Stent Coating With Titanium-Nitride-Oxide for Reduction of Neointimal Hyperplasia

Stephan Windecker, MD; Isabella Mayer, MD; Gabriella De Pasquale, BA; Willibald Maier, MD; Olaf Dirsch, MD; Philip De Groot, MD; Ya-Ping Wu, PhD; Georg Noll, MD; Boris Leskosek, BA; Bernhard Meier, MD; Otto M. Hess, MD; in collaboration with the Working Group on Novel Surface Coating of Biomedical Devices (SCOL)

Background—Coronary stents prevent constrictive arterial remodeling but stimulate neointimal hyperplasia. Stainless steel induces a metallic foreign body reaction, which is absent for titanium. The hypothesis of the present study was that titanium renders the stent surface biologically inert, with reduced platelet and fibrinogen binding.

Methods and Results—Twelve pigs were instrumented with a stainless steel and 2 titanium-nitride-oxide–coated stents (TiNOX 1, ceramic; TiNOX 2, metallic). Animals were restudied after 6 weeks. Histological specimens of stented segments were analyzed by digital morphometry. Platelet adhesion and fibrinogen binding studies were performed in the perfusion chamber. Under in vitro conditions, TiNOX 1 showed reduced platelet adhesion (65±3%) compared with TiNOX 2 (72±5%; P<0.05) and stainless steel (71±4%; P<0.05). Platelet adhesion 48 hours after incubation with human plasma, however, was not different between TiNOX 1 (17±3%) and 2 (15±3%) but was significantly higher with stainless steel (23±2%; P<0.05). Fibrinogen binding was significantly reduced with TiNOX 2 (54±3%) compared with TiNOX 1 (82±4%, P<0.05) or stainless steel (100%, P<0.05). Histomorphometry revealed a significantly larger neointimal area in stainless steel (2.61±1.12 mm²) than in TiNOX 1–coated (1.47±0.84 mm², P<0.02) or TiNOX 2–coated (1.39±0.93 mm², P<0.02) stents. The reductions were 44% and 47%, respectively.

Conclusions—TiNOX coating significantly reduces neointimal hyperplasia in stainless steel stents. The antiproliferative effect was similar for both TiNOX coatings, suggesting that the electrochemical properties are more important for attenuation of neointimal proliferation than the observed differences in platelet adhesion and fibrinogen binding.

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Key Words: stents ■ restenosis ■ titanium-nitride-oxide ■ hyperplasia
platelet and fibrinogen binding, attenuating neointimal hyperplasia.

**Methods**

The study with experimental animals was approved by the local Institutional Animal Care and Use Committee. It conforms to the guidelines established in the “Position of the American Heart Association on Research Animal Use” adopted by the American Heart Association on November 11, 1984.

**Animal Preparation and Instrumentation**

Domestic pigs of either sex (mean weight 52±9 kg) were preanesthetized with 20 mg/kg IM ketamine hydrochloride and 1.1 mg/kg acepromazine IM. Anesthesia was induced with sodium pentobarbital 10 mg/kg IV bolus and maintained by halothane inhalation. Once anesthesia was induced, the animals were endotracheally intubated and ventilated with a Harvard respirator (Harvard Apparatus Co) with supplemental oxygen. Blood pressure and surface ECG leads were continuously displayed on a physiological monitor.

**Stent Coating**

A custom-made stainless steel, slotted-tube stent (BeStent, Medtronic, Inc) of 15-mm length and 2.5- to 3.0-mm expanded diameter was used in the present study. Stent coating with TiNOX was performed by reactive physical vapor deposition in a vacuum chamber. Depending on the oxygen-nitrogen ratio, it was possible to deposit films with defined composition and resistivity. Two TiNOX compounds with different material properties were selected for this study: TiNOX 1 elicited semiconducting (ceramic) properties with a resistivity of 6×10² µΩ·m, whereas TiNOX 2 demonstrated metallic properties with a resistivity of 1×10⁰ µΩ·m. The thickness of the coating was ~500 nm. Scanning electron microscopy showed homogeneous surface coating without evidence of cracks after stent expansion (Figure 1).

**Stent Implantation**

Arterial access was established by cutdown of the right carotid artery and insertion of a 7F arterial sheath with hemostatic valve under sterile conditions. Heparin sodium (100 IU/kg IV) was administered. Diagnostic right and left coronary angiography was carried out in 2 orthogonal projections with a 6F Judkins guiding catheter with injection of nonionic, low-osmolar contrast medium (Optiray 320, Guerbet Inc). Coronary artery stenting was performed by positioning a 0.014-in guidewire in the distal coronary artery and advancing the manually crimped stent on a 3.0-mm balloon catheter under fluoroscopic control. The stent was implanted in a selected coronary artery segment with a vessel diameter of ~2.5 to 3.0 mm. Inflation pressures were chosen according to the manufacturer-derived compliance curves, aiming for overstretch-induced vascular injury and a stent-to-artery ratio of >1.2:1. Successful stent deployment was checked by repeat coronary angiography. The animals received 300 mg acetylsalicylic acid for the first 3 days.

A total of 12 pigs were instrumented, of which 1 animal died during catheter manipulation because of ventricular fibrillation. Thus, data from 11 pigs were available for the purpose of the present study. TiNOX 1– and 2–coated stents and 1 uncoated (control) stent were randomly assigned to either the left anterior descending, left circumflex, or right coronary artery. In 1 animal, the control stent was lost during stent implantation, but the loss was not realized until histological examination was performed.

**Histological Examination**

The animals were euthanized 6 weeks after stent implantation. First, a control angiogram was carried out to exclude angiographic restenosis. Then, the heart was arrested by injection of 20 mL KCl into the aortic root. The heart was excised and fixed in 300 mL buffered 4% formaldehyde. A few days later, the coronary arteries were carefully dissected free, and the stented and adjacent unstented coronary artery segments were embedded in polymethylmethacrylate. The fixed coronary artery segments were sectioned transversely, cut into 800-μm sections, and polished to a thickness of 100 μm. The tissue was prepared for light microscopy by staining with Paragon (7.3 g toluidine blue with basic fuchsin dissolved in 1000 mL 30% ethanol) under prewarming (90°C for 15 seconds). Then, the sections were analyzed on a digital system for quantitative histomorphometry (Image Pro Plus, Media Cybernetics). Histological evaluation was performed by an observer unaware of the location or coating status of the stent. Within the stented coronary segments, ≥3 sections corresponding to the proximal, middle, and distal parts of the stent were examined. The cross-sectional areas of the intima, media, and lumen were determined (Figure 2). The intima was defined as the area between the endothelial lining and the internal elastic lamina, the media as the area between the internal and external elastic laminae, and the luminal area as the area circumscribed by the endothelial layer. The total vessel area was calculated as the sum of lumen, intima, and media. Neointimal thickness was determined by calculating the ratio of neointimal area and internal elastic lamina circumference.

**Platelet Adhesion and Fibrinogen Binding**

Before the animal experiments, in vitro studies for platelet adhesion and fibrinogen binding were performed in the perfusion chamber.

**Figure 1.** Scanning electron microscope photograph of expanded TiNOX-coated stent.

**Figure 2.** Low-magnification photomicrograph of 4 different vessel segments: A, Control segment with normal 3-layer vessel structure. B, Stented segment (uncoated control) with severe neointimal hyperplasia showing near obliteration of lumen. C, Stented segment (coated with TiNOX 1) with minimal neointimal hyperplasia. D, Stented segment (coated with TiNOX 2) with mild neointimal hyperplasia.
TABLE 1. Procedural Data

<table>
<thead>
<tr>
<th></th>
<th>Uncoated</th>
<th>TiNOX 1</th>
<th>TiNOX 2</th>
</tr>
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<tbody>
<tr>
<td>Stents, n</td>
<td>10</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>LAD, n</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>LCx, n</td>
<td>4</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>RCA, n</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Balloon-to-artery ratio</td>
<td>1.54±0.46</td>
<td>1.57±0.34</td>
<td>1.57±0.35</td>
</tr>
<tr>
<td>Injury score</td>
<td>1.1±0.7</td>
<td>0.9±0.7</td>
<td>1.0±0.9</td>
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</tbody>
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LAD denotes left anterior descending coronary artery; LCx, left circumflex artery; and RCA, right coronary artery. Injury score determined according to the method described by Schwartz et al.4

P=NS for all comparisons.

with defined rheological characteristics with stainless steel L 316 and TiNOX 1 and TiNOX 2, respectively.12 Whole blood was used for perfusion, which was anticoagulated with low-molecular-weight heparin, prewarmed at 37°C for 5 minutes, and then drawn from a container through the perfusion chamber over the different coatings for 5 minutes at shear rates of 800 s⁻¹ by a syringe pump (Harvard Apparatus). Perfusion chamber experiments were carried out before and immediately after albumin incubation as well as 24 and 48 hours after replacement of albumin by fibrinogen with human plasma.13,14 Platelet adhesion was quantified by fluorescence microscopy equipped with a JAI-CCD camera coupled to a Matrox Frame grabber (Matrox Electronoc Systems, Ltd) with Optimas 6.0 software (Optimas Inc) for image analysis and expressed in percentage of surface coverage. In addition, fibrinogen binding was tested in coated and uncoated stainless steel samples, which were incubated with human plasma for 4 days. The amount of fibrinogen adhering to the stent surface was measured by a modified ELISA method.

Statistical Analysis
Data are expressed as mean±SD. Continuous variables were compared by 1-way ANOVA or an unpaired, 2-sided Student’s t test. Data were analyzed with SPSS statistical software, version 10.0. Statistical significance was assumed at a value of P<0.05.

Results
The stents were implanted in either the left anterior descending, left circumflex, or right coronary artery. Numbers and distributions of stents in the 3 segments were similar with respect to coating status (Table 1). There were no differences in balloon-to-artery ratio and injury score between the 3 groups. Angiography showed full stent expansion and no difference in vessel diameters before and after stent deployment between coated and uncoated stents. At follow-up, there were no angiographic signs of restenosis in coated and uncoated stent segments.

Platelet Adhesion and Fibrinogen Binding
Data for platelet adhesion and fibrinogen binding are depicted in Figure 3. At baseline, percentage surface coverage with fluorescent platelets was significantly less for TiNOX 1 (65±3%; P<0.05) than for TiNOX 2 (72±5%) or stainless steel (71±4%) samples. Incubation with albumin resulted in near abolition of platelet adhesion in all 3 samples. After 48 hours of incubation with human plasma, subsequent albumin replacement with fibrinogen revealed less platelet adhesion for both TiNOX 1 (17±3%) and TiNOX 2 (15±3%) than for stainless steel (23±2%; P<0.05) samples.

Fibrinogen binding was significantly reduced with TiNOX 1 (82±4%) and TiNOX 2 (54±3%) compared with stainless steel (100%, P<0.05) samples. The attenuation of fibrinogen binding, however, was significantly stronger for TiNOX 2 than TiNOX 1 (P<0.05).

Histomorphometry
Morphometric analyses of coated and uncoated coronary stent segments are summarized in Table 2. Stented coronary artery segments revealed more neointimal hyperplasia than unstented control segments. Neointimal area was significantly reduced in coated stents, by 44% (TiNOX 1, P<0.02) and 47% (TiNOX 2, P<0.02), compared with uncoated stents (Figure 4). Neointimal thickness was significantly diminished.
Table 2. Morphometric Data

<table>
<thead>
<tr>
<th></th>
<th>Uncoated</th>
<th>TiNOX 1</th>
<th>TiNOX 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neointima, mm²</td>
<td>2.61 ± 1.12</td>
<td>1.47 ± 0.84</td>
<td>1.39 ± 0.93</td>
</tr>
<tr>
<td>Media, mm²</td>
<td>2.17 ± 2.09</td>
<td>1.56 ± 0.53</td>
<td>1.33 ± 0.57</td>
</tr>
<tr>
<td>Lumen, mm²</td>
<td>3.72 ± 2.01</td>
<td>3.90 ± 1.93</td>
<td>3.93 ± 1.72</td>
</tr>
<tr>
<td>Vessel area, mm²</td>
<td>8.49 ± 2.85</td>
<td>6.93 ± 2.04</td>
<td>6.66 ± 1.47</td>
</tr>
<tr>
<td>Neointimal thickness, mm</td>
<td>0.30 ± 0.15</td>
<td>0.18 ± 0.11</td>
<td>0.17 ± 0.12</td>
</tr>
<tr>
<td>Restenosis, %</td>
<td>44 ± 26</td>
<td>29 ± 17</td>
<td>27 ± 18</td>
</tr>
</tbody>
</table>

*p<0.02; †P<0.05.

in coated (TiNOX 1, 0.18 ± 0.11 mm; TiNOX 2, 0.17 ± 0.12 mm) compared with uncoated (0.30 ± 0.15 mm, P<0.05) stents. There were no differences in media surface area between coated and uncoated stents. Luminal surface area was nonsignificantly increased in coated compared with uncoated stents. Assessment of total vessel area revealed that vascular remodeling in coated stents was mainly due to a reduction in neointimal hyperplasia without significant changes in media and luminal surface area.

**Discussion**

In-stent restenosis has been observed in 20% to 30% of patients undergoing stent implantation, depending on the length of the implanted stents and other risk factors, such as diabetes, presence of multiple stents, vessel size, amount of residual plaque burden, and extent of arterial injury. Late lumen loss after coronary artery stenting has been attributed to neointimal proliferation, as demonstrated by intravascular ultrasound (constrictive remodeling). 17, 18

**Pathophysiology**

Platelet activation and thrombus formation resulting from arterial injury are pivotal to the initiation of smooth muscle cell proliferation and extracellular matrix formation. A recent investigation on the pathobiology of in-stent restenosis in humans related neointimal hyperplasia to staged redifferentiation of migrating smooth muscle cells. 8, 19 The initial event appears to be platelet adhesion with thrombus formation adjacent to the stent struts, followed by invasion of cellular components, such as macrophages and undifferentiated spindle-shaped cells. The release of cytokines and growth factors from activated platelets and macrophages subsequently promotes smooth muscle cell migration and extracellular matrix formation.

Diminished neointimal hyperplasia has been observed in platelet-depleted rats, whereas the potential for neointimal hyperplasia was restored by reinfusion of platelets 2 weeks after the injury. 20 Conversely, thrombin plays an important role in generating the platelet-rich thrombus at the site of the vascular injury and is the most potent activator of platelets and mediator of smooth muscle cell migration. Thrombin inhibition by administration of hirudin has proved effective in reducing neointimal hyperplasia after balloon angioplasty in the animal model. 21

**Stent Coating With Titanium**

Previous examinations of the biocompatibility of various metals have indicated that in addition to their excellent mechanical performance, stainless steel and gold have an increased electrochemical surface potential, allowing the transfer of electrons to proteins, such as fibrinogen. This promotes thrombus formation and neointimal hyperplasia. In contrast, titanium, with its low electrochemical surface potential, is biologically inert and has excellent biocompatibility, exemplified by the lack of a redox and hydrolysis reaction as well as an absent complex formation (Table 3). Thus, TiNOX is a novel compound that renders stainless steel stents biologically inert and prevents metallic foreign body reaction.

Two TiNOX coatings with different material properties were investigated in this study. TiNOX 1 was characterized by ceramic (resistivity of 6x10¹⁰ Ω · m) and TiNOX 2 by metallic (resistivity of 1x10⁸ Ω · m) properties. In vitro experiments showed a reduced platelet adhesion for TiNOX 1 at baseline and for both TiNOX 1 and 2 after 48 hours’ incubation with human plasma. In addition, fibrinogen binding was reduced for both TiNOX 1 and 2, although attenuation was significantly stronger for TiNOX 2. Nevertheless, the antiproliferative effect was similar for TiNOX 1 and 2, suggesting that the electrochemical properties are more important for attenuation of neointimal proliferation than the observed differences in platelet adhesion and fibrinogen binding.

**Effect of Stent Coating on Arterial Remodeling**

Coated stents are intended to create a biologically inert barrier between stent surface and circulating blood. Several stent coatings have been tested either experimentally or clinically, including heparin, silicon carbide, gold, polymers with and without drug elution, and radioactive coatings. Heparin-coated stents have been shown to reduce subacute stent thrombosis. 22, 23 Despite an antiproliferative effect of heparin under in vitro conditions, however, no reduction in neointimal proliferation was observed in histopathological examinations of porcine arteries. Silicon carbide, a semicon-
ducteur ceramic, deposited on the surface of coronary artery stents reduces the electrochemical surface potential of stainless steel stents, which are known to be thrombogenic. In a clinical study of 165 consecutive patients implanted with 215 silicon carbide–coated stents (Tensum, Biotronik Inc), stent thrombosis and in-stent restenosis rates were 2% and 27%, respectively.24 No histopathological data on the neointimal response to silicon carbide–coated stents are available at present. Stent coating with gold has been used in 2 commercially available coronary artery stents (InFlow, Inc, and NIR Royal, Boston Scientific). A recent randomized clinical trial demonstrated significantly increased in-stent restenosis in gold-coated Inflow stents compared with identical uncoated stainless steel stents (gold 50% versus uncoated 38%, P<0.01),25 proving gold to be less suited for stent coating. Phosphorylcholine, the major phospholipid component of biological membranes, has been used as a stent coating (Biodyntivo stent, Biocompatibles, Ltd), serving as an inert membrane coverage. Polymers with and without drug elution have been used as stent coatings, with various results. A recent multicenter investigation of 8 different biodegradable and nondegradable polymers showed marked inflammatory reactions with subsequent neointimal hyperplasia.26 In contrast, Alt et al27 observed a reduction of neointimal hyperplasia by ≈25% with polyactic acid releasing recombinant polyethylene glycol (r-PEG)-hirudin and iloprost in both the sheep and pig models.

Limitations
The findings of our study have to be interpreted in light of the following limitations: (1) The study was carried out in pigs with normal coronary arteries. These data certainly require validation in future clinical trials. (2) Although TiNOX–coated stents demonstrate attenuated platelet adhesion and reduced fibrinoid binding, other mechanisms leading to reduced neointimal hyperplasia are possible. (3) The extent of neointimal hyperplasia for the control stainless steel stents in our study is comparable to that in previous reports, with several studies showing similar or even more severe neointimal hyperplasia in the porcine restenosis model.27–30 Differences in the amount of neointimal hyperplasia may be related to the degree of overstretch, the time of follow-up, and the size of the artery. To circumvent this problem, each animal served as its own control by having a control stent randomly implanted in 1 of the 3 coronary arteries in our study.

Acknowledgments
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References
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