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The pre-clinical assessment of rapamycin-eluting, durable polymer-free stent coating concepts

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ABSTRACT

All four currently FDA-approved drug-eluting stents (DESs) contain a durable polymeric coating which can negatively impact vascular healing processes and eventually lead to adverse cardiac events. Aim of this study was the pre-clinical assessment of two novel rapamycin-eluting stent (RES) coating technologies that abstain from use of a durable polymer. Two distinctive RES coating technologies were evaluated in vitro and in the porcine coronary artery stent model. The R-poly^S stent platform elutes rapamycin from a biodegradable polymer that is top coated with the resin shellac to minimize the amount of polymer. The R-pro^S stent platform allows dual drug release of rapamycin and probucol, blended by shellac. HPLC-based determination of pharmacokinetics indicated drug release for more than 28 days. At 30 days, neointimal formation was found to be significantly decreased for both DESs compared to bare-metal stents. Assessment of vascular healing revealed absence of increased inflammation in both DESs, which is commonly observed in DES with non-erodible polymeric coating. In conclusion, the pre-clinical assessment of RESs with resin-based or dual drug coating indicated an adequate efficacy profile as well as a beneficial effect for vascular healing processes. These results encourage the transfer of these technologies to clinical evaluation.

1. Introduction

The introduction of drug-eluting stents (DES) a few years ago has significantly alleviated the problem of restenosis, the major limitation of percutaneous coronary intervention (PCI) [1]. The principle of these devices is a fractionated release of anti-proliferative drugs such as rapamycin or paclitaxel [2] by means of a polymeric coating. All of the currently approved DESs utilize a polymeric coating that is not biodegradable. Since the continuous presence of a polymer in the coronary vasculature is believed to be associated with impaired vascular healing and a moderate increase of life-threatening late or very late stent thrombosis [3], substantial efforts are currently underway to identify alternatives to biostable polymeric coating.

The ISAR (Individualizable drug-eluting Stent system to Abrogate Restenosis) DES system allows on-site coating of specially designed stents consisting of 316L medical stainless steel with a relatively thin strut thickness of 87 μm and a microporous surface, thus offering considerable versatility for stent coating processes [4] such as simultaneous coating with multiple compounds as well as synthetic substances that retard drug release (e.g. polymeric or alternative substances). The ISAR system uses rapamycin as a default since rapamycin is a cytostatic drug that impacts on key mechanisms of coronary restenosis [2,5]. Polymer-free, rapamycin-coated ISAR stents have been shown to be clinically effective in terms of the prevention of restenosis [6] and are non-inferior compared to the non-erodible polymer-coated paclitaxel eluting stent as demonstrated in the randomized controlled Intracoronary Stenting and Angiographic Restenosis investigators – Test Efficacy of Rapamycin-Eluting Stents with Different Polymer Coating Strategies (ISAR-TEST) trial [7]. Recently, rapamycin-eluting ISAR stents with biodegradable polymeric coating showed non-inferiority compared to the non-erodible polymer-coated Cypher™ stent in the randomized ISAR-TEST 3 trial [8].

In search for alternative coating strategies, we now identified new approaches that could potentially add to the medical device-

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coating field. We observed that release of rapamycin from the ISAR stent platform could be significantly retarded by simultaneous coating with other lipophilic compounds such as probucol. Probucol has been previously shown to impose anti-restenotic effects through anti-oxidative [9] and direct anti-proliferative effects [10]. In particular, systemic administration of probucol can attenuate restenosis after PCI in humans [11].

Further, we hypothesized that resin-based coating of medical devices might be an alternative or additive to synthetic polymers. As a result, we identified the natural resin shellac as a potential candidate that was tested in this pre-clinical investigation.

2. Methods and materials

2.1. Stents

The DES platform used in this study consists of a premounted, sandblasted, 316L stainless steel microporous stent which allows on-site stent coating without the obligate use of a permanent polymer. A detailed description of this stent system, termed ISAR (Individualizable Drug-eluting Stent System to abrogate restenosis), in terms of coating process as well as mechanical stent surface modification for increased drug storage capacity has been published previously [4]. DESs investigated in this study were identical in stent design as the bare-metal control stents, thus differing solely in rapamycin coating technology. Stents 12 mm long and 3.0 mm (RCA) or 2.5 mm (LAD and LCx), respectively, of nominal diameter were coated within 48 h prior to intended use. Three different DES platforms were created: 1) polymer-free rapamycin-eluting stents (designated R-only) coated solely with 1% rapamycin dissolved in ethanol; 2) rapamycin-eluting stents coated with polymer and shellac (R-poly^S). This stent platform was coated with a blend of 0.4% rapamycin and 0.7% biodegradable polymer in ethyl acetate followed by a separate top coating by means of a 0.1% shellac resin in ethanol; 3) rapamycin-eluting stents coated with probucol and shellac (R-pro^S) that was produced by coating with an ethanol-based solution containing at the same time 1.0% rapamycin, 1.0% probucol and 0.4% shellac resin (see Fig. 1 for a schematic overview).

Rapamycin was purchased from the pharmaceutical distributor "cfm"(Marktredwitz, Germany). All compounds were dissolved for the coating process either in pure ethanol (#100986, Merck, Darmstadt, Germany) or in extra pure ethyl acetate (#100864; Merck).

2.2. Coating

The coating process was enabled by a coating device [4] that allows flexible adjustment of coating pressure and time.

2.3. Pharmacokinetic studies in vitro

To determine pharmacological release kinetics, coated stents ($n = 3$ per group) were deployed ex vivo and transferred in 1 ml PBS (#14190-094, Invitrogen, Karlsruhe, Germany) at 37 °C. Rapamycin release was measured at distinct time points by means of UV spectroscopy (Shimadzu UV 160, Duisburg, Germany) at a wave length of 280 nm. Stent-based rapamycin loading was determined by elution of the drug in 5 ml ethanol or ethyl acetate, respectively, overnight at 4 °C and subsequent UV spectroscopy.

2.4. Porcine coronary artery stent model

The porcine coronary stent model is considered a standard model for the pre-clinical evaluation of drug-eluting stents [12]. Thus, the model was utilized to determine in vivo rapamycin tissue concentrations at distinct time point as well as morphological assessment of healing parameters and neointimal hyperplasia. All animal experiments were in accordance with the German Animal Welfare Act. Studies were approved by the appropriate governmental agency (Regierung von Oberbayern, reference no. 55.2-1-54-2531-40-06).

One day prior stenting, juvenile farm pigs (25–28 kg) received a loading dose of 300 mg clopidogrel (Iscover, Bristol-Myers Squibb, Munich, Germany) and 250 mg aspirin (Bayer VITAL, Leverkusen, Germany). For coronary stenting, pigs were sedated with ketamin (Narketan 10, Chassot, Ravensburg, Germany) and azaperon (Stresnil, Jansen-Cilag, Neuss, Germany). General anaesthesia was maintained by intravenous administration of propofol (propofol 2%, Fresenius-Kabi, Bad Homburg, Germany). Pigs were intubated and mechanically ventilated throughout surgery. Analgesia was secured by repetitive fentanyl (DeltaSelect, Dreieich, Germany) boli. After surgical exposure of the common carotid artery, a 7F sheath was placed by Seldinger technique. Subsequently, pigs received 5000 IU heparin (Liquemin 2500, Ratiopharm, Ulm, Germany) and 250 mg aspirin intravenously. Anti-platelet therapy was maintained for 30 days consisting of 75 mg clopidogrel and 250 mg aspirin daily. Stents from all experimental subgroups were implanted according to a previously assigned list ensuring equal distribution of stents in the respective subgroups in all three major coronary arteries (right coronary artery, left anterior descending and left circumflex coronary artery). Stents were placed at a balloon-to-vessel ratio of 1.1:1 under fluoroscopic guidance (GE Medical OEC 9800 Plus Cardiac). For each group, a total of nine stents equally distributed to all three major coronary arteries were implanted. 30 days after stent placement, animals were euthanized with a lethal dose of pentobarbital. Hearts were immediately removed and coronary arteries were flushed *in situ* with 250 ml Ringer's solution (Sterofundin, Braun, Melsungen, Germany) followed by perfusion fixation with 4% buffered formaldehyde (#P33.2, Roth, Karlsruhe, Germany). Subsequently, stented artery segments were harvested and processed.

To determine the specific concentration of rapamycin in the vascular wall in a separate experiment, pigs were stented with the various rapamycin-eluting stents ($n = 3$ each group and time point) and sacrificed 10 and 20 days after stenting, respectively. Stented vascular segments were harvested immediately. Thereafter, specimens were carefully cleared from perivascular tissue under a dissecting microscope and longitudinally cut open. Subsequently, stents were cautiously removed and the vascular and perivascular tissues (harvested just adjacent to the vasculature) were snap frozen in liquid nitrogen and kept at −70 °C. For further processing, frozen tissues were weighed, placed into 1 ml PBS (# 14190-094, Invitrogen, Karlsruhe, Germany) and homogenized using a MICCRA RT mixer

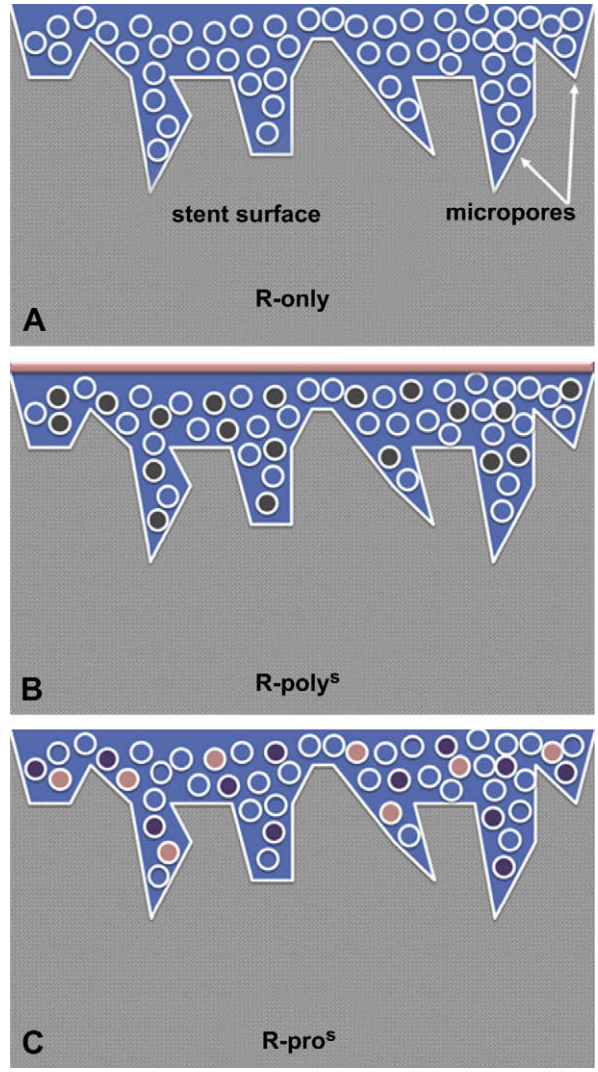

Fig. 1. Schematic assembly of differentially coated ISAR rapamycin-eluting stent platforms. Backbone stent platform for all rapamycin-coated stents is the microporous ISAR stent platform. Panel A demonstrates the assembly of the R-only stent. Rapamycin (unfilled circles) is coated into the microporous pockets by the ISAR drug coating device. Panel B depicts the design of the R-poly^S stent which consists of a blend of rapamycin (open circles) with the biodegradable polymer (grey filled circles) and a resin-based topcoat consisting of shellac (pink). Panel C illustrates the R-pro^S stent platform that consists of a blend of rapamycin (open circles), probucol (purple filled circles) and shellac (pink filled circles). Details are described in the text.

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(Art-Labortechnik, Mülheim, Germany). After 3 freeze–thaw cycles non-solvent, remaining solid components were removed by centrifugation and supernatant subjected to a HPLC-based analysis of rapamycin concentration as previously described [13].

2.5. Histomorphometric assessment

Harvested stented vascular segments were fixed for 24 h in 4% formaldehyde and then dehydrated and embedded in methyl methacrylate polymerization solution (#8.00590.250, Merck, Darmstadt, Germany). Beginning at the proximal end, four 100-μm thick sections were cut every 1000 μm with a Leitz saw microtome (Mikrotom Jung 1600, Leica Instruments, Nussloch, Germany), stained according to Paragon technique, dried, glued onto EXAKT slides (#41500, PSI Medizintechnik Grünewald, Laudenbach, Germany) and polished down to 10 μm thickness with the EXAKT-polish machine (EXAKT 400 CS, EXAKT Apparatebau, Norderstedt, Germany). Data analysis was carried out by use of an Olympus BX41 microscope and its corresponding imaging software cell^{F}. Each of the four in-stent sections was measured for luminal area, strut area, area within the internal elastic lamina (IEL) as well as area within the external elastic lamina (EEL). Neointimal area was calculated by subtraction of luminal and strut area from the area within the IEL. Percent stenosis (DS%) was calculated by dividing the neointimal area by the area within the IEL minus strut area and multiplying by 100. To assess healing parameters, a score-based system was applied by two operators' visual assessment unaware of the origin of the stented segment. Using 100–200 fold magnification, operators assessed endothelialization, inflammation and fibrin deposition for all stent groups on 10-μm thick samples according to established scores [14]. Stent endothelialization was defined as the extent of the circumference of the arterial lumen covered by endothelium: 1 = 25%; 2 = 25% to 75%; and 3 = 75% to 100% coverage. Strut-associated fibrin content was assessed as follows: 0 = no evidence of residual fibrin; 1 = focal regions of residual fibrin deposition adjacent to the strut involving <25% of the circumference of the artery; 2 = moderate fibrin involving >25% of stent struts; and 3 = heavy fibrin deposition involving the majority of stent struts. Injury score (0–3) was determined as follows: 0 = internal elastic lamina intact, media may be compressed but not lacerated; 1 = internal elastic lamina lacerated, media compressed but not lacerated; 2 = internal elastic lamina lacerated, media visibly lacerated, external elastic lamina compressed but intact; and 3 = external elastic lamina lacerated, typically large lacerations of media extending through the external elastic lamina, struts sometimes residing in adventitia [15].

2.6. Statistical analysis

Significance of variability amongst the means of the experimental groups was determined by one or two way analysis of variance (ANOVA), using SPSS for Windows V10.0 software. Differences among experimental groups were considered to be statistically significant when $P < 0.05$. Unless indicated, values are given as mean ± SD.

3. Results

3.1. Coated stents

The coating pattern of stents is depicted in Fig. 1. Rapamycin concentration was 310.2 ± 20.7 μg/cm^2 stent surface for the R-only stent, 206.5 ± 13.0 μg/cm^2 for the R-poly^S and 311.3 ± 6.1 μg/cm^2 for the R-pro^S stent.

3.2. Pharmacokinetics in vitro and in vivo

In vitro determined rapamycin release from the R-only DES platform showed expedited drug release with more than 2/3 of the total dose released within the first week (Fig. 2A). In contrast, rapamycin release from the polymer-based R-poly^S and the polymer-free R-pro^S platform showed prolonged drug release with approximately 20% of the drug still remaining on the stent after 28 days under ex vivo conditions. These results from in vitro examination of drug release kinetics were reflected by in vivo, HPLC-based rapamycin measurement 10 and 20 days, respectively, following coronary stent placement. Rapamycin concentrations were higher with the stents that provided polymer-free (R-pro^S) or biodegradable polymer-based (R-poly^S) rapamycin delivery than rapamycin only coated stents (Fig. 2B).

3.3. Efficacy and healing parameters in bare-metal compared to rapamycin-eluting stents with different coating technologies

The standard pig coronary artery stent model was used to determine both efficacy and safety of BMSs and DESs. Under dual anti-platelet therapy, no incident of death or stent thrombosis occurred in any of the various stent groups. Similarly, PCI-related as well as overall mortality was 0% in all groups.

Results of morphometric stent assessment are provided in Table 1. No statistical difference was detectable regarding IEL and mean injury score between individual groups indicating similar vascular conditions imposed by PCI. However, neointimal area was significantly lower in all three different groups of rapamycin-coated DESs, namely R-only, R-poly^S and R-pro^S. Consequently, percent diameter stenosis (%DS) was also significantly less in rapamycin-coated stent groups vs. BMSs. No individual difference could be detected between the various rapamycin-eluting stent clusters.

Vascular healing was assessed by standard protocols regarding fibrin deposition, inflammation and endothelialization at four layers within each stented vascular segment (Figs. 3 and 4). R-only coated stents revealed similar degrees of fibrin deposition, inflammation and endothial coverage compared to BMSs at the end of the 30-day post-PCI observational period. Although inflammation tended to be higher in polymer-based R-poly^S stents, it was not found to be significantly different compared to BMS, R-only as well as R-pro^S stents. However, fibrin deposition was found to be significantly higher in R-poly^S stents as well as, to a lesser degree, in R-pro^S stents.

Thus, morphometric assessment of efficacy revealed inhibition of neointimal formation and diameters stenosis in all rapamycin-coated stent groups compared to bare-metal controls. Histology-based evaluation of vascular healing indicated appropriate results for all rapamycin-eluting stents, however, fibrin deposition was highest in the biodegradable polymer-based R-poly^S stent.

4. Discussion

After the introduction of percutaneous coronary intervention in the late 1970s and stents in the late 1980s of the last century, restenosis was always considered to be the major drawback of PCI [16]. The occurrence of restenosis was significantly lowered by the introduction of DESs. However, a considerable number of clinical studies with appropriate follow-up suggest a small but significant increase of late and very late stent thrombosis associated with primary generation DESs [17], an event that is of considerable clinical importance because it is associated with high mortality [18]. Furthermore, the current use of DESs is limited by the need for protracted dual anti-platelet therapy, which is currently recommended for 12 months following PCI with DESs, compared to 4 weeks for BMSs [19].

To alleviate this problem, substantial efforts are currently undertaken to modify the coating technology of DESs. Regarding the pharmacologic compound, rapamycin appears to be associated with a high level of enduring efficacy and safety in clinical studies, regardless whether tested in randomized controlled trials [20] or observational studies [21]. To abstain from the need of a non-erodible polymeric coating, we developed two alternative coating strategies that facilitate protracted rapamycin release to the vascular wall. Importantly, we could previously show in a clinical trial that a custom-made, non-commercially available rapamycin-coated DES that resembles release kinetics of a durable polymer-based rapamycin-eluting Cypher™ stent but uses a biodegradable polymeric coating may achieve a late lumen loss that is as favorable as the one accomplished by the Cypher™ stent [8].

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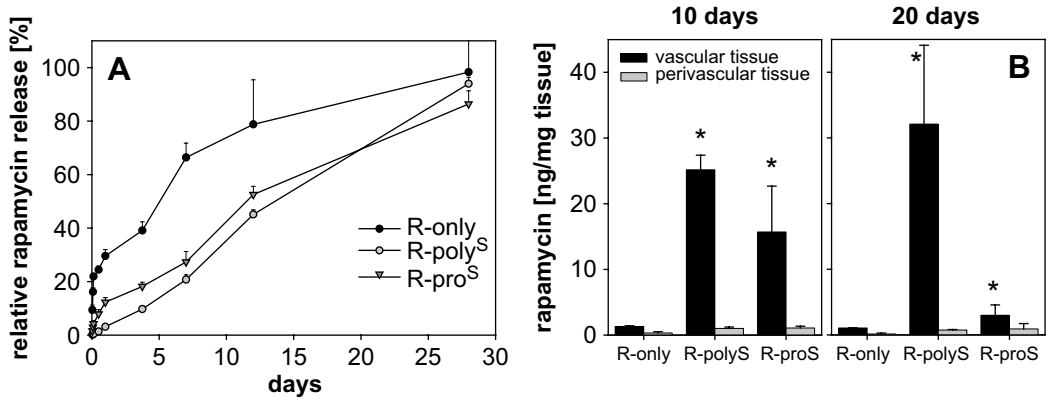

Fig. 2. In vitro and in vivo release of rapamycin by the various rapamycin-eluting stent platforms. Panel A illustrates protracted rapamycin release from the two modified rapamycin-eluting stent platforms R-poly^S and R-pro^S compared to mere rapamycin-coated stent R-only. Polymer/resin-based stent coating protracts rapamycin release most efficiently. These findings were essentially confirmed by measurement of rapamycin concentration in the adjacent vascular wall 10 and 20 days after stent placement. Vascular rapamycin concentrations reached the highest levels in R-poly^S stented vascular segments, followed by R-pro^S. Both stent coatings achieved specific rapamycin accumulation in vascular compared to adjacent, perivascular tissue at levels significantly higher than mere rapamycin-coated R-only stents. $^*p < 0.01$ compared to R-only stents.

One approach uses a biodegradable polymeric coating with a small amount of a resin-based top layer to limit the amount of biodegradable polymer necessary for prolonged drug release (R-poly^S stent) and thus, eventually, negative interference with vascular healing processes. The concept of the additional approach was to entirely omit the need of a polymer-based coating by a dual drug coating strategy with rapamycin and probucol that protracts mutual drug release, amended by a small level resin alloy by means of shellac to optimize drug release (R-pro^S stent). The advantage of probucol, although not directly shown in this study, is a complementary pleiotropic anti-restenotic pharmacological profile that is distinct to rapamycin. First, probucol, a very lipophilic drug, imposes an anti-oxidant effect that has been shown to be beneficial not only for the prevention of restenosis but as well for the deceleration of atherosclerosis progression [9,22]. Additionally, there is evidence from pre-clinical studies that probucol facilitates stent endothelialization, thereby reducing the magnitude of neointimal hyperplasia as well as the occurrence of stent thrombosis [23,24]. This property may counteract to some part endothelial dysfunction which is associated with stent-based rapamycin delivery to the vascular wall [25]. Both drugs, rapamycin as well as probucol, directly inhibit smooth muscle cell proliferation, the key event of coronary restenosis [26], yet by different mechanisms. Whereas rapamycin inhibits mTOR and thus down regulates p27^{kip1} degradation [27], probucol appears to inhibit the ERK1/2 signaling pathway [10]. Several translational clinical studies could demonstrate an anti-restenotic effect of systemic administration of probucol either after mere angioplasty [28] or bare-metal stent placement [11], although a recent study could not confirm the findings of the latter study [29].

The objective of this pre-clinical study was to investigate the safety and efficacy of these novel stent platforms in an established pre-clinical model and to compare them to control stents that were either bare-metal or rapamycin-only coated stents. Both R-poly^S and R-pro^S stents proved safe in the porcine coronary stent model while maintaining significant anti-restenotic efficacy. The considerable inflammation associated with durable polymer-based rapamycin expression [4] could not be observed in any of the tested stent platforms, albeit the usage of topical shellac is known to be possibly associated with contact dermatitis [30] although there is no study to date that studied the incidence of shellac associated contact dermatitis in dermatology prospectively. In this context, it should be mentioned that shellac-coated tablets administered orally did not exhibit relevant side effects [31]. Yet, levels of fibrin deposition were found to be significantly higher in both stent platforms compared to R-only and BMSs, with the highest degree of fibrin deposition in the polymer-based R-poly^S stent. Taken together, these findings suggest that top coating of a biodegradable polymer with a resin like shellac may inhibit inflammatory processes that can be associated with adverse outcomes [32], albeit there is an increase in fibrin deposition compared to uncoated or rapamycin only coated stents.

While the porcine coronary artery model is a decent indicator for DES efficacy and safety, it does not allow precise statements regarding the degree of efficacy of DESs, e.g. the magnitude of the reduction of neointimal hyperplasia. However, there is no other or better animal model available that closely resembles the human scenario [12]. Therefore, results of pre-clinical studies have to be regarded with caution. Yet, pre-clinical assessment of key parameters of DESs, efficacy and vascular healing, are of paramount importance prior to human application to avoid detrimental clinical outcomes [33] as far as possible.

Table 1
Histomorphometrical assessment.

	BMS	R-only	R-poly^S	R-pro^S
IEL, mm	4.84 ± 1.15	4.70 ± 1.11	5.01 ± 0.75	5.21 ± 0.79
Neointimal area, mm^2	1.39 ± 0.79	0.78 ± 0.34*	0.96 ± 0.34*	0.85 ± 0.31*
DS, %	28.8 ± 17.1	18.5 ± 10.3*	18.8 ± 5.4*	16.4 ± 5.8*
Mean injury score	1.20 ± 0.58	1.20 ± 0.29	1.10 ± 0.42	1.05 ± 0.32

IEL denotes area within internal elastic membrane, DS diameter stenosis. $^*P < 0.05$ compared to corresponding bare-metal stent values. P = N.S. between all values of the various drug-eluting stent platforms. Values are mean ± SD.

4.1. Study limitations and conclusions

As mentioned previously, findings in non-atherosclerotic animal coronary arteries are not readily adaptable to the human scenario. However, pre-clinical assessment in the porcine coronary artery stent model is considered standard prior to clinical application of new stent platforms. The two innovative stent coating concepts introduced in this study were designed to abstain from durable polymeric coating and to reduce the amount of polymer by application of resin-based top coating or, alternatively, the total abdication of polymer use by dual drug coating and resin-based modulation of rapamycin release. Whether one or both of these platforms will permit an improved clinical outcome in humans remains to be shown in clinical trials. Given the encouraging results

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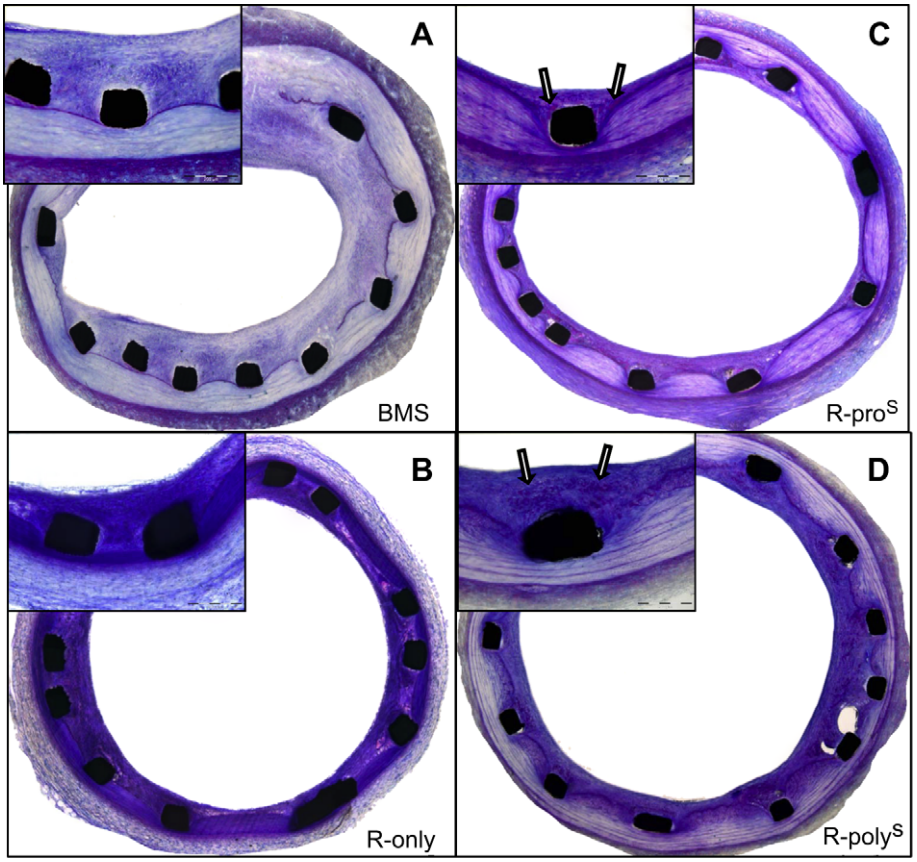

Fig. 3. All rapamycin-eluting stent platforms reduce neointimal formation and permit vascular healing. Representative images from the various stent platforms evaluated during this study. Whole stented artery segments are magnified 4×, inserts 200×. Compared to the bare-metal stent platform (A), neointimal formation is suppressed by all rapamycin-eluting stent platforms. Inflammation and endothelialization were not different between all stent platforms. The R-only stent platform showed equal levels of fibrin deposition (B), however, the latter was significantly higher in R-pro^S (C) as well as in R-poly^S (D) stents (arrows showing strut-associated fibrin deposition).

regarding efficacy and safety of the R-poly^S as well as the R-pro^S stent platform, these new concepts warrant clinical evaluation to improve outcome subsequent to stent-based treatment of coronary artery disease in humans.

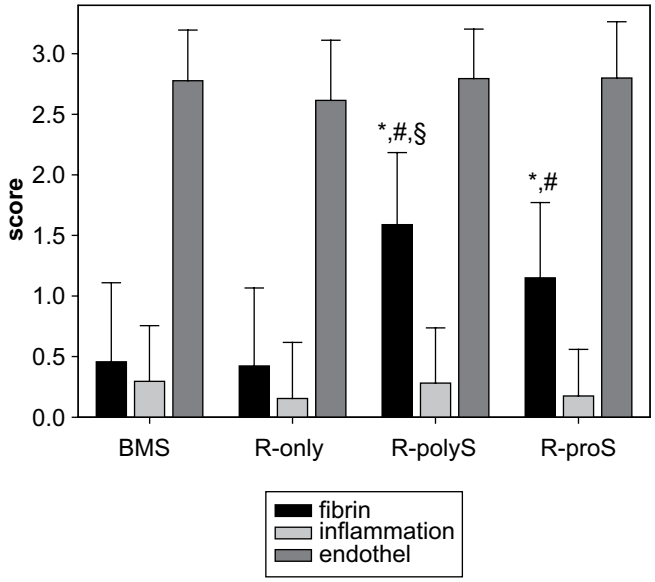

Fig. 4. Assessment of hallmarks of vascular healing 30 days after stent placement. All rapamycin-eluting stent platforms showed low level of inflammation and a high level of endothelialization, both not different from what was observed with BMS. Fibrin deposition was highest in R-poly^S followed by R-pro^S stents, levels in R-only and BMS were significantly lower. $^*P < 0.01$ vs. BMS, $\#P < 0.01$ vs. R-only, $\S P < 0.05$ vs. R-pro^S.

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Appendix

Figures with essential colour discrimination. Figure 3 in this article is difficult to interpret in black and white. The full colour image can be found in the on-line version, at doi:10.1016/j.biomaterials.2008.10.005.

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