CURRICULUM VITAE

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