Development of a Novel Prohealing Stent Designed to Deliver Sirolimus From a Biodegradable Abluminal Matrix

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Background—We aimed to demonstrate that, by separating endothelial progenitor cell capture from sirolimus delivery through the application of drug to the abluminal surface of the stent, the degree of endothelialization can be enhanced.

Methods and Results—Stainless steel R Stents, with biodegradable SynBiosys polymer coating with sirolimus abuminally applied and surface modified with anti-CD34 antibody were prepared at 2 dosages (low-dose sirolimus [LD-Combo, 2.5 μg sirolimus/mm] and full-dose sirolimus [Combo, 5 μg sirolimus/mm]). These Combo stents and the Cypher stent (10 μg sirolimus/mm) were deployed in 98 normal porcine arteries and harvested for pharmacokinetic analysis at 0.25, 1, 3, 7, 14, 28, and 35 days. The LD-Combo stents showed faster early release (50% total dose in 72 hours) than the Combo and Cypher. At 30 days, drug release was near complete with both Combo stents, whereas 20% of drug remained on the Cypher stents. To assess efficacy, a total of 50 stents (Xience V=8, Cypher=8, Genous bioengineered R stent=6, LD-Combo=14, and Combo=14) were implanted in 18 pigs for 14 and 28 days. Optical coherence tomography was performed, and stents were harvested for histology. At 28 days, there was less neointimal thickness with Combo (0.173±0.088 mm) compared with Cypher (0.358±0.225 mm), LD-Combo (0.316±0.228 mm), and Xience V (0.305±0.252 mm; P<0.00001). Immunohistochemical analysis of endothelialization showed that Genous bioengineered R stent had the highest degree of platelet endothelial cell adhesion molecule expression (87%) followed by the Combo (75%), LD-Combo (65%), and Cypher (58%).

Conclusions—Both optical coherence tomography and histology demonstrate that anti-CD34 sirolimus-eluting stents promote endothelialization while reducing neointimal formation and inflammation. (Circ Cardiovasc Interv. 2010;3:00-00.)

Key Words: stents ■ endothelium ■ anti-CD34 antibody ■ sirolimus ■ endothelial progenitor cell (EPC)

Drug-eluting stents (DESs) have been demonstrated to effectively control neointimal formation at long term in humans.\(^1\) However, the beneficial effects seen with most of the antiproliferative medications are found to be hampered by the potential of late thrombotic events that seem to be related to delayed vascular healing.\(^2,3\) Stent endothelialization after acute vascular injury results from either the local recruitment of adjacent endothelial cells\(^5\) or blood-derived endothelial progenitor cells (EPC) that adhere, differentiate, and populate the surface of the device.\(^2\) Although the predominant mechanism of stent endothelialization is still unclear, the phenomenon of vascular healing occurring after bare-metal stent implantation is linear and predictable.\(^6,7\) However, in the setting of DESs, this pattern of healing is altered as a consequence of the interaction between different factors such as initial mechanical injury, persistent cell inhibition, and the potential proinflammatory effect elicited by the polymer.\(^8,9\) Therefore, a paradox in the development of DES technology is that the beneficial effect of drug elution is overshadowed by the inhibition of stent endothelialization.

Clinical Perspective on p 000

Previous experience with stents having immobilized anti-human CD34 antibody on the device surface has shown feasibility of enhancing stent endothelialization in animals\(^10–12\) and safety of the device in the clinical setting.\(^10,13\) However, although cell recruitment achieved by the addition of the anti-CD34 coating can be nonspecific, the process of vascular healing may be heightened. Also, the addition of the anti-CD34 coating to commercially available sirolimus-eluting stents has shown to increase the degree of endothelial cell coverage compared with the original polymeric surface of the sirolimus-eluting stent.\(^14\) We hypothesized that by combining drug partitioning (abuluminal delivery) with a cell-capturing surface (anti-CD34), the antiproliferative effect of sirolimus could be maintained while enhancing surface endothelialization. In this study, we aimed to analyze the...
Phase 1: Abluminal Coating Study

Design:

36 Coronary Vessels (12 Pigs)

Comb-Abluminal (n=18)
18 Comb-Circumferential (n=18)

Histology at 3,14 and 28 days
n=6 in each group per time point

Phase 2: In Vivo PK Study Design:

98 Coronary and Internal Mammary Vessels (21 Pigs)

Combo (n=35)
LD Combo (n=35)
Cyper (n=28)

PK Analysis at 0h, 1h, 2h, 4h, 28 and 35 days
n=5 for combo and LD combo per each time point.
n=4 for Cypher per each time point.

Phase 3: Safety and Efficacy Study

Design:

50 Coronary Vessels (18 Pigs)

Combo (n=14)
LD Combo (n=14)
Cyper (n=8)
Xience (n=8)
Genous (n=6)

n=6 at 14 days
n=6 at 28 days
n=8 at 14 days
n=6 at 28 days
n=8 at 14 days
n=4 at 28 days
n=4 at 28 days
n=8 at 14 days
n=6 at 28 days

In vivo OCT analysis at 14 and 28 days
Histology at 14 and 28 days

Figure 1. Methods schematic illustrating vessel allocation and device distribution.

biological impact on restenosis and endothelialization of a novel anti-CD34 stent abluminally coated with a bioabsorbable sirolimus-containing matrix, using a swine model of restenosis.

Methods

Study Design
The present study was performed in 3 different phases (Figure 1). In the first phase, we compared the vascular effects of stainless steel stents with biodegradable polymer coating (SynBiosys, Surmodics Inc, Eden Prairie, Minn) with sirolimus either circumferentially applied or abluminally applied and surface modified with anti-CD34 antibody (OrbusNeich Medical, Fort Lauderdale, Fla). From this point on, only stents coated preferentially on the abluminal surface were used for the remainder of the studies. During the second phase, we evaluated the pharmacokinetic profile of the abluminally coated device by using 2 dosage levels. In the third phase, we evaluated the safety and efficacy of the abluminally coated device in the porcine model of restenosis by performing in vivo optical coherence tomography (OCT) and histological evaluation at 28 days.

Device Description
The Genous bioengineered R stent (Genous; OrbusNeich Medical, Fort Lauderdale, Fla) is a stainless steel stent surface modified with anti-CD34 antibody, and preliminary animal and human data have been reported previously. The coating component consists of a covalently coupled polysaccharide intermediate coating assembled with murine monoclonal anti-human CD34 (anti-hCD34) antibodies covalently attached to the stent surface (SynBiosys, 90-day degradation in porcine model; data not shown). The distribution of the anti-CD34 coating has been shown to be uniform when evaluated by fluorescence microscope and scanning electron microscope (SEM) with the use of a secondary fluorescein isothiocyanate (FITC)-labeled fluorescent antibody (anti-mouse IgG). For the present series of studies, the R stent was coated with a biodegradable polymer coating matrix containing sirolimus at 2 different concentrations (2.5 and 5 µg/mm) applied either circumferentially (Combo-C) or abluminally (Combo-A) on the surface of the stent, and each stent was surface modified with the same anti-CD34 antibody used in the Genous stent (Figure 2).

Abluminal Coating Study
A preliminary pilot study was carried out to compare the differences on neointimal proliferation of 2 different coating techniques (Combo-C versus Combo-A bioabsorbable sirolimus coating), using the porcine low-injury model. A total of 36 stents (Combo-A=18 versus Combo-C=18) were deployed in 36 coronary arteries of 12 pigs. The amount of polymer and drug coating was similar between both groups (Combo-C versus Combo-A). Animals were euthanized at 3 days (n=6 in each group), 14 days (n=6 in each group), and 28 days (n=6 in each group). Stent samples in the 3- and 14-day groups were sent for SEM and confocal microscopy, and stents for the 28-day group were subjected to light microscopy.

Pharmacokinetics Study
From this point forward, only Combo stents with drug abluminally applied were tested. We evaluated local pharmacokinetic features of Combo-A, henceforth “Combo,” up to 35 days in 21 pigs (AccelLab,
Montreal, QC, Canada). Two different doses were tested: 2.5 μg sirolimus/mm (n=5, low-dose stent [LD-Combo]) and 5 μg sirolimus/mm (n=5 [Combo]) were used and compared with the Cypher stent (10 μg sirolimus/mm, n=4). All stents were deployed using a 10% overstretch ratio using normal coronary or mammary arteries (98 vessels). Tissues containing stents were harvested for sirolimus concentration analysis at 6 hours and at 1, 3, 7, 14, 28, and 35 days. Stents were carefully removed from vessels and extracted in 100% methanol and analyzed by high-performance liquid chromatography/mass spectrometry/mass spectrometry using a validated method for sirolimus (Eurofins Medinet, Denver, Colo).15

Safety and Efficacy Porcine Study

An additional study was performed with the objective of demonstrating the effect of the 2 different sirolimus concentrations (LD-Combo versus Combo) on healing and neointimal proliferation by using in vivo OCT imaging and histological techniques. A total of 18 animals were included in this study. The animals were equally divided (n=9) into 2 groups, according to the follow-up time (14 or 28 days). The 2 different dosages (LD-Combo and Combo, n=14 in each) were compared with the Cypher (n=8, Cordis, Miami, Fla), Xience V (n=8, Abbot, Abbott Park, Ill), and Genous (n=6) stents. OCT imaging evaluation was performed at 14 (LD-Combo, Combo, Xience V, and Cypher stents) and 28 days (LD-Combo, Combo, Genous, Cypher, and Xience V stents). After euthanization, stents were harvested for light microscopy. Additional, 3 pigs were euthanized at 14 days, and vessels with LD-Combo and Combo stents (n=4 each) were harvested for SEM and confocal microscopy (platelet endothelial cell adhesion molecule [PECAM]) and compared with historical controls.14

Stent Procedure Description

All study protocols were approved by the local institutional animal care and use committee. All animals were fed a standard diet and premedicated with oral Plavix (150 mg), oral aspirin (325 mg), antiarhythmics (delivered through intravenous lactated ringers, lidocaine 200 mg/L, metoprolol 5 mg/L), and broad spectrum antibiotics (1 g IV ceftazolin). Juvenile domestic Yorkshire pigs (40 to 55 kg) were anesthetized with isoflurane (1% to 3%) through facemask. Surgical access was obtained through carotid artery prepared previously with general sterile technique. Before catheterization, heparin (5000 to 10 000 U) was injected to maintain an activated clotting time of 250 to 300 s. Nitroglycerin was administered intra-arterially to prevent or relieve vasospasm. A vascular introducer sheath was placed for advancement of guiding catheters. Vessel allocation to an experimental group was predetermined to distribute the different stent types equally in 3 different coronary arteries. All other elements of vessel allocation were randomized. The appropriate stent was delivered to the intended site over a guide wire with the use of fluoroscopic guidance, and stent deployment was performed using a 1:1:1:0 stent-to-artery diameter ratio. After the procedure, catheters were removed, the artery ligated, and the surgical incision repaired. Animals were recovered and housed until their designated day of euthanasia.

OCT Analysis Protocol

In the porcine study, 50 stents were implanted with 41 predesignated for OCT analysis, with the remainder reserved for SEM and confocal microscopy. Using conventional catheterization techniques, the M4 OCT catheter system (LightLab, Westford, Mass) was advanced distal to the stented segment. Images were obtained free of occlusion and using a continuous flush of contrast-saline mixture at a rate of 5 mL/s from the guide catheter. Motorized OCT pullbacks were performed at a rate of 10 mm/s. Images were acquired at 100 frames/s displayed with a color look-up table and digitally archived. The edges of the stent were defined as the first and last frames, which had 270 degree visibility of stent struts. Five cross-sectional frames were measured (both stent edges, middle of stent, one fourth from each edge). A series of derived measurements were made, including the following at 14- and 28-day follow-up: stent area (circumferential area limited by the contours of the struts), luminal area (circumferential dark zone in the center of the struts), neointimal area (calculated by subtracting the lumena area from the stent area), and neointimal thickness (distance from the surface of each stent strut to the lumen). Struts were also evaluated for apposition. We measured the distance between the vessel wall and the middle of stent strut surface reflection. OCT measurements were classified into 3 grades of apposition (embedded, protruding, and malapposed). Struts were classified as protruding when strut apposition does not fall within

Figure 2. Combo and LD-Combo stents. Note the way the sirolimus is exclusively eluted from the abluminal surface of the stent strut.
Histology Analysis

The animals were euthanized, while under general anesthesia, by intravenous injection of pentobarbital euthanasia solution (100 mg/kg) or potassium (40 mEq), or both. Hearts were excised and pressure perfused with 0.9% saline until cleared of blood, followed by pressure-perfusion fixation in 10% neutral-buffered formalin until hardening of the heart muscle is clearly perceptible. The stented arterial segments were then carefully dissected free from the heart. Intact stented arterial segments were bisected longitudinally to expose the lumen surface and photographed. Half of the stent was processed for SEM with the opposite side reserved for immunostaining and confocal imaging. SEM specimens were rinsed in 0.1 M sodium cacodylate buffer (pH 7.2±0.1) and then postfixed in 1% osmium tetroxide for ~30 minutes. Specimens were then dehydrated in a graded series of ethanol. After critical point drying, the tissue samples were mounted and sputter coated with gold. The specimens were visualized using a Hitachi Model 3600N (Hitachi, Tokyo, Japan) SEM. Regions of interest were photographed at incremental magnifications by SEM. From these SEM images, percent coverage between and above stent struts was reported as a visual estimate. The specimens for confocal analysis were rinsed with PBS and stained for CD31/PECAM-1 to evaluate the presence of endothelial cells. TOTO-3 (conjugated by fluor 642/660) was used as counterstain. Alexa Fluor 488 donkey anti-mouse IgG was used as secondary antibodies for CD31/PECAM-1 with concentration of 1:200. Specimens were mounted on glass slides, and representative images were acquired with a Zeiss Pascal (Carl Zeiss AG, Oberkochen, Germany) confocal microscope. The percentage of CD31/PECAM-1 stain was estimated under standard fluorescence microscopy between and above stent struts for each strut. PECAM percent expression was derived by quantifying the amount of PECAM visible as an area of the total vessel segment analyzed. For light microscopy, the stented vessels were dehydrated in a graded series of ethanol and embedded in methylmethacrylate plastic. After polymerization, 5 sections were sawed from the proximal edge (section 1), 1 quarter (2), center (3), 3 quarter (4), and distal edge (5) stent portions of each single stent to match with OCT analysis. Sections were cut on a rotary microtome at 5 to 6 μm and stained with hematoxylin–eosin and elastic Van Gieson stain. A vessel injury score was calculated according to the Schwartz method. The cross-sectional areas (external elastic lamina, internal elastic lamina, and lumen) of each section were measured with digital morphometry. Neointimal thickness was measured as the distance from the inner surface of each stent strut to the luminal border. Ordinal data were collected on each stent section and included strut apposition to the vessel wall, fibrin deposition, calcification, granuloma reactions, and hemorrhage around the stent struts and were expressed as a percentage of the total number of struts in each section. The number of uncovered struts was recorded and a percentage was given. An overall neointimal inflammation value (0 to 4) and fibrin value (0 to 3) were scored for each section. Vessel sections showing 2 or more struts with granulomatous inflammation were scored with an inflammation value of “4.” Adventitial inflammation was scored separately. Endothelial coverage was semiquantified and expressed as the percentage of the lumen circumference covered by endothelium.

Statistical Analysis

Descriptive statistics were used for the coating deposition study. Continuous data were expressed as mean±SD. For the OCT study, 1-way ANOVA analysis was performed to examine differences between the stent, lumen and neointimal areas, and neointimal thicknesses by stent group. Tukey-Kramer test was used for comparison between stent groups. Pathology continuous data were expressed as mean±SD and median with interquartile range. Statistical comparison of the parameters was performed using Kruskal-Wallis and Mann-Whitney U test for post hoc comparisons. A P value of <0.05 was considered statistically significant. A correlation between OCT parameters and histology parameters was also done.

Results

Abluminal Coating Study

A total of 36 stents (Combo-A = 18 versus Combo-C = 18) were successfully implanted and all were included in the final analysis. There were no unscheduled terminations. Qualitative evaluation of stent strut coverage analyzed by SEM did not demonstrate any differences between both coating techniques at either 3 (Combo-A: 47%±13% versus Combo-C: 45%±21%) or 14 days (Combo-A: 97%±2.4% versus Combo-C: 96%±2.6%) of follow-up (Figure 3A). However, confocal microscopy performed on the same stent segments demonstrated that the Combo-A stent had more than 2-fold

Table 1. Twenty-Eight Day Histology for Coating Study

<table>
<thead>
<tr>
<th></th>
<th>Combo-C</th>
<th>Combo-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI thickness, mm</td>
<td>0.087±0.021</td>
<td>0.094±0.068</td>
</tr>
<tr>
<td>Percent stenosis</td>
<td>17.19±2.43</td>
<td>18.57±6.43</td>
</tr>
<tr>
<td>Int. inflammatory score</td>
<td>0.94±1.14</td>
<td>0.56±0.62</td>
</tr>
<tr>
<td>Adv. inflammatory score</td>
<td>0.50±2.00</td>
<td>0.50±0.58</td>
</tr>
<tr>
<td>Fibrin score</td>
<td>1.83±0.46</td>
<td>1.56±0.54</td>
</tr>
</tbody>
</table>

There is equivalent reduction of neointimal thickness and stenosis. No statistically significant differences (P>0.05) for all results. Mann-Whitney U test. Data are presented as mean±SD followed by median (interquartile range) for each parameter. NI indicates neointimal; Int., intimal; Adv., adventitial.
increase in the cellular expression of PECAM compared with the Combo-C stent (Combo-A: 70%±35% versus Combo-C: 27%±35%; P=0.06; Figure 3B). At 28 days, histological evaluation of the stented segments (Table 1) demonstrated that the amount of neointimal thickness was similar between both coating techniques (Combo-C=0.087±0.021 mm versus Combo-A=0.094±0.068 mm). Moreover, the percentage area of stenosis by histology was similar between both groups (Combo-C=17.19%±4.35% versus Combo-A=18.57%±5.43%). In addition, there were no significant differences on any of the inflammatory score parameters. Partitioning sirolimus abluminally effectively maintains its antiproliferative effect on neointimal formation while simultaneously increasing levels of endothelialization.

Pharmacokinetic Study
All animals were successfully implanted and there were no unscheduled terminations. In vivo evaluation of drug release from explanted stents demonstrated that the LD-Combo stent (2.5 µg/mm of sirolimus) released ≈50% of its total dose in the first 72 hours and 75% of the total dose by the seventh day. After that time, there was a second period of release that reached a plateau by day 14 and later a steady but very small release occurred up to 35 days. On the other hand, the Combo stent (5 µg/mm of sirolimus) released 50% of its total dose by 7 days and 75% by day 10 days. After that period, there was a steady decline in release that progressively decreased by day 30. In addition, the Cypher stent released 50% of its total concentration (10 µg/mm) by 10 days and 75% by day 25 but continued to release after the final time point of this study (35 days; Figure 4A). Intra-arterial sirolimus concentrations were measured in the midtreated section of the coronary arteries in all animals. In these sections, the Cypher stent showed a peak in tissue levels at 24 hours (9 ng/mg tissue) and then the levels progressively steadily declined over time to ≈5 ng/mg of tissue at 35 days. Similarly, the Combo stent (5 µg/mm of sirolimus) showed a peak at 24 hours (10 ng/mm per mg of tissue) and then rapidly declined to achieve similar levels to the Cypher stent at 35 days. On the other hand, the LD-Combo (2.5 µg/mm of sirolimus) peaked at 24 hours (7 ng/mm) but rapidly declined in its tissue level values to be in the range of 1 ng/mm of tissue by 35 days of follow up (Figure 4B). LD-Combo and Combo stents were able to show faster release rates, yet an equivalent amount of drug is locally delivered to blood vessels by all stent types with less drug remaining by 30 days for LD-Combo.

Safety and Efficacy Porcine Study
OCT evaluation was performed in the 41 stents designated for OCT analysis at 14 (n=16) and 28 days (n=25). There were no unscheduled terminations. At 14 days, the Cypher stent...
seemed to have a significantly higher proportion of protruding struts (31%) compared with the Combo stent (17%), LD-Combo (25%) stent, and the Xience stent (10%; Figure 5A). Among protruding struts, there was a similar distribution of neointimal thickness ranging from 19 to 30 μm in thickness. For the struts that were embedded, the smallest neointimal thickness was found in the Combo group (0.044±0.036 mm; Figure 5B). Xience and Combo stents both show better apposition (less protruding struts) than the other test stents; however, within the categories of protruding and embedded struts, the Combo stents show the least amount neointimal formation (Figure 5A and 5B). The Cypher (0.052±0.041 mm) and the LD-Combo group (0.053±0.056 mm) had similar amount of neointimal thickness (63 μm), and the Xience displayed the highest level of neointimal formation (0.056±0.074 mm; P<0.0001; Figure 6A). At 28 days, there were no protruding struts seen by OCT in any of the stent technologies. Figure 6. In vivo OCT analysis of neointimal thickness at 14 and 28 days. *P<0.00001 for all pairwise comparisons. There was less neointimal thickness with Combo compared to Cypher, LD-Combo and Xience at 14 days (A) and at 28 days (B). *P<0.00001 for all pairwise comparisons. Data are stated as mean±SD. ANOVA followed by Tukey-Kramer post hoc test.

The results of the histology evaluation (Table 2) were consistent with results of OCT analysis. At 14 days, all the stents tested except the Xience (0.071±0.062 mm) had a comparable amount of neointimal thickness (≈0.031 to 0.035 mm). There were more giant cells in the Xience and Cypher groups compared with either the Combo or LD-Combo. Coverage rates evaluated by SEM were similar between the Combo, LD-Combo, and the Genous (≈87%) but was lower for the Cypher stent (Figure 7A).

Table 2. Fourteen-Day Histology Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cypher (n=4)</th>
<th>Combo (n=4)</th>
<th>LD-Combo (n=4)</th>
<th>Xience (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stenosis, %</td>
<td>12.01±1.44</td>
<td>9.75±2.63</td>
<td>9.99±2.15</td>
<td>12.31±6.26</td>
</tr>
<tr>
<td>Neointimal thickness, mm</td>
<td>0.035±0.007</td>
<td>0.032±0.011</td>
<td>0.031±0.006</td>
<td>0.071±0.062</td>
</tr>
<tr>
<td>Fibrin score</td>
<td>0.036±0.010</td>
<td>0.032±0.010</td>
<td>0.030±0.010</td>
<td>0.043±0.040</td>
</tr>
<tr>
<td>Int. inflammatory score</td>
<td>2.04±0.64</td>
<td>1.65±0.68</td>
<td>1.55±0.34</td>
<td>1.50±0.12</td>
</tr>
<tr>
<td>Giant cells, %</td>
<td>50.51±17.02</td>
<td>33.74±16.15</td>
<td>37.94±7.27</td>
<td>47.01±17.22</td>
</tr>
<tr>
<td>Adv. inflammatory score</td>
<td>1.05±0.19</td>
<td>0.85±0.55</td>
<td>0.85±0.19</td>
<td>1.95±1.41</td>
</tr>
<tr>
<td>Granuloma, %</td>
<td>11.0 (0.25)</td>
<td>0.90 (0.75)</td>
<td>0.90 (0.25)</td>
<td>1.40 (1.35)</td>
</tr>
<tr>
<td>EEL area, mm²</td>
<td>5.85±1.41</td>
<td>8.77±1.37</td>
<td>8.34±1.19</td>
<td>9.30±2.14</td>
</tr>
<tr>
<td>IEL area, mm²</td>
<td>8.18 (1.32)</td>
<td>8.13 (0.76)</td>
<td>8.52 (0.89)</td>
<td>9.47 (3.18)</td>
</tr>
<tr>
<td>Neointimal area, mm²</td>
<td>7.73±1.27</td>
<td>7.81±1.27</td>
<td>7.42±0.98</td>
<td>8.19±2.00</td>
</tr>
</tbody>
</table>
| No statistically significant differences (P>0.05) for all results, Kruskall-Wallis test. Data are stated as mean±SD followed by median(interquartile range) for each parameter. NI indicates neointimal; Int., intimal; Adv., adventitial; EEL, external elastic lamina; and IEL, internal elastic lamina.
Immunohistochemical analysis of device endothelialization showed that the Genous stent had the highest amount of PECAM expression (87%; Figure 7B). The Combo stent had >75% expression followed by the LD-Combo (65%) and the Cypher stent (58%). By 28 days, the lowest amount of neointimal thickness was found in the Combo group (0.12 mm). In addition, there was less inflammation in both doses of Combo stents compared with the Cypher and Genous control group (0.29±0.12 mm). In addition, there was less inflammation in both doses of Combo stents compared with the Cypher and Xience stents (Table 3). Figure 8 shows representative images of histomorphometry for each technology. OCT analysis correlated in a linear fashion with all histological parameters such as lumen area ($R^2=0.83$), neointimal area ($R^2=0.86$), stent area ($R^2=0.70$), and strut coverage ($R^2=0.90$; Figure not shown).

Therapeutic levels of sirolimus can be maintained despite the fact that the total effective dose is reduced by 75%. Both OCT and histological analyses demonstrate that Combo and LD-Combo stents promote enhanced endothelialization while reducing neointimal formation and inflammation.

**Discussion**

Endothelialization after acute vascular injury resulting from stent implantation is a critical step in the process of vascular

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**Table 3. Twenty-Eight Day Histology Results (Excluding Granulomas)**

<table>
<thead>
<tr>
<th></th>
<th>Genous (n=6)</th>
<th>Cypher (n=3)</th>
<th>Combo (n=5)</th>
<th>LD-Combo (n=5)</th>
<th>Xience (n=3)</th>
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<tbody>
<tr>
<td>Stenosis, %</td>
<td>36.55±10.38*,†</td>
<td>33.48±5.41*,†</td>
<td>19.92±5.60</td>
<td>26.04±8.74†</td>
<td>22.22±6.272†</td>
<td>0.04</td>
</tr>
<tr>
<td>NI thickness, mm</td>
<td>0.29±0.12</td>
<td>0.21±0.02</td>
<td>0.12±0.05</td>
<td>0.18±0.07</td>
<td>0.15±0.05</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.30±0.20</td>
<td>0.21 (0.02)</td>
<td>0.11 (0.08)</td>
<td>0.20 (0.02)</td>
<td>0.14 (0.05)</td>
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</tr>
<tr>
<td>Fibrin score</td>
<td>0.067±0.16*,†</td>
<td>2.00±0.72*</td>
<td>0.60±0.75</td>
<td>1.32±0.50†,¶,#</td>
<td>0.53±0.42§,¶,#</td>
<td>&lt;0.01</td>
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<tr>
<td>Int. inflammatory score</td>
<td>0.00 (0.00)</td>
<td>1.80 (0.70)</td>
<td>0.20 (1.20)</td>
<td>1.20 (0.60)</td>
<td>0.40 (0.40)</td>
<td></td>
</tr>
<tr>
<td>Giant cells, %</td>
<td>13.82±9.51*§,**</td>
<td>44.94±8.32*,††</td>
<td>10.06±7.13</td>
<td>6.04±7.55†</td>
<td>33.24±14.14§</td>
<td>0.01</td>
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<tr>
<td>Adv. Inflammatory Score</td>
<td>0.13±0.24</td>
<td>0.20±0.20</td>
<td>0.24±0.54</td>
<td>0.52±0.59</td>
<td>0.13±0.12</td>
<td>0.64</td>
</tr>
<tr>
<td>EEL area, mm²</td>
<td>8.69±2.12</td>
<td>9.72±1.15</td>
<td>9.31±1.90</td>
<td>9.82±2.15</td>
<td>7.93±1.02</td>
<td>0.37</td>
</tr>
<tr>
<td>IEL area, mm²</td>
<td>9.51 (1.97)</td>
<td>9.04 (1.91)</td>
<td>8.72 (2.90)</td>
<td>9.54 (3.47)</td>
<td>7.65 (1.04)</td>
<td></td>
</tr>
<tr>
<td>Neointimal area, mm²</td>
<td>7.61±1.89</td>
<td>8.41±0.94</td>
<td>7.99±1.56</td>
<td>8.59±1.81</td>
<td>6.71±0.79</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>8.33 (1.59)</td>
<td>7.95 (1.80)</td>
<td>7.53 (2.45)</td>
<td>8.36 (2.64)</td>
<td>6.53 (0.93)</td>
<td></td>
</tr>
</tbody>
</table>

Kruskall-Wallis test and Mann-Whitney U test for pairwise post hoc comparisons. Data are stated as mean±SD followed by median (interquartile range) for each parameter. NI indicates neointimal; Int., intimal; Adv., adventitial; EEL, external elastic lamina; and IEL, internal elastic lamina.

*P<0.05 for Genous vs Cypher.
†P<0.05 for Genous vs LD-Combo.
‡P<0.05 for Cypher vs Xience.
§P<0.05 for Genous vs Xience.
¶P<0.05 for Combo vs LD-Combo.
||P<0.05 For Combo vs Xience.
#P<0.05 for LD-Combo vs Xience.
**P<0.05 for Genous vs Combo.
††P<0.05 for Genous vs Cypher.
‡‡P<0.05 for Cypher vs LD-Combo.
healing. The origin of new endothelium may be from either the adjacent recruitment of endothelial cells or blood-derived EPC that populate the luminal surface of the stent. However, in the setting of DES implantation, this pattern of vascular healing is altered because there is nonselective inhibition of all cells involved in the process of stent healing. Therefore, a major challenge in the development of any new DES platform is to maintain sustained control of smooth cell proliferation and at the same time promote endothelial cell migration and differentiation on the stent surface. For the development of a prohealing stent that elutes medication into the vessel wall preferentially from the abluminal surface, there are several questions that need to be resolved. First of all, the potential benefit of the abluminal deposition of the coating is mainly in maintaining control of neointimal proliferation. Second, to be certain that the elution profile remains stable achieving a predictable and stable release rate is of benefit. Third, that the efficacy and safety profile of this device remains comparable with other current DES platforms is beneficial.

Previous experience with stent surfaces modified by immobilizing anti-human CD34 antibody on the device surface has shown feasibility of enhancing stent endothelialization and safety of the device in the clinical setting. The anti-CD34 Genous stent has been shown to be safe, as effective as bare-metal stents, and with similar restenosis in various studies. In vitro studies have demonstrated that the dose of sirolimus required to inhibit EPCs is 10-fold lower than the dose needed to inhibit endothelial or smooth muscle cells. Therefore, abluminal release combined with minimal sirolimus could hypothetically induce smooth muscle cell inhibition without negatively affecting EPC recruitment. Moreover, previous studies in our laboratory demonstrated the in vivo effects of sirolimus elution on endothelial cell coverage by overlapping stents with different drug-eluting platforms in normal coronary arteries. In addition, we have analyzed the synergistic effect of EPC capturing on drug elution by coating conventional Cypher stents with anti-human CD34 antibodies and assessing the degree of endothelialization. In this study, by 14 days, the endothelialization rate evidenced by confocal microscopy in this group was >80%. In a previous study, we demonstrated that endothelialization was enhanced by 1.5-fold on the Cypher stents enhanced with anti-CD34 compared with the Cypher controls.

In this particular series of studies, a new concept was tested by using stents with identical structural design and anti-CD34 coating containing an abluminal layer of a bioabsorbable sirolimus-polymer coating. As a first step, we aimed to demonstrate potential biological differences between circumferential and abluminal coating strategies. In this study, the rates of grossly estimated coverage performed by SEM were similar between both types of coatings. However, the amount of functional endothelium identified by confocal microscopy was found to be increased by almost 3-fold in the abluminal coating group. Importantly, the degree of inhibition of neointimal proliferation seemed to be maintained in the abluminally coated device. Therefore, this particular study demonstrated that neointimal proliferation control is possible while maintaining the EPC recruiting potential. In a second study, we aimed to demonstrate the differences in drug release of abluminally coated devices compared to conventional Cypher

Figure 8. Representative histomorphometric images at 14 and 28 days in Cypher, Combo, LD-Combo, and Genous stents.
stents. In this study, we demonstrated that the patterns of sirolimus release were maintained stable overtime and were comparable to the levels seen with the Cypher stent. The most effective dose (5 μg/mm) showed to release ~75% of its total dose in the first 10 days after implantation and seemed to be able to release almost all remaining drug by 28 days. The safety and efficacy study performed in pigs revealed very interesting findings. In vivo OCT evaluation of strut coverage demonstrated that at 14 days, the highest incidence of protruding struts (a sign of delayed vascular healing), was found in the Cypher stent group (~30%) followed by the LD-Combo stent dose (25%). In the pharmacokinetic study, the LD-Combo stent exhibited a relatively fast rate of drug release that may explain the relatively high degree of protruding struts compared with the Combo stent. As Aoki et al.\(^\text{29}\) report in the Paclitaxel In-Stent Controlled Elution Study trial, slower release rates are associated with better clinical outcomes. Although both the Combo and LD-Combo stent elute roughly the same amount of drug, the Combo’s slower release rate may be responsible for its better safety profile as reflected in the decreased number of protruding struts. Similarly, the incidence of protruding struts appeared to be equivalent at 14 days between the Xience (~10%) and the Combo stent (16%). Additionally, the mean neointimal thickness seen on the protruding struts was less for both doses of Combo stents compared with the Cypher and Xience stents. Similarly, the Combo stents displayed the lowest amount of neointimal formation compared with all other stent groups, when the embedded struts were analyzed. Histological evaluation demonstrated that the Combo had not only the smallest amount of neointimal hyperplasia but also overall less inflammation and fewer giant cells compared with the other DES controls. In addition, Confocal microscopy demonstrated higher PECAM expression compared with previously reported historical controls\(^\text{14}\) and similar to previous reports on the Genous.\(^\text{10,11}\)

In summary, this study demonstrated that it is feasible to combine antiatherostotic therapies with technologies designed to increase stent endothelialization. This combined technology may promote a more organized and appropriate vascular healing, a key factor associated with favorable clinical outcomes.\(^\text{19,30,31}\) We showed that partitioning drug release from EPC capture results in maintaining reduction of neointimal proliferation, while promoting the return of functional endothelium. This biological aspect is important in the era of drug elution because it creates a need for additional cell recruitment after DES implantation.\(^\text{32}\) It is important to highlight, however, that despite the fact that this combination device seems to increase endothelial cell recruitment and functionality, some delay in vascular healing could still be expected compared to bare-metal stent surfaces. Current animal models are very limited in the evaluation of device endothelialization and if this early phenomenon has any clinical implications it needs to be determined.

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Disclosures
Drs Parker and Steve Rowland are employees of OrbusNeich Medical, Fort Lauderdale, Fla. Dr Leon is a scientific advisor to OrbusNeich Medical.

References


**CLINICAL PERSPECTIVE**

Very late stent thrombosis is a devastating and potentially lethal complication occurring almost exclusively among patients treated with drug-eluting stents. Although the mechanisms responsible for this phenomenon are still unclear, it is believed that delayed vascular healing plays a major role in the development of very late stent thrombosis. Thus, there is substantial clinical potential for stent platforms that integrate the antiproliferative effect of sirolimus elution with the vascular healing/reendothelialization effect of endothelial progenitor cell (EPC) capture (anti-CD34). Functional reendothelialization through EPC capture could increase natural healing while reducing the thrombotic potential as well as the need for prolonged anti-platelet therapy. A paradox in the development of drug-eluting stent technology is that the beneficial effect of drug elution is overshadowed by the inhibition of stent endothelialization. Combining drug elution with EPC capture could potentially achieve an optimum hybrid technology harnessing the antiproliferative qualities of drug elution with the prohealing aspects of EPC capture. We found that by separating EPC capture from sirolimus delivery through application of the elution polymer to the abluminal surface of the stent, we could potentially improve the functionality of these hybrid stents.
Development of a Novel Prohealing Stent Designed to Deliver Sirolimus From a Biodegradable Abluminal Matrix
Juan F. Granada, Shigenobu Inami, Michael S. Aboodi, Armando Tellez, Krzysztof Milewski, David Wallace-Bradley, Sherry Parker, Steve Rowland, Gaku Nakazawa, Marc Vorpahl, Frank D. Kolodgie, Greg L. Kaluza, Martin B. Leon and Renu Virmani

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