

Short-term effects of fully bioabsorbable PLLA coronary stents in a porcine model

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Abstract Although metallic stents are effective in preventing acute occlusion and reducing late restenosis after coronary angioplasty, many concerns still remain. In this report, short-term effects of fully bioabsorbable sirolimus-loaded poly-L-lactic acid (PLLA) stents (Xinsorb) were evaluated in a porcine coronary model. Commercially available PLLA-coated sirolimus-eluting stents (Excel) were used as controls. The purpose of this study was to assess technical feasibility, biocompatibility, and impact on coronary stenosis of fully bioabsorbable PLLA stents. Our preliminary experience suggested that Xinsorb stents have succeeded in preventing elastic recoil and suppressing neointimal formation for the first 90 days and only mildly delayed the endothelialisation process of the stented blood vessel. Coronary stenosis following Xinsorb and Excel stent implantations after 30 and 90 days was $18.6 \pm 5.2\%$ versus $21.4 \pm 7.2\%$ and $24.5 \pm 4.7\%$ versus $27.7 \pm 5.6\%$, respectively ($p > 0.05$). Both Xinsorb and Excel stents required approximately 1–3 months for re-endothelialization of the inner wall of stented blood vessels. These data provided additional insights into the mechanism and efficacy of fully bioabsorbable PLLA stents in normal porcine coronary arteries while raising questions regarding the potential durability of this novel medical device. Long-term follow-up will be required to validate the long-term efficacy of fully bioabsorbable PLLA stents.

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Introduction

Conventional metallic, drug-eluting stents (DES) restrict restenosis after percutaneous coronary intervention (PCI) by scaffolding intimal flaps that have separated from deeper arterial layers, which controls early elastic recoil and reduces neointimal growth response to stenting by coating stents with antiproliferative drugs [1–6]. However, stents are foreign bodies, and they can generate additional foreign body reactions and consequently increase the possibility of adverse events. Indeed, a permanent metallic DES predisposes the patient to late stent thrombosis, prevents late lumen vessel enlargement, hinders surgical revascularisation, jails side branches, prevents expansive remodelling, and impairs non-invasive imaging of coronary arteries with multislice CT and MRI [7–12]. Late restenosis in DES has also been observed and attributed to an inflammatory reaction to stent materials [13]. DES has been designed to reduce in-stent neointimal growth through elution of agents that arrest the cell proliferation cycle. Restenosis commonly occurs within 3–6 months after coronary intervention, and it rarely occurs thereafter [14]. Therefore, a permanent prosthesis placed beyond this initial period has no clear function. Considering the short-term need and potential for long-term complications with metallic stents and the fact that surgical removal of an expanded metal stent in situ is extremely traumatic, biodegradable stents—that initially scaffold the vessel wall and then disappear once the acute recoil and constrictive remodelling processes have subsided—have theoretical advantages [15]. In contrast to permanent metal stents, completely absorbable stents may allow vessels to react normally to pulsatile flow, positively remodel and respond normally to factors released by endothelium [16].

Poly-L-lactic acid (PLLA) is a biodegradable material with non-toxic metabolic end products (carbon dioxide and water), which cause minimal tissue responses. PLLA stents have the potential to emerge as the ideal stent, leaving behind a natural vessel after complete resorption and restoring vasoreactivity and obviating the need for long-term antiplatelet therapy [3, 13]. Currently, they are in the early stages of development, and several improvisations are ongoing [17–19]. The landscape for bioabsorbable stents is constantly evolving through continued improvisation of existing technology and the emergence of new technology. They represent the next logical step in the advancement of PCI technology and hold a promise for the future of interventional cardiology. Nowadays, the antimigration and antiproliferation effects of sirolimus on human artery smooth muscle cells is now well accepted [20], making sirolimus one of the most promising drugs in reducing neointimal hyperplasia in coronary artery stenting process. In the present study, short-term performance of fully bioabsorbable, expandable, sirolimus-loaded PLLA coronary stents (Xinsorb) has been evaluated in a porcine coronary model. The aim of this study was to assess in vivo effects of this novel medical device with respect to biocompatibility, neointimal hyperplasia formation, and reliability.

Experimental

Stent preparation

Fully bioabsorbable, balloon expandable, PLLA stents (Xinsorb, $3.0 \times 13.4 \text{ mm}^2$, Shandong Huaan Biotechnology Co., Ltd, China, Fig. 1) were spray coated with a thin layer of PLLA containing $\approx 140 \mu\text{g}/\text{cm}^2$ of sirolimus and the total amount of sirolimus for Xinsorb stents was about $120 \mu\text{g}$ per stent. Excel stents ($3.0 \times 14 \text{ mm}^2$) were commercially available and the amount of sirolimus was about $200 \mu\text{g}$ per stent. All stents were individually packaged, coded with a serial number on the packaging label, and sterilised.

Sirolimus release profile of Xinsorb stents

In vitro release profile of Xinsorb stents and Excel stents was evaluated by immersing each stent in a 20 mL of PBS containing 4% FBS (Hyclone, pH 7.4) at 37°C in a shaking incubator ($n = 5$, 5 stents per time point). At each time point, the syringes were quickly removed from the incubator and the concentration of sirolimus in PBS was measured with High Performance Liquid Chromatography (HPLC, Agilent 1100) [21].

Stent implantation

Eighteen male pigs ($\sim 20 \text{ kg}$, weight) were obtained from the Shanghai Animal Administration Centre. All animals received daily oral antiplatelet medication with clopidogrel (75 mg) and aspirin (100 mg) for 3 days before stenting followed by the same amount of clopidogrel and less aspirin (81 mg) daily until termination. Stents were implanted according to standard procedures. Vessel diameter at the target site was measured by quantitative coronary angiography (QCA). Two different stents were randomly implanted in two coronary arteries of RCA and LAD for each pig. The stents were deployed by inflating the balloon (3.0 mm) to nominal pressure at the injury site and maintained for 20 s, and the resulting stent-to-artery ratio was approximately 1.2–1.3:1. Repeated angiograms were immediately obtained after stent implantation. Next, all equipment was retreated and the iliac artery was ligated. On the day of euthanasia, angiography was performed and a lethal amount of potassium chloride was injected. After euthanasia, the hearts were harvested and labelled. The stented arteries were carefully dissected from the myocardium and cut

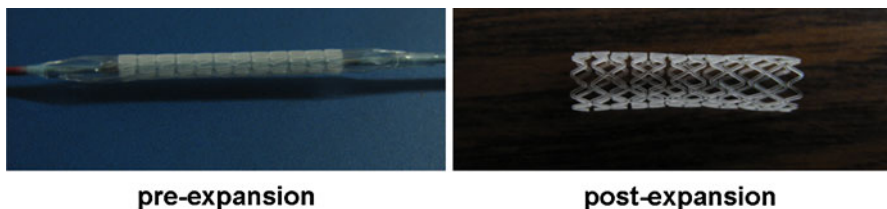


Fig. 1 Xinsorb is a premounted, balloon-expandable, sirolimus-loaded PLLA stent

into three pieces, each about 5-mm long. The proximal pieces were fixed in 10 mL of buffered formalin, embedded in methacrylate for cross section preparation using a section cutter (Leica SP1600, Germany), stained with haematoxylin and eosin for measuring vessel area and histologically analysed with Leica Qwin V3 software. Injury score was determined by the method of Schwartz et al. [22]. After peered off of the stent struts, the medial pieces from the stented arteries were fixed in buffered formalin, dehydrated, and embedded in paraffin. H-E staining was used to evaluate the inflammation formed. Inflammation was graded as follows: 0, none; 1, scattered inflammatory cells; 2, inflammatory cells encompassing 50% of a strut in at least 25–50% of the artery circumference; and 3, inflammatory cells surrounding a strut in at least 25–50% of the artery circumference [23]. The distal pieces were cut open lengthwise and fixed with 1% osmium tetroxide for SEM imaging.

QCA analysis

Coronary angiography was performed before, immediately after, 30 days after, and 90 days after the procedure. QCA was analysed using the cardiovascular measurement system (Medical Imaging Systems), which was referenced to the known diameter of the angiographic catheter. The minimal lumen diameter (MLD) of treated coronary segments, reference diameter, and percent diameter stenosis from the baseline angiogram were determined to demonstrate the lesion to be severe and not foreshortened. Baseline and follow-up cineangiograms were evaluated to demonstrate the same conclusion.

Histological analysis of neointimal hyperplasia

Vessel area was measured by tracing the external elastic lamina (EEL, mm^2), internal elastic lamina (IEL, mm^2), and lumen area (LA, mm^2). Neointimal area (NA, mm^2) was calculated as follows: $\text{NA} = \text{IEL} - \text{LA}$; mean neointimal thickness was calculated using the following equation: $\text{mean neointimal thickness} = \sqrt{\text{IEL}/\pi} - \sqrt{\text{LA}/\pi}$; the percent neointimal stenosis was calculated using the following equation: $\% \text{ stenosis} = \text{NA}/\text{IEL} \times 100$.

Statistical analysis

Numerical data were presented as mean $\pm$ standard deviation of the mean. Continuous variables were compared by ANOVA (t test with Bonferroni correction), and categorical variables were compared by χ^2 test. A value of $p \leq 0.05$ was considered significant.

Results and discussion

A total of 18 Xinsorb stents ($n = 10$ for 30 days, $n = 8$ for 90 days) and 18 Excel stents ($n = 10$ for 30 days, $n = 8$ for 90 days) were successfully implanted in the

coronary arteries of 18 pigs. All animals survived the in-life phase without clinical or angiographic stent thrombosis. The animals remained well throughout the study without abnormal temperature, weight loss, or other major health problems. A complete blood cell count showed similar results for white blood cell count, platelet count and haematocrit among all animals. All stents were angiographically patent at the time of euthanasia without evidence of migration or aneurysm formation.

Sirolimus release profile of Xinsorb stents

In vitro release profile of sirolimus from Xinsorb stents and Excel stents was obtained and shown in Fig. 2. The release of sirolimus from Xinsorb stents and Excel stents was observed to go through two stages; with an initial sirolimus of approximately 75% (78% for Xinsorb stents and 76% for Excel stents) within the first 2 weeks and subsequently a more slow release. All the loaded sirolimus reaches a release plateau of larger than 80% (83% for Xinsorb stents and 86% for Excel stents) within 4-week incubation in PBS. The sirolimus release in first stage was mainly dominated by diffusion, then shifted to a degradation-controlled stage after 2 weeks.

Quantitative coronary angiography

The baseline vessel diameter ranged from 2.44 to 2.85 mm. The balloon-to-artery ratio was approximately 1.2–1.3:1. Coronary artery angiograms taken 30 and 90 days after the implantation of Xinsorb and Excel stents are illustrated in Fig. 3. No distinctive vessel narrowing greater than 50% or remodelling effects on surrounding tissues were observed. Both Xinsorb and Excel stents had a remarkable effect on reducing neointimal hyperplasia. No in-stent thrombosis appeared in this

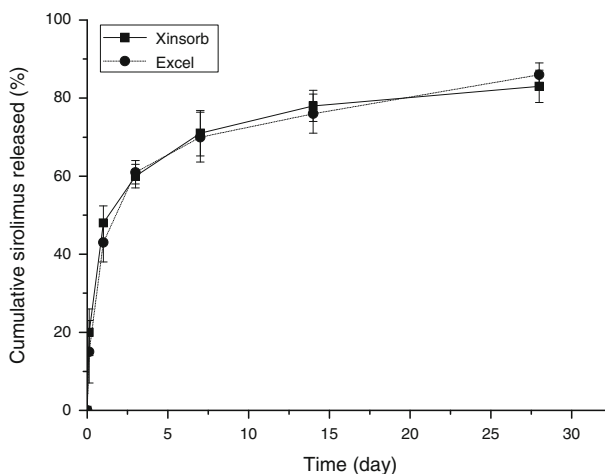


Fig. 2 In vitro sirolimus release profile from Xinsorb stents and Excel stents

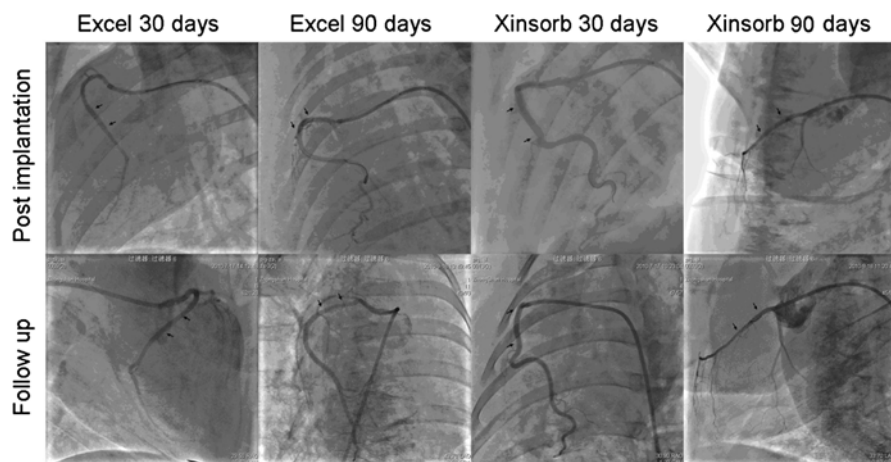


Fig. 3 Angiograms of the coronary arteries after implantation of Excel and Xinsorb stents. No distinctive vessel narrowing greater than 50% and remodelling effects on the surrounding tissue was observed

study, although late-stage thrombosis in stents usually occurs beyond the 3-month timeframe of our animal study.

Histological analysis and morphometric evaluations

The histomorphometry at 30 and 90 days for Xinsorb and Excel stents in a porcine coronary artery model is illustrated in Fig. 4. The morphometric data and

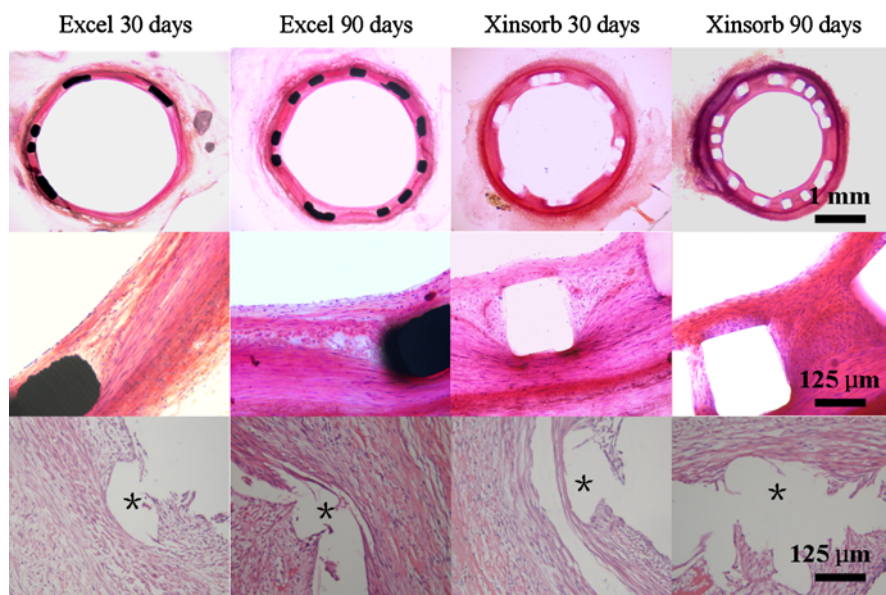
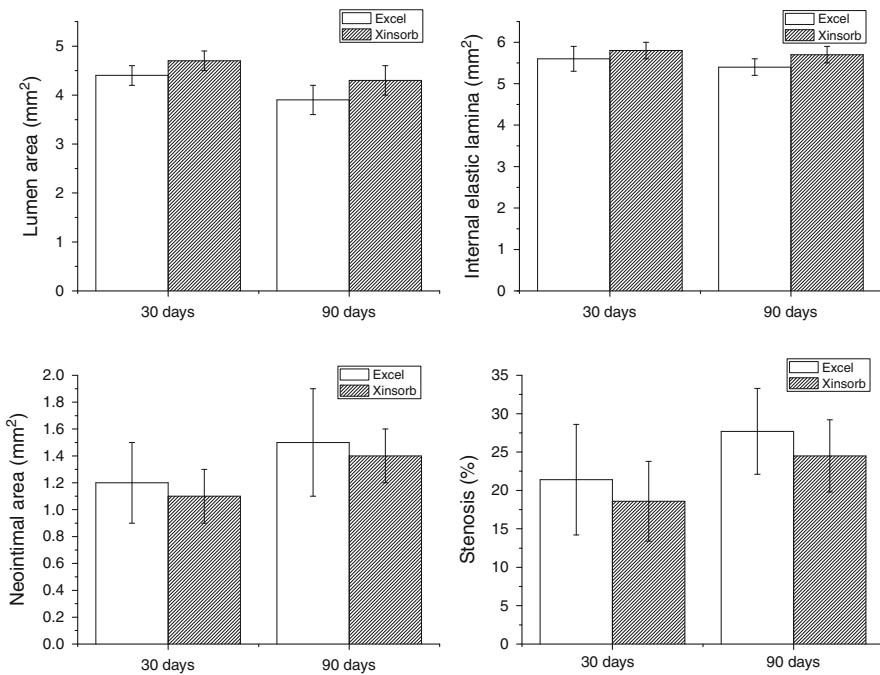


Fig. 4 Photomicrographs of blood vessel segments with haematoxylin–eosin staining 30 and 90 days post-implantation; *stent position

Table 1 Histomorphometric analysis on LA, NA, IEL, and stenosis% of the arteries treated with Excel stents and Xinsorb stents

	LA (mm ²)	IEL (mm ²)	NA (mm ²)	Stenosis (%)	<i>p</i> value
30 days					
Excel	4.4 ± 0.2	5.6 ± 0.3	1.2 ± 0.3	21.4 ± 7.2	>0.05
Xinsorb	4.7 ± 0.2	5.8 ± 0.2	1.1 ± 0.2	18.6 ± 5.2	
90 days					
Excel	3.9 ± 0.3	5.4 ± 0.2	1.5 ± 0.4	27.7 ± 5.6	>0.05
Xinsorb	4.3 ± 0.3	5.7 ± 0.2	1.4 ± 0.2	24.5 ± 4.7	

Results are presented as mean ± standard deviation of the mean. There was no significant difference in NA and stenosis% at days 30 and 90 between Excel stents and Xinsorb stents ($p > 0.05$)

**Fig. 5** Histomorphometric analysis of LA, NA, IEL, and % stenosis of the arteries treated with Excel and Xinsorb stents. Results are presented as mean ± standard deviation of the mean. There was no significant difference in NA or % stenosis at days 30 and 90 between Excel and Xinsorb stents ($p > 0.05$)

semi-quantitative scoring for injury and inflammation obtained from the treated arteries are illustrated in Table 1 and Fig. 5, respectively.

Xinsorb and Excel stents had a stronger capacity to reduce neointimal hyperplasia. As presented in Figs. 4 and 5, NAs of Xinsorb and Excel stents at 30- and 90-day post-implantation are 1.1 ± 0.2 mm² versus 1.2 ± 0.3 mm² and 1.4 ± 0.2 mm² versus 1.5 ± 0.4 mm², respectively. There was no significant difference ($p > 0.05$) in cross-sectional area stenosis after 30 days ($18.6 \pm 5.2\%$ vs.

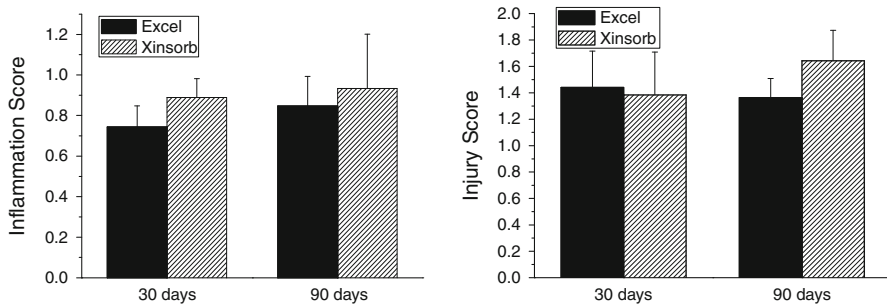


Fig. 6 Inflammation and injury scores of the arteries treated with Excel and Xinsorb stents at days 30 and 90. There was no significant difference in inflammation or injury score at days 30 and 90 ($p > 0.05$)

21.4 $\pm$ 7.2%) and 90 days (24.5 $\pm$ 4.7% vs. 27.7 $\pm$ 5.6%). The long-term inhibition of neointimal formation was most likely due to sustained sirolimus elution from the surface of these two stents. The LA and IEL at 30 and 90 days were 4.7 $\pm$ 0.2 mm² versus 4.4 $\pm$ 0.2 mm², 5.8 $\pm$ 0.2 mm² versus 5.6 $\pm$ 0.3 mm²; and 4.3 $\pm$ 0.3 mm² versus 3.9 $\pm$ 0.3 mm², 5.7 $\pm$ 0.2 mm² versus 5.4 $\pm$ 0.2 mm². No statistical difference in IEL was observed between Xinsorb and Excel stents ($p > 0.05$). The above results suggested that Xinsorb stents have succeeded in preventing elastic recoil within 90 days after stent implantation.

PLLA was expected to be a biocompatible and bioabsorbable coating material for DES. The PLLA-coated sirolimus eluting stents (Excel) have been used in China to treat coronary artery diseases. For traditional DES, the amount of coating material has played a pivotal role in foreign body reactions and inflammation formation; more coating material has caused severe inflammatory reactions. The accumulation of inflammatory cells stimulates growth factors and cytokines, which promotes neointimal formation and eventually narrows the intra-stent lumen. Therefore, it was believed that reducing the amount of coating material will improve long-term safety and efficacy of drug-eluting stents. For all bioabsorbable PLLA stents in the present study, the PLLA matrix was predicted to induce severe inflammation due to foreign body reactions. On the contrary, only a mild inflammatory response with few inflammatory cells surrounding the stent struts is observed, as shown in Fig. 4. The inflammation scores at 30 and 90 days for Xinsorb and Excel stents were 0.84 $\pm$ 0.15 versus 0.74 $\pm$ 0.10 and 0.93 $\pm$ 0.26 versus 0.88 $\pm$ 0.10, respectively (Fig. 6). There was no significant difference between Xinsorb and Excel stents ($p > 0.05$). The above result was thought to be related to the relatively slower degradation rates of PLLA struts of Xinsorb stents. The in vitro weight loss of Xinsorb stents in PBS at 37 °C (not shown) was less than 10% within 90 days. The injury scores for Xinsorb and Excel stents at 30 and 90 days were 1.36 $\pm$ 0.14 versus 1.44 $\pm$ 0.28 and 1.64 $\pm$ 0.23 versus 1.38 $\pm$ 0.32, respectively. Also, there was no significant difference ($p > 0.05$).

Endothelialisation of stented arteries

The endothelialization of the stented arteries was examined using SEM at 30 and 90 days after stent implantation. As shown in Fig. 7, the areas between struts shows

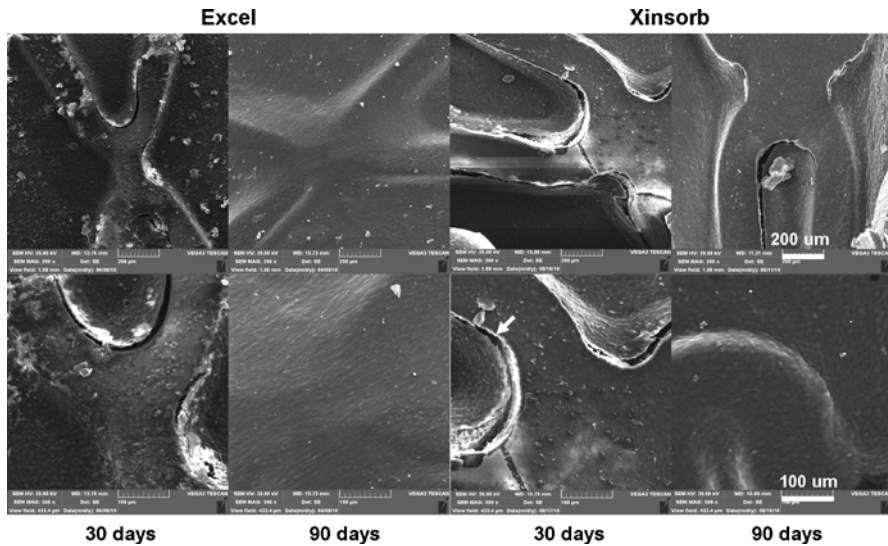


Fig. 7 SEM images of the porcine coronary arteries 30 and 90 days after stent implantation. The lumen surface of the stented blood vessel above the struts was not fully covered by endothelial cells at day 30. After 90 days, the arteries treated with Xinsorb and Excel stents were fully endothelialised; the vessel wall lumen surfaces and stent struts were covered by confluent endothelial cells

>90% surface area coverage by endothelial cells for fully bioabsorbable PLLA stents at 30 days. In contrast, endothelial coverage above the struts was less than 30%, which probably resulted from relatively poor cell affinity and PLLA matrix proliferation [24]. Also, it was found that endothelial coverage was generally more complete at the extreme proximal and distal stent regions versus the middle segments. Areas above the struts lacking endothelial cells coverage showed focal aggregates of platelets and inflammatory cells on bare surfaces. In most reports, complete endothelialisation of strut surfaces for both sirolimus and paclitaxel polymer-coated DES was observed at 28-day post-implantation in both rabbit and porcine models [25, 26]. In Excel stents, 60–70% of the stented lumen surface was covered by endothelial cells after 30 days, which probably resulted from the relatively high drug content (200 µg per stent). At 90 days, the arteries treated with Xinsorb and Excel stents were completely endothelialised. The lumen surface of the vessel wall and stent struts were covered by confluent endothelial cells with a cobblestone structure, and few inflammatory cells and platelets were found on the endothelial surfaces. Thus, there was enough evidence to conclude that fully bioabsorbable Xinsorb stents mildly delay the endothelialisation process in porcine coronary arteries, especially on the stent struts. Both Xinsorb and Excel stents took approximately 1–3 months to heal the blood vessel inner walls.

Conclusions

Xinsorb stents reliably supported blood vessels and favourably modulated neointimal formation for 90 days in a porcine coronary model. Long-term inhibition

of neointimal hyperplasia was sustained, presumably due to delayed cellular proliferation by the sirolimus elution. Our data highlighted the necessity to improve our understanding regarding the performance of fully bioabsorbable DES.

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References

1. Sousa JE, Costa MA, Sousa AGMR (2003) Two-year angiographic and intravascular ultrasound follow-up after implantation of sirolimus-eluting stents in human coronary arteries. *Circulation* 107:381–383
2. Stone GW, Ellis SG, Cox DA (2004) A polymer-based, paclitaxel-eluting stent in patients with coronary artery diseases. *New Eng J Med* 350:221–231
3. Serruys PW, Kutryk KJ, Ong AT (2006) Coronary-artery stents. *N Engl J Med* 354:483–495
4. Tamai H, Igaki K, Kyo E et al (2000) Initial and 6-month results of biodegradable poly-L-lactic acid coronary stents in humans. *Circulation* 102:399–404
5. Serruys PW, Ormiston JA, Onuma Y et al (2009) A bioabsorbable everolimus-eluting coronary stent system (ABSORB): 2-year outcomes and results from multiple imaging methods. *Lancet* 373: 897–910
6. Mintz GS, Popma JJ, Pichard AD et al (1996) Arterial remodeling after coronary angioplasty: a serial intravascular ultrasound study. *Circulation* 94:35–43
7. Joner M, Finn AV, Farb A et al (2006) Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. *J Am Coll Cardiol* 48:193–202
8. Luscher TF, Steffel J, Eberli FR et al (2007) Drug-eluting stent and coronary thrombosis: biological mechanisms and clinical implications. *Circulation* 115:1051–1058
9. Kotani J, Awata M, Nanto S et al (2006) Incomplete neointimal coverage of sirolimus-eluting stents: angioscopic findings. *J Am Coll Cardiol* 47:2108–2111
10. Hofma SH, van der Giessen WJ, van Dalen BM et al (2006) Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation. *Eur Heart J* 27:166–170
11. Ako J, Morino Y, Honda Y et al (2005) Late incomplete stent apposition after sirolimus-eluting stent implantation: a serial intravascular ultrasound analysis. *J Am Coll Cardiol* 46:1002–1005
12. Wilson GJ, Nakazawa G, Schwartz RS et al (2009) Comparison of inflammatory response after implantation of sirolimus- and paclitaxel-eluting stents in porcine coronary arteries. *Circulation* 120:141–149
13. Balla S, Aggarwall K, Nistala R (2010) Bioabsorbable coronary stents—are these the next big thing in coronary angioplasty? *Recent Pat Cardiovasc Drug Discov* 5:86–90
14. Kimura T, Yokoi H, Nakagawa Y et al (1996) Three-year follow-up after implantation of metallic coronary-artery stents. *N Engl J Med* 334:561–566
15. Ormiston JA, Serruys PW, Regar E et al (2008) A bioabsorbable everolimus-eluting coronary stent system for patients with single de-novo coronary artery lesions (ABSORB): a prospective open-label trial. *Lancet* 371:899–907
16. Ormiston JA, Webster MWI, Armstrong G (2007) First-in-human implantation of a fully bioabsorbable drug-eluting stent: the BVS poly-L-lactic acid everolimus-eluting coronary stent. *Catheter Cardiovasc Interv* 69:128–131
17. Bünger CM, Grabow N, Christina Kröger C et al (2006) Iliac anastomotic stenting with a sirolimus-eluting biodegradable poly-L-lactide stent: a preliminary study after 6 weeks. *J Endovasc Ther* 13: 630–639
18. Onuma Y, Serruys PW, Perkins LEL et al (2010) Intracoronary optical coherence tomography and histology at 1 month and 2, 3, and 4 years after implantation of everolimus-eluting bioresorbable vascular scaffolds in a porcine coronary artery model: an attempt to decipher the human optical coherence tomography images in the ABSORB trial. *Circulation* 122:2288–2300
19. Bünger CM, Grabow N, Sternberg K (2007) Sirolimus-eluting biodegradable poly-L-lactide stent for peripheral vascular application: a preliminary study in porcine carotid arteries. *J Surg Res* 139:77–82

20. Marx SO, Jayaraman T, Go LO et al (1995) Rapamycin-FKBP inhibits cell cycle regulators of proliferation in vascular smooth muscle cells. *Circ Res* 76:412–417
21. Huang YY, Venkatraman SS, Boey FYC et al (2010) In vitro and in vivo performance of a dual drug-eluting stent (DDES). *Biomaterials* 31:4382–4391
22. Schwartz RS, Huber KC, Murphy JG et al (1991) Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. *J Am Coll Cardiol* 19:267–274
23. Carter AJ, Aggarwal M, Kopiac GA et al (2004) Long-term effects of polymer-based, slow-release, sirolimus-eluting stents in a porcine coronary model. *Cardiovasc Res* 63:617–624
24. Gong FR, Cheng XY, Wang SF et al (2009) Biodegradable comb-dendritic tri-block copolymers consisting of poly(ethylene glycol) and poly(L-lactide): synthesis, characterizations, and regulation of surface morphology and cell responses. *Polymer* 50:2775–2785
25. Joner M, Nakazawa G, Finn AV et al (2008) Endothelial cell recovery between comparator polymer-based drug-eluting stents. *J Am Coll Cardiol* 52:333–342
26. Finn AV, Kolodgie FD, Harnek J et al (2005) Differential response of delayed healing and persistent inflammation at sites of overlapping sirolimus- or paclitaxel-eluting stents. *Circulation* 112:270–278