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The paradigm of endothelium and stent thrombosis in DES

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Abstract

Drug eluting stents (DES) have attracted considerable attention due to concerns of late stent thrombosis (LST) thought to be related to delayed endothelialisation. This hypothesis is based on clinical autopsy studies indicating an association between lack of endothelium and LST. However, meta-analysis of clinical trials does not support the notion that all DES induce more late stent thrombosis than BMS. In addition, preclinical data using animal models also do not necessarily support this hypothesis. Most animal models using single non-overlapping stents show no signs of delayed endothelialisation at all. Experiments with several DES in our laboratory using the porcine coronary artery model also suggest that DES show no differences in re-endothelialisation. They can however induce (late) differences in endothelial function, depending on the DES of choice. These phenomena are also described clinically in coronary segments distal from the DES. We hypothesise that DES do not necessarily delay endothelialisation but more likely induce late endothelial dysfunction that varies between DES.

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Introduction

Sirolimus (SES) and paclitaxel eluting stents (PES) have markedly reduced the rate of in-stent restenosis and late lumen loss compared to bare-metal stents (BMS)[1-3], resulting in a significant reduction in the need for repeat revascularisation. However, enthusiasm for this technology has been dampened by concerns about late thrombosis (LST), an event often with serious consequences[4]. Delayed re-endothelialisation and endothelial dysfunction after drug-eluting stent (DES) have been suggested as the cause of LST and this has attracted considerable attention recently[5]. The current hypothesis is derived mainly from autopsy studies and clinical studies using intracoronary angioscopy that support delayed re-endothelialisation. However, this is still controversial, and furthermore, not all DES may induce LST (Figure 1)[6].

Our understanding of the pathophysiology of late DES stent thrombosis stems from both clinical, experimental and pathological studies. This review discusses these studies, focusing on re-endothelialisation and endothelial dysfunction. In addition, we present our perspective derived from experience with stented porcine coronary arteries.

Animal studies

In porcine coronary arteries early re-endothelialisation is usually studied at five days after stenting. A time point early enough to assess differences in healing before endothelialisation is complete, mostly using scanning EM and computerised planimetric analysis[7]. To understand the role of the endothelium we restudied tissues from the first generation BMS, the Palmaz-Schatz stent in comparison to the newer designs[8]. We found significant differences in the percentage endothelialisation of the stent struts at five days in the bare Palmaz-Schatz stent (60±27%; n=10) and in the bare divYsio stent (91±12%; n=10, p<0.05, T-test). While this difference is quite large, it had no effect on the amount of intimal thickening at 90 days, being 0.2±0.05 mm and 0.2±0.1 mm respectively[7,8]. It is not surprising that early presence of endothelium does not affect healing at 90 days as BMS have been known to interfere with endothelial function and induce chronic injury[9,10]. It raises the question whether this is different in DES. Indeed, current DES yield similar endothelialisation rates to BMS (Figure 2; 82±1, 80±22 and 83±25 in tacrolimus, sirolimus and paclitaxel eluting stents, versus 93±8% in BMS, ANOVA, p=0.6).

While this contradicts the current hypothesis of delayed endothelialisation, our data are in accordance with other animal studies. In a study by Klugherz[11] using SES in rabbit iliac arteries, little evidence of increased inflammation and delayed endothelialisation was noted with 28-day SES as compared to polymer-coated stents or BMS. And although Suzuki[12] reported higher amounts of accumulated fibrin with SES than BMS at 28-days in porcine coronary arteries, the degree of re-endothelialisation was similar among groups. Indeed, most papers describe a fairly high rate of endothelialisation in single DES and BMS that appear strikingly similar (80-90%). Only in segments of overlapping DES with presumably high local drug levels, can an effect be seen (60-70%).[13]

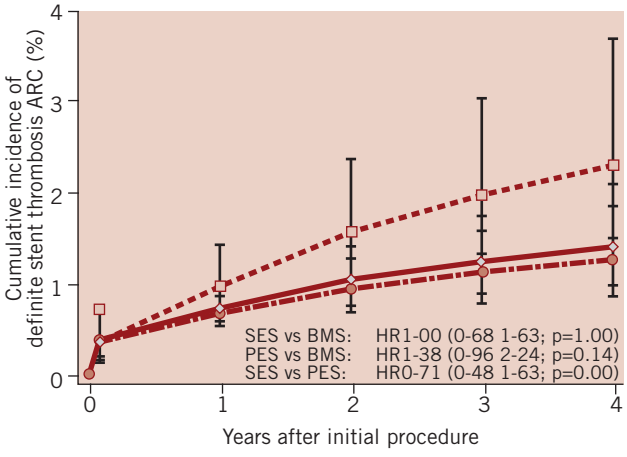

Figure 1. When plotting the incidence of definite stent thrombosis, the incidence of B<S and SES are perfectly superposed, while PES shows a higher cumulative incidence. This difference is however not statistically significant. Reproduced with permission[6].

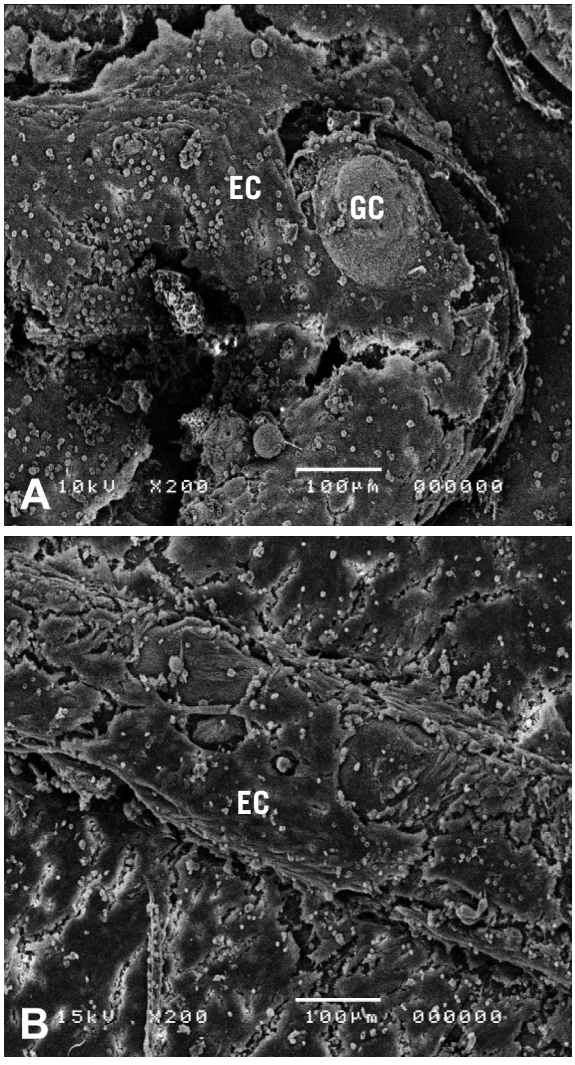

Figure 2. Current bare (A, Kaneka stainless steel) and DES (B, Taxus) also show a high percentage (80-90%) endothelial coverage (EC) of stent struts as early as 5 days. Presence of endothelium does not, however, implicate functionality. GC: macrophage giant cell.

Overlap stenting is however rare in daily practice (0.9%) and although in both BMS and DES this is associated with a high rate of revascularisation[14], overlap stenting has not been identified as a predictor of LST[15].

Absence of neointima cannot be ascertained by angioscopy

It should be noted that in clinical studies it is especially difficult to determine the difference between lack of endothelialisation, endothelial contraction and endothelial dysfunction. Several clinical angioscopic studies have described delayed endothelialisation after implantation of DES with SES showing incomplete neointimal coverage (NI) three to six months after implantation. This was associated with presence of thrombi and yellow plaques even as much as two years after implantation[16-18].

We doubt whether angioscopy can really distinguish complete from incomplete endothelial and thin NI-coverage. In swine coronary arteries, we have often observed stents that look like non-covered stents as seen by macroscopy (Figure 3). Light and scanning electron microscopy however show that these stents are not at all naked, but completely covered by endothelium as well as neointimal tissue. This tissue is however completely translucent to the extent that red thrombus can be easily observed surrounding stent struts[10].

Autopsy studies

Autopsy studies in humans have advocated the link between delayed endothelialisation (delayed healing) and LST, showing an especially strong association between lack of neointimal strut coverage in DES and LST[15,19,20]. Markers of delayed healing have also been observed in atherectomy specimen in a study that specifically excluded stent thrombosis[21] and seems unrelated to LST. Of course both approaches have clear drawbacks. Atherectomy specimen represent cases of restenosis and pertain to small pieces of tissue, while autopsy cases only cover a discrete subpopulation of DES implantation and complete clinical data are not always possible to obtain. With autopsy, there is also an inevitable delay between the time of LST and tissue retrieval at autopsy. Whether the endothelium underneath a thrombus, often rich in neutrophils, can survive this delay and remain present for assessment is unclear[22]. This cautions us to apply this data to all "real-world" cases of stent thrombosis.

Endothelial dysfunction: abnormal response to vasodilators or exercise

If mere presence of endothelium does not affect healing, assessment of endothelial function seems more appropriate and can be studied both *in vivo* and *in vitro*. Indeed, recent clinical reports suggest that DES may impair endothelial responses to acetylcholine (Ach) or exercise-mediated vasodilation in humans[23-26].

Hofma and Shin both showed that coronary segments adjacent and distal to DES showed vasoconstriction in response to Ach in SES but not BMS (Figure 4), both without impaired endothelial independent vasodilator responses to nitroglycerin. Endothelial dysfunction was corroborated by experimental studies in swine showing impaired dilation in response to Bradykinin which was more evident in PES

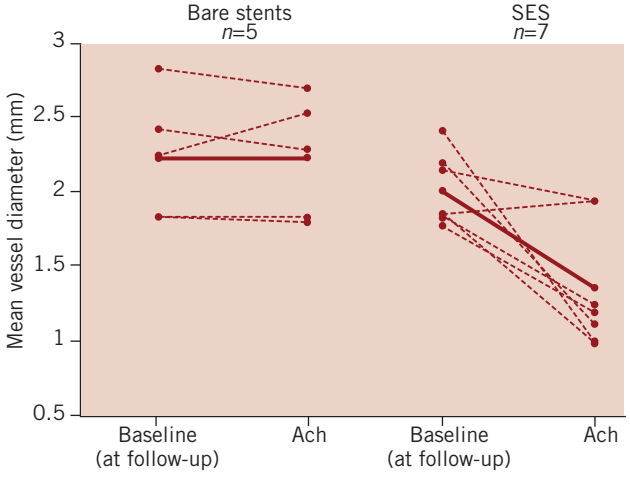

Figure 4. Sirolimus eluting stents induce chronic endothelial dysfunction in the coronary segment immediately distal from the stent as shown by a vasoconstrictive response to an acetylcholine (10^{-6}M) challenge at follow-up (P=0.03). From Hofma et al[23].

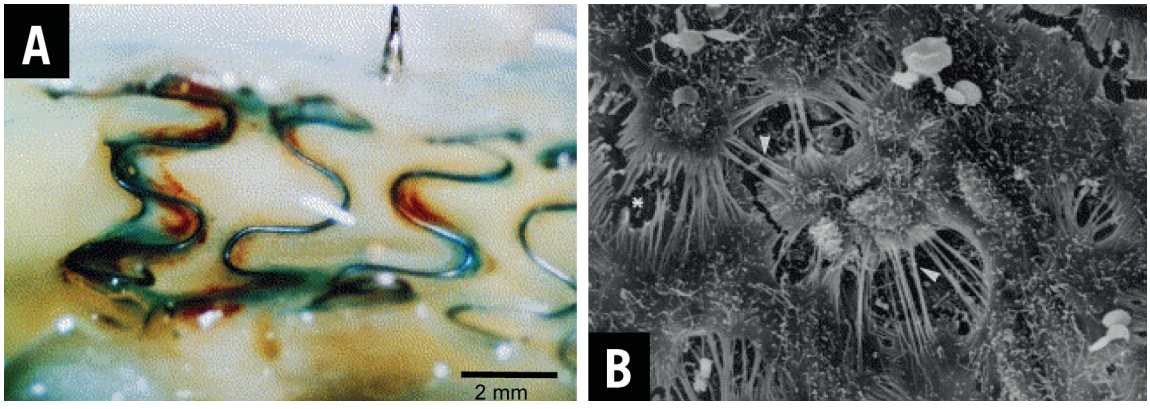

Figure 3. Although stent struts have the macroscopic appearance of a completely non-covered Medtronic Wiktor stent at 14 days after stent implantation (angioscopic grade 0, stent struts fully visible[16] [A]), scanning electron microscopy shows that the struts are actually completely covered by endothelium (right, modified from van Beusekom et al[10] [B]).

than SES[27]. Togni evaluated coronary vasomotion with quantitative coronary angiography at rest and during exercise in patients receiving SES, PES and BMS at six 2-12 (two to twelve) months after implantation. Both SES and PES showed exercise-induced vasoconstriction of the proximal and distal vessel segments adjacent to DES, while in BMS, the adjacent segments proximal and distal to the stent showed exercise-induced vasodilation. They concluded that DES are associated with exercise-induced paradoxic coronary vasoconstriction of the adjacent vessel segments, despite a maintained vasodilatory response to nitroglycerin.

Endothelial function within stents themselves is most easily studied in animal models using immunohistochemistry. Our data from the animal lab also indicate that DES are not so much associated with endothelial absence but more with a more pronounced endothelial dysfunction as shown by a decrease in eNOS expression in Paclitaxel but not Sirolimus eluting stents as compared to BMS[28].

Endothelial dysfunction or endothelial absence

Acetylcholine acts as a vasodilator by stimulating the release of NO by the endothelium. An abnormal Ach test is usually interpreted as a form of endothelial dysfunction. However, endothelium that has a normal NO release but is contracted and thus leaky to many substances (Figure 3B)[10,29], can probably also cause vasoconstriction via a direct exposure of smooth muscle cells to Ach. In that sense, it is not easy to distinguish between diminished NO release, endothelial leakage or absence of endothelium. The absence of endothelium *in vivo* is nearly impossible to assess in clinical research.

Conclusion

Although current hypotheses propose delayed endothelialisation after DES implantation, evidence is still controversial. Both clinical studies as well as our own data from *in vivo* and *in vitro* porcine coronary studies[27], suggest that DES do not necessarily show differences in re-endothelialisation. They do underscore that there are late effects of stents, DES in particular, on endothelial function. These effects could well be responsible for a pro-thrombogenic nature in some but certainly not all DES or BMS.

References

1. Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnar F, Falotico R. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med.* 2002;346(23):1773-1780.

2. Moses JW, Leon MB, Popma JJ, Fitzgerald PJ, Holmes DR, O'Shaughnessy C, Caputo RP, Kereiakes DJ, Williams DO, Teirstein PS, Jaeger JL, Kuntz RE. Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. *N Engl J Med.* 2003;349(14):1315-1323.

3. Park SJ, Shim WH, Ho DS, Raizner AE, Park SW, Hong MK, Lee CW, Choi D, Jang Y, Lam R, Weissman NJ, Mintz GS. A paclitaxel-eluting stent for the prevention of coronary restenosis. *N Engl J Med.* 2003;348(16):1537-1545.

4. McFadden EP, Stabile E, Regar E, Cheneau E, Ong AT, Kinnaird T, Suddath WO, Weissman NJ, Torguson R, Kent KM, Pichard AD, Satler LF, Waksman R, Serruys PW. Late thrombosis in drug-eluting coronary stents after discontinuation of antiplatelet therapy. *Lancet.* 2004; 364(9444): 1519-1521.

5. Collected_DES_papers. Sections "perspectives", "original articles" and "editorial". *N Engl J Med.* 2007;356(10):981-1039, 1059-1060.

6. Stettler C, Wandel S, Allemann S, Kastrati A, Morice MC, Schomig A, Pfisterer ME, Stone GW, Leon MB, de Lezo JS, Goy JJ, Park SJ, Sabate M, Suttorp MJ, Kelbaek H, Spaulding C, Menichelli M, Vermeersch P, Dirksen MT, Cervinka P, Petronio AS, Nordmann AJ, Diem P, Meier B, Zwahlen M, Reichenbach S, Trelle S, Windecker S, Juni P. Outcomes associated with drug-eluting and bare-metal stents: a collaborative network meta-analysis. *Lancet.* 2007;370(9591):937-948.

7. Whelan DM, van der Giessen WJ, Krabbendam SC, van Vliet EA, Verdouw PD, Serruys PW, van Beusekom HM. Biocompatibility of phosphorylcholine coated stents in normal porcine coronary arteries. *Heart.* 2000;83(3):338-345.

8. Hardhammar 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(3):423-430.

9. Hofma SH, Whelan DM, van Beusekom HM, Verdouw PD, van der Giessen WJ. Increasing arterial wall injury after long-term implantation of two types of stent in a porcine coronary model. *Eur Heart J.* 1998; 19(4): 601-609.

10. 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(4):1109-1117.

11. Klugherz BD, Llanos G, Lieuallen W, Kopia GA, Papandreou G, Narayan P, Sasseen B, Adelman SJ, Falotico R, Wilensky RL. Twenty-eight-day efficacy and phamacokinetics of the sirolimus-eluting stent. *Coron Artery Dis.* 2002;13(3):183-188.

12. Suzuki T, Kopia G, Hayashi S, Bailey LR, Llanos G, Wilensky R, Klugherz BD, Papandreou G, Narayan P, Leon MB, Yeung AC, Tio F, Tsao PS, Falotico R, Carter AJ. Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. *Circulation.* 2001; 104(10): 1188-1193.

13. Finn AV, Kolodgie FD, Harnek J, Guerrero LJ, Acampado E, Tefera K, Skorija K, Weber DK, Gold HK, Virmani R. Differential response of delayed healing and persistent inflammation at sites of overlapping sirolimus- or paclitaxel-eluting stents. *Circulation.* 2005;112(2):270-278.

14. Aoki J, Kirtane AJ, Dangas GD, Lansky AJ, Morales A, Kimura M, Kim YH, Moussa I, Weisz G, Kreps EM, Collins M, Frankin-Bond T, Stone GW, Moses JW, Leon MB, Mehran R. Clinical outcomes after heterogeneous overlap stenting with drug-eluting stents and bare-metal stents for de novo coronary artery narrowings. *Am J Cardiol.* 2008;101(1):58-62.

15. Finn AV, Joner M, Nakazawa G, Kolodgie F, Newell J, John MC, Gold HK, Virmani R. Pathological correlates of late drug-eluting stent thrombosis: strut coverage as a marker of endothelialization. *Circulation.* 2007;115(18):2435-2441.

16. Awata M, Kotani J, Uematsu M, Morozumi T, Watanabe T, Onishi T, Iida O, Sera F, Nanto S, Hori M, Nagata S. Serial angioscopic evidence of incomplete neointimal coverage after sirolimus-eluting stent implantation: comparison with bare-metal stents. *Circulation.* 2007;116(8):910-916.

17. Kotani J, Awata M, Nanto S, Uematsu M, Oshima F, Minamiguchi H, Mintz GS, Nagata S. Incomplete neointimal coverage of sirolimus-eluting stents: angioscopic findings. *J Am Coll Cardiol.* 2006;47(10):2108-2111.

18. Oyabu J, Ueda Y, Ogasawara N, Okada K, Hirayama A, Kodama K. Angioscopic evaluation of neointima coverage: sirolimus drug-eluting stent versus bare metal stent. *Am Heart J*. 2006;152(6):1168-1174.

19. Farb A, Burke AP, Kolodgie FD, Virmani R. Pathological mechanisms of fatal late coronary stent thrombosis in humans. *Circulation*. 2003;108(14):1701-1706.

20. Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, Kutys R, Skorija K, Gold HK, Virmani R. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. *J Am Coll Cardiol*. 2006;48(1):193-202.

21. van Beusekom HM, Saia F, Zindler JD, Lemos PA, Swager-Ten Hoor SL, van Leeuwen MA, de Feijter PJ, Serruys PW, van der Giessen WJ. Drug-eluting stents show delayed healing: paclitaxel more pronounced than sirolimus. *Eur Heart J*. 2007;28(8):974-979.

22. Westlin WF, Gimbrone MA, Jr. Neutrophil-mediated damage to human vascular endothelium. Role of cytokine activation. *Am J Pathol*. 1993;142(1):117-128.

23. Hofma SH, van der Giessen WJ, van Dalen BM, Lemos PA, McFadden EP, Sianos G, Ligthart JM, van Essen D, de Feyter PJ, Serruys PW. Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation. *Eur Heart J*. 2006;27(2):166-170.

24. Togni M, Windecker S, Cocchia R, Wenaweser P, Cook S, Billinger M, Meier B, Hess OM. Sirolimus-eluting stents associated with paradoxic coronary vasoconstriction. *J Am Coll Cardiol*. 2005;46(2):231-236.

25. Shin DI, Kim PJ, Seung KB, Kim DB, Kim MJ, Chang K, Lim SM, Jeon DS, Chung WS, Baek SH, Lee MY. Drug-eluting stent implantation could be associated with long-term coronary endothelial dysfunction. *Int Heart J*. 2007;48(5):553-567.

26. Togni M, Raber L, Cocchia R, Wenaweser P, Cook S, Windecker S, Meier B, Hess OM. Local vascular dysfunction after coronary paclitaxel-eluting stent implantation. *Int J Cardiol*. 2007;120(2):212-220.

27. Sorop O, Batenburg WW, Koopmans S-J, Dekker R, Duncker D, Danser A, Beusekom Hv, Giessen Wvd. Taxus but not Cypher Drug Eluting Stents induce Endothelial Dysfunction in the Distal Coronary Microvasculature. 2007;116:II:293.

28. Van Beusekom HM, Sorop O, Weymaere M, Duncker D, van der Giessen WJ. The neointimal response to stents eluting Tacrolimus from a degradable coating depends on the balance between polymer degradation and drug release. *Eurointervention*. 2008;4:139-147.

29. Van der Giessen WJ, Danser A, Van Beusekom HM, Derkx FHM, Verdouw PD, Lamers JMJ, Serruys PW. Enhanced angiotensin II degradation in porcine coronary neointimal hyperplasia induced by stent implantation. *Coronary Artery Dis*. 1992;3:730-737.